Section A-Research paper
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Development, Characterization, and Study of Topical Gel Containing a Non-Selective COX inhibitor: Exploring Ketorolac Potential

Anasuya Patil[1], Nilesh O. Chachda[2*], Vema Kiran[3], Vivekanand A Kashid[4], Anagha V Baviskar[5], Veena Kalyani S[6], Barkale Rushikesh S[7], Priya Arya[8]

[1]Department of Pharmaceutics, KLE College of Pharmacy, Bengaluru

[2]Shri Chhatrapati Shahu Maharaj Shikshan Sanstha's Institute of Pharmacy Maregaon, Yavatmal, Maharashtra, India

[3]M B School of Pharmaceutical Sciences, Mohan Babu University, A. Rangampeta, Tirupati

[4.5]Dr. Kolpe Institute of Pharmacy, Kolpewadi, Tal. Kopargaon, Dist. Ahmednagar.

[6]Department of Pharmaceutics, Vivekananda College of Pharmacy, Rajajinagar, Bengaluru & Research Scholar, Sri Adichunchanagiri College of Pharmacy, Nagamangala, B G Nagara

[7]Y. B. Chavan College of Pharmacy Aurangabad, Maharashtra, India

[8]Faculty of Pharmacy, Dr APJ Abdul Kalam Technical University, Jankipuram, Lucknow.

Main Author: Anasuya Patil

anusuya.raghu@gmail.com

Corresponding Author: Nilesh O. Chachda*

nileshchachda@gmail.com

Abstract: This study introduces a novel approach involving the formulation of controlled-release ketorolac gels, which have demonstrated efficacy when administered topically. The investigation delves into the potential of these gels as carriers for the delivery of ketorolac. The release pattern of the drug in vitro, across all formulations, followed a diffusion-controlled mechanism characterized by a zero-order pattern. Furthermore, these formulations displayed compatibility with skin application and stability congruent with ICH guidelines. The optimized ketorolac gel stood out with a substantial reduction in paw edema, achieving a noteworthy 77.02% reduction after 6 hours. Nonetheless, it was observed that the optimized gel did not exert a significant effect on the inhibition of 5-lipoxygenase, even at higher doses. Based on these findings, the

ketorolac gel comprising 0.5 g of Carbopol emerged as the optimized formulation for addressing localized inflammation.

Introduction

In the realm of administering medications through the skin, a formulation containing the active drug is applied directly onto the skin surface to address skin-related symptoms arising from a broader internal condition. This approach provides notable benefits, including bypassing initial metabolic processes, reducing potential stomach irritation, mitigating the risk of degradation often linked with oral ingestion, offering a non-greasy texture, and enabling effortless removal from the skin. To tackle the associated challenges, the utilization of gel-based formulations has emerged as a promising strategy for enhancing topical drug delivery. These gels incorporate gelling agents that, when dispersed within a suitable solvent, intertwine or mesh together to form a three-dimensional colloidal network structure. This intricate framework effectively hinders the movement of fluids by capturing and immobilizing solvent molecules (1-5)

NSAIDs (non-steroidal anti-inflammatory drugs) exert their effects by disrupting two key pathways, cyclooxygenase (COX) and lipoxygenase (LOX), which hold pivotal roles in inflammatory disorders. The conversion of arachidonic acid (AA) into prostaglandins (PGs) is facilitated by enzymes known as cyclooxygenases (COX-1 and COX-2). COX-1 is distributed widely in various tissues and maintains regular bodily functions, while COX-2 is responsible for generating PGs during inflammatory responses. On a parallel note, the lipoxygenase pathway involves the enzymatic conversion of arachidonic acid into leukotrienes (LTs), and this process is primarily governed by the enzyme 5-lipoxygenase. This enzymatic conversion significantly contributes to the development of chronic inflammatory conditions. Ketorolac, a non-selective COX inhibitor, acts on both COX-1 and COX-2, exhibiting anti-inflammatory, analgesic, and antipyretic properties (6, 7)

However, it's important to note that oral administration of COX-2 inhibitors, including Ketorolac, has been linked to an elevated risk of thrombotic events, myocardial infarction (MI), and stroke. To address this concern, current research is delving into the topical application of Ketorolac, as illustrated in Figure 1, through the creation of diverse polymeric gels. This investigation aims to ascertain the potential of these gels in inhibiting both the COX and LOX

pathways, thereby providing an alternative route for administering Ketorolac with potential benefits in terms of reduced systemic risks (8, 9).

Experimental Section

The study utilized analytical-grade chemicals without requiring further purification. UV analysis and in vitro release investigations were conducted using double-distilled water.

Preparation of Gels

To create a spectrum of gel formulations, polymeric dispersion techniques were put into action. The drug's requisite amount was dissolved in propylene glycol, and then supplemented with a hydro alcoholic blend at a 2:1 ratio. This resulting solution was employed to distribute non-toxic gelling agents-namely, MC, Carbopol 934, and HPMC at varied concentrations (0.5 g, 1 g, and 2 g) to achieve distinct, uniform gels. Concurrently, penetration enhancers and other excipients, including methyl paraben and propyl paraben, were introduced while ensuring continuous agitation. To align with the skin's pH, the pH of all formulations was harmonized using TEM. The removal of any entrapped air bubbles was carried out by placing the gels in a vacuum oven for duration of 2 hours (10-14)

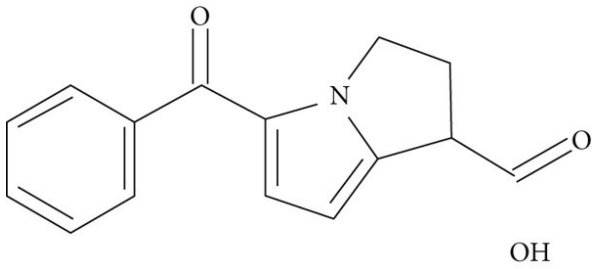

Fig 01: Ketorolac

Evaluation of Gels

The physical attributes of the formulated gels were evaluated through visual inspection to assess their appearance, the presence of potential obstructions, and any abrupt shifts in viscosity. A thin layer of the gels was applied onto a glass slide and scrutinized under a microscope to detect any obstructions. To gauge sensory properties, the formulations were administered to the skin, and psycho rheological techniques were employed for evaluation. Quantification of the drug content was achieved using a spectrophotometric approach following a procedure outlined previously. In

this method, a sample of the formulation (0.5 g) was mixed with a 1% w/v solution of sodium lauryl sulfate (SLS) and agitated to ensure complete dissolution. The resultant solution was then filtered through a Whatman filter paper No.1, and the filtrate was subjected to spectrophotometric analysis at a wavelength of 239 nm (lmax). Viscosity measurements were conducted at room temperature (RT) using a programmable cone and plate rheometer (Model: DV-III ULTRA, Brookfield Engineering Lab; Inc; Middleboro, USA) equipped with a Cp-52 cone spindle. The pH of the formulations was ascertained using a pH meter (Model: 7007, Digisun electronics, Hyderabad, India) (15-19)

Spreadability

To assess the spreadability of the developed gels, a specialized apparatus was fabricated. This setup featured a wooden block with a pulley affixed at one end. Positioned firmly on the block was a ground glass slide, onto which an excess quantity (2 g) of the formulated gel was applied. Another glass slide, matching the dimensions of the fixed ground slide and fitted with a hook, was employed to encase the gel. Applying a 1 kg weight on both sides for a brief period ensured the expulsion of trapped air and the formation of a uniform gel layer between them. Any surplus gel along the edges was meticulously removed. The hook on the enclosed slides was linked to the pulley, and an additional weight was suspended from it. The time taken for the upper slide to traverse a distance of 7.5 cm was meticulously documented (20-24)

***In-vitro* Drug Diffusion Studies**

The investigation into the drug diffusion rate from diverse gel formulations was carried out utilizing a modified Keishery Chein Cell. A cellophane membrane (with a molecular weight cutoff of 12-14 kD) served as a barrier within the diffusion cell. This diffusion cell, along with the diffusion membrane boasting an effective surface area of 3.8 cm^2, was assembled atop a magnetic stirrer. The membrane functioned as a segregator between the donor and recipient compartments. The receptor compartment housed a 1% w/v aqueous solution of SLS, serving as the diffusion medium, and was maintained at a temperature of 32±2°C for 30 minutes to establish equilibrium. In the donor compartment, 1 g of the gel was placed onto the membrane.

At specific intervals, a 5 ml sample was extracted from the receptor compartment over a span of 6 hours. The extracted sample was replaced with an equivalent volume of fresh medium. The absorbance of these samples was gauged spectrophotometrically at a wavelength of 239 nm, utilizing a UV-VIS double-beam spectrophotometer (Model: Shimadzu-1700, Shimadzu Corp.,

Japan). The cumulative release (%) of Ketorolac from the diverse gel formulations was determined through calculations based on these measurements (25-29).

Skin Compatibility Studies

The research protocols received approval from the Institutional Animal Ethical Committee, ensuring compliance with ethical standards. Male albino rats, weighing between 200-250 g on average, were divided into three groups, each comprising five animals. The control group was labeled as Group I, while the test groups were named Group II and III. In preparation for the application of gel formulations, the ventral surface of the rats was subjected to depilation. The gel formulations were administered twice daily over a span of one week. Throughout the study's duration, vigilant observation was maintained to detect any signs of allergic reactions or related manifestations among the animals (30-33)

Stability Studies

The study was executed in adherence to the established protocol delineated in previous records. All formulations were subjected to a cyclic temperature exposure regimen, encompassing five cycles that ranged from -5°C to 25°C, each cycle persisting for 24 hours. The gels underwent evaluation for their physical stability and susceptibility to syneresis. Moreover, following the guidelines outlined by the ICH, a three-month stability assessment was carried out. Throughout this period, the gels were subjected to varying temperatures (ranging from 25±2°C to 40±2°C) and relative humidity (maintained at 75±5% RH). Regular monthly assessments were conducted to gather data pertaining to drug content, viscosity, pH, and in vitro diffusion (34-37).

Cycloxygenase-2 Inhibition Study

The primary objective of this study was to assess the anti-inflammatory efficacy of the developed gels, considering the involvement of the COX-2 enzyme in the generation of PGs, which contribute to inflammatory responses. To achieve this goal, albino rats within the weight range of 200 to 250 g were segregated into two groups, each comprising six animals. Edema was induced by injecting a 1% w/v Carrageenan solution (0.1 ml) into the plantar surface of the hind paw. The volume of the paw was measured via immersion in a mercury column plethysmometer. Following this, the optimized gel formulation was gently applied with a rubbing motion. By comparing the paw edema against the variation in the height of the mercury column between the control and experimental groups, the anti-inflammatory potential was evaluated. The measurement of paw volume was taken at hourly intervals for a span of six hours, covering

seven days. The control group rats solely received the gel base without the drug, using the same application method. The inhibition percentage of edema was determined through the formula: (Vc - Vt / Vc) x 100, where Vc represents the average paw volume in the control group, and Vt symbolizes the average paw volume in the drug-treated group (38-41).

Results and Discussion

Diverse gelling agents-namely, MC, Carbopol 934, and HPMC-were employed in the development of topical gels containing Ketorolac. This choice aimed to attain the desired texture and visual appeal of the gels. Propylene glycol (PG) played a dual role as a solvent and humectants, contributing to drug solubility within the formulation while preventing gel desiccation. The hydro alcoholic vehicle, with a ratio of 2:1, played a pivotal role by hydrating the gelling agents. This hydration facilitated the formation of the gel's structural integrity and its overall stability. It also played a key part in the gelation process and doubled as a co-solvent for the drug.

Inclusion of elevated alcohol concentrations led to reduced viscosity in the gels, which could be attributed to a potential disruption of polymer cross-linking. Addressing the skin's outermost layer—the stratum corneum—was crucial for efficient topical drug delivery. By incorporating menthol into the formulation, alterations in this barrier were initiated, enhancing drug penetration. The presence of hydrogen bonding functional groups and lipophilic characteristics in menthol facilitated drug permeation through both lipid and pore pathways. Moreover, the application of the formulated gel with menthol yielded a refreshing sensation on the skin. Preservative aspects were also tackled by introducing methyl and propyl paraben to prevent microbial growth.

The process involved developing various gel formulations, each utilizing distinct concentrations (0.5, 1, and 2 g) of MC (A1, A2, and A3), Carbopol 934 (B1, B2, and B3), and HPMC (C1, C2, and C3). Optimization was based on parameters such as consistency and in vitro drug release, as summarized in Table 1. Notably, the formulated gels exhibited no issues with clogging or unexpected alterations in viscosity. The tactile experience post-application was consistently perceived as smooth and comfortable. Transparency characterized MC and Carbopol 934-based gels, whereas HPMC-based gel displayed a whitish hue, possibly due to HPMC's robust cross-linking tendency. Minor variations were noted in drug content, and it's crucial to acknowledge

that viscosity inversely correlates with in vitro drug release, potentially affecting product efficacy (42-46).

The viscosity of the formulations is under the influence of various factors, including polymer concentration, molecular weight, and the extent of cross-linking. Notably, the gels formulated with Carbopol 934 demonstrated elevated viscosities compared to those formulated with MC and HPMC, as outlined in Table 2. This distinction can be attributed to the higher molecular weight of Carbopol 934, along with its more pronounced cross-linking, effectively impeding fluid flow. A noteworthy observation was that the viscosities of these gels were in harmony with the skin's pH, minimizing the risk of skin irritation. A vital prerequisite for achieving the intended therapeutic outcome at the targeted area is the spreadability of the gel to that localized site.

The spreadability of the gel is inherently linked to its viscosity, a range seen from 18.39 ± 0.12 to 36.72 ±0.45 gcm/s across all formulations (as indicated in Table 2). Analysis of cumulative drug release (%) over a 6-hour span from diverse gels (also outlined in Table 2) highlighted an inverse relationship between in vitro drug release and polymer concentration. The presence of water and propylene glycol aids in hydrating the stratum corneum, facilitating the creation of conduits through which the drug can permeate into deeper skin layers. Concurrently, alcohol evaporation on the skin's surface escalates the drug concentration on the skin, generating a concentration gradient that propels drug penetration.

Furthermore, alcohol has been recognized to render the skin surface more fluid, effectively enhancing drug permeation. However, prudence in alcohol concentration is vital to prevent any potential harm to the skin. In terms of drug release, the Carbopol 934-based gels displayed higher rates in comparison to their MC and HPMC counterparts. The drug release kinetics (outlined in Table 2) signaled that all formulations adhered to zero-order kinetics. Upon evaluating drug release mechanisms based on Higuchi data, diffusion was established as the rate-limiting step in drug permeation. Among the various batches, formulation B1 (0.5 g, Carbopol 934) aligned with the critical criteria of an optimized formulation (as shown in Table 2), thereby showcasing desirable in-vitro release. It is worth noting that all the gels incorporated pharmaceutically approved excipients in appropriate proportions, ensuring their non-immunogenicity and biocompatibility. Nevertheless, it's important to acknowledge the potential for allergic manifestations post-application.

The investigation into skin compatibility brought to light initial behavioral shifts in rats upon the initial application, likely attributed to the cooling effect of menthol and alcohol. However, with subsequent applications, the rats exhibited an increasing tolerance to these effects. Notably, no allergic manifestations surfaced during the 7-day observation period. During the stability assessment, the formulated gels underwent cyclic temperature variations (25°C and -5°C) without manifesting any alterations in their physical stability. Impressively, all formulations upheld the standards of stability tests aligned with ICH guidelines for three-month duration. Noteworthy stability persisted across drug content, viscosity, pH, and in-vitro drug release, as demonstrated in Table 3. This collective evidence underscored the enduring stability of the formulated gels.

The optimized gel formulation (B1) demonstrated significant anti-inflammatory potential, as shown in Table 4. It exhibited a maximum inhibition of rat paw edema of up to 77.02%, which aligns with the reported values. This indicates that the optimized formulation (B1) possesses potent topical activity in inhibiting edema. The in vitro 5-lipoxygenase inhibition by the optimized Ketorolac gel was assessed using increasing dosages.

Conclusions:

The utilization of topical ketorolac administration presents a safer option compared to oral intake, reducing the potential for adverse effects like abdominal discomfort, nausea, dyspepsia, and headaches. The research at hand focused on the development of ketorolac gels employing distinct gelling agents such as methyl cellulose, carbopol, and HPMC. Further exploration in this avenue holds promise for effectively addressing localized inflammatory conditions in a precise and focused manner.

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Table 1: Formulation table for Ketorolac gels

Ingredients in gm	**Formulation Code**								
	A1	A2	A3	B1	B2	B3	C1	C2	C3
Methyl Cellulose	0.5	01	02	-	-	-	-	-	-
Carbapol 934	-	-	-	0.5	01	02			
HPMC	-	-	-	-	-	-	0.5	01	02

Every formulation code has: Ketorolac, 0.5 g; mentha oil, 1 g; prostaglandin (PG), 5 g; methyl paraben, 0.32; propyl paraben, 0.62; triethanolamine (TEM), Q.S and hydroalcoholic vehicle (2:1), Q.S. to 50 g

Table 2—Evaluation parameters of Ketorolac gels [values are represented as Mean±SD, (n=3)]

Formulation Code	Drug Content %	Viscosity CPS	pH	Spreadability gcm/s	Drug release in 6 hr % Q	Cumulative drug release in 6h % Q/A	First Order R^2	Zero Order R^2	Higuchi Model R^2
A1	97.70± 0.25	1180.1 ± 0.03	6.33±0.12	18.39 ± 0.12	31.41± 0.02	14.02± 0.03	0.9657	0.9723	0 .9632
A2	97.21± 0.22	2955.3 ± 0.02	6.54±0.04	31.44 ± 0.12	41.23± 0.13	12.33± 0.05	0.9345	0.9756	0 .9646
A3	98.22± 0.44	4111.3 ± 0.04	6.34±0.11	33.01 ±0.03	32.52± 0.21	9.23 ± 0.02	0.9768	0.9734	0 .9756
B1	97.53± 0.33	3222.6 ± 0.03	7.01±0.10	37.52 ±0.04	57.72± 0.09	14.11± 0.06	0.9456	0.9797	0 .9645
B2	97.26± 0.44	3243.3 ± 0.02	6.47±0.03	36.72 ±0.45	42.33± 0.03	9.32 ± 0.04	0.9532	0.9743	0.9633
B3	97.72± 0.12	4644.3 ± 0.11	6.57±0.04	34.44 ±0.05	40.32± 0.22	7.25 ± 0.05	0.9373	0.9757	0.9612
C1	96.52± 0.54	1323.5 ± 0.03	7.22±0.03	21.85 ±0.12	41.22± 0.73	12.34± 0.04	0.9855	0.9745	0.9820
C2	97.45± 0.52	1945.5 ± 0.05	6.32±0.11	23.41 ±0.45	40.22± 0.06	13.12± 0.02	0.9637	0.9723	0.9434
C3	96.31± 0.82	2846.5 ± 0.21	7.01±0.02	27.70 ±0.02	39.12± 0.54	12.34± 0.01	0.9634	0.9722	0.9501

Table 3—Stability studies data [values are represented as Mean±SD, (n=3)]

Formulation code	Drug content %	Viscosity cps	pH	Cumulative drug release in 6 h, % Q/A
A1	98.33 ± 0.34	1141.9 ± 0.02	6.22 ± 0.03	13.20 ± 0.03
A2	98.23 ± 0.31	2924.5 ± 0.01	6.33 ± 0.09	11.70 ± 0.04
A3	97.14 ± 0.22	4132.7 ± 0.04	6.42 ± 0.11	10.12 ± 0.06
B1	97.12 ± 0.32	2322.5 ± 0.05	6.79 ± 0.12	12.32 ± 0.08
B2	98.42 ± 0.34	3418.1 ± 0.01	6.77 ± 0.08	13.21 ± 0.08
B3	95.42 ± 0.33	4545.2 ± 0.04	6.56 ± 0.04	9.90 ± 0.05
C1	97.11 ± 0.11	1533.3 ± 0.03	6.44 ± 0.11	12.97 ± 0.04
C2	95.34 ± 0.05	1424.5 ± 0.03	6.51 ± 0.07	12.31 ± 0.03
C3	98.89 ± 0.90	2726.4 ± 0.10	6.46 ± 0.10	11.20 ± 0.03

Table 4—Anti-inflammatory study of Ketorolac Gel (B1)

Group	Time (hr)	Mean paw edema volume	Inhibition of edema (%)
Control	1	0.167	-
	2	0.275	-
	3	0.234	-
	4	0.323	-
	5	0.411	-
	6	0.445	-
Test	1	0.123	20.71
	2	0.135	44.42
	3	0.119	55.14
	4	0.107	64.44
	5	0.103	71.01
	6	0.101	77.02