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*Research paper*

# Comparison and evaluation of the efficacy of topical drug formulations containing diclofenac

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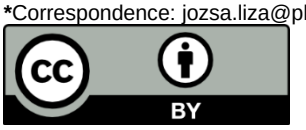


## Abstract

The choice of dermal dosage form is critical for therapeutic success, as it directly influences drug absorption, bioavailability, and overall efficacy. The skin possesses distinct physiological and anatomical characteristics that determine the performance of topical formulations. In addition to efficacy, the selected dosage form affects patient comfort, treatment adherence, and overall therapeutic outcomes. Therefore, optimizing dermal formulations is essential. This study aimed to compare different diclofenac sodium-containing dermal dosage forms in terms of drug release, bioavailability, cytotoxicity, and anti-inflammatory activity. Three formulations were evaluated: cream, foam, and gel. Texture analysis was performed to predict bioavailability. Drug release and membrane penetration were assessed using a Franz diffusion cell combined with ultraviolet–visible spectrophotometry. Biocompatibility was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on HaCaT human keratinocyte cells. The in vitro anti-inflammatory effect was evaluated by measuring tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) levels using enzyme-linked immunosorbent assay (ELISA). Franz diffusion studies demonstrated that the foam formulation exhibited the most favorable penetration profile, followed by the cream and gel. MTT results confirmed acceptable biocompatibility for all formulations; however, cream and gel showed a greater reduction in cell viability, likely due to excipients. The foam achieved the greatest reduction in inflammatory markers, while no significant difference was observed between cream and gel. Based on the results, it can be concluded that the foam formulation containing diclofenac sodium is the most advantageous in terms of drug release, bioavailability, toxicity, and anti-inflammatory effect.

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## 1. Introduction

Understanding the structure and physiology of the skin is fundamental for the rational design of dermal medicinal products, as the primary physiological role of the skin is to provide protection against chemical and physical external influences (1, 2). This barrier function represents the main challenge in topical and transdermal drug delivery (1, 3). The skin comprises of three main layers, first the epidermis, dermis, and hypodermis. The primary barrier to percutaneous absorption is the *stratum corneum*, the outermost layer of the epidermis, composed of terminally differentiated keratinocytes embedded in a highly organized lipid matrix (1, 4). Current knowledge in dermal drug delivery highlights that successful topical therapy requires not only an active compound with suitable physicochemical properties, but also a dosage form capable of controlling drug release, skin penetration, local retention, and tolerability (5, 6). Drug transport across the epidermis occurs predominantly via passive diffusion (3, 4). Penetration may occur through transcellular, intercellular, follicular, or appendageal pathways, with transcellular and intercellular routes being most relevant (1). Diffusion is influenced by concentration gradient, molecular size, lipophilicity, ionization, and skin condition (4). Because many active pharmaceutical ingredients show limited skin permeability, penetration enhancers are frequently incorporated into dermal formulations (7, 8). These agents transiently modify the barrier properties of the *stratum corneum* by disrupting lipid organization, increasing hydration, altering protein conformation, or improving drug solubility and partitioning (4, 8). Common penetration enhancers include sulfoxides, fatty acids, alcohols, glycols, and surfactants (7, 8). Surfactants enhance permeability by fluidizing intercellular lipids, although certain anionic surfactants may impair barrier integrity (9). Nonionic surfactants are generally preferred due to lower irritancy potential (7, 8).

Topical dosage forms provide localized therapeutic action while minimizing systemic exposure, gastrointestinal involvement, and first-pass metabolism (10). However, the therapeutic performance of a topical product cannot be predicted solely from the active ingredient or its concentration. Previous studies on diclofenac containing formulations demonstrated that the vehicle type, excipient composition, microstructure, viscosity, and rheological behavior can substantially influence drug release and skin permeation (6, 11, 12). Most dermal formulations are semisolid systems exhibiting pseudoplastic behavior, characterized by high viscosity at rest and reduced viscosity under shear stress (13, 14). These mechanical characteristics are relevant for dermal application

because they influence spreadability, residence time on the skin, patient acceptability, and the diffusion of the active substance from the formulation (6, 12). Therefore, mechanical characterization can provide useful information for interpreting in vitro release and penetration data (12).

Creams are multiphase emulsions consisting of aqueous and lipophilic phases, formulated as oil-in-water (o/w) or water-in-oil (w/o) systems depending on drug properties and desired release characteristics (13, 14). Their favorable cosmetic properties and spreadability contribute to improved patient adherence (14).

Gels are polymer-structured liquid systems, typically based on water, glycerin, or propylene glycol, and are widely used due to ease of application (15).

Medicinal foams are colloidal systems in which gas is dispersed in a liquid phase, stabilized by surfactants (16). They allow rapid coverage of sensitive skin areas but require careful stabilization due to thermodynamic instability (16, 17). In recent years, dermal foams have received increasing attention because their porous structure, large surface area, rapid spreading, and low need for rubbing may promote faster drug release and improve patient comfort, particularly on inflamed, sensitive, or hairy skin surfaces (18).

Diclofenac sodium, a phenylacetic acid derivative non-steroidal anti-inflammatory drug (NSAID), was selected as the model drug due to its analgesic, antipyretic, and anti-inflammatory effects. It exhibits relative cyclooxygenase-2 selectivity and inhibits prostaglandin synthesis. After systemic administration, diclofenac undergoes rapid absorption, extensive protein binding, and significant first-pass metabolism, resulting in approximately 50% bioavailability. It is metabolized primarily by CYP2C enzymes and eliminated via renal and biliary pathways. Systemic use is associated with gastrointestinal, cardiovascular, hepatic, and renal adverse effects (10). Therefore, topical delivery is a rational approach to achieve local anti-inflammatory activity while reducing systemic exposure (19).

Several diclofenac-containing topical formulations have already been investigated, including gels, emulsions, emulgels, ointments, solutions, patches, hydrogels, cream-gels, gel-in-oil emulsions, and foams (6, 11, 12, 18). These studies confirm that dosage form selection is a key determinant of diclofenac release and penetration (6, 11, 12). Nevertheless, the available studies have mainly focused on physicochemical characterization, *in vitro* release, or permeation behavior, while fewer studies have integrated these data with cellular biocompatibility and anti-inflammatory activity. In

particular, a direct comparison of diclofenac sodium containing cream, gel, and foam formulations under the same experimental conditions, combined with mechanical characterization, Franz diffusion studies, keratinocyte viability testing, and cytokine-based anti-inflammatory evaluation, remains insufficiently explored.

Therefore, the aim of the present study was to formulate cream, gel, and foam preparations containing diclofenac sodium and to compare them regarding physicochemical suitability, mechanical properties, in vitro drug release, membrane penetration, anti-inflammatory efficacy, and biocompatibility. The scientific novelty of this work lies in the integrated comparison of three dermal dosage forms with different structural and mechanical characteristics, and in the evaluation of how these formulation dependent properties are related to diclofenac sodium release, penetration behavior, cytocompatibility, and inflammatory cytokine reduction in HaCaT human keratinocyte cells. Physicochemical suitability was evaluated by pH assessment, while mechanical properties were determined by penetration testing. *In vitro* penetration was examined using a Franz diffusion cell system (4). Anti-inflammatory activity was assessed on HaCaT human keratinocytes, and biocompatibility was evaluated using cell viability assays. This comparative approach enabled identification of the most advantageous dermal dosage form based on physicochemical behavior, biological activity, and safety profile.

## 2. Materials and methods

### *2.1 Materials*

Sucrose ester SP70 used as a nonionic emulsifier was supplied by Sisterna B.V. (Roosendaal, The Netherlands). Glycerol used as humectant and co-solvent was purchased from Molar Chemicals Ltd. (Halásztelek, Hungary). Pemulen™ TR-1 used as a polymeric gelling agent and viscosity enhancer was obtained from Lubrizol Advanced Materials (Cleveland, OH, USA). Diclofenac sodium used as the active pharmaceutical ingredient, cetyl stearyl alcohol used as a consistency enhancer and lipophilic structuring agent, stearic acid used as a lipophilic structuring agent and co-emulsifier, trolamine used as a neutralizing and pH-adjusting agent, xanthan gum used as a viscosity enhancer and stabilizer, isopropyl myristate used as a lipophilic excipient and penetration enhancer, Triton X-100 used as a positive control in the cytotoxicity assay, MTT reagent (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) used for cell viability determination, phosphate-buffered saline (PBS) used as receptor

medium and dilution medium, heat-inactivated fetal bovine serum (FBS) used as a cell culture supplement, L-glutamine used as a nutrient supplement, non-essential amino acids solution used as a cell culture supplement, penicillin-streptomycin solution used as an antimicrobial supplement, recombinant human interleukin-6 (IL-6) used as an inflammatory stimulus, tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) enzyme-linked immunosorbent assay (ELISA) kits were obtained from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Transcutol® HP used as a co-solvent and penetration enhancer and Labrasol® ALF used as a nonionic surfactant, solubilizer, and foaming agent were supplied by Gattefossé (Saint-Priest, France), and Kolliphor® EL used as a nonionic surfactant, solubilizer, and foam-stabilizing excipient by BASF SE (Ludwigshafen, Germany). TrypLE™ Express Enzyme used for cell detachment and Dulbecco's Modified Eagle's Medium (DMEM) used as the cell culture medium were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Acidified isopropanol used for dissolving the formazan crystals in the MTT assay was prepared freshly before use. The HaCaT cell line used as an immortalized human keratinocyte model was obtained from Cell Lines Service (CLS, Heidelberg, Germany), and 96-well plates and culture flasks used for cell culture experiments from VWR International (Debrecen, Hungary).

### *2.2 Formulation of Topical Dosage Forms*

All formulations contained 1% w/w diclofenac sodium to ensure comparability.

#### 2.2.1 Oil in Water Cream formulation

The base cream is an o/w emulsion, in which sucrose ester SP70 was used as a nonionic surfactant. The lipophilic and aqueous phases were heated separately to 60 °C and emulsified under continuous stirring. After cooling, diclofenac sodium dissolved in water and preservative solution were incorporated under homogenization. The formulation is shown in Table 1.

| | |
|---|---|
| Diclofenac sodium | 1 g |
| Cetyl stearyl alcohol | 10 g |
| Stearic acid | 12.5 g |
| Sucrose ester SP70 | 1.5 g |
| Glycerol | 5 g |
| Preservative solution | 1 g |
| Distilled water | 69 g |

**Table 1.**: Composition of the o/w cream

*2.2.2. Gel formulation*

Pemulen TR-1 and Transcutol HP were dispersed in a glycerol, water mixture under magnetic stirring (350 rpm, 24 °C, 4 h), followed by 2 h swelling. Neutralization was performed using aqueous trolamine to induce gel formation. Diclofenac sodium and preservative solution were incorporated under continuous mixing. The formulation is shown in Table 2.

| | |
|---|---|
| Diclofenac sodium | 1 g |
| Pemulen TR-1 | 0.5 g |
| Trolamin | 1 g |
| Transcutol HP | 1.5 g |
| Glycerol | 10 g |
| Preservative solution | 1 g |
| Distilled water | 85 g |

**Table 2.**: Composition of the gel

*2.2.3. Foam formulation*

Foams are thermodynamically unstable systems, in which the dispersed gas bubbles may coalesce over time, increase in size, and eventually collapse. Therefore, foam-stabilizing excipients were incorporated into the formulation. Polymers can increase the viscosity of the aqueous phase and thereby contribute to improved foam stability. In this study, xanthan gum, a natural polymer, was used as a viscosity enhancing and foam-stabilizing agent. Kolliphor® EL and Labrasol® ALF were applied as nonionic surfactants and foaming agents. In addition to promoting foam formation, these excipients may also enhance dermal penetration by improving drug solubilization and modifying membrane fluidity. Due to their nonionic character, they are suitable for the formulation of skin-friendly dermal preparations(17). The formulation is shown in Table 3.

| | |
|---|---|
| Diclofenac sodium | 1 g |
| Kolliphor® EL | 0.5 g |
| Labrasol® ALF | 2 g |
| Xanthan gum | 2 g |
| Glycerol | 10 g |
| Preservative solution | 1 g |
| Distilled water | 83.5 g |

**Table 3.**: Composition of the foam

The foam formulation was prepared in several steps. First, the polymer containing phase was prepared by dispersing xanthan gum in distilled water and allowing it to swell for 2 h. Subsequently, the second phase, consisting of Kolliphor® EL, Labrasol® ALF, glycerol, diclofenac sodium, preservative solution, and the remaining water, was added to the hydrated polymer phase under continuous mixing. The bulk liquid was foamed for 10 min using an IKA mechanical stirrer at 2000 rpm. The foaming parameters were selected based on the results of the preformulation mechanical characterization.

*2.3. Determination of pH*

The pH of the formulations was measured using a portable digital pH meter (Mettler-Toledo, Zurich, Switzerland). For each determination, 5 g of the formulation was dispersed in 20 mL of distilled water and stirred at 300 rpm for 10 min at 37.5 °C. After cooling to room temperature, the dispersion was filtered and the pH of the filtrate was recorded.

*2.4. Mechanical Characterization of the Formulations*

The mechanical properties of the formulations were assessed on the day of preparation and after 60 days of storage. Measurements were performed at room temperature using a CT3 Texture Analyzer (Brookfield Engineering Laboratories, Middleboro, MA, USA) equipped with a TA5 cylindrical probe (35 mm length, 12.7 mm diameter). Data were recorded and analyzed with Texture Pro CT Software version 1.3 (Brookfield Engineering Laboratories, Middleboro, MA, USA).

Penetration tests were carried out using a trigger load of 4.0 g, a target distance of 10.0 mm, and a test speed of 0.50 mm/s. The penetration force, reflecting the resistance of the formulation to probe penetration, was recorded in mN.

*2.5. In Vitro Diffusion Study*

In vitro diffusion studies were performed using a Phoenix™ 6-Station Vertical Diffusion Cell system (Phoenix Research Laboratories, Inc., USA). Cellulose acetate membranes were pretreated with isopropyl myristate for 10 min to simulate a lipophilic skin surface. One gram of each formulation was applied onto the membrane surface as donor phase. PBS served as receptor medium. The system was sealed to prevent evaporation and ensure stability. The receptor phase was maintained at 32 °C and

continuously stirred at 350 rpm to provide homogeneous drug distribution and maintain sink conditions. Samples (1.0 ml) were withdrawn from the receptor compartment at 15, 30, 60, 90, and 120 min and immediately replaced with an equal volume of fresh PBS. Diclofenac sodium concentrations were quantified using a UV–Vis spectrophotometer (Shimadzu, Tokyo, Japan) at 275 nm.

2.6. HaCaT cell line culturing

The immortalized human keratinocyte cell line HaCaT was used for the in vitro experiments. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 3.7 g/L sodium bicarbonate, 10% (v/v) fetal bovine serum, 1% (v/v) nonessential amino acids, 1% (v/v) L-glutamine, 100 IU/mL penicillin, and 100 µg/mL streptomycin. Cultures were maintained at 37 °C in a humidified atmosphere containing 5% $CO_2$. The cells were weekly passaged to maintain exponential growth. Cells between passages 20 and 30 were used for cytotoxicity and anti-inflammatory assays.

*2.7. In Vitro Cytotoxicity Assay*

Cell viability was determined using the MTT assay, a colorimetric method based on the metabolic activity of viable cells (20). In this assay, the yellow tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide is reduced by mitochondrial and cytosolic oxidoreductase enzymes of metabolically active cells to insoluble purple formazan crystals. The amount of formazan formed is proportional to the number of viable cells.

Diclofenac sodium containing and placebo formulations were prepared for the cytotoxicity assay by dilution with PBS. The formulations were diluted to final concentrations of 0.1%, 0.5%, and 1% w/v immediately before the experiment.Triton X-100 served as the positive control and PBS as the negative control.

HaCaT cells were seeded in 96-well plates at 1 × $10^4$ cells per well and incubated for 4 days at 37 °C in 5% $CO_2$. The culture medium was then removed, and 200 µL of each diluted sample was added. After 120 min exposure, samples were removed and 100 µL of MTT solution (0.5 mg/mL) was added. Plates were incubated for 180 min at 37 °C. Formazan crystals were dissolved in 100 µL of acidified isopropanol, and absorbance was measured at 570 nm with a reference wavelength of 690 nm using a

FLUOstar OPTIMA microplate reader (BMG LABTECH, Offenburg, Germany). Cell viability was expressed as a percentage relative to the negative control (100%).

### *2.8. In Vitro Anti-Inflammatory Effect*

To assess the in vitro anti-inflammatory activity of the formulations, human TNF-α (Sigma—RAB0476) and IL-1β (Sigma—RAB0273) ELISA assays were performed. HaCaT cells (1 × $10^4$/well) were stimulated with IL-6 (20 ng/mL), then treated with diluted formulations (0.1–1% w/v). After 12 h, supernatants were collected and TNF-α and IL-1β levels were quantified by ELISA according to manufacturer instructions.

## 3. Results

### *3.1 Topical Formulations pH measurement*

All formulations exhibited pH values within the physiologically acceptable dermal range. The initial pH values were mildly acidic, consistent with skin compatibility. After 60 days of storage, no relevant shifts in pH were observed, indicating acceptable pH stability of the preparations. Results are summarized in Table 4.

| Composition | pH Value ± SD | |
|---|---|---|
| | 0. day | 60. day |
| **Cream** | 5.95 ± 0.12 | 5.99 ± 0.14 |
| **Gel** | 6.32 ± 0.16 | 6.52 ± 0.12 |
| **Foam** | 5.89 ± 0.09 | 6.01 ± 0.15 |

***Table 4****.:* The pH values immediately after formulation and after 60 days of storage at 21 °C. Values represent mean ± standard deviation (S.D.), n = 3.

### *3.2. Mechanical Characterization of the Formulations*

Mechanical characterization revealed measurable differences among the investigated dermal formulations. The penetration force required for probe insertion varied according to the dosage form and reflected the resistance of the formulations to penetration. The foam formulation exhibited the lowest resistance to penetration, indicating reduced structural rigidity and higher spreadability. The cream demonstrated intermediate resistance, while the gel showed the highest penetration force values.

Following 60 days of storage, no marked structural collapse was observed in any formulation. Minor variations in penetration force were detected. However, no drastic loss of consistency or structural integrity occurred, indicating acceptable physical stability over the test period. The results are shown in Figure 1.

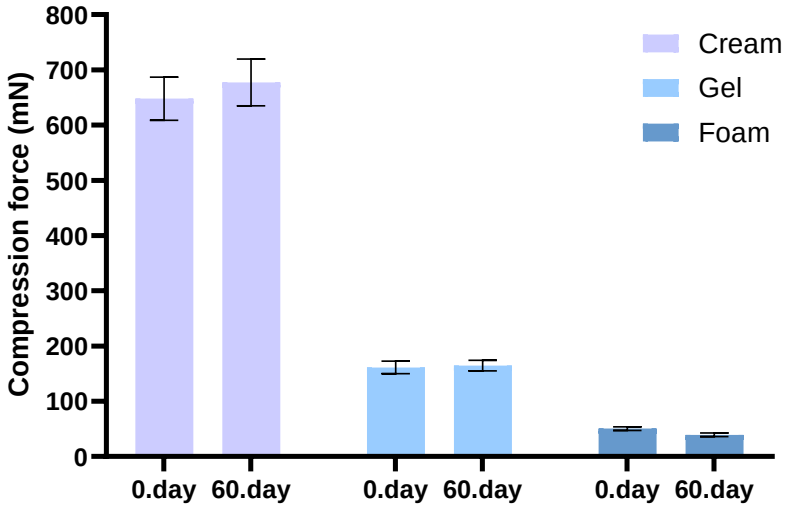


**Figure 1**.: Mechanical characterization of the formulations at 25 °C, expressed as penetration force. Data are expressed as means ± S.D., n=3.

*3.3. In Vitro Permeation Study*

Franz diffusion cell studies demonstrated distinct permeation profiles among the formulations. The cumulative amount of diclofenac sodium permeated increased over time in all cases. The foam formulation showed the most favorable penetration profile, characterized by the highest cumulative permeated amount at each measured time point. The cream exhibited intermediate permeation, while the gel showed the lowest diffusion efficiency. These findings indicate that formulation type significantly influences diclofenac sodium release kinetics and membrane permeation behavior. The results are shown in Figure 2.

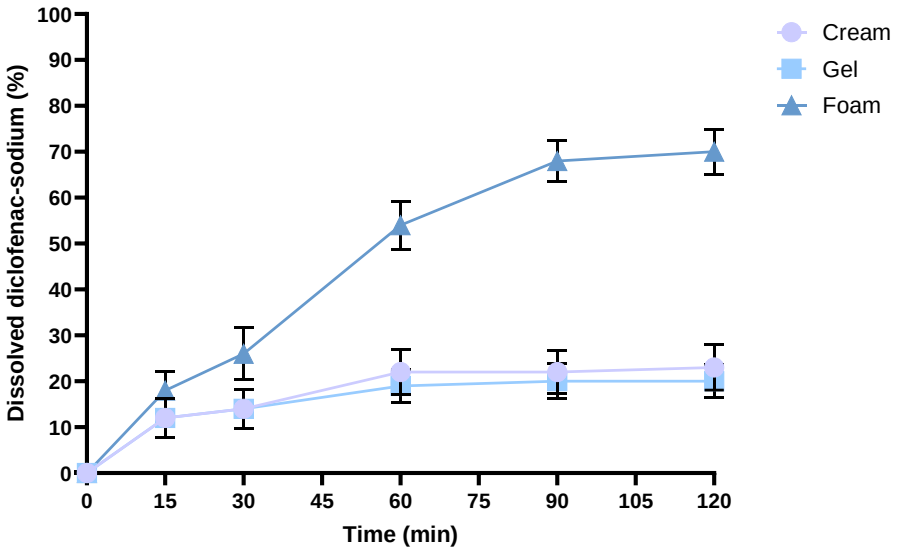


**Figure 2**.: The in vitro diffusion profiles of the cream, gel, foam containing diclonfenac-sodium. Bars represent the mean ± S.D., n = 3.

*3.4.In Vitro Cytotoxicity Assay*

Cell viability results confirmed the acceptable cytocompatibility of all investigated formulations. The PBS-treated negative control showed approximately 100% viability, whereas Triton X-100, used as the positive control, markedly reduced cell viability, confirming the sensitivity and validity of the assay. All diclofenac sodium-containing and placebo formulations maintained HaCaT cell viability above the 70% threshold, indicating cytocompatibility under the applied experimental conditions.

A concentration dependent tendency was observed, particularly in the case of the cream and gel formulations. The highest formulation concentration, 1%, resulted in the lowest viability values, while the 0.1% samples showed higher cell viability. The cream and gel formulations caused a more pronounced reduction in cell viability than the foam, especially at higher concentrations. In contrast, the foam formulation maintained the highest viability values among the investigated dosage forms, with values remaining close to 85–90% at all tested concentrations.

No relevant difference was observed between the diclofenac sodium containing formulations and their corresponding placebo formulations. This indicates that the slight decrease in cell viability was more likely associated with the vehicle composition and excipient concentration than with diclofenac sodium itself. Overall, the results demonstrate that all formulations were cytocompatible, while the foam formulation showed the most favorable cell viability profile.

These findings support the suitability of the foam formulation for further dermal application, as it combined favorable cytocompatibility with the highest cell viability values among the tested dosage forms.The results are shown in Figure 3.

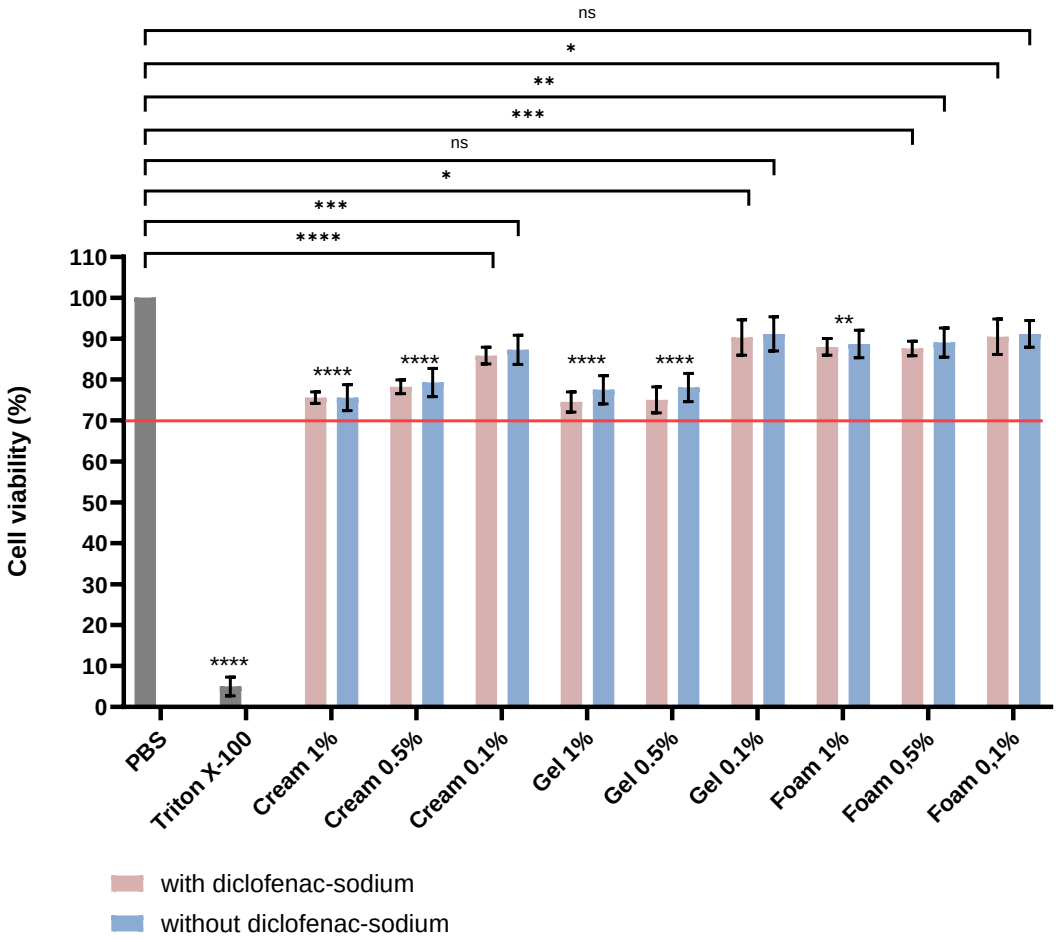


**Figure 3**.: HaCaT cell viability after incubation of compositions Preparations resulting in cell viability above the red line (70%) are considered biocompatible according to the ISO 10993-5 recommendation. Data represent the mean ± S.D., n = 6. *, **, ***, and **** for $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$, respectively, while “ns” indicates no significant difference.

### *3.5. In Vitro Anti-Inflammatory Effect*

ELISA analysis confirmed that all diclofenac sodium containing formulations exerted an anti-inflammatory effect by reducing TNF-α and IL-1β levels in HaCaT cells. In contrast, the corresponding placebo formulations did not markedly reduce cytokine levels, indicating that the observed anti-inflammatory activity was primarily related to diclofenac sodium rather than to the vehicle itself.

For TNF-α, all active formulations decreased cytokine levels compared with the PBS treated control. The foam formulation showed the most pronounced reduction among the investigated dermal dosage forms. At all tested concentrations, TNF-α levels after treatment with the diclofenac sodium containing foam were lower than those measured after treatment with the cream and gel formulations. The effect of the foam approached that of the diclofenac sodium solution used as a positive control, particularly at the higher tested concentrations. The cream showed moderate TNF-α reduction, while the gel generally produced a weaker inhibitory effect.

A similar tendency was observed for IL-1β. Diclofenac sodium containing formulations reduced IL-1β levels compared with the untreated control and their corresponding placebo formulations. The foam formulation again produced the greatest decrease in IL-1β levels among the semisolid and foam based preparations. Cream and gel formulations showed moderate anti-inflammatory activity, with relatively similar cytokine reducing effects. The placebo formulations maintained cytokine levels close to the control values, supporting that the reduction in IL-1β was mainly associated with the released diclofenac sodium.

The anti-inflammatory results showed good agreement with the in vitro permeation findings. The foam formulation, which exhibited the most favorable diclofenac sodium permeation profile in the Franz diffusion cell study, also produced the strongest reduction in both TNF-α and IL-1β levels. This suggests that the enhanced release and membrane penetration of diclofenac sodium from the foam contributed to its stronger biological activity. In contrast, the cream and gel showed lower permeation efficiency and a less pronounced cytokine-reducing effect. Therefore, the rank order observed in the anti-inflammatory assay was consistent with the permeation results, indicating that formulation dependent drug release and penetration behavior strongly influenced the anti-inflammatory performance of the preparations. The results are shown in Figure 4.

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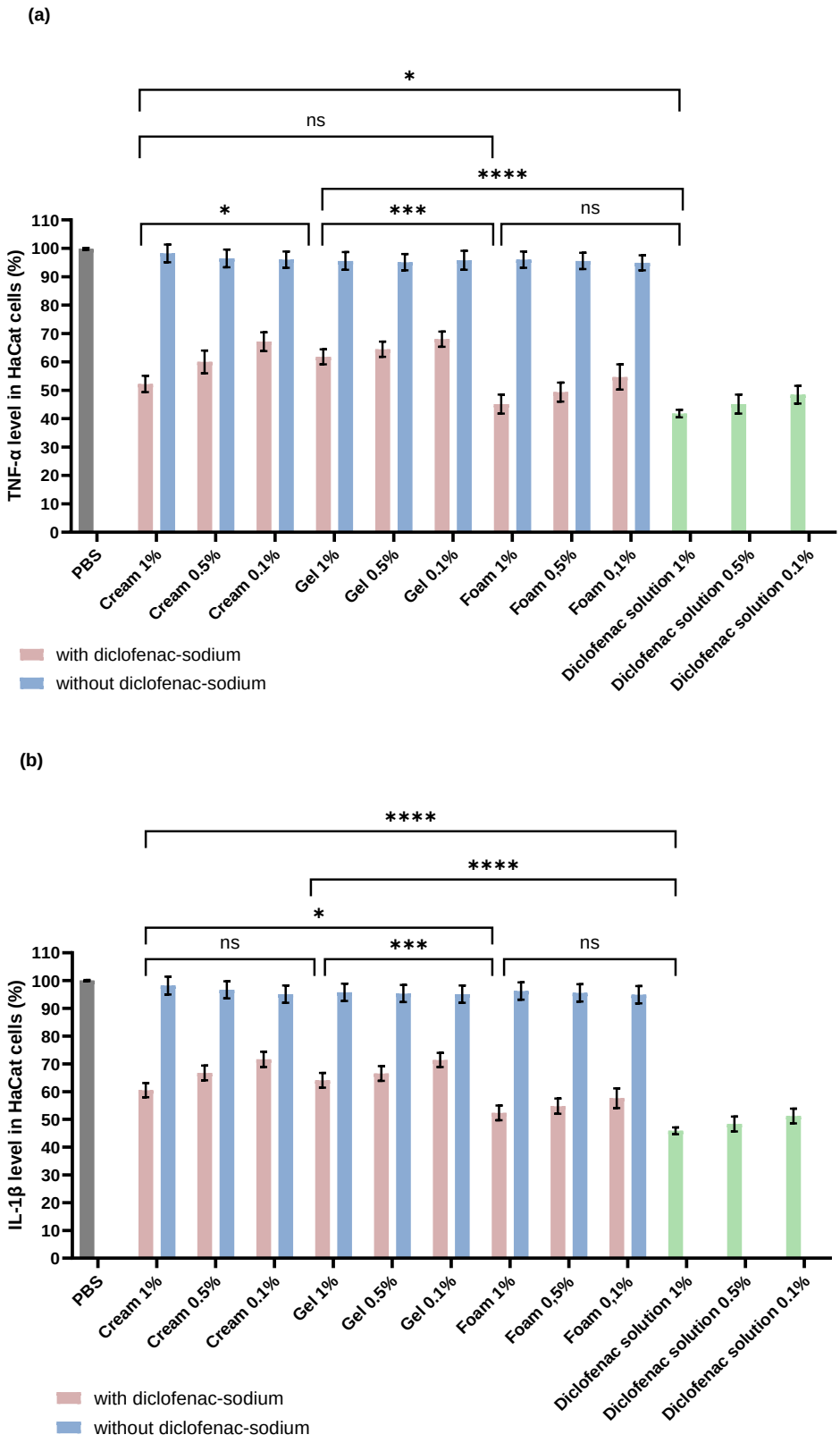


**Figure 4**.: Human TNF-α (a) and IL-1β (b) ELISA tests on HaCaT cell line. Data represent the mean ± S.D., n = 6. *, **, ***, and **** for $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$, respectively, while “ns” indicates no significant difference.

## 4. Discussion

Topical dosage forms are widely represented on the pharmaceutical market due to their ease of application, generally favorable tolerability profile, reduced systemic adverse effects, and high patient acceptability (14). The aim of the present research was to compare two conventional and widely used dosage forms, cream and gel, with a newer and increasingly popular formulation type, foam. Three formulations with different compositions were developed, one cream, one gel, and one foam. Each preparation contained diclofenac sodium at a concentration of 1% (10 mg/g). Emulsifiers, gelling agents, penetration enhancers, and a preservative were used as excipients. The main novelty of this work is the integrated comparison of diclofenac sodium containing cream, gel, and foam formulations under the same experimental conditions. Although several diclofenac containing topical systems have already been investigated, including emulsions, emulgels, gels, ointments, hydrogels, cream-gels, gel-in-oil emulsions, and foams, most studies have focused mainly on physicochemical characterization, drug release, or permeation behavior (12, 18). In contrast, the present study connects formulation dependent mechanical properties with membrane penetration, HaCaT keratinocyte viability, and cytokine-based anti-inflammatory activity.

The pH measurements revealed that the formulations were slightly acidic, which is favorable given the mildly acidic physiological pH of the skin (21). The gel exhibited a higher pH than the cream and foam, which was attributed to the addition of trolamine required for stabilization of the gelling polymer (13). The stable pH values observed after 60 days indicate that the formulations maintained their physicochemical characteristics during storage.

Mechanical characterization demonstrated that all formulations exhibited appropriate consistency. The cream showed the highest firmness, whereas the foam displayed the lowest resistance to penetration. All preparations retained their consistency during 60 days of storage, indicating adequate physical stability. These differences in mechanical behavior may also contribute to the different release and permeation profiles of the formulations. A higher resistance to penetration may indicate a more structured formulation matrix, which can slow the mobility of the active substance. In contrast, the lower resistance to penetration observed for the foam suggests a less rigid and more easily dispersible structure, which may facilitate diclofenac sodium release from the formulation. This is in agreement with published data showing that formulation type,

rheological behavior, and microstructure can substantially affect diclofenac sodium release and diffusion from topical semisolid systems (12).

Drug release from the vehicle and membrane permeation were evaluated using a Franz-type vertical diffusion cell and UV–Vis spectrophotometry. Franz diffusion cells are commonly employed to compare in vitro release/permeation performance of topical semisolid formulations (22). During the 120-minute study, the cream and gel approached their maximum cumulative permeated amounts at 60 minutes, whereas the foam reached near-maximum permeation at 90 minutes. The foam demonstrated the most favorable permeation profile, releasing more than three times the amount of diclofenac sodium compared to the cream and gel. This superior performance may be explained by the specific structural properties of the foam. The foam system provides a large interfacial surface area, which can increase the contact between the formulation and the membrane. In addition, the incorporated nonionic surfactants, Kolliphor® EL and Labrasol® ALF, may have contributed to improved diclofenac sodium solubilization and enhanced membrane transport. The lower resistance to penetration of the foam may also indicate that its structure does not strongly restrict drug diffusion.Similar findings were reported for diclofenac sodium-containing foam systems, where faster release was observed compared with hydrogel formulations (18).

The cream containing sucrose ester SP70 exhibited a more favorable diffusion profile than the gel containing Transcutol HP as a penetration enhancer, despite literature reports generally describing gels as efficient drug delivery systems. Further optimization of gel formulations is therefore warranted (13).

This fining highlights that the performance of a topical dosage form cannot be generalized solely based on its pharmaceutical category. For example, Manian et al. reported favorable release and permeation behavior for certain diclofenac sodium-containing gel and emulgel systems, whereas in the present study the gel showed the lowest dissolved diclofenac sodium values. This difference does not contradict the literature, but rather confirms that excipient composition, polymer type, viscosity, microstructure, and the applied membrane model strongly influence the final performance of topical formulations (12).

The favorable behavior of the foam is also consistent with recent findings on diclofenac sodium-containing foams. Falusi et al. reported that foam systems can provide rapid diclofenac sodium release compared with hydrogel systems and attributed this effect partly to the porous structure and large surface area of the foam (12) . The present

results support this observation, as the foam formulation showed clearly higher dissolved diclofenac sodium values than both the cream and gel formulations.

Biocompatibility was assessed using an MTT assay on HaCaT cell line. Both active and placebo formulations were tested at different dilutions. All preparations met the cytocompatibility criteria, and no significant differences were observed between active and inactive formulations. The foam yielded the highest cell viability, approaching 90%, which exceeds the 70% threshold commonly reported in the literature. Overall, non-toxic and dermally compatible formulations were successfully developed. The similar viability values observed for diclofenac sodium-containing and placebo formulations suggest that the minor reduction in cell viability was more likely associated with the vehicle composition and excipient concentration than with diclofenac sodium itself. This is relevant because topical diclofenac products may still cause local tolerability issues despite their lower systemic risk. Therefore, the favorable cytocompatibility profile of the foam supports its suitability for dermal application. Overall, non-toxic and dermally compatible formulations were successfully developed. Nevertheless, in vivo biocompatibility studies remain necessary, as cellular responses under in vitro conditions may differ from in vivo behavior (13).

Anti-inflammatory activity was evaluated using in vitro TNF-α and IL-1β ELISA assays. The foam exhibited the greatest anti-inflammatory effect, reducing cytokine levels to less than 50% of control values at 0.5% and 1.0% dilutions. The results were comparable to those obtained with the diclofenac sodium solution used as positive control. The cream and gel also demonstrated notable anti-inflammatory effects, although to a lesser extent than the foam. These biological results can be correlated with the Franz diffusion cell findings. The foam formulation, which showed the most favorable dissolved diclofenac sodium profile, also produced the strongest reduction in TNF-α and IL-1β levels. This suggests that the enhanced release and membrane penetration of diclofenac sodium from the foam contributed to its stronger anti-inflammatory activity. In contrast, the gel showed lower diclofenac sodium release and a weaker cytokine-reducing effect, which may be related to its higher structural resistance and slower drug diffusion.

Overall, the foam formulation provided the most balanced performance among the investigated dermal dosage forms. It combined suitable pH stability, low resistance to penetration, superior diclofenac sodium release and membrane penetration, favorable cytocompatibility, and strong cytokine reducing activity. The comparison with published

studies supports the view that foam systems may represent promising alternatives to conventional semisolid dermal dosage forms, especially when rapid drug release and local anti-inflammatory activity are desired (18). The novelty of the present work is that the foam was not evaluated only as a physicochemical carrier, but was directly compared with cream and gel formulations in a complex experimental system including mechanical, permeation, cytotoxicity, and anti-inflammatory endpoints.

Nevertheless, the study has limitations. The permeation experiment was performed using an artificial membrane model, which is useful for comparative formulation screening but cannot fully reproduce the complexity of human skin. In addition, HaCaT keratinocyte assays provide relevant information about cytocompatibility and inflammatory marker modulation, but they cannot replace in vivo tolerability and efficacy studies. Therefore, further investigations using ex vivo human or animal skin models, followed by in vivo studies, are necessary to confirm the dermal performance and safety of the developed foam formulation.

## 5. Conclusions

In this study, diclofenac sodium-containing cream, gel, and foam formulations were successfully prepared and compared using an integrated in vitro approach. All formulations showed acceptable pH stability, cytocompatibility, and anti-inflammatory activity. Among the investigated dosage forms, the foam formulation demonstrated the most favorable overall performance, including the lowest resistance to penetration, the highest diclofenac sodium release and membrane penetration, the best cell viability profile, and the strongest reduction in inflammatory cytokine levels. The results suggest that the foam formulation may be a promising dermal dosage form for topical diclofenac sodium delivery. However, further *ex vivo* and *in vivo* studies are required to confirm its applicability under more physiologically relevant conditions.

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## Data Availability Statement:

All measurement data are available at the corresponding author upon request.

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