ORIGINAL ARTICLE

Enhancement of Solubility, Dissolution, and Anti-Inflammatory Activity of Aceclofenac via Multicomponent Crystal Formation with Tromethamine

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ABSTRACT

Aceclofenac (ACE) is a non-steroidal anti-inflammatory drug (NSAID) widely used for osteoarthritis and rheumatoid arthritis, yet its therapeutic performance is limited by poor aqueous solubility as a Biopharmaceutics Classification System (BCS) Class II drug. This study aimed to enhance the physicochemical and pharmacological properties of ACE by forming a multicomponent crystal (MC) with tromethamine (TRIS). The MC was produced via solvent-dropped grinding in a 1:1 molar ratio and characterized by differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD), and Fourier-transform infrared spectroscopy (FTIR). DSC and PXRD results indicated the formation of a eutectic system with reduced crystallinity, while FTIR showed intermolecular hydrogen bonding, evidenced by the broad OH stretching band near 3000 cm^{-1}, without new covalent bond formation. Solubility increased 35.8-fold relative to pure ACE, and dissolution testing showed a markedly improved dissolution efficiency (DE_{60}) of 75.33 ± 1.57% compared with 49.10 ± 0.54% for ACE alone. In vivo anti-inflammatory assessment using a carrageenan-induced granuloma pouch model further demonstrated superior pharmacological activity of the MC, significantly reducing exudate volume (0.56 mL) and TNF-α levels (25.26 pg/mL) versus pure ACE (0.82 mL and 29.70 pg/mL). Overall, the ACE–TRIS multicomponent crystal effectively enhances solubility, dissolution, and anti-inflammatory efficacy, offering a promising approach for improving ACE's therapeutic performance.

Keywords: Aceclofenac, Tromethamine, Multicomponent crystal, Solubility, Dissolution rate

Introduction

Aceclofenac (ACE) is a commonly utilized non-steroidal anti-inflammatory agent (NSAID) that is routinely prescribed for managing conditions such as osteoarthritis, and ankylosing spondylitis and rheumatoid arthritis. Its pharmacological activity involves the inhibition of cyclooxygenase enzymes (COX-1 and COX-2), which leads to a reduction in both inflammation and pain [1,2]. Although ACE is known for its therapeutic effectiveness, it falls under Class II of the Biopharmaceutics Classification System (BCS), which is defined by its poor solubility in water and high permeability across biological membranes [3]. This poor solubility significantly limits its dissolution rate, leading to suboptimal bioavailability and inconsistent therapeutic effects. To compensate for this limitation, higher doses are often required, which increases the risk of gastrointestinal and renal adverse effects, thereby posing safety concerns for long-term use [4–6].

Improving the solubility and dissolution behavior of drugs with limited water solubility remains a key focus in pharmaceutical formulation. Numerous strategies, including salt modification, nanoparticle-based systems, and the induction of amorphous states have been investigated to enhance the dissolution performance of BCS Class II compounds [7,8]. For ACE, some strategies including salt formation [3], solid dispersions

[2], and multicomponent crystal formation with different coformers [1,9] have been investigated to overcome its solubility limitations. Among these approaches, solid dispersions have shown potential in improving dissolution; however, their reliance on polymeric carriers can lead to increased molecular weight and particle size, which may compromise drug bioavailability and therapeutic efficacy [10]. In contrast, multicomponent crystal formation emerges as a superior alternative, enhancing solubility and dissolution without the need for polymeric carriers, thereby avoiding potential pharmacokinetic complications [5,11]. The technique, which utilizes a coformer to adjust the physicochemical characteristics of the active pharmaceutical ingredient (API) without impacting its pharmacological function, has received considerable interest for its effectiveness in improving solubility, dissolution rate, and stability while preserving the molecular structure of the drug [12,13]. Consequently, multicomponent crystal formation represents a promising strategy for optimizing the physicochemical and biopharmaceutical performance of ACE.

ACE is a widely prescribed non-steroidal anti-inflammatory drug (NSAID) commonly indicated for the treatment of osteoarthritis and rheumatoid arthritis. Its therapeutic action is mediated through inhibition of cyclooxygenase isoenzymes (COX-1 and COX-2), resulting in attenuation of inflammation and pain [1,2]. Despite its proven clinical efficacy, ACE is classified as a Biopharmaceutics Classification System (BCS) Class II compound, characterized by low aqueous solubility and high membrane permeability [3]. This intrinsic solubility limitation restricts its dissolution rate, leading to suboptimal bioavailability and variable therapeutic responses. Consequently, higher doses are often required to achieve therapeutic levels, which may elevate the risk of gastrointestinal and renal adverse events, posing safety concerns for prolonged administration [4,5].

Enhancing the solubility and dissolution profile of poorly water-soluble drugs remains a central challenge in pharmaceutical development. Various formulation strategies, such as salt modification, nanoparticle-based systems, and amorphization, have been explored to improve the dissolution performance of BCS Class II drugs [7,8]. For ACE, approaches including salt formation [3], solid dispersion systems [2], and multicomponent crystal with diverse coformers have been evaluated [1,9]. While solid dispersions can improve dissolution, their dependence on polymeric carriers often results in increased molecular weight and particle size, potentially compromising drug bioavailability and therapeutic efficiency [10]. In contrast, multicomponent crystal formation offers a more advantageous alternative, improving solubility and dissolution without the use of polymeric excipients, thereby mitigating associated pharmacokinetic drawbacks [5,11]. This technique involves the incorporation of a suitable coformer to modulate the physicochemical properties of the active pharmaceutical ingredient (API) without altering its pharmacological activity, and has garnered significant attention for its ability to enhance solubility, dissolution rate, and stability while maintaining the drug's structural integrity [12,13]. Accordingly, multicomponent crystal holds substantial promise for optimizing the physicochemical characteristics and pharmacokinetic profile of ACE.

Tromethamine (TRIS; 2-amino-2-(hydroxymethyl)propane-1,3-diol) is an FDA-approved GRAS excipient with high water solubility [14,15], a pKa of ~8.07, and a melting point of 171 °C [16], widely employed as an alkalizing agent [17] and effective salt co-former for weakly acidic drugs, including mefenamic acid [18], ketoprofen [14], gliclazide [19], indomethacin [15], and fosfomycin (marketed as Monurol® for urinary tract infections) [20], owing to its ability to enhance solubility, dissolution rate, and bioavailability, as exemplified by >1000-fold solubility improvement in indomethacin–mefenamic acid multicomponent crystals [15] and a 2.5-fold dissolution efficiency increase in mefenamic acid–TRIS salts [18]; its physicochemical versatility, structural transitions (orthorhombic to body-centered cubic at 134.3 °C), and compatibility with physiological pH make it a promising pharmaceutical coformer [16].

The multicomponent crystal was prepared using the solvent-drop grinding method, a widely utilized technique that facilitates intermolecular interactions between the API and coformer. The confirmation of multicomponent crystal formation and evaluation of its physicochemical behavior were carried out using solid-state techniques including differential scanning calorimetry, powder X-ray diffraction, and Fourier

transform infrared spectroscopy. In addition, evaluations of solubility and in vitro dissolution rates were performed to determine the extent of improvement in the drug's bioavailability profile. This study aims to demonstrate that multicomponent crystal formation with TRIS can serve as an effective strategy to increase the solubility and dissolution rate of ACE, potentially leading to improved bioavailability and therapeutic efficacy.

METHODS

Materials

ACE (Bocsci Inc., USA), Tromethamine (TCI, Tokyo, Japan), analytical grade ethanol and methanol, as well as carbon dioxide–free distilled water were employed in this study. All other reagents used were of pharmaceutical grade quality.

Preparation of Multicomponent Crystal of ACE-TRIS Using Solvent Dropped Grinding Method

ACE (354.18 mg) and TRIS (121.14 mg) were combined in a 1:1 equimolar ratio. The mixture was subjected to manual grinding in a mortar for 15 minutes, with ethanol gradually added as a grinding solvent. The resulting powder was subsequently stored at ambient temperature until further characterization.

Differential Scanning Calorimetry (DSC) Analysis

The thermal behavior of the sample was evaluated using DSC (Shimadzu DSC-60 Plus, Japan) with a pre-calibrated temperature program. Approximately 5 mg of the sample was accurately weighed, sealed in an aluminum pan, and subjected to heating from 20 °C to 180 °C at a constant rate of 10 °C/min.

Powder X-Ray Diffraction (PXRD)

PXRD analysis was performed at ambient temperature using a PANalytical X'Pert Pro diffractometer (MPD PW3040/60, The Netherlands) equipped with a Cu-Kα radiation source. The instrument was operated at 40 kV and 40 mA, and diffraction patterns were recorded over a 2θ range of 5°–50°, with the sample carefully mounted on a glass sample holder.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy was performed using a Thermo Fisher Scientific spectrophotometer (USA) over a wavenumber range of 4500–500 cm^{-1}. Spectra were obtained for pure ACE, pure TRIS, and the synthesized ACE–TRIS multicomponent crystal, with each sample appropriately mounted on the sample holder prior to scanning.

Scanning Electron Microscopy (SEM)

For morphological analysis, the samples were mounted onto aluminum stubs and examined using a scanning electron microscope (JEOL JSM-6360 LA, Japan) at various magnifications. Observations were conducted under an accelerating voltage of 15–20 kV with a beam current of 12 mA.

Solubility Study

The solubility of pure ACE and its multicomponent crystal was evaluated using an orbital shaker (Memmert WNB 29, Germany). A sample quantity equivalent to 20 mg of ACE was dispersed in 100 mL of CO_2-free distilled water within an Erlenmeyer flask and agitated at 25 °C for 24 h under controlled room temperature. Following agitation, the suspensions were filtered through a 0.45 μm Whatman membrane filter, and the drug concentration was determined via UV-Vis spectrophotometry at 275 nm. Solubility values were calculated from a standard calibration curve prepared using known ACE concentrations. All experiments were performed in triplicate to ensure accuracy and reproducibility. Statistical comparisons between multicomponent crystal formulations were conducted using one-way ANOVA in SPSS (version 26.0, IBM Corp., Armonk, NY, USA), with significance accepted at $p < 0.05$. The methodology followed established protocols for assessing solubility enhancement in pharmaceutical systems.

Dissolution Rate Profile

The dissolution profile was evaluated using a paddle-type dissolution apparatus (USP Apparatus II; Hanson SR8 Plus, USA). Each vessel contained 900 mL of CO_2-free distilled water, maintained at 37 ± 0.5 °C, and stirred at a paddle speed of 50 rpm. At predetermined time intervals (5, 10, 15, 30, 45, and 60 minutes), 5 mL aliquots were withdrawn and immediately replaced

with an equal volume of preheated fresh dissolution medium to maintain sink conditions. All experiments were conducted in triplicate to ensure reproducibility. The collected samples were transferred into glass vials and analyzed using a UV-Vis spectrophotometer. This method was applied to both pure ACE and the ACE–TRIS multicomponent crystal for comparative analysis of their dissolution performance.

Anti-Inflammatory Activity Assay

Animals and Experimental Design

Male white mice (*Mus musculus*), weighing approximately 25 g and drug-naïve, were used in this study (n = 12). The animals were housed under standard laboratory conditions and acclimatized for seven days prior to the experiment to allow environmental adaptation, health assessment, body weight monitoring, and dietary standardization. The mice were randomly assigned into three experimental groups (n = 4 per group): control (0.5% NaCMC), test formulations (multicomponent crystals of ACE-TRIS), and reference drug (pure ACE).

Preparation of 0.5% NaCMC Suspension and Test Formulations

A 0.5% NaCMC suspension was prepared by dispersing 0.5 g of NaCMC in 20 mL of hot water, followed by the addition of distilled water to a final volume of 100 mL. Test formulations were prepared by dispersing the predetermined dose of the active compound in 0.5% NaCMC suspension. The dosing concentration was calculated using the following equation:

$$\text{Concentration}\left(\frac{\text{mg}}{\text{mL}}\right) = \frac{\text{Dose}\left(\frac{\text{mg}}{\text{kg}}\right) \text{x Body weight (kg)}}{\text{Administration volume (mL)}}$$

Dose Calculation for ACE

The human therapeutic dose of ACE is 100 mg/day. Conversion to the equivalent mouse dose was performed using the human-to-mouse conversion factor of 0.026 [21], resulting in a dose of 2.6 mg per 25 g body weight.

Preparation of Carrageenan-Inducing Solution

A 2% carrageenan suspension was prepared by triturating 1 g of carrageenan with 50 mL sesame oil in a mortar until homogeneous.

Induction of Inflammation (Granuloma Pouch Method)

Twenty-four hours prior to the experiment, the dorsal hair of each mouse was shaved (~3 cm diameter). Inflammation was induced by subcutaneous injection of 5 mL sterile air into the shaved dorsal region to form a pouch, followed immediately by the injection of 0.1 mL of 2% carrageenan suspension. After 24 hours, the air was aspirated from the pouch and replaced with 0.5 mL of 2% carrageenan to sustain the inflammatory response.

Drug Administration

Test formulations and controls were administered intraperitoneally immediately after carrageenan injection (Day 0) and subsequently on Days 2, 4, and 6, once daily.

Measurement of Inflammatory Parameters

Inflammation was quantified by aspirating exudate from the granuloma pouch using a syringe and measuring the volume on Days 2, 4, and 6.

Evaluation of Anti-Inflammatory Efficacy via TNF-α Assay

Cell Culture and Induction of Inflammation

Murine macrophage RAW 264.7 cells were seeded at a density of 5×10^5 cells/well in a 96-well plate and incubated at 37°C for 24 h. The medium was then replaced with fresh medium containing the test compounds at three concentrations (x1, x2, x3 μg/mL) or dexamethasone (2.5 and 5 μg/mL) as the positive control. Cells without treatment served as the negative control. Following 2 h of treatment, lipopolysaccharide (LPS) was added to a final concentration of 1 μg/mL, and incubation was continued for 24 h.

Sample Collection and Storage

After incubation, the culture medium was collected and centrifuged at 2000 × g for 20 min at 2–8°C. The supernatant was collected and stored at −80°C until further analysis.

TNF-α Quantification (ELISA)

TNF-α levels were quantified using a commercial

sandwich ELISA kit (Elabscience) according to the manufacturer's protocol. Briefly, 100 µL of standards and samples were added to wells pre-coated with a TNF-α–specific antibody and incubated at 37°C for 90 min. After washing, 100 µL of biotinylated detection antibody was added and incubated at 37°C for 1 h. Following additional washing steps, 100 µL of HRP conjugate was added and incubated at 37°C for 30 min. Wells were washed, and 90 µL of substrate reagent was added, incubated for 15 min in the dark at 37°C, followed by 50 µL stop solution. Absorbance was measured immediately at 450 nm using a microplate reader. All measurements were performed in triplicate, and results were expressed as mean ± SD.

RESULT AND DISCUSSION

Differential Scanning Calorimetry (DSC)

DSC is a thermal analysis technique employed to characterize solid-state materials by detecting heat flow variations associated with temperature or time. This method is particularly effective for identifying thermal transitions in multicomponent crystals, such as melting, phase transformations, and crystallization. In this study, DSC analysis was performed in the temperature range of 20-180 °C at a heating rate of 10 °C/min, and the resulting thermograms are presented in **Figure 1**.

The DSC thermogram of pure ACE (Figure 1A) exhibited a sharp endothermic peak at 154.77 °C, characteristic of its crystalline structure. Pure TRIS (Figure 1B) showed a distinct melting endotherm at 141.45 °C, with a broader peak profile compared to ACE, suggesting differences in crystal packing and molecular interactions. In contrast, the ACE-TRIS multicomponent crystal (Figure 1C) displayed a melting endotherm at 151.09 °C, lower than that of pure ACE but higher than TRIS. The observed shift in melting temperature, along with the maintained sharpness of the peak, suggests the formation of a eutectic-type system. In such systems, the interaction between the two components leads to a decrease in lattice stability, thereby lowering the melting point relative to the pure compounds. This reduction in melting point is consistent with enhanced molecular mobility, which may contribute to improved dissolution behavior of the multicomponent crystal [8,22].

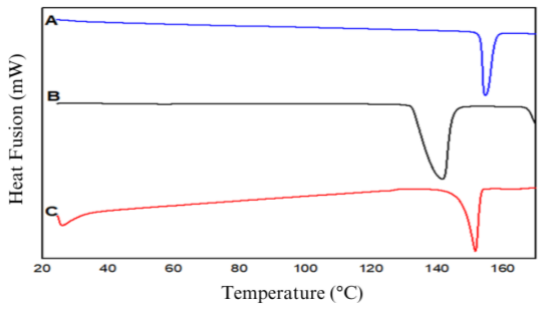

Figure 1. DSC Thermograms of (A) ACE, (B) TRIS, and (C) Multicomponent crystal of ACE-TRIS

Powder X-Ray Diffraction (PXRD)

PXRD is a powerful analytical technique used to assess solid-state interactions and detect the formation of new crystalline phases. In this study, PXRD analysis was conducted to compare the diffraction patterns of pure ACE, pure TRIS, and the ACE–TRIS multicomponent crystal. The obtained diffractograms are presented in **Figure 2**.

Pure ACE (Figure 2A) exhibited sharp and intense peaks at characteristic 2θ positions, confirming its high crystallinity. Similarly, pure TRIS (Figure 2B) displayed multiple well-defined peaks, also indicative of its crystalline nature. In contrast, the diffraction pattern of the ACE–TRIS multicomponent crystal (Figure 2C) retained some of the characteristic peaks of both components but showed a noticeable reduction in peak intensity and partial broadening, suggesting decreased overall crystallinity. No new diffraction peaks were detected, indicating the absence of a novel crystalline phase formation. Instead, the data suggest the formation of a eutectic-type system, where both ACE-TRIS

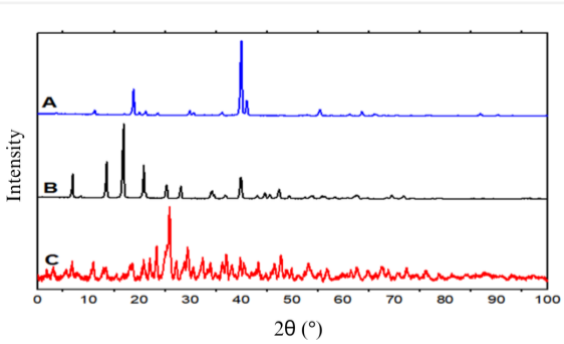

Figure 2. PXRD Patterns of (A) ACE, (B) TRIS, (C) Multicomponent crystal of ACE-TRIS

maintain their individual crystalline structures while exhibiting physical mixing at the molecular level [8,22,23]. This interpretation is consistent with the DSC results, which demonstrated a melting point depression, further supporting the eutectic nature of the system.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy was employed to investigate potential intermolecular interactions within the ACE–TRIS multicomponent crystal by comparing its vibrational spectra with those of the pure components. The spectra are depicted in **Figure 3**.

To assess possible intermolecular interactions in the multicomponent crystal, FTIR analysis focused on detecting changes in the transmission bands of the infrared spectra [24]. The spectral data of the multicomponent crystal were compared with those of pure ACE-TRIS to identify any potential changes in functional group vibrations. As illustrated in Figure 3, the FTIR spectra of the multicomponent crystal exhibit slight shifts in specific absorption bands when compared to those of pure ACE-TRIS. These shifts, however, remain within the expected range for functional groups present in the individual components, suggesting that no significant chemical transformation has occurred

Pure ACE (**Figure 3A**) displayed characteristic absorption bands corresponding to its functional groups, including a sharp C=O stretching band and aromatic C–H vibrations. TRIS (**Figure 3B**) exhibited distinctive –OH and –NH stretching vibrations, as well as C–N and C–O bands. In the spectrum of the ACE–TRIS multicomponent crystal (**Figure 3C**), most characteristic peaks of the parent compounds were retained; however, a notable broadening and smoothing of the absorption band around ~3000–3500 cm^{-1} was observed. This broadened band is attributed to –OH stretching, indicating the presence of strong hydrogen bonding interactions between ACE-TRIS molecules in the solid state.

The minor shifts in certain characteristic bands, alongside the broad OH peak, suggest that the interaction in the multicomponent crystal is predominantly mediated through non-covalent hydrogen bonding rather than covalent bond formation. This observation supports the

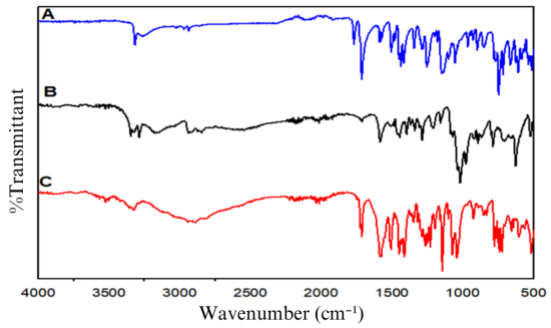

Figure 3. FTIR Morphology of (A) ACE, (B) TRIS, and (C) Multicomponent crystal of ACE-TRIS

Multicomponent crystal of ACE-TRIS

conclusion that the system represents a physical modification, likely a eutectic or co-crystalline arrangement, rather than a chemically altered compound.

The observed spectral shifts are primarily attributed to hydrogen bonding interactions between ACE-TRIS in the solid-state system. Hydrogen bonding can influence molecular vibrations by altering bond strength, leading to minor frequency shifts in the FTIR spectrum. This finding indicates that the formation of the multicomponent crystal involves non-covalent interactions rather than the creation of new chemical bonds, further supporting the conclusion that the system represents a physical modification rather than a chemical alteration [23,25].

Solubility Study

The solubility outcomes for pure ACE and its multicomponent crystal are presented in **Table 1**. The findings demonstrate that the multicomponent crystal achieved a 35.8-fold increase in solubility compared to pure ACE. This significant enhancement can be attributed to the formation of a eutectic system, which is

Table 1. Solubility data of ACE and Multicomponent crystal of ACE-TRIS

Sample	Solubility (mg/100mL) ± SD	Solubility enhancing
ACE	6.057 ± 0.845	-
Multicomponent crystal of ACE-TRIS	216.839 ± 4.117	35.8-fold

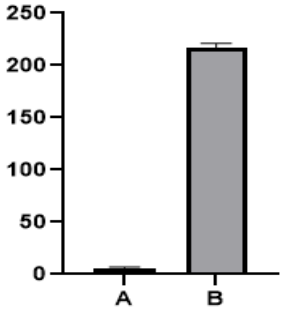

Figure 4. Solubility comparison of (A) ACE and (B) multicomponent crystal of ACE-TRIS

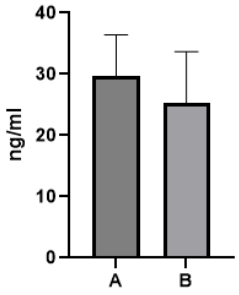

Figure 8. TNF-α levels after treatment (A) ACE and (B) Multicomponent crystal ACE-TRIS

associated with a reduced melting point, reflecting lower lattice energy and decreased enthalpy of fusion. As a result, the overall crystallinity of the material diminishes, leading to a less rigid molecular framework. The reduced rigidity facilitates easier disruption of the crystal lattice, thereby promoting faster dissolution and improved solubility performance [1,8]. Furthermore, statistical analysis using an independent *t*-test at the 60-minute time point confirmed a significant difference between the dissolution of pure ACE (mean = 63.99 ± 1.68) and the multicomponent crystal (mean = 83.37 ± 1.87), with $p < 0.005$. These results clearly indicate that the multicomponent crystal formulation exhibits superior dissolution efficiency compared to pure ACE.

Dissolution Rate Profile

The dissolution study demonstrated a consistent enhancement in drug release behavior, aligning with the solubility findings (**Figure 5**). After 60 minutes, pure ACE exhibited a dissolution of approximately 63%, whereas the ACE–TRIS multicomponent crystal achieved a markedly higher dissolution of about 83%. Furthermore, the dissolution efficiency at 60 minutes (DE60) increased significantly from 49.10 ± 0.54% for pure ACE to **75.33 ± 1.57%** for the multicomponent crystal (**Figure 6**, **Table 2**).

This pronounced improvement is attributed to the formation of a eutectic mixture, which reduces lattice energy and crystallinity, while simultaneously improving wettability and molecular mobility, thereby facilitating a faster dissolution process [13]. Since dissolution rate is a critical determinant of oral bioavailability for poorly water-soluble drugs, these findings highlight the potential of multicomponent crystal formation as a promising approach to overcoming solubility-related limitations of ACE [1,2,8].

Anti-Inflammatory Study

Effect on Exudate Volume (Granuloma Pouch Model)

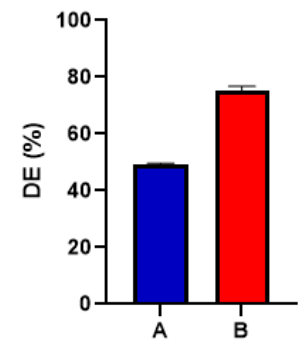

Figure 6. Dissolution efficiency (DE60) comparison between (A) ACE and (B) multicomponent crystal of ACE-TRIS.

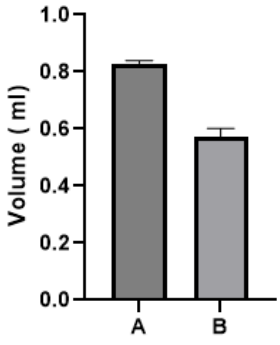

Figure 7. Exudate volume after treatment (A) ACE and (B) Multicomponent crystal ACE-TRIS

Table 2. Dissolution efficiency (DE60) values for ACE and ACE–TRIS multicomponent crystal.

Sample	Dissolution Efficiency			DE_{60}
	1	2	3	
ACE	48.610	49.682	49.108	49.103±0.541
Multicomponent crystal of ACE-TRIS	76.531	73.477	75.648	75.326±1.572

The present study investigated the anti-inflammatory potential of ACE and its multicomponent crystal with TRIS using a carrageenan-induced granuloma pouch model in mice. Exudate volume was measured as a physical marker of inflammation. As shown in Figure 1, administration of the MC led to a more pronounced reduction in exudate volume (0.56 mL) compared with pure ACE (0.82 mL). This enhanced inhibition of fluid accumulation suggests that the MC improves the systemic bioavailability of ACE, likely due to its superior solubility and dissolution profile [26]. Since fluid accumulation within the pouch reflects vascular permeability and plasma exudation, the observed reduction indicates a stronger suppression of inflammatory mediator activity by the MC formulation [27].

Effect on TNF-α Levels (ELISA Assay)

In parallel, TNF-α concentration was assessed as a biochemical marker of cytokine-mediated inflammation. Carrageenan control animals exhibited elevated TNF-α evels (55.63 pg/mL), confirming the successful induction of inflammation. Treatment with pure ACE reduced TNF-α to 29.70 pg/mL, whereas the MC achieved a further decrease to 25.26 pg/mL (**Figure 2**, **Table 2**). As TNF-α plays a central role in the later phase of carrageenan-induced inflammation by driving cytokine cascades and leukocyte recruitment, this reduction highlights the amplified anti-inflammatory effect of the MC [28,29].

The superior activity may not only derive from improved drug release but also from modulation of upstream regulators such as NF-κB, which governs cytokine gene transcription. Taken together, the dual-parameter evaluation, physical (exudate volume) and biochemical (TNF-α level), provides compelling evidence that the MC consistently outperforms pure ACE. These findings underscore its therapeutic promise for cytokine-driven chronic inflammation, although further studies are required to assess its pharmacokinetics, long-term efficacy, and safety profile [26,28,29].

Table 3. TNF-α levels in male white mice induced with carrageenan

No	Treatment	Absorbance			Mean±SD
		1	2	3	
1	Carrageenan	0.126	0.124	0.109	0.1197±0.009
2	ACE	0.102	0.09	0.097	0.0963±0.006
3	ACE-TRIS	0.102	0.089	0.087	0.0927±0.008

Table 1. TNF-α levels (pg/mL) in carrageenan-induced inflammation model in male white mice following treatment with ACE and its MC

No	Treatment	TNF-α (pg/mL)			Mean±SD
		1	2	3	
1	Carrageenan	62.667	60.444	43.778	55.630±10.324
2	ACE	36	22.667	30.444	29.704±6.697
3	ACE-TRIS	34.889	21.556	19.333	25.259±6.869

CONCLUSION

The ACE–TRIS multicomponent crystal significantly improved the physicochemical performance of aceclofenac, as evidenced by reduced melting point, eutectic behavior, and intermolecular hydrogen bonding without the formation of new covalent bonds. These solid-state modifications resulted in a substantial enhancement of solubility (35.8-fold) and dissolution efficiency (DE_{60} of 75.33 ± 1.57%) compared with pure ACE. Moreover, the improved dissolution translated into superior in vivo anti-inflammatory efficacy, demonstrated by reduced exudate volume and TNF-α levels in a carrageenan-induced granuloma pouch model. Overall, the ACE–TRIS multicomponent crystal represents a promising strategy to overcome solubility-related limitations and enhance the therapeutic potential of ACE, although further pharmacokinetic studies are warranted to confirm these findings.

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation.

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