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Characterization and *in vitro* stability of tolfenamic acid-loaded carboxymethylcellulose ethanolic hydrogels

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Abstract

Tolfenamic acid (TA) is a fenamate nonsteroidal anti-inflammatory drug with expanding therapeutic potential, but its clinical use is limited by systemic side effects and the lack of topical formulations. Developing a stable topical gel may enable localized delivery of TA while minimizing systemic exposure. In the present study, simple hydroalcoholic gel formulations of TA with carboxymethylcellulose sodium (CMC) were prepared, with or without propylene glycol (PG), at pH 6.5. Before the formulation step, the interactions and compatibility of the TA/CMC (1:1) physical mixtures were studied using Fourier transform infrared spectroscopy and high-performance liquid chromatography. The formulated gels were stored at room temperature (27 ± 2°C) for six months and evaluated for their physical and chemical stability. The results indicated that gels prepared without PG showed greater moisture evaporation, resulting in higher viscosity, spreadability, and swelling index, with lower weight loss. No syneresis was observed in either gel formulation under different conditions, except under acidic conditions, where syneresis was observed. Microscopic evaluation of the formulations revealed polymorphic changes of the drug in the gels during storage. Both gels demonstrated optimal chemical stability, with negligible degradation throughout the study. Release kinetic studies indicated that TA followed the Higuchi model, with almost 100% drug release within 2.5 h.

Keywords: Tolfenamic acid; Gel formulation; Humectant; Stability; Interaction; Release kinetics

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1. Introduction

Tolfenamic acid (TA) is an anthranilic acid derivative that belongs to the fenamates group of nonsteroidal anti-inflammatory drugs (NSAIDs).[1-3] It is traditionally used as an anti-inflammatory drug for the treatment of migraine, dysmenorrhea, rheumatoid arthritis, and osteoarthritis.[1,4,5] Recent studies have also shown that TA possesses potent

anticancer activity against several cancers.[6–16] In addition, it is effective in Alzheimer's disease when administered systemically[17–18] and has been reported to exhibit antibacterial activity against *Helicobacter pylori* when combined with bismuth III.[19,20] All these findings regarding TA's anticancer and antimicrobial activities have increased its clinical significance. TA can partially aid in the recovery of precancerous and cancerous lesions by controlling microbial colonization through its antimicrobial activity. Moreover, it can promote wound healing in cutaneous carcinoma lesions without disrupting healthy cells and can limit the adverse effects associated with cancer therapy. Currently, only the tablet dosage form of TA is available for human use[4] and a solution for injection for veterinary use,[21] with very little information regarding its topical use. The DrugBank reports that TA has fewer reported side effects compared to the other fenamates. Systemic absorption of TA is associated with gastrointestinal discomfort, nausea, occasional bleeding, ulceration, and others.[1,4] The literature lacks information on the formulation of different TA dosage forms, such as gels.

Keeping in mind recent advances in TA use, this study was designed to develop a topical gel that is advantageous for treating localized inflammation and reducing NSAID-associated side effects. As a Biopharmaceutics Classification System Class II drug, TA is inherently well-suited for topical delivery, where, due to its higher permeation, it can cross the stratum corneum without the solubility limitations encountered in oral administration. Such a detailed formulation-based study has not been conducted previously, and no gel formulation is currently available in the market for human use. The data obtained in this study would be beneficial to pharmaceutical scientists in formulating topical TA preparations for human use.

2. Materials and methods

2.1. Materials

Tolfenamic acid (99.97%), carboxymethylcellulose (CMC) sodium salt (medium viscosity), and ethanol (>99.1%) were purchased from Sigma-Aldrich Company Ltd. (United Kingdom [UK]). Propylene glycol (PG; >99.5%) was procured from Merck Chemicals (Germany). All other chemicals used in this study were of analytical grade, with the highest purity available from Sigma-Aldrich or Merck. The distilled water was deionized and then sterilized before use.

2.2. Methods

2.2.1. Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) was used to determine the purity of the active drug and to assess any interactions between the drug and the polymer. The TA–CMC powder mixture was prepared in a 1:1 ratio. The sample was thoroughly ground and mixed in a mortar with a pestle for 5 min before analysis. All spectra were collected using a diamond crystal (Smart iTR) sampling assembly attached to a Thermo Scientific Nicolet iS10 spectrometer (Thermo Fisher Scientific Inc., United States of America [USA]). The spectrum of each sample ($n = 3$) was collected after 64 scans over 4000–700 cm^{-1} with a resolution of 4 cm^{-1}. The data were analyzed using Omnic software (version 8.1).

2.2.2. Thin-layer chromatography

Thin-layer chromatography (TLC) was performed according to the method described in the British Pharmacopoeia[2] to confirm the purity of TA in the raw material and gel formulations. Pure TA (25 mg) was dissolved in a 1:3 volume/volume (v/v) mixture of methanol and methylene chloride, while an equivalent amount of drug was extracted from gel formulations using ethanol. A 10 µL solution was applied to precoated silica gel GF_{254} plates (Merck, Germany) with a thickness of 250 µm. The spot was allowed to dry in a warm-air current. The plate was then placed in the TLC tank and developed using a mobile phase of glacial acetic acid, dioxane, and toluene (1:25:90, v/v/v) at 27 ± 2 °C. The plate was allowed to dry, and the spot was detected under an ultraviolet (UV) lamp (Uvitech, UK) at 254 and 365 nm. The purity was further confirmed using another TLC method reported by Abdelwahab *et al.*[22] The mobile phase consisted of hexane, chloroform, acetone, and glacial acetic acid (75:25:20:0.1, v/v/v/v) at 27 ± 2 °C. The plates were prepared and used as described above, and the spots were detected in a similar manner. Each sample was run in triplicate by either method. Moreover, control excipients, either pure or from placebo gel formulations, were also run each time to verify whether the spots obtained were from the pure drug, its degradation products, or impurities in the excipients.

2.2.3. Compatibility study

Dried powders of TA (100 mg) and CMC (100 mg) in a 1:1 ratio were thoroughly mixed in a mortar with a pestle for 15 min and transferred to glass vials (Pyrex) (Normax, Portugal). One set of vials was placed in a stability chamber for six months at a temperature of 30 °C and 65% relative humidity (Model NEC 2530RS, Newtronic Lifecare Equipment Pvt. Ltd., India), while another set was stored under accelerated conditions of 40 °C/75% (Model YWER-A1001P, Dongguan Yuanyao Electronics Technology Co., Ltd., China). The samples were assayed periodically in triplicate at 0, 1, 2, 3, 4, 5, and 6 months. Control samples containing pure TA were also stored under the same

conditions and assayed accordingly.

2.2.4. Gel Formulation

The following formulation for a simple hydroalcoholic gel of TA was developed (Gel 1):

Constituents	Percentage (weight/weight)
TA	0.5%
CMC	3%
Ethanol	31.5%
Water	65%

Abbreviations: CMC: Carboxymethylcellulose sodium; TA: Tolfenamic acid.

To study the effect of humectant on the stability of TA in gel formulations, PG was added, and the formulation was adjusted accordingly (Gel 2):

Constituents	Percentage (weight/weight)
TA	0.5%
CMC	3%
PG	15%
Ethanol	31.5%
Water	50%

Abbreviations: CMC: Carboxymethylcellulose sodium; PG: Propylene glycol; TA: Tolfenamic acid.

2.2.5. Preparation of gels

To prepare the gel, a weighed amount of TA was dissolved in ethanol using a magnetic stirrer for 30 min in a stoppered glass container to form a clear solution. In this solution, a small amount of water was added, and the mixture was continuously stirred with a mechanical mixer fitted with a glass stirrer at approximately 500 rpm for 5 min. Similarly, CMC was added in small portions to the same solution and mixed for 10 min until a thick mass formed. The remaining water and humectant were added, and the mixture was stirred for a further 30 min. The pH of the gels was adjusted to about 6.5, and the mixture was mixed thoroughly for 15 min. The formulated gels were allowed to settle for 24 h before analysis. Placebo gels (without TA) with and without PG were prepared similarly at the same pH and used as controls.

2.2.6. pH measurements

The pH of the gel formulations was determined using a digital pH meter (Accumet® AR10, Fisher Scientific, USA). All measurements were performed in triplicate using a glass electrode, and the formulation temperature was recorded with a probe attached to the pH meter. The instrument was calibrated at room temperature (27 ± 2 °C) using commercially available buffer tablets (Merck, Germany) at pH 4.0 and 7.0, each diluted to 100 mL with distilled water. The glass electrode was immersed directly into the gel formulation and allowed to equilibrate for a few seconds.[2,23,24] The pH of the formulations was adjusted during preparation to approximately 6.5 using a few drops of phosphoric acid (1.0 M) or sodium hydroxide solution (1.0 M).

2.2.7. Storage of gels and stability studies

All gels were prepared under uniform conditions, stored at room temperature (27 ± 2 °C) in airtight glass containers, and evaluated periodically for any changes in their physical characteristics. The stability of the gels was determined by measuring TA over six months.

2.2.8. Determination of tolfenamic acid in gel formulations

(a) High-performance liquid chromatography

The assay of TA in the gel formulations was carried out using a previously developed and validated high-performance liquid chromatography (HPLC) method reported by Kazi *et al.*.[25] An ultra HPLC system (Dionex Ultimate 3000, ThermoFisher, Germany), equipped with a UV detector (VWD-3100, ThermoFisher, Germany) and a dual gradient analytical binary pump (HPG-3200SD, ThermoFisher, Germany), was used. In this study, a C18 column (5 µm, 250 × 4.6 mm) was used, and the solvent system was a mixture of acetonitrile and water (90:10, v/v) adjusted to pH 2.5.

(b) Assay method

The assay of TA was performed in triplicate by accurately weighing 1 g of the gel into a 25 mL beaker. To extract the drug from the gel matrix, 10 mL of ethanol was added and the mixture was thoroughly mixed with a glass rod for 5 min, and then filtered through a Whatman number 41 filter (Schleicher & Schuell, UK). The filtrate was collected in a screw-cap tube, tightly capped to prevent solvent evaporation, and then analyzed by HPLC.

2.2.9. Organoleptic studies

All gel formulations were physically evaluated for organoleptic properties, including color, odor, tactile characteristics (smoothness and thickness), visual changes or growth, and homogeneity throughout the study. Smoothness and thickness of the formulations were assessed by applying 1 g of the gel to the back of the hand, while all other observations were made through visual inspection. A total of 10 healthy volunteers (five males and five females) with no known disease and normal eyesight participated in the study.

2.2.10. Viscosity measurements

The viscosity of gels was measured using a calibrated digital viscometer (Model NDJ-8SN, WestTune, China). The instrument was calibrated using the manufacturer's standard. An accurately weighed gel was diluted with water at a 1:1 ratio and transferred to a beaker. The beaker was tapped several times and uniformly compressed from the top to remove voids within the gel. The spindle (no. 4) was adjusted to the mark in the sample and rotated at 0.3 rpm at 27 ± 2 °C. Each reading was taken in triplicate, and the average viscosity was recorded.

2.2.11. Spreadability

The spreading coefficient, or spreadability, of the gel formulations was determined using an apparatus described by Mutimer and colleagues.[26] A fixed glass slab mounted on a wooden base was fitted with a pulley at one end. A similar movable glass slab, with a hook at one end, was placed over the fixed glass slab. A string tied to a pan was attached to the hook to displace the movable slab. To perform the spreadability test, 1 g of gel was placed between the fixed and movable glass slabs. A 500 mg weight was placed on the glass slabs for 10 min to remove air bubbles from the gel. A weight of 125 mg was then placed on the pan attached to the movable slab. The time required to displace the movable slab by 0.15 m (i.e., 15 cm) was measured with a stopwatch. Each sample was performed in triplicate, and the spreadability was calculated using the following formula:[27–30]

$$S = (W_p \times D_m)/T \quad (1)$$

where S is the spreadability of the formulation, W_p is the weight placed on the pan, D_m is the distance (m) travelled by the movable slab after putting the weight, and T is the time (s) taken for the slab to move after applying the weight.

2.2.12. Moisture content

The moisture content of the gels was determined by placing 1 g of the gel in a 25 mL beaker and heating it in a water bath at 100 °C until it dried, then placing it in a hot-air oven at 50 °C for 15 min to ensure complete moisture removal. The percentage moisture content of each sample was determined in triplicate and calculated using the following equation:[31–33]

$$MC\ (\%) = [(W_{gel} - W_{dry})/W_{gel}] \times 100 \quad (2)$$

where MC (%) is the percentage moisture content, W_{gel} is the weight of the gel before drying, and W_{dry} is the weight of the gel after drying in an oven.

2.2.13. Swelling index

The swelling index was measured by weighing 1 g of gel and drying it in a water bath at 100 °C until completely dry. The dried mass was then placed in a hot-air oven at 50 °C for 15 min to remove residual moisture. The dried gel mass was weighed accurately and transferred onto aluminium foil with equidistant perforations, which served as a sieve. This sieve was placed in a petri dish containing water and allowed to stand (swell) for 4 h. The sample was reweighed after the excess water was removed. The swelling index of each sample was measured in triplicate, and the percentage was calculated using the following formula:[34–38]

$$S_w = [(W_{swell} - W_{dry})/W_{dry}] \quad (3)$$

where S_w is the ratio of swelling, W_{swell} is the weight of the swollen gel, and W_{dry} is the weight of the dried gel.

2.2.14. Syneresis

Syneresis for each sample was performed in triplicate by adding 1 g of each gel into a centrifuge tube under the following conditions, followed by centrifugation (Model 80-2S, Labfuge, Taiwan) at 4000 rpm for 60 min. The conditions were as follows:[39,40]

(i) Formulated gels with no variation.
(ii) Gels were heated in a water bath at 70 °C for 1 h and tested after equilibration to room temperature.
(iii) Gels refrigerated for 24 h and used after equilibration to room temperature.
(iv) Gels acidified using 1 M phosphoric acid (H_3PO_4) at pH 2.5–3.0 and used after 24 h.
(v) Gels alkalinized using 1 M sodium hydroxide (NaOH) at pH 9.5–10 and used after 24 h.

2.2.15. Microscopic examination of the gel formulations

A compound microscope (Eclipse E200LED MV, Nikon, Japan) equipped with a camera (DS-L3 control unit and DS-Fi2 head, Nikon, Japan) was used to visually examine the gel formulations. A thin film of gel was prepared by spreading 500 mg of gel onto a glass slide (7.5 × 2.5 cm) and observed under 10× and 40× magnification after air drying.

2.2.16. Release study

The release of TA from the gels was studied using a digital six-cell diffusion apparatus (Franz diffusion cell system) (Orchid Scientific, India) connected to a thermostatically controlled water circulation tank (Model No. EMFDC 06, Orchid Scientific, India). The body of each diffusion cell was filled with 5 ± 0.1 mL of hydroalcoholic solution in a 50:50 (v/v) ratio. Nylon membranes (Membrane Solutions, USA) with a diameter of 13 mm (1.69 cm^2) and a pore size of 0.45 μm were placed between the cap and the body of

the Franz diffusion cell. The membranes were soaked in the receptor medium for 30 min before use. 300 mg of gel was placed on the membrane, and the cells were covered with parafilm to prevent evaporation of the vehicle. The receptor medium was continuously rotated with a magnetic bead at 100 rpm and maintained at 32 ± 0.5 °C. Samples were withdrawn at predetermined time intervals of 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 h, and an equal volume of fresh receptor medium was added after each sampling to maintain the sink conditions. The withdrawn samples were subjected to UV spectrometric assay, and the amount of TA released from each gel was determined in triplicate. The release kinetics of TA from each gel were determined using various mathematical models, including zero-order, first-order, and Higuchi models, according to the following formulas:[41–44]

Zero-order model: $Q_t = k_0 t$ (4)

where Q_t is the amount of drug release at time t; k_0 is the zero-order release rate constant (concentration/time), and t is the time in hours.

First-order model: Log Q = Log Q_0 – $kt/2.303$ (5)

where Q_0 is the initial concentration of the drug, k is the first-order release rate constant, and t is the time in hours.

Higuchi model: $Q = k_H t$ (6)

where Q is the amount of drug release, k_H is the Higuchi release rate constant, and t is the time in hours.

3. Results and discussion

Since TA has emerged as a viable alternative to the most commonly used fenamates, owing to its relatively lower toxicity and less toxic metabolites, the lack of formulation development remained a significant limitation preventing its use as a dosage form. Therefore, a stable gel matrix embedded with TA was developed as a potent local anti-inflammatory drug delivery system.

To create a stable gel formulation, excipients and the polymer were selected based on their suitability, formulation roles, and cost. The gels were prepared in a defined mixing order and subjected to confirmation of ingredient purity, interaction, and compatibility, as well as physicochemical characterization, including release profiling, as discussed in the following sections.

3.1. Confirmation of purity of tolfenamic acid

The purity of TA was confirmed through FTIR. The principal peaks obtained for the pure drug (Figure 1) were compared with those reported in the literature.[45–50] TA contains a chloro, methyl phenyl ring and a benzoic acid ring in its structure, which are linked together by a secondary amino group (Figure 1). It exhibits characteristic peaks at 3342–3340 cm^{-1} (amino group stretching), 1661 cm^{-1} (ring carbonyl group stretching), 1582 cm^{-1} (benzene ring stretching), 1500 cm^{-1} (amino group bending), 1440–1400 cm^{-1} (methyl group stretching), 1267 cm^{-1} (C–H, stretching) and 749 cm^{-1} (C–N, stretching).[45–47,51–53] All principal peaks were consistent with the reported values, with no additional peaks observed, indicating that the material was pure and free of impurities.

Moreover, the purity was confirmed by TLC. TLC was performed according to the methods reported in the British Pharmacopoeia[2] and Abdelwahab *et al.*[22] No spots other than TA were detected on the TLC plates using either method, and the retention factor values were consistent with those reported for the pure compound (0.81 and 0.49 by each method, respectively). These findings further confirm the purity of the active drug.

3.2. Interaction and compatibility studies of tolfenamic acid and carboxymethylcellulose sodium

3.2.1. Interaction study

To identify possible molecular interactions between the drug and the polymer, FTIR spectroscopy was performed on a physical mixture of TA and CMC. This was done before formulation to identify potential physical and/or chemical instability between TA and CMC. The FTIR spectra of pure TA and CMC were compared with that of their 1:1 physical mixture (Figure 1). No significant changes in the characteristic absorption bands of TA were observed, indicating the absence of molecular interaction between the two compounds. However, due to overlapping band regions in TA and CMC, minor peak overlaps were observed.

Tolfenamic acid is a crystalline drug that exhibits polymorphism. Any polymorphic changes can be detected by FTIR spectroscopy in the regions 3350–3300 cm^{-1} and 1700–1300 cm^{-1}.[45,47,48,50] No shift or change in the peak of TA was observed, indicating that physical mixing of the two compounds did not induce any polymorphic change in the crystalline form (Figure 1).

3.2.2. Compatibility test

The possibility of chemical interaction between TA and CMC was investigated through a compatibility study. This study was performed to identify potential chemical risks between TA and CMC prior to gel formulation. The assay results showed no significant chemical changes in the 1:1 physical mixtures of TA and CMC, stored under either real-time (30 °C/75%) or accelerated conditions (40 °C/75%) (Table 1). These findings indicate that the two compounds are chemically compatible and suitable for use

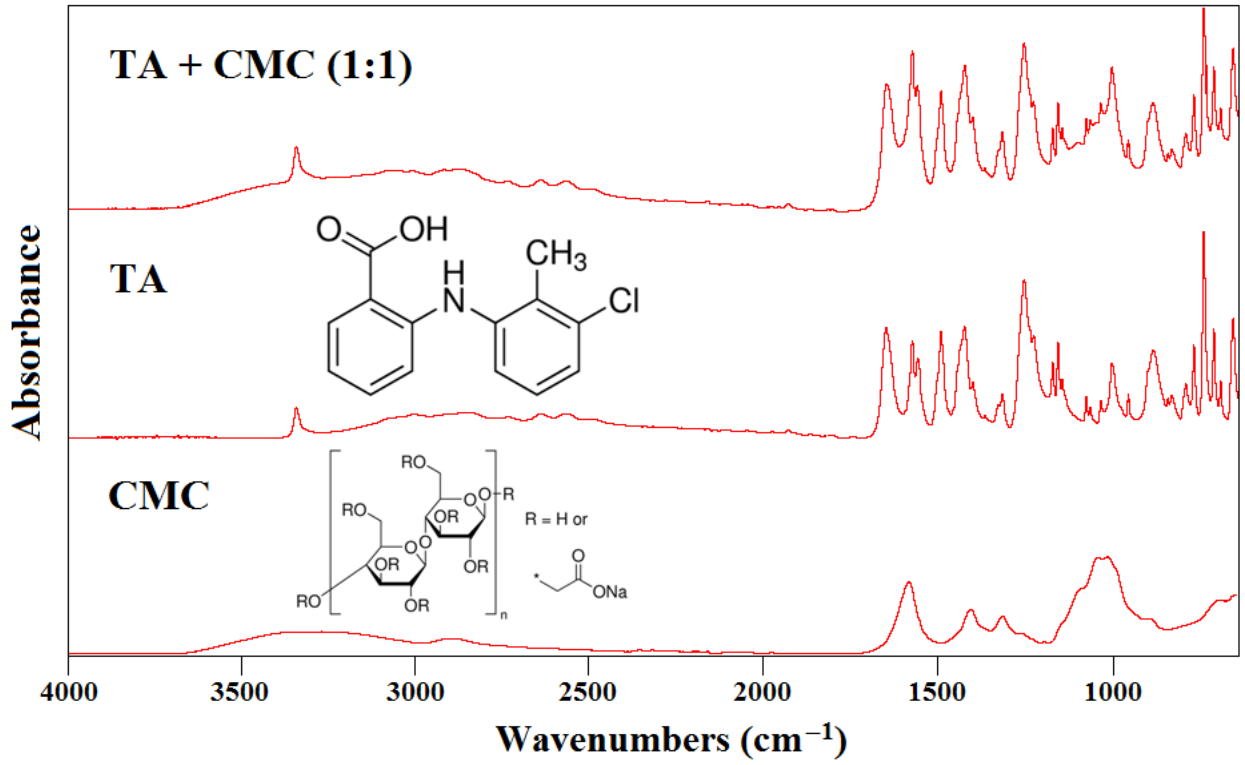

Figure 1. FTIR spectra of pure TA, CMC sodium, and their physical mixture
Abbreviations: CMC: Carboxymethylcellulose; FTIR: Fourier transform infrared spectroscopy; TA: Tolfenamic acid.

together in gel formulations.

3.3. Formulation of the gels

Simple hydroalcoholic gel formulations of TA were prepared using CMC sodium as the gelling agent. The medium viscosity grade is recommended for use as a gel-forming agent at concentrations of 3–6%.[54] Therefore, the recommended minimum concentration (i.e., 3%) was used to formulate the gels. CMC is widely used in pharmaceutical, cosmetic, and food products due to its non-toxic, non-irritant nature. It is also known to provide cytoprotective activity[54] and is stable over a wide pH range of 2–10. CMC can precipitate when mixed with alcohol[54]; therefore, during preparation, water was added to the drug–ethanol solution before the addition of the polymer. The gradual addition of CMC in small portions to the hydroalcoholic solution resulted in the formation of a thick and uniform gel. Another gel was prepared with PG to assess the effect of the humectant on the formulation stability. PG was selected based on its good chemical stability when mixed with ethanol or water.[54] It was used as a humectant, and the minimum recommended concentration for such purposes in gels is about 15%.[54] Additionally, PG is known to provide antimicrobial preservation when used at a concentration of at least 15%.[54] Therefore, due to this dual action, PG was added to the gel formulations at a 15% concentration. Gels prepared without and with PG are designated as Gel 1 and Gel 2, respectively, in this study.

The concentration of TA in the gels was fixed at 0.5%. Given the high potency and favorable safety profile of TA, a 0.5% concentration represents a conservative yet therapeutically meaningful dose suitable for first-in-class topical development. Although no commercial TA gel is currently available, the selected concentration aligns well with established topical NSAID formulations, such as diclofenac gel (0.5–1%), ketoprofen gel (0.5–2.5%), mefenamic acid, and other fenamates reported in experimental topical systems (~0.5–1%). Ethanol was added not only to solubilize TA in the gel system but also to act as a penetration enhancer.[54] All formulations were prepared using identical protocols to avoid any physical discrepancies. Placebo gels have also been prepared to compare the results with the formulated gels and to observe any changes in TA. Various physicochemical properties of the gel formulations were studied and are discussed in the following sections.

3.4. pH of the formulations

Table 1. Compatibility data of the physical mixture (1:1) of tolfenamic acid (TA) and carboxymethylcellulose sodium (CMC) at real and accelerated temperatures (n = 3).

Time (months)	TA (Pure) (%) ± SD		TA + CMC (1:1) (%) ± SD	
	30 °C	40 °C	30 °C	40 °C
0	99.97 ± 0.14	99.97 ± 0.14	99.97 ± 0.14	99.97 ± 0.14
1	100.10 ± 0.10	100.15 ± 0.15	99.41 ± 0.10	100.41 ± 0.15
2	100.05 ± 0.06	100.01 ± 0.10	101.16 ± 0.15	99.66 ± 0.08
3	99.80 ± 0.20	99.94 ± 0.11	99.66 ± 0.11	99.46 ± 0.25
4	99.90 ± 0.09	99.80 ± 0.12	99.67 ± 0.05	99.50 ± 0.18
5	100.25 ± 0.06	99.71 ± 0.06	98.91 ± 0.19	99.35 ± 0.07
6	100.10 ± 0.10	99.77 ± 0.12	99.16 ± 0.12	98.47 ± 0.13

A solution of CMC has a pH of approximately 6–8, with optimum stability near neutral pH.[54] The pH of both formulations was adjusted to 6.5, which lies within the optimal stability range for CMC. The pK_a of TA is reported to be 3.7–4.3,[55–57] while that of CMC is 4.3.[54] At this pH, both compounds are approximately 99% ionized. The increased ionization of both the drug and the polymer improves their miscibility, resulting in a uniform distribution of TA throughout the gel matrix, as evidenced by studies discussed later.

3.5. Quantification of tolfenamic acid in gels

The TA content in gel formulations was determined using the HPLC assay method described by Kazi *et al.*[25] As the reported method was originally developed for the assay of TA in pure solutions, the accuracy and precision of the method in gel formulations were determined before its application. The recovery results indicated that the method is highly accurate and precise for the assay of TA in gel formulations (Table S1). No interference from formulation excipients was observed. The chromatogram of TA in gel formulation is shown in the supplementary file (Figure S1).

3.6. Organoleptic properties of gel formulations

Organoleptic studies are considered essential not only for formulation identification and stability assessment but also for consumer acceptance.[58] The gel formulations were evaluated for color, odor, tactile characteristics, visual changes, and homogeneity. Observations related to these parameters studied are summarized in Table 2.

The formulated gels are highly opaque at the time of preparation and become slightly less opaque after 24 h. The appearance of both types of gels became translucent (i.e., semi-transparent) within one month, with no further changes observed thereafter. Uniformly mixing ingredients at a constant speed is an important step in gel preparation, as slight variations can lead to a yellow coloration due to polymorphic changes. TA is known to exhibit polymorphism with 11 distinct polymorphic forms identified to date, among which the white and yellow forms are the two most distinct.[45,48,50,51,59–66] Occasionally, during the preparation of trial batches, the gels turned yellow during ingredient mixing. However, this yellow coloration disappeared within 1–2 days, possibly because of the reversion of the yellow polymorphic form to the white form of TA.[48,50]

No characteristic odor was detected in either gel throughout the study. Moreover, no visual signs of mold/fungal growth were observed, which may be attributed to the presence of ethanol and PG in the formulations, both of which possess antimicrobial properties.[54] Both gel formulations appeared to produce a cooling effect when applied to the skin, likely due to the ethanol content. It was observed that the gels became thicker and stickier over time, requiring more friction during application on the back of the hand. This change was more pronounced in gels prepared without PG than in those prepared with PG (Table 2). Initially, some entrapped air bubbles were present in both formulations. The formation of air bubbles in gel preparations usually arises from vortex mixing during preparation.[67] Over time, the number of air bubbles decreases, possibly due to moisture evaporation from the formulations. The remaining entrapped bubbles persisted due to the increase in viscosity resulting from water loss.

3.7. Effect on viscosity

The viscosity of a gel determines its consistency and affects its spreadability.[68] It also indicates how long the gel will remain at the application site. Viscosity is known to increase with the concentration of the gelling agent.[69] In this study, a medium-viscosity grade of CMC sodium was used at 3% concentration to prepare gels at pH 6.5. This

Table 2. Organoleptic observations of gel formulations (n = 10)

Time (months)	Gel	Color	Odor	Tactile characteristics[a]	Visual changes/Mold growth	Homogeneity[b]
0	1	Opaque	–	S, C^+, +	–	+++
	2	Opaque	–	S, C^+, +	–	+++
1	1	Translucent	–	S, C^+, +	–	++
	2	Translucent	–	S, C^+, +	–	++
2	1	Translucent	–	S, C^+, ++	–	++
	2	Translucent	–	S, C^+, ++	–	++
3	1	Translucent	–	S, C^+, ++	–	++
	2	Translucent	–	S, C^+, ++	–	++
4	1	Translucent	–	S, C^+, +++	–	++
	2	Translucent	–	S, C^+, ++	–	+
5	1	Translucent	–	S, C^+, +++	–	+
	2	Translucent	–	S, C^+, +++	–	+
6	1	Translucent	–	S, C^+, +++	–	+
	2	Translucent	–	S, C^+, +++	–	+

Notes: [a] S = smooth; cooling sensation = C^+ (present) or C^- (absent); degree of thickness = + (thick), ++ (thicker), +++ (thickest). The degree of thickness is in comparison to the thickness at zero time. [b] Homogeneity (presence of air bubbles); high (+++), medium (++), low (+).

amount is recommended for topical gel preparation and provides maximum viscosity at this pH.[54] However, at this concentration, the gels' viscosity exceeded the measuring range of the viscometer (i.e., 0.01–2000 Pa·s). Therefore, uniform dilutions in a 1:1 ratio were performed prior to viscosity measurements, and the results are reported in Table 3.

The viscosity of gels was found to change over time (Figure S2). Initially, an increase in viscosity was observed for the first three months in Gel 1 and four months in Gel 2. Thereafter, a slight decrease or negligible change was noted in both formulations (Table 3). The initial increase in viscosity could be due to moisture evaporation from the gels, as evidenced by the weight-loss values (Table 3). In contrast, the subsequent decrease could be due to moisture absorption from the atmosphere. CMC is hygroscopic and can dry out during storage.[54] The change in viscosity was more pronounced in gels prepared without PG (Figure S2). The presence of a humectant in the other gel may have slowed evaporation.[54]

3.8. Spreadability of the gels

Spreadability refers to the thickness of a gel to cover an area of application. Generally, the lower gel thickness results in better spreadability. Increasing the polymer concentration decreases spreadability, whereas increasing ethanol concentration usually improves it.[70] Spreadability is measured as the time (s) taken by the gel to slide a known distance (m) under an applied weight. Faster coverage indicates better spreadability.[68]

Low spreadability values were initially observed for both gels, indicating good adhesion at the site of application (Table 3). A gradual increase in the spreadability values of both gels was observed over several months, after which the rate of increase slowed (Figure S3). Initially, Gel 1 showed better spreadability than Gel 2, likely due to its lower viscosity. By the end of six months of storage, the gel prepared with PG showed better spreadability than those prepared without it (Table 3). This indicates that humectants play a vital role in maintaining the physical stability of gel formulations.

3.9. Determination of moisture content

Moisture content refers to the amount of water present in a formulation, including both free and bound water molecules. The moisture content of a gel affects its biocompatibility and formulation performance. The amount of water absorbed by a gel is directly proportional to the concentration of the hydrophilic gelling agent.[33,38,71]

In the present study, both gel formulations showed a gradual loss in moisture content, indicating solvent evaporation during storage (Table 3). Theoretically, both gels contained approximately 96.5% solvent. However, the change in moisture content values of Gel 2 highlights the role of PG in slowing moisture evaporation, as gels

Table 3. Details of the physical parameters studied for the characterization of gels[a]

Time (months)	Gel	Viscosity × 104(Pa·s)[b,c]	Spreadability(g·m/s)[b]	Moisture content (%)[b]	Weight loss (%)[d]	Swelling index[b]
0	1	3.25 ± 0.16	11.31 ± 1.05	94.47 ± 2.83	0.00	2.66 ± 0.08
	2	3.55 ± 0.21	12.64 ± 1.09	89.64 ± 3.43	0.00	5.87 ± 0.19
1	1	3.92 ± 0.22	18.93 ± 0.95	90.74 ± 3.25	3.88	15.73 ± 0.55
	2	3.72 ± 0.26	15.35 ± 1.06	88.76 ± 2.76	1.56	9.05 ± 0.27
2	1	4.40 ± 0.23	28.53 ± 1.05	85.11 ± 1.92	7.50	36.79 ± 1.21
	2	4.05 ± 0.19	21.38 ± 0.78	85.76 ± 3.08	3.25	18.42 ± 0.63
3	1	4.73 ± 0.30	34.19 ± 0.94	77.45 ± 2.47	17.55	41.70 ± 1.55
	2	4.19 ± 0.25	24.80 ± 1.09	82.70 ± 2.25	5.45	24.27 ± 0.80
4	1	4.61 ± 0.29	34.77 ± 0.10	79.15 ± 2.99	17.20	39.25 ± 1.35
	2	4.25 ± 0.23	27.65 ± 1.03	80.50 ± 2.75	8.89	30.60 ± 0.92
5	1	4.65 ± 0.27	36.21 ± 1.11	78.70 ± 3.35	16.90	40.50 ± 1.19
	2	4.12 ± 0.25	27.50 ± 1.05	81.16 ± 2.21	8.85	27.80 ± 0.95
6	1	4.70 ± 0.31	37.66 ± 01.04	74.65 ± 2.86	18.15	42.15 ± 1.18
	2	4.22 ± 0.21	28.05 ± 0.97	80.35 ± 2.46	10.35	28.90 ± 1.15

Notes: [a] $n = 3$ in each case. [b] The ± values are standard deviations. [c] Viscosity measurements after 1:1 dilution. [d] Weight loss = (initial weight−weight after a month)/(initial weight) × 100.

prepared without PG showed a higher percentage of moisture loss (Figure S4). The slightly increased moisture content observed after the third and fourth months in Gel 1 and 2, respectively, may have resulted from absorption of atmospheric moisture (Table 3), as CMC can absorb large quantities of water at temperatures up to 37 °C.[54]

3.10. Determination of swelling index

The swelling index measures a gel's ability, in its dried state, to absorb water upon immersion for a defined period. The degree of swelling depends on the amount of gelling agent used in the formulation[26] and can provide insights into drug release from the gel matrix.[38] Dried gel samples rapidly absorb water upon immersion, resulting in weight gain. Along with the weight gain, a considerable increase in volume was also observed with increasing immersion time. Swelling is known to be associated with the pK_a values. Water absorption increases in the polymeric network when the medium pH exceeds the polymer's pK_a.[38] Since the gel formulations were prepared at pH 6.5, well above the pK_a values, high swelling index values were observed (Table 3).

Over the first month, the swelling index of both gels increased markedly (Figure S5), likely due to moisture loss during storage, as evidenced by the viscosity data. This loss of moisture increased the relative concentration of the gelling agent, leading to higher swelling index values. Minor fluctuations throughout the study may reflect absorption of environmental moisture, consistent with viscosity and moisture content observations.

The increase in swelling index values was more pronounced in gels prepared without PG (Gel 1) as compared to those prepared with PG (Gel 2) (Table 3). Gel 1 showed a 15-fold increase in swelling after 6 months relative to the initial value. In contrast, only a 4-fold increase has been observed in Gel 2, indicating less moisture evaporation from the polymeric network. These findings thus further confirm the advantage of using humectants in CMC gel formulations.

After drying, the gels differed physically: Gel 1, in its dried state, became hard and brittle, while Gel 2 formed a stretchy, rubbery mass. This difference may be due to PG's boiling point (188 °C).[54] Heating at 100 °C easily removed water, producing a hard mass in Gel 1. In contrast, residual PG in Gel 2 likely imparted flexibility, resulting in lower swelling index values.

3.11. Syneresis under different storage conditions

Syneresis is a macroscopic phenomenon in which liquid exudes from a gel, causing deswelling.[40,72] It is a form of instability in gel formulations[39] that typically occurs due to an insufficient amount of the gelling agent.[73]

In the present study, the syneresis of the formulated gels was determined over time and under different conditions (Table 4). Both gels showed good stability

under all conditions except under acidic conditions, where syneresis was observed (Table 4). This indicates that the concentration of each ingredient in the gel formulations was optimal with respect to syneresis, and that the formulated gels were stable under refrigeration, heating, and alkaline exposure. In an acidic medium, small amounts of liquid were exuded, likely due to the instability of CMC under highly acidic conditions.[54]

3.12. Physical parameters of placebo gels

The physical changes observed in the TA gel formulations were also compared with those of the placebo gels. A set of placebo gels, both with and without PG, was prepared and stored under conditions similar to those used for the TA gels. Measurements of viscosity, spreadability, moisture content, swelling index, and syneresis showed almost negligible differences compared with the TA gels. Likewise, the organoleptic properties of the placebo gels were comparable to those of the drug-loaded gels, except that no yellow polymorphic changes appeared in the placebos. This indicates that the presence of the drug in the polymeric network does not alter the physical parameters; these effects are solely related to the properties of the gelling agent and solvents employed.

3.13. Microscopic studies of the gel formulations

The gel formulations were subjected to optical microscopy to evaluate the distribution of TA particles within the gel matrix and to detect any changes in their physical appearance during storage. Both gels initially exhibited a homogeneous distribution of TA crystals (Figure 2A,B), indicating uniform mixing and consistent formulation. Over time, however, the distribution of TA in the gel matrix became more clustered or crowded (Figure 2C,D), which could be due to water evaporation, which increased the relative drug mass compared with the gel weight, consistent with the observations from the swelling index studies. However, the distribution of TA is denser in Gel 1 (Figure 2C) than in Gel 2 (Figure 2D), further confirming the previously discussed effects of PG.

TA is known to exhibit polymorphism.[45,47,48,50] Initially, no physical or polymorphic changes were observed in TA particles (Figure 2A–D). Over time, the yellow polymorphic form of TA has been observed in both gel formulations (Figure 2E,F), with the form more prominent in Gel 1 (Figure 2E). This yellow form was identified and confirmed by FTIR spectroscopy, as previously described.[25,46,47,50,51,53]

3.14. Stability of tolfenamic acid in gel formulations

Both gel formulations were stored at room temperature (27 ± 2 °C) for six months. The assay of each gel has been performed using HPLC, and the results are reported in Table 5. The results are consistent with the interaction and compatibility studies, as both formulations retained almost 100% content after six months of storage. These findings were further confirmed by TLC, which shows no spots other than TA, indicating negligible or no formation of degradation products.

Table 4. Effect of different conditions on the syneresis of the gels (n = 3)

Time (months)	Gel	No variation	Heated	Refrigerated	Acidified	Alkalinized
0	1	–	–	–	+	–
	2	–	–	–	+	–
1	1	–	–	–	+	–
	2	–	–	–	+	–
2	1	–	–	–	+	–
	2	–	–	–	+	–
3	1	–	–	–	+	–
	2	–	–	–	+	–
4	1	–	–	–	+	–
	2	–	–	–	+	–
5	1	–	–	–	+	–
	2	–	–	–	+	–
6	1	–	–	–	+	–
	2	–	–	–	+	–

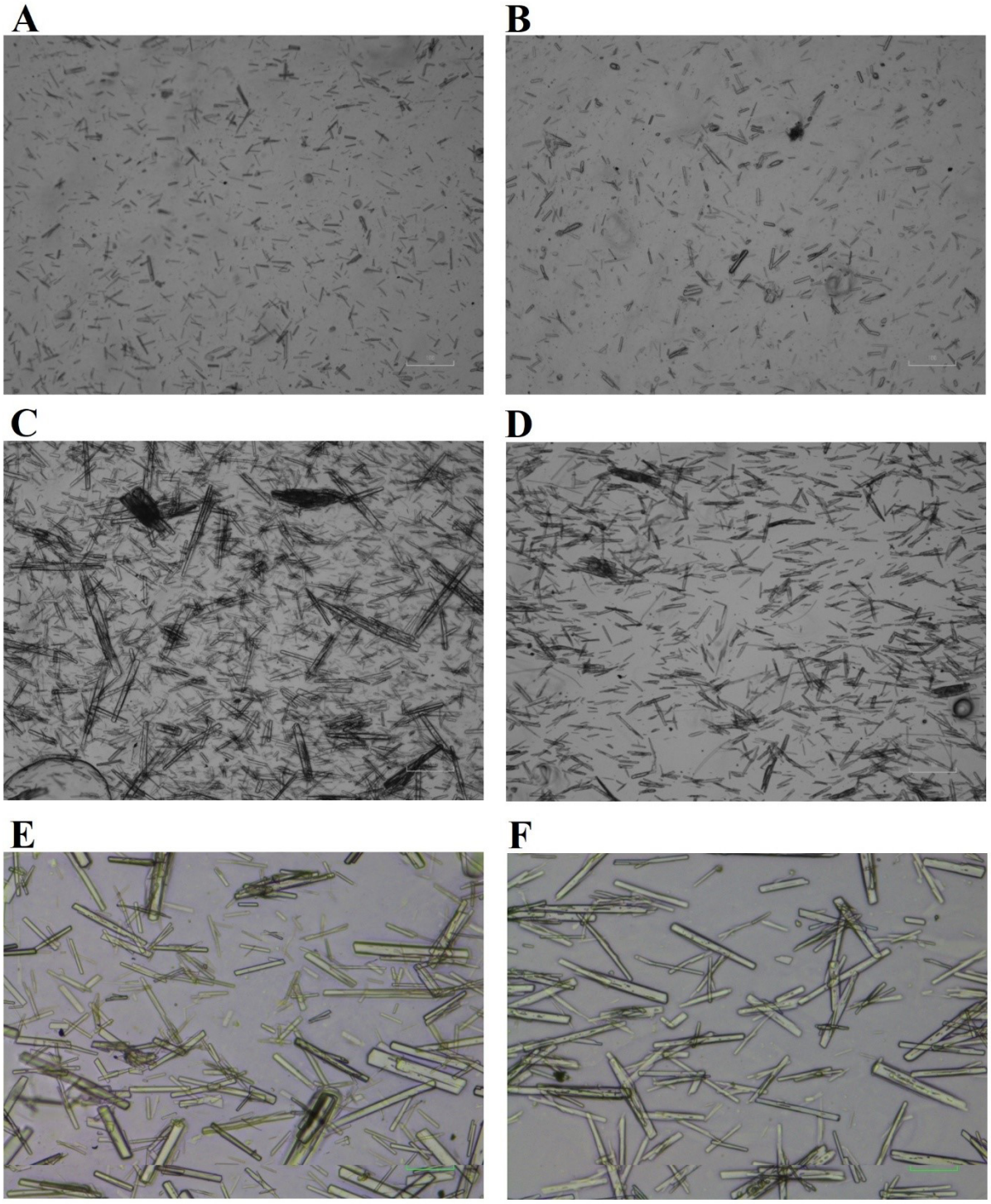

Figure 2. Distribution of tolfenamic acid: At zero time in Gel 1 (A) and Gel 2 (B); after 6 months in Gel 1 (C) and Gel 2 (D); physical appearance of TA crystals after 6 months in Gel 1 (E) and Gel 2 (F)

Table 5. Stability of tolfenamic acid in gel formulations stored at 30 ± 1 °C for 6 months (n = 3)

Time (month)	Recovery (%) ± SD	
	Gel 1[a]	Gel 2[a]
0	101.25 ± 1.52	99.63 ± 0.80
1	100.90 ± 1.22	101.40 ± 1.06
2	100.55 ± 0.85	100.05 ± 1.15
3	101.14 ± 1.32	102.03 ± 1.58
4	99.75 ± 1.02	101.25 ± 1.09
5	100.47 ± 0.78	99.85 ± 1.64
6	98.88 ± 1.24	100.35 ± 0.91

Note: [a]The weight of gel taken from each formulation is corrected for the values of weight loss during storage.

As discussed earlier under the swelling index and observed in the microscopic studies, solvent evaporation increased the relative concentration of both the polymer and the drug. The results obtained for 1 g of gel over 1–6 months were consistently above 100%. Therefore, the calculations were adjusted based on the gels' monthly weight loss during storage (Table 3). The assay values of approximately 99% and 100% for Gels 1 and 2, respectively, after six months of storage indicate good stability of TA in the gel formulations.

3.15. Release of tolfenamic acid from gel formulations

Determining the pattern and rate of drug release from a formulation is essential for its optimization.[74] In the present study, the performance of the formulated gels in releasing the drug from the polymeric network was evaluated using a Franz diffusion apparatus in accordance with United States Food and Drug Administration guidelines.[75] Both gel formulations exhibited moderate TA release, with almost 100% of the drug released within 2.5 h (Table 6). The drug release was faster in gels prepared without PG (Gel 1) compared with gels containing PG (Figure S6). The slight delay in TA release from Gel 2 is likely attributable to the increased viscosity resulting from the addition of PG (Table 3).

The release kinetics of TA from both gel formulations were investigated using zero-order, first-order, and Higuchi models. In the zero-order model, a plot of concentration versus time yields a straight line with a slope equal to k_0 and an intercept at the origin. In a first-order model, a plot of the logarithm of the remaining drug concentration versus time produces a straight line, with k calculated by multiplying the slope by 2.303. In the Higuchi model, a plot of cumulative percent drug release versus the square root of time is made, and the slope corresponds to k_H. When all three models were applied to the data, the Higuchi model provided the best fit, indicating that the drug release rate from CMC gels is proportional to the inverse square root of time. The regression coefficients and rate constants for all models are presented in Table 7. The Higuchi model provided the best fit. The first-order model also showed a moderate fit for both gels (Table 7), indicating good drug release from the gels.

In vitro release data demonstrated that the 0.5% gel delivered approximately 1400 µg/1.69 cm² within 2.5 h (Table 6), a range considered sufficient to achieve localized

Table 6. Percentage release of tolfenamic acid from gel formulations at different time intervals (n = 3)

Gel	% Drug release ± SD at different time intervals (h)[a]						
	0	0.5	1.0	1.5	2.0	2.5	>2.5
1	0.00	34.93 ± 1.71 (524)	62.87 ± 2.51 (943)	79.47 ± 4.77 (1,192)	90.67 ± 4.63 (1,360)	98.27 ± 4.37 (1,474)	100.00
2	0.00	30.87 ± 0.91 (463)	56.07 ± 2.44 (841)	73.33 ± 3.56 (1,100)	85.40 ± 4.02 (1,281)	94.67 ± 2.87 (1,420)	100.00

Note: [a]The values in parentheses are the drug release in µg/1.69 cm².

Table 7. Release kinetics of tolfenamic acid from gel formulations (n = 3).

Gel	Zero-order		First-order		Higuchi model	
	R^2	k_0 (h^{-1})	R^2	k (h^{-1})	R^2	k_H (h$^{-1/2}$)
1	0.928	38.58	0.932	1.53	0.987	64.89
2	0.951	37.38	0.966	1.13	0.983	61.99

Abbreviations: k: First-order release rate constant; k_0: Zero-order release rate constant; k_H: Higuchi release rate constant; R^2: Coefficient of determination.

anti-inflammatory activity without excessive systemic exposure. Higher drug loading may not be required in such cases, as near-complete release was achieved with predictable Higuchi kinetics, indicating that diffusion through the hydrated CMC matrix was the dominant release mechanism. However, further optimization through modification of formulation excipients and investigation of controlled or sustained-release systems should be explored in future studies.

4. Conclusion

The gel formulations prepared in this study demonstrated good chemical stability, attributed to the good compatibility between the drug and the polymer. Changes in the gels' physical parameters were primarily associated with moisture loss during storage. Approximately 45% and ~20% changes in viscosity were observed for Gel 1 and Gel 2, respectively, over six months. Similar trends were noted for other related parameters during the storage period. Although incorporation of TA into the gels did not significantly alter the physical characteristics of the formulations—as confirmed by placebo gel studies—the observed physical changes in the gels may have influenced the drug's physicochemical characteristics. The polymorphic transitions may have occurred as a result of gel drying due to water evaporation. The use of a humectant during the preparation of CMC gels loaded with TA is of great importance, as these gels exhibit better physical stability than those prepared without it. However, the release of TA from such gels is slower than from gels prepared without a humectant, but the difference is not significant.

Overall, moisture evaporation was identified as a key factor affecting all parameters related to the gels' physical stability. Moisture absorption is evident due to repeated container opening for analysis or use. An increase in spreadability would improve the uniformity of gel distribution across the skin, potentially influencing drug absorption. In such cases, either a higher amount of the drug may be delivered unintentionally or a lower applied dose may be sufficient to achieve the desired therapeutic effect. Therefore, the use of improved packaging materials—such as airtight glass jars or high-density polyethene tubes—is recommended to control moisture evaporation and absorption. Also, prolonged exposure of the container to the open environment should be avoided to avoid unnecessary moisture loss. Further investigations into the microbiological stability and photostability of TA gel formulations are warranted to support the development of a more stable and effective topical formulation.

Tolfenamic acid was incorporated at a concentration of 0.5%. This concentration was selected to ensure adequate local drug availability while maintaining formulation stability and acceptable rheological properties. The selected strength allowed uniform drug distribution within the gel matrix and enabled diffusion-controlled release suitable for topical delivery. Although satisfactory *in vitro* performance was demonstrated, future studies should explore concentration-response relationships and sustained skin permeation to further optimize the topical dosing of TA.

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Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

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Availability of data

The data will be made available on reasonable request to the corresponding author.

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