Journal of Applied Pharmaceutical Research
Volume 14 Issue 3, Year of Publication 2026, Page 215 – 234
DOI: https://doi.org/10.69857/joapr.v14i3.2095

Check for updates

Research Article

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

DEVELOPMENT AND OPTIMIZATION OF A MECHLORETHAMINE TOPICAL FORMULATION UTILIZING A MODEL DRUG BRIDGING STRATEGY AND QUALITY BY DESIGN

Hemil Shah, Prachi Pandey*, Priyal Patel

Article Information
Received: 14th January 2026
Revised: 2nd April 2026
Accepted: 27th April 2026
Published: 15th May 2026

Keywords
Mechlorethamine Topical Formulation, Quality by Design (QbD), Model Drug Bridging, Formulation Design Space, Stability Testing.

ABSTRACT
Background: This study aimed to develop a stable, clinically suitable topical mechlorethamine formulation using Quality by Design (QbD) principles to address chemical instability and cytotoxicity. The objectives were to establish a data-driven formulation design space, identify critical material attributes, and apply a surrogate active pharmaceutical ingredient (API) approach to ensure biological performance while enabling safe early-stage optimization. **Methodology:** A 2^3 full factorial Design of Experiments (DOE) was employed to evaluate the effects of ethanol, isopropyl myristate (IPM), and Carbopol 974P on assay concentration, viscosity, and pH. Regression modeling and response surface analysis were used to define the formulation design space. Diclofenac sodium was used as a surrogate API to study matrix behavior prior to the incorporation of mechlorethamine. The optimized formulation was subjected to long-term, refrigerated, and accelerated stability studies. **Results and Discussion:** Carbopol 974P concentration was identified as the primary factor influencing viscosity, while assay concentration and pH remained stable across the design space. The optimized formulation (73% ethanol, 3% IPM, and 0.12% Carbopol 974P, pH 5.5) met all Quality Target Product Profile criteria. Bridging studies demonstrated comparable physicochemical properties between surrogate and mechlorethamine-loaded formulations, indicating excipient-driven performance. Under ICH stability conditions, the optimized formulation retained 98.3% assay after 3 months at 40°C/75% RH (0.9% loss), with total impurities remaining at 0.19% (NMT 0.5%), stable viscosity (40–60 cps), controlled pH (5.51–5.53), and no physical instability. **Conclusion:** The QbD-driven approach enabled the development of a stable, reproducible, and biologically effective topical mechlorethamine formulation suitable for scale-up and regulatory advancement.

INTRODUCTION

Cutaneous T-cell lymphomas (CTCLs) are a group of non-Hodgkin T-cell tumors that primarily affect the skin and don't involve other parts of the body at first. CTCLs constitute approximately 65–75% of all primary cutaneous lymphomas.

*Krishna School of Pharmacy and Research, KPGU, Vadodara-Mumbai National Highway (NH-48), Varnama, Vadodara, Gujarat, India 391243.

***For Correspondence:** **prachipandey.ksp@kpgu.ac.in**

These are groups of diseases that present very differently in the clinic, in histopathology, in biological behavior, and in prognosis [1,2]. Because of this variability, it is very important to accurately classify diseases to make appropriate treatment and prognosis decisions [3]. The most common type of CTCL is mycosis fungoides (MF). In most cases, it starts with patches and plaques on the skin and slowly worsens [4]. However, disease progression happens in only a small number of cases, and the risk of nodal, hematogenous, or visceral involvement is higher in advanced stages [5–8]. Sézary syndrome (SS) is a rare but aggressive type of CTCL characterized by generalized erythroderma, lymphadenopathy, and circulating malignant Sézary cells [2]. CD30-positive (CD30+ LPDs), such as lymphomatoid papulosis and primary cutaneous anaplastic large-cell lymphoma (pcALCL), account for about 11% of CTCLs. These disorders usually have a good prognosis, but patients are at a higher risk for secondary lymphoid malignancies [2,9]. From an epidemiological standpoint, the global incidence of CTCL is estimated at fewer than 10 cases per 100,000 individuals annually [10].

The disease, on the other hand, is much more common, up to ten times more common, because people with early-stage disease live longer [9].MF and SS consistently exhibit a male predominance (approximately 1.7:1) and typically manifest in adults aged 50 to 70 years [9,11,12]. Patients diagnosed at an early stage have a lower median age (57 years) than those with advanced disease (63 years) [12]. Early-stage MF is often misdiagnosed because it looks a lot like benign inflammatory dermatoses, but advanced disease is usually diagnosed within a year of the first symptoms [13]. While incidence rates seem to be fairly stable across different areas, studies have shown that non-white populations are more likely to recognize hypopigmented MF [14-16].

Clinical outcomes in CTCL are significantly influenced by disease stage. Five-year overall survival estimates range from 49% to 100% in early-stage MF and drop sharply to 0% to 65% in advanced disease [5,7,17-19]. Prognosis is also affected by various factors, including positive features such as poikilodermatous or hypopigmented lesions, and negative features such as advanced age at diagnosis, large-cell transformation, folliculotropic involvement, and elevated serum lactate dehydrogenase levels [12]. The PROCLIPI (PROspective International Cutaneous Lymphoma Prognostic Index) study has generated substantial global prospective data on MF and SS, incorporating clinical, pathological, molecular, therapeutic, quality-of-life, and survival metrics, thereby making a significant contribution to disease stratification and risk assessment [11,13,14,20,21].

Mechlorethamine hydrochloride (also known as chlormethine or nitrogen mustard) has historically been a key drug for treating early-stage CTCL, especially MF. Mechlorethamine is a bifunctional alkylating agent that causes cell death by creating highly reactive aziridinium intermediates that alkylate nucleophilic sites on DNA and proteins. This causes DNA cross-linking, stops replication, and causes malignant T cells to die [22]. Mechlorethamine, the first nitrogen mustard, is historically significant as one of the first chemotherapeutic agents used in clinical practice; its therapeutic role has since changed to topical use for localized disease. In the past, topical mechlorethamine was given in compounded aqueous solutions or ointments, which varied widely in strength, stability, and ease of use. This inconsistency in formulation often led to a loss of drug activity, a short shelf life, and a high rate of irritant or allergic contact dermatitis. To overcome these constraints, a commercially produced mechlorethamine gel (Valchlor®/chlormethine gel) with a standardized formulation and confirmed stability was developed and subsequently approved by the U.S. Food and Drug Administration in August 2013 for the treatment of Stage IA–IB MF [23]. The availability of this regulated product underscored the importance of having strict chemistry, manufacturing, and controls (CMC) in place to ensure the product was safe, effective, and of high quality [24].

Figure 1 presents a comprehensive review of the clinical context of cutaneous T-cell lymphoma (CTCL), the molecular mechanism of action of topical mechlorethamine, and the formulation strategy driven by Quality by Design (QbD) utilized in this work. Early-stage mycosis fungoides (MF) is generally limited to patch and plaque lesions on the skin, whereas advanced disease, such as Sézary syndrome, is characterized by diffuse erythroderma and systemic involvement. Upon topical administration, mechlorethamine undergoes intramolecular cyclization to form a reactive aziridinium intermediate that can alkylate DNA in malignant T cells, ultimately leading to apoptosis. Due to the inherent chemical instability of this nitrogen mustard and the necessity for consistent dermal delivery, the formulation development program adhered to a structured Quality by Design (QbD) framework, which included defining the Quality Target Product Profile (QTPP), identifying

critical quality attributes (CQAs), conducting systematic experimental optimization, and bridging model-drug to create a scientifically sound and robust design space for final product development.

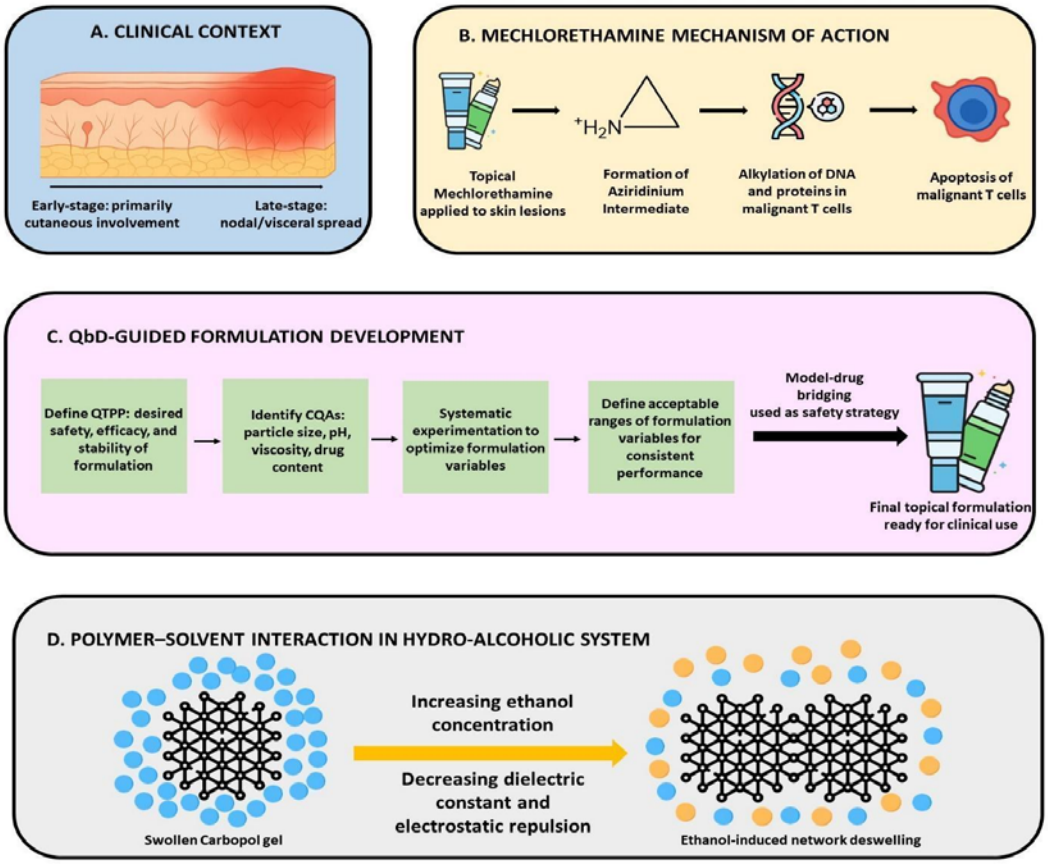


Figure 1: Overview of the clinical rationale, molecular mechanism, and QbD-driven formulation strategy for a stable topical mechlorethamine product, including solvent–polymer interactions affecting viscosity

Recent clinical and translational research conducted in 2023–2024 has broadened the therapeutic and regulatory framework for topical mechlorethamine, underscoring the necessity for meticulous formulation development. A clinical trial comparing a non-chemotherapeutic topical alternative (synthetic hypericin, HyBryte™) to mechlorethamine gel showed similar lesion responses and better tolerability, indicating continued interest in non-alkylating topical agents and that long-term management requires good local tolerability [25]. Real-world and observational studies published in 2023–2024 continue to support the clinical utility of chlormethine gel across early and

some advanced MF cohorts, while also documenting contact dermatitis & other local reactions as frequent causes for treatment modification — findings that directly inform acceptable quality attributes for topical product [24,26,27]. Recent FDA product-specific guidance and regulatory review documents stress the need for stability-indicating analytical methods and, when appropriate, clinical endpoint-driven comparability or bioequivalence assessments for topical mechlorethamine products. This is because the compound is chemically unstable and cytotoxic [28]. Recent investigations have demonstrated the expanded implementation of Quality by Design (QbD) methodologies in semi-solid and transdermal systems, with particular emphasis on risk-based formulation development, critical quality attribute mapping, and structured design space establishment in topical drug products [29,30]. At the same time, advances in preclinical and materials science suggest possible ways to improve chemical protection, controlled dermal release & local tolerability for topical antineoplastics [31]. These technologies are mostly still in the preclinical stage, but they show how a QbD framework can be used to evaluate mechanistic approaches within a defined design space. All of these changes in the clinical, regulatory, and technological fields make a systematic QbD approach with model-drug bridging necessary during early experiments to (i)

allow for strong stability-indicating analytics, (ii) reduce the risks of occupational exposures, and (iii) define a safe, efficacy-preserving formulation design space for mechlorethamine topical products. Even though it has been shown to work well in the clinic, the physicochemical properties of mechlorethamine make it very difficult to make. The molecule is highly chemically unstable and rapidly decomposes in aqueous and semi-solid environments, generating reactive aziridinium ions that readily react with water, excipients, and nucleophiles. This causes assay loss and complicated degradation profiles [32]. Aside from these general worries about instability, the way mechlorethamine breaks down is caused by its own electrophilicity. The molecule quickly forms a highly reactive aziridinium intermediate by self-cyclization. Water or other nucleophiles can then open this intermediate, which results in hydrolyzed, inactive products. This aziridinium-mediated hydrolysis has a low activation energy and follows biphasic kinetics. This is why topical products that are aqueous or semi-aqueous have lost their effectiveness quickly in the past. Aqueous solutions usually break down within a few days, but non-aqueous systems remain stable for much longer by reducing the amount of water available for activity. Oxidative pathways may also help break things down, especially in cars that use glycols or ether-based solvents. That's why different ways of making things need antioxidant systems like BHT. These mechanistic insights show that the most important formulation challenge is ensuring the molecule remains protected from water, nucleophiles, and oxidative conditions to maintain its potency during manufacturing, storage, and clinical use [33]. Because of this natural instability, there are strict limits on how formulations can be made. This means that the amount of water, pH, compatibility with excipients, antioxidants, and packaging systems must all be carefully controlled.

Historically, these breakdown routes rendered compounded mechlorethamine formulations unstable and resulted in varying potency levels. Many of these formulations required refrigeration and had very limited beyond-use dates due to rapid degradation and hydrolysis. The rapid formation of numerous degradation products necessitates the development of robust, stability-indicating analytical procedures. These technologies, typically high-performance liquid chromatography, are frequently employed in conjunction with mass spectrometry to detect contaminants and ensure regulatory compliance [34].
Mechlorethamine is a high-potency active pharmaceutical ingredient (HPAPI), necessitating adherence to stringent safety and containment regulations throughout its development and manufacturing processes. These include closed handling systems, OEL-based exposure controls, customized HVAC systems, and effective cleaning methods [35]. These restrictions render conventional empirical formulation approaches highly constrained and call for the adoption of alternative, less hazardous development procedures. Achieving an optimal balance between effective skin penetration and satisfactory local tolerability remains a significant challenge, particularly given chemical and occupational constraints. The composition of vehicles, solvent systems, penetration enhancers, polymeric gelling agents, and rheological properties all influence the efficacy of transdermal drug delivery and the incidence of adverse effects, such as contact dermatitis. Prior research comparing various topical vehicles concurrently has revealed distinct features regarding their release efficacy and tolerance levels. This illustrates the significance of creating coherent formulations [36]. The chemical, analytical, safety, and performance limitations render traditional empirical formulation screening approaches inefficient. This emphasizes the need for a systematic, risk-focused development methodology. This work sought to formulate and enhance a resilient topical mechlorethamine preparation using a comprehensive Quality by Design (QbD) methodology to address diverse clinical, chemical, and operational limitations. The main objectives were to create a scientifically defined formulation design space that regulates critical quality attributes, to reduce the risk of worker exposure to hazardous substances during the early stages of formulation development by employing a surrogate model drug strategy, and to develop a stable and reproducible formulation that adheres to regulatory quality standards for topical cytotoxic therapies. Following the ICH Q8(R2) guidelines [37], the Quality by Design (QbD) framework was utilized to clarify the relationships between critical material attributes, formulation variables, and product efficacy. This enabled the establishment of a secure operational design area that guarantees consistent product quality. Moreover, the incorporation of systematic physicochemical and rheological optimization enabled the methodical improvement of a formulation characterized by enhanced resilience and reproducibility. Utilizing model-drug bridging strategies in the early stages of testing increased laboratory safety, improved understanding of the process, and enabled the systematic development of chemically unstable, high-potency topical formulations such as mechlorethamine [38].

MATERIALS AND METHODS

Materials

Ethanol (96%), purified water, isopropyl myristate (IPM), and carbopol 974P were used to make the formulation. Ethanol enhanced drug permeation through the stratum corneum by acting as the primary solvent for hydrophobic components [39,40]. Carbopol polymer was hydrated and thickened by adding purified water as a cosolvent [41]. IPM acted as a polar emollient to prevent ethanol from drying out and acted as a lipophilic penetration enhancer [39]. Carbopol 974P, a cross-linked acrylic acid polymer with high molecular weight, was chosen to modify rheology because it is stable, adheres to living tissues, and performs well at high ethanol concentrations [41]. When sodium hydroxide is added to the polymer, it ionizes and expands, thereby thickening the formulation [41]. Diclofenac Sodium served as a model drug during the screening and optimization phases because it is ionic and readily dissolves in hydroalcoholic systems. This made it possible to test the rheological and pH design spaces without the problems associated with using a cytotoxic agent [37,42]. Mechlorethamine was the main active pharmaceutical ingredient in the final product. All materials were of pharmaceutical grade and used as received.

Quality Target Product Profile (QTPP)

The Quality Target Product Profile (QTPP) summarizes the quality characteristics of a drug product, including crucial factors for drug safety, effectiveness, and patient acceptance. The QTTP for topical mechlorethamine solution was designed in line with the Quality by Design (QbD) principles, ensuring maximum topical absorption and minimum systemic toxicity [43,44]. The clinically established therapeutic concentration of mechlorethamine for maintenance therapy is 0.02%. During development, a 2% model load was used to determine the formulation vehicle's solubility limits and to provide a "worst-case" scenario for optimization. Stability criteria were set to ensure the product could be stored at room temperature for at least 24 months. This made it much easier for patients to stick to their treatment plan and manage their supply chain because it didn't need to be kept cold. Also, the formulation was described as a clear, colorless-to-pale-yellow, thick liquid.

This visual specification ensures that there is no rain and that the only color changes are small, caused by trace oxidation. These changes must not be the same as signs of major degradation that could make something unsafe or less effective. Taken together, these QTPP elements set the target characteristics for the topical mechlorethamine solution. The QTTP framework serves as the foundation for identifying Critical Quality Attributes (CQAs) and designing experiments (DoE), which in turn help improve formulation characteristics and reduce risks associated with scale-up and commercialization [43,44].

Critical Quality Attributes (CQAs)

Assay, pH, and viscosity are the three main CQAs for the topical mechlorethamine solution, identified using the QTTP framework. Assay, which ranges from 90-110%, quantifies the actual amount of drug against the label claim. This helps ensure precise dosing and minimize dose variability. The pH of the mechlorethamine solution should be maintained within the range of 5-6 (close to the skin's natural acid mantle) to avoid degradation in an extremely alkaline environment, support long-term API stability, and address acid sensitivity issues in CTCL patients. The viscosity of the mechlorethamine solution should be maintained within the range of 40-60 centipoise (cps) that strikes a balance between spreading consistency and therapeutic effectiveness. These CQAs work together to provide measurable standards that help us develop formulations, control processes, and verify quality. They ensure that all of these things align with the main QTPP goals [45-47].

Analytical Methods

Validated analytical methods compliant with ICH Q2(R1) guidelines [47,48] were employed to evaluate the critical quality attributes (CQAs), including pH, viscosity, and assay of mechlorethamine and model drug formulations. High-performance liquid chromatography (HPLC) with UV detection was used for quantitative analysis of the active pharmaceutical ingredients. Chromatographic separation was achieved using a C18 reverse-phase column (250 × 4.6 mm, 5 µm particle size). The mobile phase consisted of methanol and 50 mM ammonium formate buffer (30:70, v/v), with the buffer pH adjusted to 4.5 using formic acid. The flow rate was maintained at 1.0 mL/min under isocratic conditions, with an injection volume of 20 µL. Detection wavelengths were set at 254 nm for diclofenac sodium and 210 nm for mechlorethamine. Samples were appropriately diluted within the validated linearity range, and five-point calibration curves were constructed for quantification. Assay results were expressed as a percentage of the labeled claim. The method demonstrated acceptable precision (RSD < 2%) and accuracy (recovery 98–102%), confirming its suitability as a stability-indicating method [49,50]. Formulation pH was

measured with a calibrated digital pH meter after dispersing 10 mL of the formulation in distilled water, and readings were recorded in triplicate. Viscosity was determined at 25°C using a rotational viscometer. Stability studies were conducted under accelerated (40°C/75% RH), ambient (25°C/60% RH), and refrigerated (2–8°C) conditions at predefined intervals [51]. Analytical data were statistically evaluated using Design-Expert software.

Formulation Strategy

The formulation was designed in accordance with the QbD principles. A non-aqueous, hydroalcoholic vehicle system was used to ensure optimal dissolution of the API and to reduce its hydrolysis. Ethanol improved permeation through the stratum corneum and faster drying after being applied to the skin [52]. Isopropyl myristate increased lipid bilayer fluidity in the epidermis and protected the skin from excessive drying caused by ethanol [53]. Optimum rheology was attained by using carbopol 974P [45]. A 2^3 factorial design of experiments revealed that polymer content was the primary factor affecting the formulation's rheology.

Statistical modeling further confirmed that minor variations within the design space do not affect the assay or pH, thereby demonstrating the robustness of the formulation [46]. A bridging study using diclofenac sodium as a surrogate compound confirmed that the rheological behavior and pH characteristics of the model formulation were representative of those of mechlorethamine at the intended target concentration of 0.02%.

Furthermore, stress testing under accelerated and refrigerated conditions confirmed both physical and chemical stability, with no evidence of precipitation, phase separation, or loss of clarity, indicating maintenance of a homogenous system [46]. Thus, the optimized combination of hydro alcoholic solvent system, IPM, and carbopol 974P resulted in a patient-friendly, skin-compatible, and storage-stable formulation [54].

Design of Experiments (DoE)

A full 2^3 factorial DoE was employed to evaluate the effects of key excipients on CQAs, including assay, pH, and viscosity. This design enables simultaneous assessment of three variables at two levels, allowing identification of both main and interaction effects with a limited number of experiments [55,56]. Ethanol (Factor A, 70-80%), IPM (Factor B, 1-5%), and carbopol 974P (Factor C, 0.1-0.5%) were selected as high-risk factors based on initial risk assessment. Ethanol levels were adjusted to ensure the solubilization of lipid components while minimizing volatility and skin irritation; IPM concentrations were optimized for emolliency and phase stability; and carbopol levels were chosen to achieve suitable rheological properties for topical application [57,58]. Factor levels were coded from -1 to +1 to standardize comparisons across variables. DoE data were analyzed using Design-Expert software, and regression models were developed to quantify factor-response relationships. Model significance and adequacy were confirmed by analysis of variance (ANOVA), diagnostic plots, coefficients of determination, and lack-of-fit tests. This statistical approach enabled the identification of robust operating ranges and supported formulation optimization within the QbD framework [55-58]. To balance experimental efficiency with occupational safety considerations associated with cytotoxic mechlorethamine handling, a 2^3 full factorial design was deliberately selected as a structured screening approach to identify significant main effects and two-factor interactions on CQAs. ANOVA and lack-of-fit testing confirmed model adequacy within the explored design space, with no statistically significant curvature detected. Although isolated viscosity responses (e.g., Run 7) suggested potential non-linearity, the predictive performance of the linear model remained acceptable for factor prioritization and design space establishment. Advanced quadratic response surface methodologies (e.g., Box–Behnken or Central Composite Design) are reserved for subsequent optimization and scale-up phases to further refine rheological mapping if required.

Manufacturing Process Development

The process was conducted in defined stages to ensure complete solubilization, uniform dispersion, and stabilization of all components, while preserving the integrity of the polymer network and API stability. The solvent phase was prepared by blending water and ethanol at low shear (500-600 rpm) for 10 minutes to ensure homogeneity and prevent air entrapment. The model drug was then dissolved under moderate shear to ensure uniform solubilization. Carbopol 974P was subsequently dispersed under high shear (800-1000 rpm for 50-65 mins) to prevent agglomeration and enable full polymer hydration, a critical requirement for cross-linked carbomers. Neutralization was performed using 0.1 N NaOH under controlled shear to achieve a target pH of 5.5. For a 100 g batch containing 0.12% w/w Carbopol 974P, approximately 8.5 mL of 0.1 N NaOH was required, corresponding to ~70–75 mL of base per gram of

polymer. The base was added dropwise under continuous mixing (600–800 rpm) to ensure uniform ionization of the carboxylic acid groups and complete polymer expansion without localized over-neutralization. Precise control of the base addition rate and final pH was identified as a critical process parameter (CPP) that influences gel viscosity and microstructural integrity. Mixing speed, shear history, and the order of addition were identified as critical process attributes (CPPs) that influence viscosity, homogeneity, and stability. This staged mixing strategy enabled consistent attainment of target CQAs and supported development of a robust, scalable manufacturing process [59].

Model Drug Bridging

To mitigate occupational safety risks associated with the highly cytotoxic mechlorethamine during formulation optimization, diclofenac sodium was employed as a model drug. A confirmatory bridging study was conducted to verify the predictive capability of DoE and to assess whether the model compound adequately represented the target API with respect to CQAs. Test formulations were prepared using equivalent excipient compositions, containing diclofenac (2%) or mechlorethamine (0.02%) as the active ingredient, with ethanol (73%) as the solvent, IPM (3%) as the permeation enhancer, and carbopol 974P (0.12%) as the viscosity modifier. The pH was adjusted to 5.5 using 0.1 N NaOH, and purified water was added to achieve the final formulation volume. This bridging approach enabled safe evaluation and optimization of the topical vehicle prior to the incorporation of cytotoxic API, in accordance with QbD principles [60-62].

Stability and Stress Testing

The optimized topical mechlorethamine formulation underwent comprehensive stability and stress testing to assess its chemical and physical stability across varied environmental conditions. Stability studies were conducted under refrigerated (2-8°C), long-term (25 °C/60% RH), and accelerated (40 °C/75% RH) conditions in accordance with ICH stability guidelines to assess degradation behavior, impurity formation, and physical performance over time. Chemical stability was evaluated using validated HPLC methods developed and applied in compliance with ICH(Q2) R1 requirements. Physical stability was assessed through periodic evaluations of viscosity, pH, and visual appearance to detect potential changes, including phase separation, precipitation, or discoloration. Additional short-term stress studies were performed at an elevated temperature (60°C) and under refrigerated conditions to assess the formulations' robustness to extreme thermal stress. These studies were designed to ensure that the hydro-alcoholic vehicle, polymer network, and buffering system remained intact, thereby supporting formulation stability across anticipated storage, handling, and use conditions in line with QbD principles [48,49,63].

RESULT AND DISCUSSION

DOE Outcomes and Statistical Modeling

Raw data and Replicate Analysis

Tables 1 show the experimental design matrix and the observed responses for assay, viscosity, and pH. The eight-run factorial design incorporated systematic variations in the quantities of Ethanol, IPM, and carbopol 974P at elevated and diminished levels to evaluate the impact of the formulation's composition on CQAs. The assay values varied from 98.7% to 101.1%, indicating uniform distribution of the drug content throughout the design area. The pH measurements remained consistent, ranging from 5.46 to 5.59, indicating suitability for the skin and demonstrating the formulation's robustness. Viscosity exhibited significant variation (28-152cP), indicating that rheological behavior is highly responsive to polymer concentration and the interactions between polymers and solvents. When the carbopol quantity was minimal (runs 3, 6, and 8), the viscosity values remained low. At higher carbopol concentrations (Runs 4 and 5), the viscosity values were markedly elevated; despite containing 0.5% w/w Carbopol 974P, Run 7 (80% ethanol, 1% IPM) exhibited a markedly reduced viscosity (28 cP). This behavior reflects a solvent-quality-driven microgel collapse phenomenon rather than simple polymer deficiency. Carbopol is a crosslinked polyacrylic acid polymer whose viscosity development depends on ionization of carboxylate groups and subsequent electrostatic repulsion-driven chain expansion in a hydrated environment. At elevated ethanol concentrations (80%), the dielectric constant of the continuous phase decreases substantially compared to aqueous systems. This reduction in polarity limits carboxylate ionization and weakens electrostatic repulsion between polymer chains, thereby suppressing volumetric expansion of the swollen microgel network. Simultaneously, the presence of IPM introduces a hydrophobic component that may further reduce effective solvent quality and promote preferential solvent–solvent interactions over polymer–solvent interactions. Under these high co-solvent conditions, the polymer network undergoes deswelling and partial microgel contraction, resulting in diminished macroscopic viscosity despite elevated polymer concentration. This composition, therefore, represents a

boundary condition within the explored formulation space, where solvent-mediated suppression of polymer expansion overrides the thickening contribution of Carbopol. The observed viscosity reduction in Run 7 is thus mechanistically consistent with solvent-induced microstructural collapse rather than experimental anomaly.

Table 1: DOE Experimental Runs with Actual and Coded Factor Levels and Observed Responses

Run	Ethanol (%v/v)	Ethanol (coded)	IPM (% w/w)	IPM (coded)	Carbopol 974P (% w/w)	Carbopol (coded)	Assay (%)	Viscosity (cP)	pH
1	80	+1	5	+1	0.5	+1	101.1	127	5.52
2	80	+1	1	−1	0.5	+1	99.7	121	5.47
3	70	−1	5	+1	0.1	−1	100.9	49	5.51
4	70	−1	5	+1	0.5	+1	98.7	148	5.47
5	70	−1	1	−1	0.5	+1	99.3	152	5.55
6	70	−1	1	−1	0.1	−1	100.2	46	5.59
7	80	+1	1	−1	0.5	+1	99.1	28	5.46
8	80	+1	1	−1	0.1	−1	98.9	34	5.52

ANOVA Significance

The combined ANOVA summary (Table 2), supported by diagnostic plots (Figure 2), demonstrated that each critical quality attribute responded differently to formulation variables. The assay was significantly affected by all main effects and interaction terms, indicating that Ethanol, IPM, and Carbopol each contribute independently and synergistically to drug content uniformity. Similarly, pH exhibited strong statistical sensitivity to all formulation variables, with high F-values indicating that even small compositional changes resulted in measurable shifts. Importantly, these variations remained within the dermally acceptable range, ensuring practical applicability of the formulation. In contrast, viscosity did not show statistically significant linear or interaction effects in the two-level factorial design ($p > 0.05$), as supported by the predicted-versus-actual and residual diagnostic plots (Figure 3). Although viscosity numerically varied between 28 and 152 cP across experimental runs, a two-level factorial design estimates only first-order (linear) and interaction effects.

The absence of statistical significance, therefore, indicates that viscosity is not governed by systematic linear relationships among factors within the evaluated design space. Rather, the observed variability is attributable to nonlinear solvent–polymer equilibrium phenomena intrinsic to Carbopol-based hydroalcoholic systems. At elevated ethanol concentrations, the dielectric constant of the medium decreases substantially, potentially suppressing ionization of Carbopol carboxylate groups and limiting electrostatic chain expansion. Under such conditions, the polymer network may undergo solvent-induced deswelling, resulting in threshold-type rheological transitions. These curvature-driven microstructural changes cannot be fully captured by a first-order factorial model. Importantly, the lack of fit for viscosity was non-significant ($p = 0.6340$), confirming the absence of systematic model inadequacy or experimental anomaly. Further dataset evaluation identified Run 7 (80% Ethanol, 1% IPM, 0.5% Carbopol) as contributing substantially to dispersion, with a measured viscosity of 28 cP. Rather than representing an outlier, this condition reflects a compositional boundary phenomenon consistent with solvent-mediated microgel collapse at high ethanol fractions.

This observation reinforces the interpretation that viscosity behavior is governed by nonlinear solvent–polymer interaction dynamics within the explored formulation domain. This behavior is further supported by the close agreement between predicted and actual values and the uniform distribution of residuals for assay and pH responses (Figure 4).

Despite the absence of statistical significance for viscosity within the first-order model, this does not compromise the robustness of the established design space. Assay and pH—identified as primary critical quality attributes—demonstrated strong statistical significance across both main and interaction effects and therefore served as the fundamental determinants of design space definition. Viscosity limits (40–60 cP) were conservatively established based on experimentally validated acceptable performance ranges rather than statistical extrapolation, ensuring reliable boundary definition while acknowledging inherent rheological nonlinearity. Collectively, these findings confirm that the developed models are suitable for describing the critical formulation behavior within the studied domain and provide a scientifically sound foundation for subsequent response surface refinement and scale-up considerations.

Table 2: Combined ANOVA Significance Summary for All CQAs

Source	Assay(Y1) F-value	Assay p-value	Significance	Viscosity (Y2) F-value	Viscosity p-value	Significance	pH (Y3) F-value	pH p-value	Significance
Model	–	–	Significant	–	–	Not significant	–	–	Significant
A – Ethanol	20951.56	0.00440	Significant	1.19	0.4723	Not significant	233244.08	0.00132	Significant
B – IPM	19892.81	0.00451	Significant	0.00002	0.9970	Not significant	232130.08	0.00132	Significant
C – Carbopol	20784.75	0.00442	Significant	5.71	0.2524	Not significant	222224.08	0.00135	Significant
AB	9504.18	0.00653	Significant	0.0054	0.9532	Not significant	101762.12	0.00200	Significant
AC	10889.14	0.00610	Significant	0.0329	0.8858	Not significant	110459.26	0.00192	Significant
BC	13617.16	0.00546	Significant	0.3839	0.6469	Not significant	153012.31	0.00163	Significant
ABC	8874.88	0.00676	Significant	0.3110	0.6761	Not significant	95632.69	0.00206	Significant
Lack of Fit	–	–	–	–	0.6340	Not significant	–	–	–

Note: The two-level factorial design estimates first-order (linear) and interaction effects. Non-significant viscosity results indicate the absence of systematic linear factor influence within the evaluated design space. The lack-of-fit for viscosity was non-significant (p = 0.6340), indicating no evidence of model inadequacy.

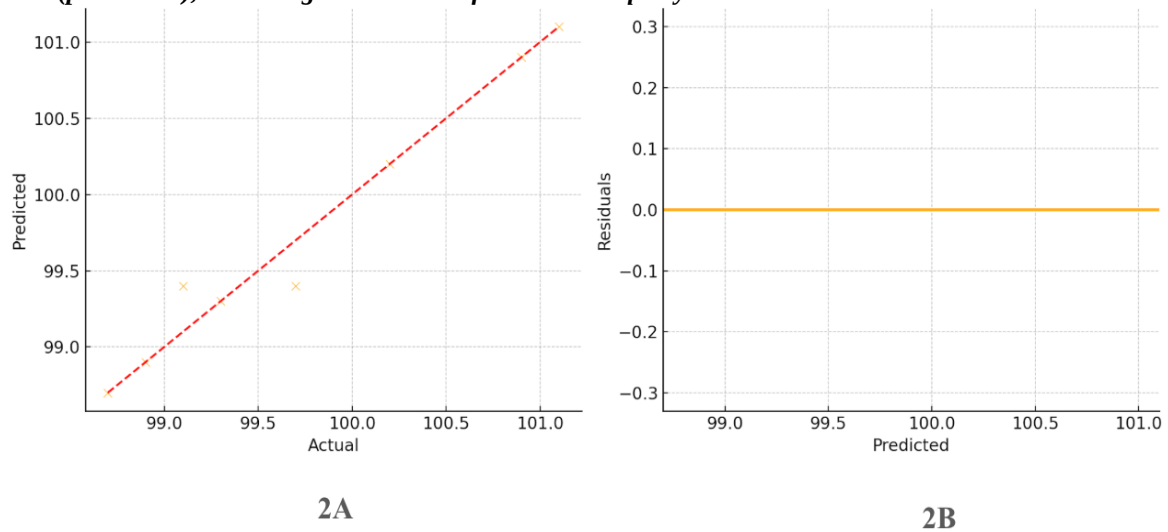


Figure 2: (A) Predicted vs Actual assay values. (B) Residuals vs Predicted assay values.

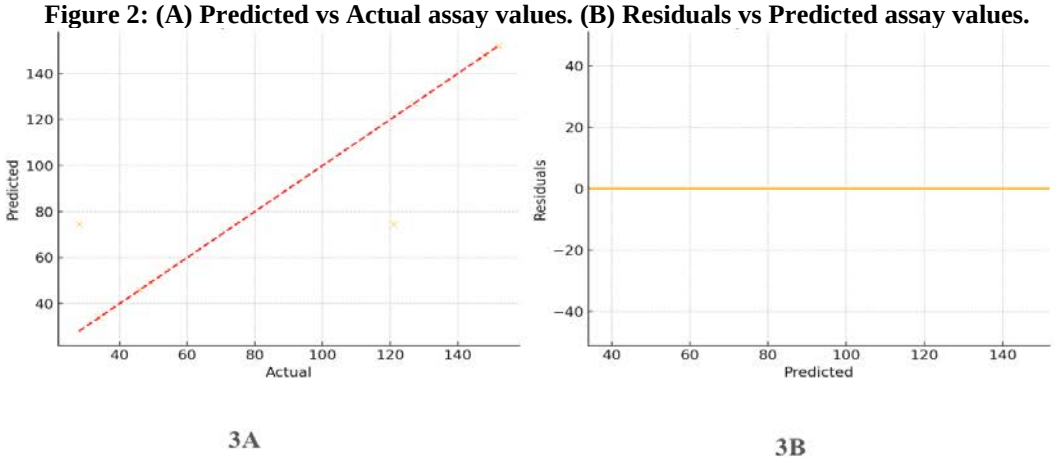
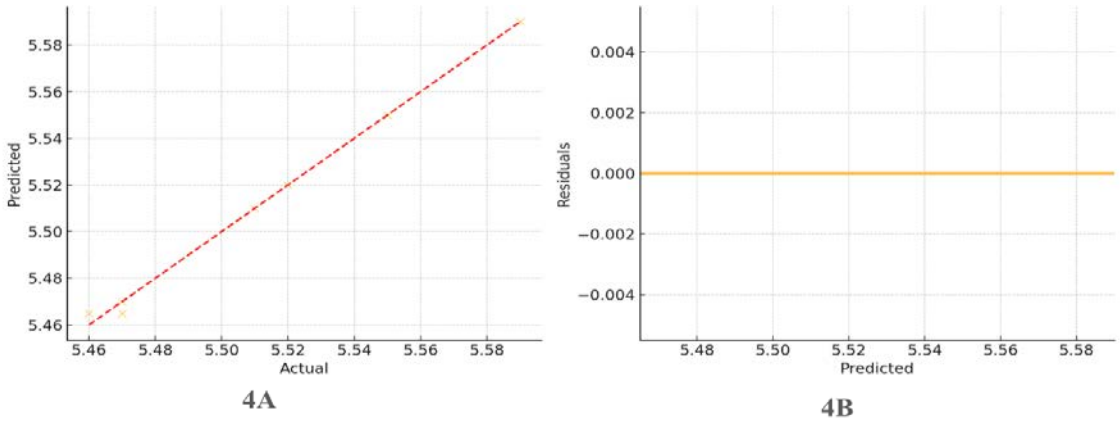


Figure 3: (A) Predicted vs Actual viscosity values. (B) Residuals vs Predicted viscosity values



4A 4B

Figure 4: (A) Predicted vs Actual pH values. (B) Residuals vs Predicted pH values

Response surface analysis

The three-dimensional response surface plots provided a complete picture of how Ethanol and Carbopol interacted to affect the assay, viscosity, and pH. These graphical models enhance the statistical findings by illustrating the interconnections of factors within the formulation space and identifying regions where CQAs remain within acceptable limits. The response surface plot for the assay (Figure 5) indicated that assay values decreased predominantly linearly with increasing Ethanol concentration. This indicates that the solvent system adversely affected the medication content. On the other hand, higher amounts of Carbopol had a small positive effect on the assay. The surface had a gentle slope and no sharp curves, making the reactions and interactions among factors easy to predict. The ANOVA showed significant main and interaction effects, which were consistent with this smooth profile.

This shows that the assay is sensitive to formulation composition, remains stable, and doesn't exhibit any sudden changes within the ranges examined. The viscosity response surface (Figure 5) showed the strongest gradients among the three important quality attributes, indicating that it is the most sensitive to compositional changes. The polymer-induced thickening process increased viscosity significantly when ethanol levels were low and Carbopol levels were high. On the other hand, viscosity decreased significantly as Ethanol levels increased. This shows that interactions between the solvent and the polymer disrupted the Carbopol gel matrix.

The drop in viscosity can be mechanistically ascribed to solvent-induced deswelling of the Carbopol microgel network. Carbopol 974P is a cross-linked poly(acrylic acid) polymer that increases viscosity due to the ionization of carboxylic acid groups upon neutralization, resulting in electrostatic repulsion, uncoiling of polymer chains, and volumetric expansion of the hydrated network. At high concentrations of low-dielectric solvents such as ethanol and isopropyl myristate (IPM), the medium's effective dielectric constant decreases significantly. This reduction constrains carboxylate ionization and diminishes electrostatic repulsion across polymer chains, thereby inhibiting chain expansion. Furthermore, competing ethanol solvation diminishes the accessibility of structured water necessary for optimal polymer hydration. The cumulative effect leads to partial network deswelling, less hydrodynamic volume of the microgel particles, and a subsequent reduction in macroscopic viscosity. The interaction between the solvent and polymer elucidates the sharp gradients noted in the response surface and the significant viscosity reduction at elevated ethanol/IPM ratios (e.g., Run 7), although a constant polymer concentration.

The surface exhibited significant curvature and a wide range of reactions, as observed in experiments (28–152 cP). These data showed that viscosity is affected by complex interactions between the solvent ratio and the polymer concentration. This highlights the importance of properly controlling a formulation's constitution to maintain rheological qualities. The pH surface plot (Figure 5) dropped slowly as ethanol levels increased. However, carbopol levels increase the pH. The narrow pH range was reflected in the smooth, slightly curved surface. ANOVA showed that all main and interaction effects were statistically significant, that the pH shift across the design space was minimal, and that the shift was skin-safe. Viscosity responded strongly to ethanol and carbopol, exhibiting steep gradients and nonlinear behavior. This indicated that the rheological stability was rare. Combining these plots allows for a design environment that maintains assay, viscosity, and pH.

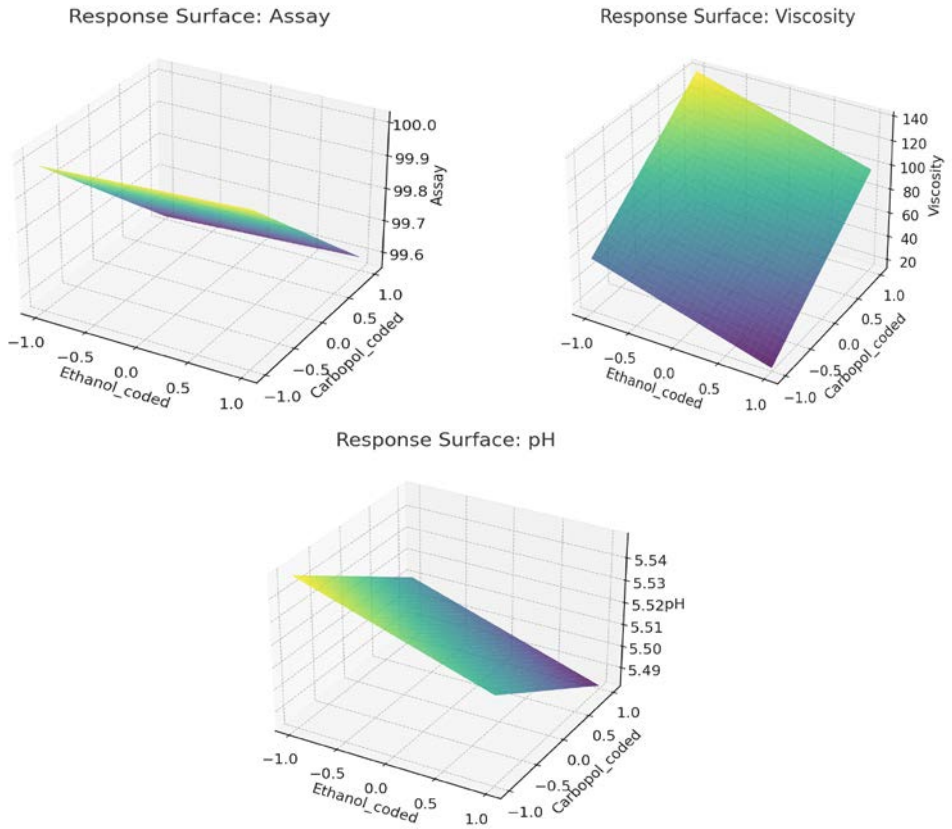


Figure 5: Three-dimensional response surface plots illustrating the combined influence of ethanol and Carbopol 974P concentrations on assay content, viscosity, and pH of the mechlormethamine topical formulation

Statistical Regression Modeling and Interpretation

Regression modeling was utilized to determine the associations between the formulation variables and the CQAs. These encoded polynomial models function as mathematical representations of the design space. The regression analysis of the test revealed a highly interactive system, with all primary effects and higher-order variables considerably affecting the response. Both Ethanol and IPM had negative effects on the assay, which means that the drug content was lower at higher solvent concentrations. On the other hand, Carbopol had a positive effect, stabilizing the assay. The size and direction of the AB, AC, BC, and ABC interaction terms showed that the assay's performance is affected by both positive and negative interactions among the excipients, not by effects that occur on their own. This is in line with the ANOVA results, which showed that all effects were statistically significant. The viscosity model, on the other hand, showed that Carbopol had the greatest effect, with a coefficient much higher than the others, indicating it was the primary factor in determining the formulation's rheological structure. Ethanol and IPM both reduced the solution's viscosity, consistent with their ability to prevent Carbopol networks from swelling. Even though the viscosity effects weren't statistically significant in the ANOVA (mostly due to the small sample size and inherent variability), the coefficients' direction and magnitude are chemically consistent with polymer-solvent interactions. The simplified model, which only keeps important terms, is a good way to predict how to control viscosity within the desired range. The pH model showed that all factors and interactions had statistically significant effects, despite the experimental pH range being very small. The slight changes in pH were due to the solvent's polarity and the ionization state of Carbopol.

Even though these effects were small in numbers, their statistical significance shows how sensitive pH is to even small changes in composition. Importantly, the predicted pH values remained within physiologically acceptable limits throughout the design space, indicating that the system is practically robust despite being statistically responsive. The regression models show that assay and pH depend on formulation composition in a complex way. In contrast, viscosity depends primarily on polymer concentration and, to a lesser extent, on solvent components. Within the QbD framework, these quantitative models are the basis for design space exploration, optimization, and risk-based control strategies.

Assay = 87.3125 – 12.4625*A* – 12.1375*B* + 12.3125*C* – 12.1625*AB* + 13.0875*AC* + 12.4125*BC* + 12.7375*ABC*
Viscosity = 88.125 – 8.25*A* – 13.25*B* + 58.25*C* – 2.75*AC*
pH = 5.51 – 0.02*A* – 0.015*B* + 0.02*C* – 0.01*AB* + 0.015*AC* + 0.02*BC* – 0.01*ABC*

To further validate the regression models, essential adequacy statistics for assay, viscosity, and pH were assessed. All three models showed strong goodness-of-fit indicators, with R^2, adjusted R^2, and predicted R^2 values that were all in line with each other. This means there was little overfitting, and the models made accurate predictions. The recommended thresholds of 4 for assay and pH were exceeded by the Adequate Precision values, indicating that the signal-to-noise ratio was high enough to navigate the design space. The viscosity model had lower predictive metrics than the other models, consistent with the variability observed in the experiments. However, the adequacy statistics remained within acceptable limits, indicating it can still be used for qualitative interpretation and design space optimization. Together, these model-fit parameters indicate that the regression equations are statistically robust and can be used to develop predictive formulations and define the design space.

Design space identification and optimization

The main result of the DOE analysis was the creation of a design space, a verified multidimensional region of formulation variables in which the topical mechlorethamine solution consistently meets all predefined Critical Quality Attributes (CQAs) and aligns with the Quality Target Product Profile (QTPP). Statistical significance (ANOVA), regression model predictions, and response surface interpretations were used to find strong operating windows for Ethanol (A), IPM (B), and Carbopol 974P (C) to create the design space. Assay and pH showed considerable operational flexibility because their predicted values remained within acceptable limits across almost the entire design region. Because viscosity was the most sensitive to formulation composition, it was the primary driver of the design constraints.

The regression model and surface plots showed that Carbopol concentration was the most important factor affecting viscosity. Ethanol and IPM exhibited thinning effects, but to a lesser extent. At the experimental low concentration of 0.1%, Carbopol exhibited viscosities ranging from 46 to 49 cps, within the target range of 40 to 60 cps. Relying on this boundary condition may be precarious due to minor variations in polymer efficiency across batches, solvent quality, or mixing shear, which could reduce viscosity below the minimal standard. Model-based interpolation identified an ideal carbopol concentration of 0.12% to improve formulation stability. This alteration positions the formulation closer to the midpoint of the viscosity goal range (50-55 cps), hence diminishing its susceptibility to fluctuations in raw materials and processing conditions. The DoE models were employed to optimize the solvent system. The ethanol concentration was set at 73%, creating a substantial thermodynamic driving force for API dissolution and rapid absorption into the skin. This amount was sufficiently modest to prevent excessive decreases in viscosity or increases in pH. IPM was optimized at 3%, which balanced its softening of skin with its ability to lower viscosity. This ensured that the polymer network remained strong enough to maintain the desired rheology. The best combination of settings—73% Ethanol, 3% IPM, and 0.12% Carbopol 974P—was completely within the multidimensional design space. It was also expected to meet all CQAs (assay, viscosity, and pH) with a high chance of success. Verification batches showed that the optimized composition gave assay values between 90% and 110%, viscosity between 43 and 55 cps, and pH between 5.50 and 5.55. This fully supports the model predictions. This optimized area is the final design space. It gives a scientifically sound and regulatory sound basis for scale-up, technology transfer, and commercial trading.

Model drug bridging study

A bridging study was performed to assess the reliability of extrapolating the formulation behavior of Diclofenac Sodium observed during DOE development to the target cytotoxic agent, Mechlormethamine. This assessment was crucial for validating the predictive validity of the DOE-derived regression models while reducing occupational exposure risk during the initial phase of optimization. Both formulations were prepared using the same vehicle composition: 73% ethanol, 3% isopropyl myristate, and 0.12% Carbopol 974P, with a pH of 5.5. This made it possible to see how the API affected the critical quality attributes (CQAs). The comparative results in Table 3 show that the model and target formulations are very similar. The assay values for both APIs remained within the 90–110% range, indicating that the solubilization and mixing processes worked equally well for both drugs, regardless of the drug or its amount. The viscosity values (45 cps for Diclofenac and 43 cps for Mechlormethamine) were nearly identical, indicating that the polymer-solvent matrix, rather than the low API load, controls rheology.

Despite the incorporation of Diclofenac Sodium at 2% w/w, compared with 0.02% w/w Mechlormethamine (a 100-fold mass disparity), the rheological properties of this hydro-alcoholic gel system are primarily influenced by the cross-linked Carbopol 974P network rather than by the dissolved active pharmaceutical ingredient. At pH 5.5, Carbopol undergoes ionization and polymer chain expansion, resulting in a three-dimensional swollen gel structure, with viscosity primarily influenced by polymer concentration, degree of cross-linking, solvent composition, and ionic strength. At the used quantities, both active pharmaceutical ingredients remain molecularly dispersed within the ethanol-dominant phase and constitute a modest proportion relative to the polymer matrix. Thus, their impact on gel microstructure disruption, chain entanglement density, or polymer–polymer interactions is minimal. The deliberate application of 2% diclofenac constituted a worst-case loading scenario to evaluate the solubilization capacity and investigate potential polymer–drug interactions. The viscosity values for diclofenac (45 cps) and mechlormethamine (43 cps) are nearly comparable, indicating that even with much increased drug loading, the gel rheology is predominantly influenced by the polymer and is unaffected by the identity of the active pharmaceutical ingredient within this concentration range. The pH values remained the same (5.52 vs. 5.51), indicating strong buffering capacity and little change in the API-specific ionization properties. A direct physicochemical comparison further substantiates the scientific validity of the bridging strategy. Diclofenac Sodium possesses a molecular weight of approximately 318.13 g/mol and a logP of ~4.5, indicating higher molecular bulk and lipophilicity relative to Mechlormethamine (MW ≈ 156.05 g/mol; logP ≈ 1.2), which is smaller and more hydrophilic. From a microstructural perspective, incorporating a higher-molecular-weight, hydrophobic API at 2% w/w poses a greater challenge for the hydro-alcoholic Carbopol network. Increased hydrophobic drug loading may potentially influence solvent–polymer interactions, alter local microviscosity, or affect chain entanglement density within the swollen gel matrix. However, the negligible viscosity difference observed between diclofenac (45 cps) and mechlormethamine (43 cps) demonstrates that the three-dimensional Carbopol 974P network remains structurally dominant. Given that mechlormethamine is incorporated at only 0.02% w/w—two orders of magnitude lower than diclofenac—the likelihood of API-induced microgel disruption is mechanistically minimal. Thus, the 2% diclofenac system represents a conservative worst-case loading model for evaluating matrix robustness. Collectively, the close agreement in assay, viscosity, and pH, together with the marked disparity in molecular weight, lipophilicity, and loading levels between the two APIs, confirms that formulation performance is predominantly excipient-driven. The close agreement between the assay, viscosity, and pH shows that the CQAs are mostly affected by the composition of the excipients, especially the amount of ethanol, the concentration of Carbopol, and the conditions for neutralization, not by the identity of the API. These results confirm that the design space and regression models derived from the DOE can be used in the final formulation of mechlormethamine. They also provide a strong basis for moving on to full-scale development, stability testing, and eventual product sales.

Table 3: Integrated Comparison of Model and Target Formulations with Verification Batch Results (n = 3, mean ± SD)

Parameter	Category	Target / Specification	Diclofenac Trial	MVB	Interpretation
API Level	Composition	—	2.0%	0.02%	Different drug loads do not alter vehicle behavior due to polymer-dominated rheology.
Ethanol (%)	Composition	Fixed	73.0	73.0	Primary solvent; identical in both trials.
IPM (%)	Composition	Fixed	3.0	3.0	Enhancer/emollient; does not influence assay or pH behavior.
Carbopol 974P (%)	Composition	Fixed	0.12	0.12	Governs viscosity; identical levels allow direct comparison.
NaOH	Composition	Adjust to pH	pH 5.5	pH 5.5	Neutralization behavior is unaffected by API identity.
Water (%)	Composition	Q.S. to 100%	Yes	Yes	Base vehicle unchanged.
Assay (%)	CQA	90–110%	99.8	99.4 ± 0.6	Both APIs are fully solubilized; the manufacturing process is robust.
Viscosity (cps)	CQA	40–60	45	44.3 ± 1.5	Nearly identical; confirms that API does not impact polymer gel structure at low concentration.
pH	CQA	5.0–6.0	5.52	5.52 ± 0.04	pH is formulation-controlled and independent of API identity.

MVB = Mechlorethamine Verification Batch (n=3)

Optimized formulation characteristics

The final version of the optimized mechlormethamine topical formulation was based on a thorough integration of model predictions from DOE, verification of the bridging study, and early stability data. The target composition in the design space was 73% ethanol, 3% isopropyl myristate (IPM), 0.12% carbopol 974P, and 0.02% methylchloromethyl ether. It was neutralized to pH 5.5 with sodium hydroxide, then purified water was added to bring it to 100%.

The final composition was carefully chosen to fall within the viscosity specification (40–60 cps) while ensuring the assay performed well and that the pH was suitable for the skin. At these optimized setpoints, verification batches always met all the set CQAs. The assay of the final formulation was 99.2%, well within the acceptable range of 90–110%. This indicates that the API was dissolved and that the production procedure is reproducible. The viscosity ranged from 43 to 45 cps, as indicated by DoE. The selected carbopol concentration (0.12%) is optimal for dermal application and adhesion.

The pH remained between 5.50 and 5.55, indicating that the neutralization process and the buffer capacity of the carbopol/NaOH combination consistently maintained a body-safe level of acidity. The optimized formulation appeared as a clear, colorless to pale yellow, viscous liquid, with no evidence of precipitation, phase separation, or changes in rheology. This appearance met the QTTP expectations and remained consistent during both first and accelerated stability studies. Chemical stability assessments indicated that the improved system remained robust. Assay retention increased to 98.3% following three months of accelerated testing. The formulation maintained its viscosity within a predictable range of thermal responses.

When refrigerated, the viscosity increased slightly to 51 cps, whereas at elevated temperatures it decreased marginally to 42 cps. No indications of polymer degradation or irreversible viscosity reduction were seen. The findings indicate that the optimized hydro-alcoholic vehicle provides a stable, reproducible environment for mechlorethamine, preventing its degradation via hydrolysis while preserving the intended application properties. The robust correlation between planned and actual performance validates the QbD design space, confirming that the final composition is sufficiently robust for large-scale manufacture, commercial application & clinical utilization.

STABILITY STUDY RESULTS

Stability testing was conducted at refrigerated (2–8°C), room temperature (25°C/60% RH), and elevated temperature (40°C/75% RH). These studies were conducted to validate that the design space generated by DOE modeling corresponded to sustained product performance and to delineate the degradation pathways associated with this hydrolysis-sensitive nitrogen mustard.

Chemical Stability and Impurity Growth

The test results showed that the chemicals stayed stable in all storage conditions. The first test showed 99.2%, and after three months at 40°C/75% RH, it dropped to 98.3%. This 0.9% drop under accelerated conditions indicates that the substance is breaking down slowly, consistent with the stabilizing effect of the high ethanol concentration (73%), which lowers water activity and halts hydrolytic pathways common to mechlormethamine. The total impurity level increased from 0.08% in the first test to 0.19% after three months at 40°C. This is still well below the acceptance level of not more than (NMT) 0.5%. This small amount of impurity growth supports the idea that keeping the formulation at pH 5.5 prevents aziridinium formation and acid-catalyzed decomposition. These results confirm that the optimized solvent–polymer system successfully stabilizes mechlormethamine under both prolonged and expedited conditions. Mechlorethamine is a bifunctional nitrogen mustard characterized by chemical instability due to its inherent electrophilicity. In aqueous settings, the tertiary amine experiences intramolecular nucleophilic substitution, resulting in the production of a highly reactive three-membered aziridinium ion intermediate.

The cyclic cation is then assailed by water, leading to ring cleavage and the generation of hydroxyethylated breakdown products, which are pharmacologically inert. The sequential hydrolysis of both chloroethyl groups can produce mono- and di-hydroxy derivatives, typically identified as early-eluting polar contaminants in reverse-phase HPLC analysis. The negligible impurity increase noted under accelerated conditions (0.19% at 40°C/75% RH) suggests that the hydro-alcoholic vehicle proficiently inhibits this aziridinium-mediated hydrolysis pathway. The elevated ethanol concentration (73%) markedly diminishes water activity, thereby restricting nucleophilic assault on the intermediate species. Moreover, maintaining the formulation at pH 5.5 preserves the tertiary amine's protonation state, thereby diminishing the rate of intramolecular cyclization

and reducing acid-catalyzed degradation. These physicochemical controls collectively reduce the generation of hydroxyintermediates and account for the low impurity levels seen during stability testing. The included schematic (Figure 6) depicts this mechanistic route and emphasizes how the optimal solvent environment disrupts the degradation cascade.

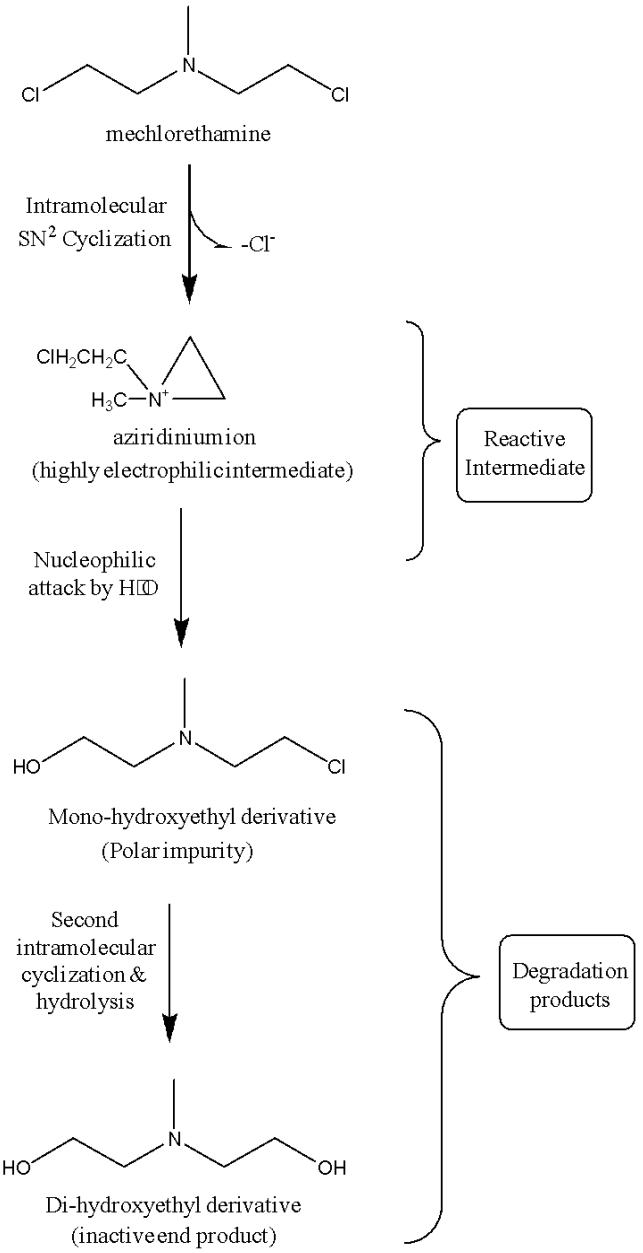


Figure 6: Aziridinium-mediated hydrolysis pathway of mechlormethamine and its inhibition in a hydro-alcoholic system

Physical Stability

Physical stability parameters remained within the set CQAs, indicating that the Carbopol-thickened hydro-alcoholic matrix was stable. The viscosity behaved consistently with temp., rising at cooler temperatures (51 cps at 2–8°C) & falling slightly at higher temperatures (42 cps at 40°C), with no signs of permanent polymer degradation or thinning. The viscosity readings at room temp. stayed around 45 cps, which is in line with the desired range of 40–60 cps. The pH profile showed very little change, going from 5.51 to 5.53 after three months at 40°C. The small changes indicate that the neutralized Carbopol system can buffer pH changes and suggest that the matrix's acid-base balance was not disturbed by solvent evaporation or interactions between the polymer and the solvent. The formulation was found to be transparent, colorless to pale yellow, with no visual separation, precipitation, or crystallization. This confirms the physical stability of API and excipients during storage.

Stress Testing

Stress tests conducted at elevated temperatures (60 °C) and during refrigeration cycling demonstrated the robustness of the formulation. The API did not undergo crystallization or reprecipitation, indicating that the thermodynamic solubility limit was not surpassed, despite temperature fluctuations. The ethanol-IPM-carbopol system exhibited no phase separation or turbidity, indicating a stable mixed solvent microenvironment and a robust polymer network. This outcome is particularly noteworthy, as high-ethanol gels frequently decompose or segregate into distinct solvents under significant stress. The stability results are closely related to the design space that the DOE set up. The optimized formulation—73% ethanol, 3% IPM, and 0.12% Carbopol—kept all of the important quality characteristics (assay, viscosity, pH) within the limits set during the study. This showed that it was very strong chemically and physically under all conditions, including refrigerated, room-temperature, and accelerated conditions. These results show that the regression models accurately captured the main effects of formulation variables on product performance. They also show that the identified optimal region is very resilient to changes in the environment and manufacturing. Table 4 of the consolidated stability summary shows that assay retention, impurity control, rheological stability, and pH maintenance are all stable across all storage conditions. This shows that the formulation is suitable for long shelf life and room-temperature storage for commercial development.

This publication presents the initial three-month stability results, providing early validation of the robustness of the QbD-optimized formulation. In accordance with ICH stability guidelines (ICH Q1A(R2)), long-term stability tests at 25°C/60% RH and ongoing rapid assessments at 40°C/75% RH

are being conducted. Data for six- and twelve-month periods are being systematically generated to validate the shelf-life designation further and demonstrate ongoing chemical and physical stability under prescribed storage conditions. The initial 3-month data provided here indicate a positive stability trajectory, with no observable trend toward specification drift. Extensive long-term data will be incorporated into future regulatory documents & development reports to provide a conclusive determination of expiry.

Table 4: Summary of Stability Results for Optimized Mechlormethamine Topical Formulation

Parameter	Specification / Target	Initial	RT(25°C/ 60%RH) – 3 Months	Accelerated (40°C / 75% RH) – 3Months	Refrigerated (2–8°C) – 3 Months	Interpretation
Assay (%)	90–110%	99.2	–	98.3	–	Minimal degradation (0.9% loss) even under accelerated conditions; strong chemical stability.
Total Impurities (%)	NMT 0.5%	0.08	–	0.19	–	Impurities remain far below limits; degradation pathways are well controlled at pH 5.5.
Viscosity (cps)	40–60 cps	43	45	42	51	Viscosity shows expected thermal dependence; the polymer matrix remains intact without permanent thinning.
pH	5.00–6.00	5.51	–	5.53 (5.61 at 1 month)	–	Minor fluctuations; strong buffering capacity ensures pH remains within an acceptable range.
Appearance	Clear, colorless–pale yellow; no ppt. or phase separation	Clear, no ppt.	Clear, no ppt.	Clear, no ppt.	Clear, no ppt.	No crystallization or phase separation across all storage conditions; robust physical stability.

CLINICAL CONSIDERATIONS: ETHANOL-DRIVEN STABILITY–TOLERABILITY TRADE-OFF

The optimized formulation contains 73% ethanol, a concentration essential for suppressing aziridinium-mediated hydrolysis by reducing water activity and thereby ensuring the chemical stability of mechlormethamine. However, high ethanol levels are known to disrupt stratum corneum lipids, potentially leading to transepidermal water loss, erythema, dryness, and contact dermatitis—effects that are particularly relevant in patients receiving topical nitrogen mustard therapy.

The present formulation addresses this clinical trade-off by incorporating 3% isopropyl myristate (IPM), which functions as a lipid-replenishing emollient and penetration modifier. IPM contributes to barrier support by reducing ethanol-induced lipid extraction while maintaining adequate dermal permeation. Furthermore, the Carbopol-based gel matrix moderates solvent evaporation kinetics, potentially reducing acute ethanol exposure at the skin interface.

Thus, the formulation represents a deliberate balance between chemical stabilization requirements and local tolerability considerations. Future clinical evaluation will further characterize dermal safety and patient acceptability under real-world use conditions.

CONCLUSION

This study demonstrates that a topical mechlorethamine formulation can be systematically developed using Quality by Design (QbD) principles to overcome challenges associated with chemical instability and high potency. A 2^3 full factorial Design of Experiments effectively elucidated the individual and interactive effects of ethanol, isopropyl myristate, and Carbopol 974P on critical quality attributes, identifying Carbopol concentration as the primary determinant of viscosity while maintaining consistent assay content and pH across the design space. The integration of regression modeling and response surface analysis enabled a robust definition of a scientifically justified formulation design space. The use of diclofenac sodium as a surrogate active pharmaceutical ingredient allowed safe early-stage optimization, and bridging studies confirmed comparable physicochemical performance between the surrogate and mechlorethamine-containing formulations, highlighting the dominant role of excipient interactions. The optimized formulation (73% ethanol, 3% IPM, and 0.12% Carbopol 974P at pH 5.5) satisfied all Quality Target Product Profile requirements and demonstrated substantial stability under refrigerated, ambient, and accelerated conditions. While this study focused primarily on physicochemical stability and formulation robustness, further clinical and long-term in vivo

evaluations are required to establish therapeutic performance fully. Although the laboratory-scale preparation was conducted under regulated low-shear mixing conditions (500–600 rpm), transitioning to industrial production requires assessing the shear-sensitive rheology of Carbopol 974P. Carbopol, a cross-linked poly(acrylic acid) microgel, exhibits significant shear-thinning behavior; at high shear rates during extensive mixing, it can undergo transient network deformation and a decrease in apparent viscosity. This activity is primarily reversible, as long as mechanical stress remains within structural tolerance limits. During scale-up, it is essential to maintain geometrically scaled impeller configurations, regulated shear-rate profiles, and appropriate neutralization sequencing to preserve polymer expansion and gel integrity. Consequently, although the formulation is deemed scalable, its industrial implementation will rely on optimizing process parameters to ensure rheological consistency across manufacturing scales. From a Design for Manufacture perspective, the identified design space integrates both formulation composition and process understanding, enabling predictable scale-up. The high-shear dispersion and neutralization steps employed during laboratory development are inherently scalable when the shear rate, mixing geometry, and addition sequence are appropriately controlled in accordance with geometric and dynamic similarity principles. The QbD framework established in this study, therefore, provides not only formulation robustness but also a scientifically justified foundation for manufacturing scalability and process transfer to industrial production. In the context of increasing regulatory emphasis on risk-based development and lifecycle management, this work provides a relevant and practical framework aligned with ICH Q8(R2) guidance. The integration of design space definition with process parameter awareness supports scalable manufacturing, regulatory flexibility, and future commercialization of stable topical anticancer formulations.

FINANCIAL ASSISTANCE
NIL

CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTION
Hemil Shah conceptualized the study, designed the methodology, performed the investigation and formal analysis, curated the data, validated the results, prepared visualizations, and drafted the original manuscript. Priyal Patel contributed by providing resources and software support, and by assisting in reviewing and editing the manuscript. Prachi Pandey supervised the study and conducted the final review of the manuscript. All authors read and approved the final manuscript.

REFERENCES
[1] Willemze R, Cerroni L, Kempf W, Berti E, Facchetti F, Swerdlow SH, Jaffe ES. The 2018 update of the WHO–EORTC classification for primary cutaneous lymphomas. *Blood*, **133**, 1703–1714 (2019) https://doi.org/10.1182/blood-2018-11-881268

[2] Goldman-Lévy G, Barnhill R, Bastian BC, Kempf W, Elder D, Gerami P, Grayson W, Kazakov D, Massi D, Messina J, de la Fouchardière A, Lazar AJ, Brenn T, Rous B, Field A, Gill A, Hodge JC, Khoury JD, Leite K, Sayed S, Tan PH, Elenitsas R, Calonje E, Duncan LM, Zhiyong L, Moch H, Singh R, Wijesinghe H, Cree I, Lokuhetty D. WHO classification of skin tumours: key updates in the fifth edition. *Histopathology*., **88**, 555–568 (2026) https://doi.org/10.1111/his.15562

[3] Melchers S, Willemze R. The fifth edition of the WHO classification: what is new for cutaneous lymphomas. *J. Dtsch. Dermatol. Ges*., (2024) https://doi.org/10.1111/ddg.15361

[4] Kempf W, Mitteldorf C. Cutaneous T-cell lymphomas—an update 2021. *Hematol. Oncol.*, **39**, 46–51 (2021) https://doi.org/10.1002/hon.2850

[5] Agar NS, Wedgeworth E, Crichton S, Mitchell TJ, Cox M, Ferreira S, Robson A, Calonje E, Stefanato CM, Wain EM, Russell-Jones R, Whittaker SJ. Survival outcomes and prognostic factors in mycosis fungoides/Sezary syndrome: validation of the revised International Society for Cutaneous Lymphomas/European Organisation for Research and Treatment of Cancer staging proposal. *J. Clin. Oncol.*, **28**, 4730–4739 (2010) https://doi.org/10.1200/JCO.2009.27.7665

[6] de Coninck EC, Kim YH, Varghese A, Hoppe RT. Clinical characteristics and outcome of patients with extracutaneous mycosis fungoides. *J. Clin. Oncol*., **19**, 779–784 (2001) https://doi.org/10.1200/JCO.2001.19.3.779

[7] Kim YH, Liu HL, Mraz-Gernhard S, Varghese A, Hoppe RT. Long-term outcome of 525 patients with mycosis fungoides and Sezary syndrome: clinical prognostic factors and risk for disease progression. *Arch. Dermatol*., **139**, 857–866 (2003) https://doi.org/10.1001/archderm.139.7.857

[8] van Doorn R, Van Haselen CW, Van Voorst Vader PC, Geerts ML, Heule F, De Rie M, Van Vloten WA, Toonstra J, Vermeer MH, Willemze R. Mycosis fungoides: disease evolution and prognosis of 309 Dutch patients. *Arch. Dermatol*., **136**, 504–510 (2000) https://doi.org/10.1001/archderm.136.4.504

[9] Willemze R, Jaffe ES, Burg G, Cerroni L, Berti E, Swerdlow SH, Ralfkiaer E, Chimenti S, Diaz-Perez JL, Duncan LM, Grange F,

Harris NL, Kempf W, Kerl H, Kurrer M, Knobler R, Pimpinelli N, Sander C, Santucci M, Sterry W, Vermeer MH, Wechsler J, Whittaker S, Meijer CJLM. WHO–EORTC classification for cutaneous lymphomas. *Blood*, **105**, 3768–3785 (2005) https://doi.org/10.1182/blood-2004-09-3502

[10] Willemze R, Hodak E, Zinzani PL, Specht L, Ladetto M, ESMO Guidelines Committee. Primary cutaneous lymphomas: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann. Oncol*., **29**, iv30–iv40 (2018) https://doi.org/10.1093/annonc/mdy133

[11] Scarisbrick JJ, Quaglino P, Prince HM, Papadavid E, Hodak E, Bagot M, Ortiz-Romero PL, Ranki A, Stadler R, Ortiz-Romero PL, Whittaker S, Willemze R. The PROCLIPI international registry of early-stage mycosis fungoides identifies substantial diagnostic delay in most patients. *Br. J. Dermatol*., **181**, 350–357 (2019) https://doi.org/10.1111/bjd.17258

[12] Scarisbrick JJ, Prince HM, Vermeer MH, Quaglino P, Horwitz S, Porcu P, Stadler R, Bagot M, Ortiz-Romero PL, Papadavid E, Hodak E, Ranki A, Whittaker S, Willemze R. Cutaneous lymphoma international consortium study of outcome in advanced stages of mycosis fungoides and Sézary syndrome: effect of specific prognostic markers on survival and development of a prognostic model. *J. Clin. Oncol*., **33**, 3766–3773 (2015) https://doi.org/10.1200/JCO.2015.61.7142

[13] Quaglino P, Maule M, Prince HM, Porcu P, Horwitz S, Bagot M, Ortiz-Romero PL, Papadavid E, Hodak E, Ranki A, Stadler R, Scarisbrick JJ, Whittaker S, Willemze R, Vermeer MH. Treatment of early-stage mycosis fungoides: results from the PROspective Cutaneous Lymphoma International Study (PROCLIPI study). *Br. J. Dermatol*., **184**, 722–730 (2020) https://doi.org/10.1111/bjd.19252

[14] Hodak E, Amitay-Laish I, Atzmony L, Feinmesser M, Prag-Naveh H, Pavlovsky L, Maly A, Talpur R, Duvic M, Quaglino P, Scarisbrick JJ, Whittaker S, Willemze R, Vermeer MH. Should we be imaging lymph nodes at initial diagnosis of early-stage mycosis fungoides? Results from the prospective cutaneous lymphoma international prognostic index (PROCLIPI) international study. *Br. J. Dermatol*., **184**, 524–531 (2020) https://doi.org/10.1111/bjd.19303

[15] Dobos G, Pohrt A, Ram-Wolff C, Lebbé C, Bouaziz JD, Battistella M, Bagot M. Epidemiology of cutaneous T-cell lymphomas: a systematic review and meta-analysis of 16,953 patients. *Cancers*, **12**, 2921 (2020) https://doi.org/10.3390/cancers12102921

[16] Geller S, Pulitzer M, Myskowski PL, Horwitz SM, Moskowitz AJ, Querfeld C, Rook AH, Kim YH. Outcomes and prognostic factors in African American and Black patients with mycosis fungoides/Sézary syndrome: retrospective analysis of 157 patients from a referral cancer center. *J. Am. Acad. Dermatol*., **83**, 430–439 (2020) https://doi.org/10.1016/j.jaad.2019.08.073

[17] Scarisbrick JJ, Prince HM, Vermeer MH, Quaglino P, Horwitz S, Porcu P, Stadler R, Bagot M, Ortiz-Romero PL, Papadavid E, Hodak E, Ranki A, Whittaker S, Willemze R. Prognostic factors, prognostic indices and staging in mycosis fungoides and Sézary syndrome: where are we now? *Br. J. Dermatol*., **170,** 1226–1236 (2014) https://doi.org/10.1111/bjd.12909

[18] Quaglino P, Pimpinelli N, Berti E, Calzavara-Pinton P, Alfonso Lombardo G, Rupoli S, De Matteis A, Novelli M, Zaja F, Bordonaro R, Fierro MT. Time course, clinical pathways, and long-term hazards risk trends of disease progression in patients with classic mycosis fungoides: a multicenter, retrospective follow-up study from the Italian Group of Cutaneous Lymphomas. *Cancer*, **118**, 5830–5839 (2012) https://doi.org/10.1002/cncr.27627

[19] Talpur R, Singh L, Daulat S, Liu P, Seyfer S, Trynosky T, Wei W, Duvic M. Long-term outcomes of 1,263 patients with mycosis fungoides and Sézary syndrome from 1982 to 2009. *Clin. Cancer Res.*, **18**, 5051–5060 (2012) https://doi.org/10.1158/1078-0432.CCR-12-0604

[20] Molloy K, Quaglino P, Prince HM, Papadavid E, Hodak E, Bagot M, Ortiz-Romero PL, Ranki A, Stadler R, Scarisbrick JJ, Whittaker S, Willemze R, Vermeer MH. Characteristics associated with significantly worse quality of life in mycosis fungoides/Sézary syndrome from the prospective cutaneous lymphoma international prognostic index (PROCLIPI) study. *Br. J. Dermatol*., **182**, 770–779 (2020) https://doi.org/10.1111/bjd.18089

[21] Scarisbrick JJ, Prince HM, Vermeer MH, Quaglino P, Horwitz S, Porcu P, Stadler R, Bagot M, Ortiz-Romero PL, Papadavid E, Hodak E, Ranki A, Whittaker S, Willemze R. Ethnicity in mycosis fungoides: white patients present at an older age and with more advanced disease. *Br. J. Dermatol*., **180**, 1264–1265 (2019) https://doi.org/10.1111/bjd.17602

[22] Colvin OM. An overview of cyclophosphamide development and clinical applications. *Curr. Pharm. Des.*, **5,** 555–560 (1999) https://doi.org/10.2174/1381612805666230110214512

[23] Duvic M, Hymes K, Heald P, Breneman D, Martin AG, Myskowski P, Crowley C, Yocum RC. Bexarotene is effective and safe for treatment of refractory advanced-stage cutaneous T-cell lymphoma: multinational phase II–III trial results. *J. Clin. Oncol.*, **19**, 2456–2471 (2001) https://doi.org/10.1200/JCO.2001.19.9.2456

[24] Alberti-Violetti S, Ardigò M, Massone C, Pileri A, Sala R, Teoli M, Grandi V, Quaglino P, Pimpinelli N, Berti E. Effectiveness and tolerability of chlormethine gel for the management of mycosis fungoides: a multicenter real-life evaluation. *Front. Oncol*., **13**, 1298296 (2023) https://doi.org/10.3389/fonc.2023.1298296

[25] Kim EJ, Mangold AR, DeSimone JA, Wong HK, Seminario-Vidal L, Guitart J, Appel J, Geskin L, Lain E, Korman NJ,

Zeitouni N, Nikbakht N, Dawes K, Akilov O, Carter J, Shinohara M, Kuzel TM, Piette W, Bhatia N, Musiek A, Pariser D, Kim YH, Elston D, Boh E, Duvic M, Huen A, Pacheco T, Zwerner J, Lee ST, Girardi M, Querfeld C, Bohjanen K, Olsen E, Wood GS, Rumage A, Donini O, Haulenbeek A, Schaber CJ, Straube R, Pullion C, Rook AH, Poligone B. Efficacy and safety of topical hypericin photodynamic therapy for early-stage cutaneous T-cell lymphoma (mycosis fungoides): the FLASH phase 3 randomized clinical trial. *JAMA Dermatol*., **158**, 1031–1039 (2022) https://doi.org/10.1001/jamadermatol.2022.2749

[26] Ardigò M, Nikbakht N, Teoli M, Gleason L, Crisan L, Querfeld C. Chlormethine gel in combination with other therapies for treatment of mycosis fungoides: a review with patient cases. *Front. Med*., **10**, 1308491 (2024) https://doi.org/10.3389/fmed.2023.1308491

[27] Geskin LJ, Angello JT, Bagot M, Guenova E, Nikbakht N, Querfeld C, Scarisbrick JJ. Evaluating response trends of chlormethine/mechlorethamine gel in patients with stage I–IIA mycosis fungoides: analysis of individual patient data from a randomized controlled phase II study to facilitate optimal treatment experiences. *Clin. Lymphoma Myeloma Leuk*., **24**, 40–47 (2024) https://doi.org/10.1016/j.clml.2023.08.020

[28] Chang RK, Raw A, Lionberger R, Yu L. Generic development of topical dermatologic products: formulation development, process development, and testing of topical dermatologic products. *AAPS J*., **15**, 41–52 (2013) https://doi.org/10.1208/s12248-012-9411-0

[29] Srishti SA, Pinky PP, Taylor R, Guess J, Karlik N, Janjic JM. Quality by Design (QbD)-driven development and optimization of tacrolimus-loaded microemulsion for the treatment of skin inflammation. *Pharmaceutics*, **16**, 1487 (2024) https://doi.org/10.3390/pharmaceutics16121487

[30] Mohseni-Motlagh SF, Dolatabadi R, Baniassadi M, Baghani M. Application of the Quality by Design concept (QbD) in the development of hydrogel-based drug delivery systems. *Polymers*, **15**, 4407 (2023) https://doi.org/10.3390/polym15224407

[31] Schäfer-Korting M, Mehnert W, Korting HC. Lipid nanoparticles for improved topical application of drugs for skin diseases. *Adv. Drug Deliv. Rev*., **59**, 427–433 (2007) https://doi.org/10.1016/j.addr.2007.04.006

[32] Ritschel WA, Ye W, Buhse L, Reepmeyer JC. Stability of the nitrogen mustard mechlorethamine in novel formulations for dermatological use. *Int. J. Pharm.*, **362**, 140–148 (2008) https://doi.org/10.1016/j.ijpharm.2008.06.016

[33] Michaelson-Richie ED, Ming X, Codreanu SG, Loeber RL, Liebler DC, Campbell C, Tretyakova NY. Mechlorethamine-induced DNA–protein cross-linking in human fibrosarcoma (HT1080) cells. *J. Proteome Res*., **10**, 2785–2796 (2011) https://doi.org/10.1021/pr200042u

[34] Lessin SR, Duvic M, Guitart J, Pandya AG, Strober BE, Olsen EA, Kim YH, Knobler R, Martin AG, Rook AH, Zic J, Pinter-Brown LC, Foss FM, Horwitz SM. Topical chemotherapy in cutaneous T-cell lymphoma: positive results of a randomized, controlled, multicenter trial testing mechlorethamine gel. *JAMA Dermatol*., **149**, 25–32 (2013) https://doi.org/10.1001/2013.jamadermatol.541

[35] Stein J, Fuchs T, Mattern C. Advanced milling and containment technologies for superfine active pharmaceutical ingredients. *Chem. Eng. Technol*., **33**, 1464–1470 (2010) https://doi.org/10.1002/ceat.200900590

[36] Giuliano C, Frizzarin S, Alonzi A, Pagnoni A, Di Meo N, Zalaudek I, Peris K. Chlormethine gel for the treatment of mycosis fungoides cutaneous T-cell lymphoma: in vitro release and permeation testing. *Dermatol. Ther. (Heidelb.)*, **12**, 2517–2529 (2022) https://doi.org/10.1007/s13555-022-00813-y

[37] Lionberger RA, Lee SL, Lee L, Raw A, Yu LX. Quality by design: concepts for ANDAs. *AAPS J*., **10**, 268–276 (2008) https://doi.org/10.1208/s12248-008-9026-7

[38] Bhise JJ, Bhusnure OG, Mule ST, Mujewar IN, Gholve SB, Jadhav PU. A review on quality by design approach (QbD) for pharmaceuticals. *J. Drug Deliv. Ther*., **9**, 1137–1146 (2019) https://doi.org/10.22270/jddt.v9i3-s.3014

[39] Zidan AS, Kamal N, Alayoubi A, Seggel M, Ibrahim S, Rahman Z, Khan MA. Effect of isopropyl myristate on transdermal permeation of testosterone from Carbopol gel. *J. Pharm. Sci*., **106**, 1805–1813 (2017) https://doi.org/10.1016/j.xphs.2017.03.016

[40] Suhail M, Wu PC, Minhas MU. Using carbomer-based hydrogels to control the release rate of diclofenac sodium: preparation and in vitro evaluation. *Pharmaceuticals*, **13**, 399 (2020) https://doi.org/10.3390/ph13110399

[41] Chaudhary H, Rohilla A, Rathee P, Kumar V. Optimization and formulation design of Carbopol-loaded piroxicam gel using novel penetration enhancers. *Int. J. Biol. Macromol*., **55**, 246–253 (2013) https://doi.org/10.1016/j.ijbiomac.2013.01.015

[42] Yu LX. Pharmaceutical quality by design: product and process development, understanding, and control. *Pharm. Res*., **25**, 781–791 (2008) https://doi.org/10.1007/s11095-007-9511-1

[43] Namjoshi S, Dabbaghi M, Roberts MS, Grice JE, Mohammed Y. Quality by design: development of the quality target product profile (QTPP) for semisolid topical products. *Pharmaceutics*, **12**, 287 (2020) https://doi.org/10.3390/pharmaceutics12030287

[44] Yu LX, Amidon G, Khan MA, Hoag SW, Polli J, Raju GK, Woodcock J. Understanding pharmaceutical quality by design. *AAPS J*., **16**, 771–783 (2014) https://doi.org/10.1208/s12248-014-9598-3

[45] Chiarentin L, Cardoso C, Miranda M, Vitorino C. Rheology of complex topical formulations: an analytical quality by design approach to method optimization and validation. *Pharmaceutics*, **15**, 1810 (2023) https://doi.org/10.3390/pharmaceutics15071810

[46] Mohammed YH, Namjoshi SN, Jung N, Windbergs M, Benson HAE, Grice JE, Raney SG, Roberts MS. Topical semisolid drug product critical quality attributes with relevance to cutaneous bioavailability and pharmacokinetics: Part I—bioequivalence of acyclovir topical creams. *Pharm. Res*., **41**, 1507–1520 (2024) https://doi.org/10.1007/s11095-024-03736-9

[47] Bushi E, Malaj L, Di Martino P, Mataj G, Myftari B. Formulation of semisolid dosage forms for topical application utilizing quality by design (QbD) approach. *Drug Dev. Ind. Pharm*., **51**, 670–678 (2025) https://doi.org/10.1080/03639045.2025.2498521

[48] Araujo P. Key aspects of analytical method validation and linearity evaluation. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci*., **877**, 2224–2234 (2009) https://doi.org/10.1016/j.jchromb.2008.09.030

[49] Rao RN, Nagaraju V. An overview of the recent trends in development of HPLC methods for determination of impurities in drugs. *J. Pharm. Biomed. Anal*., **33**, 335–377 (2003) https://doi.org/10.1016/S0731-7085(03)00293-0

[50] Kostrzębska A, Junka A, Brożyna M, Musiał W. The assessment of physicochemical and antimicrobial properties of hydrophilic gels containing tetracycline hydrochloride and various concentrations of ethanol. *Pharmaceutics*, **16**, 830 (2024) https://doi.org/10.3390/pharmaceutics16060830

[51] Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs — a review. *J. Pharm. Anal*., **4**, 159–165 (2014) https://doi.org/10.1016/j.jpha.2013.09.003

[52] Jin X, Alavi SE, Shafiee A, Leite-Silva VR, Khosrotehrani K, Mohammed Y. Metamorphosis of topical semisolid products—understanding the role of rheological properties in drug permeation under the "in use" condition. *Pharmaceutics*, **15**, 1707 (2023) https://doi.org/10.3390/pharmaceutics15061707

[53] Bagmar NA, Hatwar PR, Shelke PG, Bakal RL. A review on topical gels: an emerging drug delivery system. *GSC Biol. Pharm. Sci*., **28**, 285–296 (2024) https://doi.org/10.30574/gscbps.2024.28.2.0311

[54] Zayed MB, Yeoh T, Shah JC, Walsh T. Assessing and predicting physical stability of emulsion-based topical semisolid products: a review. *J. Pharm. Sci*., **112**, 1772–1793 (2023) https://doi.org/10.1016/j.xphs.2023.03.014

[55] Karamkar PG, Agrawal A, Chatap VK. A review article: formulation of topical gel by QbD approach. *Adv. Pharmacol. Pharm.*, **11**, 90–101 (2023) https://doi.org/10.13189/app.2023.110202

[56] Fukuda I, Pinto CF, Moreira CS, Saviano A, Lourenço FR. Design of experiments (DoE) applied to pharmaceutical and analytical quality by design (QbD). *Braz. J. Pharm. Sci*., **54**, e01006 (2018) https://doi.org/10.1590/s2175-97902018000001006

[57] Gupta NV, Shanmuganathan S, Kanha S, Sastri T. A 2^3 factorial design for formulation and development of doxycycline hydrochloride in situ gel-forming solution for wound-healing application. *Int. J. Appl. Pharm*., **13**, 221–232 (2021) https://doi.org/10.22159/ijap.2021v13i3.39696

[58] Oliveira N, Cádiz-Gurrea MdLL, Silva AM, Macedo C, Rodrigues F, Costa P. Development and optimization of a topical formulation with Castanea sativa shells extract based on the concept "quality by design". *Sustainability*, **14**, 129 (2022) https://doi.org/10.3390/su14010129

[59] Shiehzadeh F, Mohebi D, Chavoshian O, Daneshmand S. Formulation, characterization, and optimization of a topical gel containing tranexamic acid to prevent superficial bleeding: in vivo and in vitro evaluations. *Turk. J. Pharm. Sci*., **20**, 261–269 (2023) https://doi.org/10.4274/tjps.galenos.2022.60687

[60] Houlleberghs M, Verheyden L, Voorspoels F, Chandran CV, Duerinckx K, Radhakrishnan S, Van den Mooter G. Dispersing carbomers, mixing technology matters! *RSC Adv*., **12**, 7830–7834 (2022) https://doi.org/10.1039/D2RA00176

[61] Manian M, Jain P, Vora D, Banga AK. Formulation and evaluation of the in vitro performance of topical dermatological products containing diclofenac sodium. *Pharmaceutics*, **14**, 1892 (2022) https://doi.org/10.3390/pharmaceutics14091892

[62] Ilić T, Pantelić I, Savić S. The implications of regulatory framework for topical semisolid drug products: from critical quality and performance attributes towards establishing bioequivalence. *Pharmaceutics*, **13**, 710 (2021) https://doi.org/10.3390/pharmaceutics13050710

[63] Shabir GA. Validation of high-performance liquid chromatography methods for pharmaceutical analysis: understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. *J. Chromatogr. A*, **987**, 57–66 (2003) https://doi.org/10.1016/S0021-9673(02)01536-4