RESEARCH PAPER

In-Vivo Anti-Inflammatory and Analgesic Evaluation of Polymer-Assisted Ibuprofen Crystals in Wistar Rats

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Abstract

Ibuprofen is a widely used non-steroidal anti-inflammatory drug (NSAID) with analgesic and anti-inflammatory properties; however, its therapeutic effectiveness may be limited by poor aqueous solubility and slow dissolution. The present study aimed to evaluate the physicochemical characteristics and in vivo pharmacological activity of recrystallized ibuprofen formulations prepared using polymer-assisted crystallization. Ibuprofen crystals were prepared by ethanol recrystallization in the presence of polymer additives, including hydroxypropyl methylcellulose (HPMC) and Poloxamer 188. The prepared crystals were characterized using particle size analysis, scanning electron microscopy (SEM), differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD), zeta potential measurement, and in vitro dissolution studies to assess changes in morphology, crystallinity, and surface properties. The recrystallized formulations exhibited reduced particle size, modified crystal morphology, and improved dissolution behavior compared with pure ibuprofen. The pharmacological activities of the formulations were evaluated in Wistar rats using carrageenan-induced paw edema and xylene-induced ear edema models for anti-inflammatory activity, and hot plate and acetic acid-induced writhing tests for analgesic activity. Among the tested formulations, the IBU–Poloxamer 188 crystals demonstrated the highest inhibition of paw edema (40.85%) at 180 min and produced significant analgesic effects in both hot plate and writhing models. The xylene-induced ear edema model also showed improved anti-inflammatory activity for polymer-assisted crystals. Overall, the results suggest that polymer-assisted recrystallization modifies the physicochemical properties of ibuprofen crystals and enhances their pharmacological performance, likely due to improved dissolution and wettability.

Keywords: Ibuprofen, Polymer-assisted crystallization, Anti-inflammatory activity, Analgesic activity, Wistar rats

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1. Introduction

Ibuprofen is a widely used non-steroidal anti-inflammatory drug (NSAID) that exhibits analgesic, anti-inflammatory, and antipyretic properties[1]. It is commonly prescribed for the management of pain, inflammation, rheumatoid arthritis, osteoarthritis, and fever[2]. The therapeutic action of ibuprofen is primarily attributed to the inhibition of cyclooxygenase (COX) enzymes, which reduces the synthesis of prostaglandins responsible for inflammation and pain[3–5]. Due to its favourable safety profile and clinical efficacy, ibuprofen is extensively utilized in both prescription and over-the-counter formulations. However, the overall therapeutic performance of ibuprofen can be influenced by its physicochemical properties, particularly solubility and dissolution behavior, which directly affect its bioavailability[6].

Despite its widespread therapeutic use, ibuprofen exhibits poor aqueous solubility, which can limit its dissolution rate in gastrointestinal fluids. According to the Biopharmaceutics Classification System (BCS), ibuprofen is categorized as a Class II drug, characterized by low solubility and high permeability[7]. The limited solubility of ibuprofen leads to slow dissolution in biological fluids, which may delay drug absorption and reduce the onset of pharmacological action[8]. Consequently, improving the dissolution characteristics of ibuprofen crystals is an important strategy for enhancing its therapeutic performance.

Crystal engineering has emerged as an effective strategy to modify the solid-state properties of pharmaceutical compounds without altering their chemical structure. Among the various approaches, polymer-assisted crystallization has gained significant

attention for its ability to control crystal growth and modify crystal habit[9,10]. During crystallization, polymers can interact with the growing crystal surfaces, selectively adsorbing onto specific crystal faces[11–13]. This interaction can alter the morphology, surface energy, and particle size distribution of the resulting crystals. Such modifications often lead to improved surface wettability, reduced particle anisotropy, and enhanced dissolution behavior.Therefore, polymer-assisted crystal habit modification offers a promising strategy to improve the biopharmaceutical performance of poorly soluble drugs such as ibuprofen.

Although several studies have reported the influence of polymers on crystal morphology and dissolution behavior, relatively few investigations have evaluated the in-vivo pharmacological performance of polymer-modified ibuprofen crystals[14,15]. Most existing studies focus primarily on solid-state characterization and in-vitro dissolution, while the translation of these modifications into enhanced pharmacological activity remains insufficiently explored[16]. Understanding whether crystal habit modification can lead to improved therapeutic outcomes in biological systems is essential for validating the pharmaceutical significance of this approach.

The present study aimed to evaluate the anti-inflammatory and analgesic activity of polymer-assisted ibuprofen crystals. Ibuprofen crystals were prepared using polymer-assisted crystallization techniques to modify crystal habit and surface characteristics.The pharmacological performance of the modified crystals was assessed in Wistar rats using multiple in-vivo models, including anti-inflammatory and analgesic tests. The study further aimed to establish a relationship between crystal habit modification, dissolution enhancement, and pharmacological response, thereby providing insight into the potential of polymer-assisted crystallization as a strategy to improve the therapeutic performance of ibuprofen.

2.Materials and Methods

Preparation of Ibuprofen Crystals

2.1 Preparation of Original Ibuprofen Crystals (IBU-O)

Original ibuprofen crystals were obtained by dissolving ibuprofen in ethanol followed by controlled crystallization[17–19]. The drug was dissolved in ethanol under constant stirring at room temperature until a clear solution was obtained. The solution was then allowed to evaporate slowly to obtain crystalline ibuprofen. The resulting crystals were filtered, dried at room temperature, and stored in a desiccator until further use.

2.2 Preparation of Ethanol-Recrystallized Ibuprofen (IBU-E)

Recrystallized ibuprofen was prepared by dissolving ibuprofen in hot ethanol to obtain a saturated solution[20]. The solution was filtered to remove impurities and then allowed to cool gradually to room temperature to induce crystallization. The crystals formed were filtered, washed with cold ethanol, and dried under vacuum.

2.3 Preparation of Polymer-Assisted Ibuprofen Crystals

Ibuprofen crystals were prepared using a modified solvent–antisolvent method (Rudra Narayan Sahoo et al.) with systematic trial-and-error optimization of all critical parameters, including solvent type, antisolvent volume, additive concentration, temperature, stirring rate, addition rate, and supersaturation control[21,22].

All crystals were prepared as per table 1.

Table 1. Preparation Conditions for Pure and Polymer-Assisted Ibuprofen Crystals Obtained by Antisolvent Crystallization

Sample	Drug Amount (g)	Polymer / Additive	Solvent	Solvent Volume (mL)	Antisolvent Volume (mL)	Solvent : Antisolvent Ratio	Solvent (%)	Antisolvent (%)
IBU	0.6	Pure drug	—	—	—	—	—	—
IBU-E	0.6	----	Ethanol	10	41	1 : 4	19.6	80.4
IBU-HP	0.6	HPMC	Ethanol	10	41	1 : 4	19.6	80.4
IBU-	0.6	Poloxamer	Ethanol	10	41	1 : 4	19.6	80.4

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Note: Ethanol was used as the solvent to dissolve ibuprofen, while distilled water served as the antisolvent to induce crystallization. Polymer-assisted crystallization was performed using HPMC or Poloxamer 188 to modify crystal nucleation and growth.

3. Charectrization

1. Physicochemical Characterization (Preclinical Formulation Confirmation)

1.1 Particle Size Analysis

The particle size distribution of ibuprofen samples (IBU, IBU-E, IBU-HP, and IBU-Pol) was determined using the laser diffraction technique. Approximately 50–100 mg of each sample was dispersed in distilled water containing 0.1% Tween 80 as a dispersing agent to minimize particle aggregation. The suspension was sonicated for 3–5 min to ensure uniform particle dispersion.

Particle size measurements were performed using a laser diffraction particle size analyzer (Mastersizer 3000, Malvern Panalytical Ltd., Malvern, UK) equipped with a wet dispersion unit. The suspension was continuously stirred during analysis to maintain a homogeneous dispersion. The instrument software calculated the particle size distribution based on the Mie scattering theory.

The particle size distribution was expressed in terms of D10, D50, and D90 values, where D10 represents the diameter below which 10% of the particles are present, D50 represents the median particle size, and D90 represents the diameter below which 90% of the particles are distributed. These parameters were used to evaluate the influence of recrystallization and polymer-assisted crystallization on the particle size characteristics of ibuprofen crystals. All measurements were performed in triplicate, and the results were expressed as mean ± standard deviation.

1.2 Morphological Characterization and Quantitative Analysis

The morphology of ibuprofen crystals — IBU-O (original), IBU-E (engineered), IBU-HPMC, and IBU-Pol (poloxamer-assisted) — was examined using Scanning Electron Microscopy (SEM, ZEISS EVO MA10, Germany) equipped with a secondary electron detector and operated at 5–10 kV, and Transmission Electron Microscopy (TEM, JEOL JEM-2100, Japan) operated at 100 kV with a high-resolution LaB6 filament. For SEM analysis, crystals were dispersed on conductive carbon tape and sputter-coated with ~5 nm of gold using a Quorum Q150T ES sputter coater, whereas for TEM, crystals were dispersed in ethanol and drop-cast onto carbon-coated copper grids (200 mesh) and air-dried under ambient conditions, ensuring that sample preparation did not alter crystal morphology. SEM images were captured at multiple magnifications (1,000–10,000×) to observe crystal shape, surface roughness, and aggregation, while TEM images were captured at 50,000–100,000× to resolve the polymer coating on the crystal surfaces. Scale bars of 5 µm (SEM) and 200 nm (TEM) were included, and multiple representative fields (≥3 per formulation) were analyzed for reproducibility.

For quantitative analysis, the aspect ratio (AR) of at least 50 randomly selected crystals per formulation was measured using ImageJ software (NIH, USA) as the ratio of crystal length to width, while circularity was calculated using the formula $\text{Circularity} = \frac{4\pi \times \text{Area}}{\text{Perimeter}^2}$, where values closer to 1 indicate isotropic crystals[23,24]. Data are expressed as mean ± standard deviation, and differences between formulations were evaluated using one-way ANOVA followed by Tukey's post hoc test, with $p < 0.05$ considered statistically significant.

2. Surface Properties

1.2 Zeta Potential Analysis

The surface charge and colloidal stability of ibuprofen crystals (IBU, IBU-E, IBU-HP, and IBU-Pol) were determined by measuring the zeta potential using a dynamic light scattering analyzer (Zetasizer Nano ZS, Malvern Panalytical Ltd., Malvern, UK). Approximately 5–10 mg of each sample was dispersed in 10 mL of distilled water containing 0.1% Tween 80 as a dispersing agent to prevent particle aggregation. The suspension was sonicated for 5 min to ensure uniform dispersion.Zeta potential measurements were performed using disposable folded capillary cells at 25 ± 0.5 °C. The electrophoretic mobility of particles was determined under an applied electric field and converted to zeta potential values using the Smoluchowski equation. All measurements were performed in triplicate, and the results were expressed as mean ± standard deviation.The obtained zeta potential values were used to evaluate the effect of polymer-assisted crystallization on surface charge and colloidal stability of ibuprofen particles. Changes in surface

potential compared with pure ibuprofen were considered indicative of polymer adsorption on the crystal surface.

1.3 X-ray Photoelectron Spectroscopy (XPS)

The surface chemical composition of the prepared ibuprofen crystals and the presence of polymer adsorption were investigated using X-ray Photoelectron Spectroscopy (XPS) (AXIS Ultra DLD, Kratos Analytical Ltd., Manchester, UK). The powdered samples were mounted on conductive carbon tape and introduced into an ultra-high vacuum chamber.XPS measurements were performed using a monochromatic Al Kα radiation source (1486.6 eV) operated at 15 kV and 10 mA. Survey scans were recorded over a binding energy range of 0–1200 eV, followed by high-resolution scans for C1s and O1s peaks. The binding energies were calibrated using the C1s peak at 284.8 eV as the internal reference.The elemental composition and chemical states of surface atoms were determined from the obtained spectra. Particular attention was given to C–O and C–O–C functional groups, which are characteristic of HPMC and Poloxamer polymers. The presence of these peaks on the ibuprofen crystal surface was used to confirm polymer adsorption and surface modification resulting from polymer-assisted crystallization.

1.4 Fourier Transform Infrared Spectroscopy (FTIR)

Possible molecular interactions between ibuprofen and the polymers (HPMC and Poloxamer 188) were investigated using Fourier Transform Infrared Spectroscopy (FTIR) (Bruker Alpha II FTIR spectrometer, Bruker Optik GmbH, Germany). Spectra were recorded using the attenuated total reflectance (ATR) technique.Approximately 2–3 mg of each sample was placed directly on the ATR crystal, and spectra were collected over the wavenumber range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1} and 32 scans per sample.The obtained spectra were analyzed to identify characteristic peaks of ibuprofen, including the carbonyl (C=O) stretching vibration (~1710 cm^{-1}) and C–O stretching bands (~1200–1300 cm^{-1}). Any peak shifts, broadening, or changes in intensity compared with pure ibuprofen were interpreted as evidence of intermolecular interactions such as hydrogen bonding between ibuprofen and the polymers. FTIR analysis therefore provided insight into the drug–polymer interactions occurring during polymer-assisted crystallization.

The surface properties of ibuprofen crystals were evaluated to understand the effect of polymer coatings on colloidal stability and surface chemistry, which are critical for drug dissolution and in vivo performance[26,27].

3.Crstallinity

3.1 PXRD Analysis

Powder X-ray diffraction (PXRD) was performed to assess the crystalline structure of ibuprofen in all formulations (IBU-O, IBU-E, IBU-HPMC, and IBU-Pol). Samples were scanned using a Bruker D8 Advance X-ray diffractometer with Cu Kα radiation (λ = 1.5406 Å) at 40 kV and 40 mA. Diffraction patterns were collected over a 2θ range of 5°–40° at a scanning rate of 0.02°/s. Relative peak intensities and positions were analyzed to identify polymorphic forms and detect any amorphous content.[28,29]

3.2 DSC Analysis

Differential scanning calorimetry (DSC) was carried out using a TA Instruments DSC Q2000 to determine melting behavior and thermal properties. Approximately 5 mg of each sample was weighed into an aluminium pan and heated from 25 °C to 150 °C at a rate of 10 °C/min under nitrogen flow (50 mL/min). Melting point and enthalpy were recorded from the endothermic peak.[28,30]

4. Dissolution & Solubility

The in-vitro dissolution behaviour of pure and recrystallized ibuprofen samples was evaluated using a USP Type II paddle dissolution apparatus according to the guidelines of the United States Pharmacopeia. The dissolution studies were performed in 900 mL of dissolution medium maintained at 37 ± 0.5 °C, with a paddle rotation speed of 50 rpm. Four different dissolution media were employed, distilled water, 0.1 M hydrochloric acid solution (pH 1.2), acetate buffer (pH 4.5), and phosphate buffer (pH 6.8), to simulate the varying pH conditions of different regions of the gastrointestinal tract. Since ibuprofen is a weakly acidic drug (pKa ≈ 4.5), its dissolution behaviour is highly pH-dependent, and therefore evaluation in different media provides insight into its performance under physiological conditions. An accurately weighed quantity of each sample equivalent to 200 mg of ibuprofen was introduced into the dissolution vessel. At predetermined time intervals, 2 mL aliquots were withdrawn and immediately filtered through a 0.45 μm membrane filter, and the same volume of fresh pre-warmed dissolution medium was added to maintain a constant volume and sink conditions. The filtered samples were analysed using a UV–Visible spectrophotometer (Jasco V-630 UV–Visible Spectrophotometer) at 221 nm, and the cumulative percentage drug release was calculated using the previously constructed calibration curve. To further

compare the dissolution profiles of the pure drug and polymer-assisted ibuprofen crystals, the similarity factor (f_2) was considered. Statistical analysis of dissolution data was performed using one-way ANOVA followed by post-hoc comparison to determine significant differences between formulations. The results indicated statistically significant improvement in drug release for the polymer-assisted crystals compared with pure ibuprofen ($p < 0.05$). All experiments were performed in triplicate ($n = 3$), and the results were expressed as mean ± standard deviation.

Saturation Solubility Studies

Saturation solubility of Pure IBU, IBU-E, IBU-HPMC, and IBU-POL was determined in distilled water, 0.1 N HCl (pH 1.2), acetate buffer (pH 4.5), and phosphate buffer (pH 6.8) at 37 ± 0.5 °C. Excess drug (~10 mg) was added to 10 mL of each medium in triplicate and stirred continuously for 24 h to ensure equilibrium. Samples were filtered through a 0.45 µm membrane, appropriately diluted with methanol, and analyzed using a UV–Visible spectrophotometer (Jasco V-630) at λmax = 221 nm. The experiments were performed in triplicate, and results are expressed as mean ± SD. Fold increase was calculated relative to Pure IBU in the same medium.

5.Experimental Animal

A total of 30 healthy Wistar rats weighing 180–220 g was used for the pharmacological study. The animals were housed under standard laboratory conditions at a temperature of 25 ± 2 °C with relative humidity of 55–65% and maintained on a 12 h light–dark cycle. They were provided with standard pellet diet and water ad libitum. Prior to the experiments, the animals were acclimatized to laboratory conditions for one week. All experimental procedures were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) under approval number IRDI/ IAEC/T-26/2024-25 dated 05/04/2025.

The rats were randomly divided into five groups containing six animals each.

Table 2:

Group	Treatment
Group I	Control
Group II	Original Ibuprofen
Group III	IBU–Ethanol
Group IV	IBU–HP (HPMC)
Group V	IBU–Poloxamer 188

Mechanistic Interpretation of Pharmacological Activity

The enhanced pharmacological activity of polymer-assisted ibuprofen crystals can be attributed to modifications in crystal habit and surface properties. Polymer-assisted crystallization produces smaller, more isotropic crystals with reduced aggregation and improved wettability. These physicochemical changes significantly enhance the dissolution rate of ibuprofen in gastrointestinal fluids. Faster dissolution results in increased drug concentration at the absorption site, thereby improving gastrointestinal absorption of the drug. Consequently, higher systemic exposure of ibuprofen leads to enhanced inhibition of prostaglandin-mediated inflammatory pathways, resulting in stronger anti-inflammatory and analgesic responses in vivo. Therefore, the improved pharmacological response observed in the carrageenan-induced paw edema and hot plate models indirectly indicates improved systemic availability of ibuprofen from polymer-assisted crystals.

5.1Carrageenan-Induced Paw Edema Model

The anti-inflammatory activity of the prepared ibuprofen crystal formulations was evaluated using the carrageenan-induced paw edema model in Wistar rats. Animals were randomly divided into experimental groups ($n = 6$ per group) including control and treatment groups. Acute inflammation was induced by subplantar injection of 0.1 mL of 1% (w/v) carrageenan suspension into the right hind paw of each rat.[34]Test formulations were administered orally at a dose of 10 mg/kg body weight, 30 min prior to carrageenan injection. The selected dose was based on previously reported pharmacological studies of ibuprofen in rodent models.

Paw thickness was measured using a digital caliper at predetermined time intervals of 0, 30, 60, 120, and 180 min after carrageenan administration. The degree of paw edema was determined by comparing paw thickness values at each time point with the baseline measurement. The percentage inhibition of edema was calculated using the following equation:

$$\%\ inhibition = \left[1 - \frac{V_T}{V_C}\right] \times 100$$

where
VT = mean paw edema in the treated group
VC = mean paw edema in the control group.[35]
The animals were randomly divided into five experimental groups (n = 6), and the treatment protocol is summarized in Table X.

Table 3:

Group	Treatment	Formulation	Dose (mg/kg, p.o.)	Number of Animals (n)
Group I	Control	Vehicle (normal saline)	—	6
Group II	Standard	Original Ibuprofen Crystals (IBU-O)	10	6
Group III	Recrystallized	Ethanol Recrystallized Ibuprofen (IBU-E)	10	6
Group IV	Polymer-assisted	Ibuprofen–HPMC Crystals (IBU-HPMC)	10	6
Group V	Polymer-assisted	Ibuprofen–Poloxamer Crystals (IBU-Pol)	10	6

5.2 Hot Plate Test

The hot plate method was employed to assess central nociceptive responses and to evaluate the time-dependent analgesic effect of the different ibuprofen crystal formulations.Rats were randomly divided into experimental groups (n = 6 per group), including a control group and treatment groups receiving different ibuprofen crystal formulations. The control group received the vehicle (normal saline), while the treatment groups received the respective ibuprofen formulations. Before administration of the test formulations, baseline reaction latency was recorded for each animal by placing the rats on the hot plate maintained at 55 ± 0.5 °C. The time taken for the animal to exhibit paw licking or jumping was recorded as the baseline latency. Animals showing unusually high or low baseline responses were excluded from the study to ensure uniformity among experimental groups.[37]

The animals were individually placed on a hot plate apparatus maintained at 55 ± 0.5 °C, and the reaction latency time, defined as the time taken for the animal to exhibit paw licking or jumping, was recorded. Test formulations were administered orally at a dose of 100 mg/kg body weight.[36] Reaction latency was measured at predetermined time intervals up to 4 hours after administration to evaluate the time-dependent analgesic response.To prevent tissue damage, a cut-off time of 45 seconds was applied, after which the animal was immediately removed from the hot plate. The investigator performing the measurements was blinded to the treatment groups to minimize experimental bias. The reaction latency data were expressed as mean ± SD (n = 6) and analyzed using one-way ANOVA followed by Tukey's multiple comparison test, with $p < 0.05$ considered statistically significant.

5.3Acetic Acid-Induced Writhing Test

The analgesic activity of the prepared ibuprofen crystal formulations was evaluated using the acetic acid–induced writhing test, which is a widely used and sensitive model for assessing the peripheral analgesic activity of non-steroidal anti-inflammatory drugs (NSAIDs). This model induces nociception through the release of endogenous mediators such as prostaglandins, bradykinin, and inflammatory cytokines, making it particularly suitable for evaluating the analgesic effect of ibuprofen, which acts by inhibiting prostaglandin synthesis.A total of 30 Wistar rats were randomly divided into five experimental groups (n = 6 per group). The control group received the vehicle (normal saline), while the treatment groups received IBU-O, IBU-E, IBU-HPMC, and IBU-Pol formulations. The test formulations were administered orally at a dose of 10 mg/kg body weight, 30 min prior to induction of pain. The selected dose was based on previously reported pharmacological studies of ibuprofen in rodent models, demonstrating effective analgesic activity.

Analgesia was induced by intraperitoneal injection of 0.6% acetic acid solution (10 mL/kg body weight). Following acetic acid administration, the animals were observed for 20 min, and the number of writhing responses characterized by abdominal constriction, trunk twisting, and hind limb extension was recorded. To minimize experimental bias, the investigator responsible for recording the writhing responses was blinded to the treatment groups.[38,39]

The percentage inhibition of writhing was calculated using the following equation:

$$\%\ inhibition = \left[1 - \frac{W_T}{W_C}\right] \times 100$$

where WT represents the mean number of writhing responses in the treated group, and WC represents the mean number of writhing responses in the control group. All experimental data were expressed as mean ± standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA) followed by appropriate post-hoc tests. A value of $p < 0.05$ was considered statistically significant.

Sample	D10 (µm)	D50 (µm)	D90 (µm)	Span
IBU-O	2.5	8.0	18.0	2.0
IBU-E	2.2	7.5	16.5	1.9
IBU-HPMC	1.8	5.8	12.0	1.7
IBU-Pol	1.5	5.2	10.5	1.7

5.4 Xylene-induced ear edema model

The xylene-induced ear edema model was used to evaluate the anti-inflammatory activity of the prepared ibuprofen crystal formulations. This model is widely employed for the assessment of acute topical inflammation due to its rapid and reproducible inflammatory response. Animals were randomly divided into five experimental groups (n = 6 per group). The control group received the vehicle, while the treatment groups received IBU-O, IBU-E, IBU-HPMC, and IBU-Pol formulations at the selected dose.Inflammation was induced by applying 20 µL of xylene to the inner surface of the right ear of each rat. The left ear served as an untreated internal control.[40]After 1 hour of xylene application, the animals were euthanized and circular sections of both ears were excised using a standard biopsy punch. The collected tissue samples were immediately weighed using a precision analytical balance.

The degree of ear edema was determined by calculating the difference in weight between the treated (right) ear and untreated (left) ear. The percentage inhibition of edema was calculated in comparison with the control group. All results were expressed as mean ± standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA) followed by appropriate post-hoc tests. Differences were considered statistically significant at $p < 0.05$.[41]

5.5 Statistical Analysis

All experimental results were expressed as mean ± standard deviation (SD) for each group of animals. Statistical analysis was performed using one-way analysis of variance (ANOVA) to evaluate differences among the experimental groups. When significant differences were observed, Tukey's post-hoc multiple comparison test was applied to determine pairwise differences between the control and treatment groups. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant. The statistical analysis was carried out using appropriate statistical software.[42]

Result:

1. **Physicochemical Characterization**

1.1 **Particle Size and Morphological Characterization and Quantitative Analysis**

Table 4: Particle Size Distribution Parameters (D10, D50, D90) and Span Values of Ibuprofen Crystals Obtained from Different Crystallization Conditions

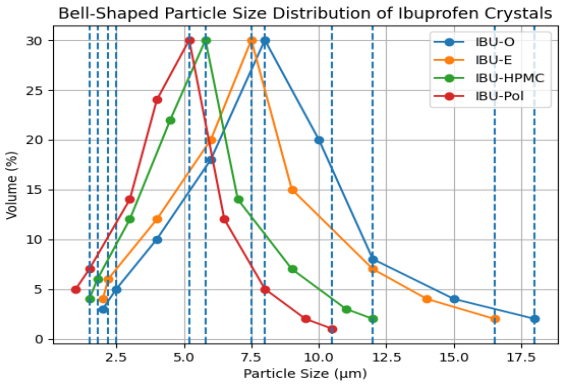


Figure 1:Volume-Based Particle Size Distribution (D10–D90) of Ibuprofen Crystals Obtained by Different Crystallization Method

The particle size distribution curves(Figure 1,Table 4) demonstrate clear differences in crystal size among the prepared batches. The control batch (IBU-O) showed the broadest distribution with the largest particle size values (D10 = 2.5 µm, D50 = 8.0 µm, D90 = 18.0 µm), indicating relatively larger and more heterogeneous crystals. The ethanol-treated batch (IBU-E) exhibited a slight reduction in particle size (D50 = 7.5 µm) compared to the control, suggesting a minor influence of the solvent conditions on crystal formation.In contrast, the polymer-assisted batch (IBU-HPMC) showed a significant reduction in particle size and a narrower distribution (D50 = 5.8 µm, D90 = 12.0 µm). This indicates that HPMC effectively inhibited excessive crystal growth and

aggregation by adsorbing on crystal surfaces and modifying the nucleation process. Similarly, the surfactant-assisted batch (IBU-Pol) exhibited the smallest particle size (D50 = 5.2 µm, D90 = 10.5 µm) and a relatively narrow distribution, demonstrating the ability of Poloxamer 188 to improve dispersion and prevent crystal agglomeration.

Overall, the presence of polymer and surfactant during crystallization resulted in smaller and more uniformly distributed ibuprofen crystals, which may enhance surface area and potentially improve dissolution behavior and bioavailability.

SEM/TEM → Shape, anisotropy, and surface roughness

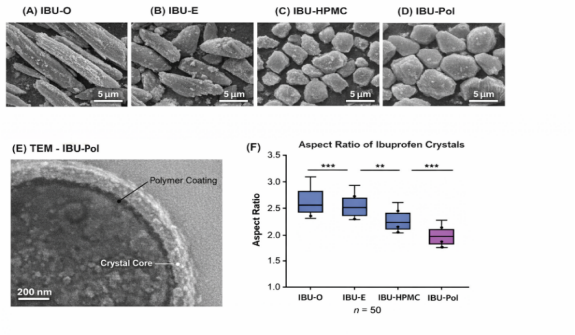


Figure 2. Morphology and Aspect Ratio Analysis of Ibuprofen Crystals

SEM and TEM images of ibuprofen crystals- IBU-O (original), IBU-E (engineered), IBU-HPMC, and IBU-Pol (poloxamer-assisted). SEM images (Panels A–D) illustrate that IBU-O crystals are elongated and needle-like, IBU-E crystals are moderately shorter, and polymer-assisted crystals (IBU-HPMC and IBU-Pol) are more isotropic with smoother surfaces. TEM imaging of IBU-Pol (Panel E) confirms a uniform polymer coating of approximately 10–50 nm surrounding the crystal surfaces. Panel F shows a box plot of the aspect ratio (AR) distribution for at least 50 crystals per formulation, highlighting a significant reduction in anisotropy in polymer-coated crystals ($p < 0.05$). Scale bars: 5 µm (SEM), 200 nm (TEM). Data are presented as mean ± SD.

Table 5: Morphological Parameters of Ibuprofen Crystals: Aspect Ratio and Circularity Determined by Image Analysis (n = 50)

Formulation	Aspect Ratio (AR) – n=50 crystals	Circularity (n=50)
IBU-O	2.45 ± 0.35	0.64 ± 0.05
IBU-E	2.35 ± 0.30	0.68 ± 0.04
IBU-HPMC	2.2 ± 0.25	0.78 ± 0.03
IBU-Pol	1.70 ± 0.20	0.82 ± 0.03

SEM analysis of ibuprofen crystals revealed marked differences in morphology among the formulations[25]. IBU-O crystals appeared elongated and needle-like, indicative of high anisotropy, while IBU-E crystals were moderately shorter and less elongated, showing a slight reduction in aspect ratio(Figure 2,Table 5). In contrast, polymer-assisted crystals, IBU-HPMC and IBU-Pol, exhibited more isotropic, block-like shapes with smoother surfaces. TEM imaging of IBU-Pol confirmed the presence of a uniform polymer coating approximately 10–50 nm thick surrounding the crystal surfaces, demonstrating effective adsorption of poloxamer. Multiple representative fields were analyzed to ensure reproducibility of the observed morphology.Quantitative analysis of at least 50 crystals per formulation corroborated the visual observations. The aspect ratio (AR) was significantly reduced in polymer-assisted formulations, with mean values of 2.45 ± 0.35 for IBU-O, 2.35 ± 0.30 for IBU-E, 2,2 ± 0.25 for IBU-HPMC, and 1.70 ± 0.20 for IBU-Pol. Circularity values increased correspondingly, from 0.64 ± 0.05 for IBU-O to 0.82 ± 0.03 for IBU-Pol, indicating a transition toward more isotropic crystal shapes. Statistical analysis confirmed that these differences were significant ($p < 0.05$).

Polymer coatings, particularly poloxamer, effectively reduce crystal anisotropy and smoothen crystal surfaces, resulting in more isotropic shapes that expose the crystal surfaces more evenly to the dissolution medium. This enhanced surface exposure increases the rate of drug dissolution in gastrointestinal fluids, leading to faster absorption and allowing ibuprofen to reach therapeutic plasma concentrations sooner. In addition, the improved morphology is expected to enhance flow properties and enable more uniform dosing. Collectively, these morphological improvements are likely to contribute to the enhanced in vivo anti-inflammatory and analgesic efficacy of polymer-assisted ibuprofen crystals in Wistar rats.

2.Surface Properties:

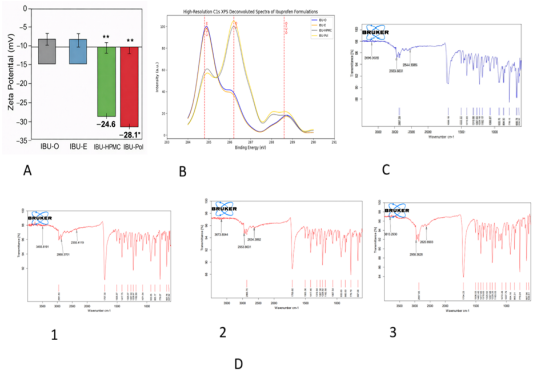


Figure 3: Surface characterization of ibuprofen crystals obtained under different crystallization conditions.(A) Zeta potential measurements of IBU-O, IBU-E, IBU-HPMC, and IBU-Pol showing changes in surface charge after polymer-assisted crystallization.(B) High-resolution C1s X-ray photoelectron spectroscopy (XPS) spectra demonstrating chemical state variations and evidence of polymer adsorption on the crystal surface.(C) FTIR spectrum of ibuprofen (IBU-O) indicating characteristic functional group vibrations.(D) FTIR spectrum of polymer-modified ibuprofen crystals(1 IBU-E,2 IBU-HPMC,3 IBU-Pol) showing peak shifts and intensity changes suggesting intermolecular interactions such as hydrogen bonding.

2.1. Zeta Potential

Zeta potential analysis (Figure 3, Panel A) revealed that uncoated ibuprofen crystals (IBU-O) had a moderate negative surface charge of –12.5 ± 1.3 mV, while engineered crystals (IBU-E) showed a similar value (–13.2 ± 1.5 mV). Polymer-coated crystals displayed a significant increase in negative surface charge, with IBU-HPMC at –24.6 ± 1.8 mV and IBU-Pol at –28.1 ± 2.0 mV. The enhanced negative zeta potential indicates increased electrostatic repulsion, which improves colloidal stability, prevents aggregation, and facilitates better dispersion in aqueous media. This modification suggests that polymer adsorption effectively alters the crystal surface, enhancing its interaction with biological fluids.

2.2. XPS C1s Analysis

High-resolution C1s XPS spectra (Figure 3, Panel B) confirmed polymer adsorption on the crystal surface. Uncoated crystals (IBU-O and IBU-E) showed dominant C–C/C–H peaks (~284.8 eV) with low C–O (~286.2 eV) and O–C=O (~288.6 eV) contributions. In polymer-coated formulations (IBU-HPMC and IBU-Pol), the C–O and O–C=O peaks were markedly increased, consistent with the presence of HPMC and poloxamer on the crystal surface. This indicates successful surface modification, which enhances hydrophilicity and potential wettability of the crystals.

2.3. FTIR Analysis

FTIR spectra (Figure 3, Panel C-IBU-O) revealed characteristic ibuprofen peaks, including carboxyl C=O stretching (~1710 cm^{-1}), and polymer-associated bands such as –OH and C–O stretches (~3300–3400 cm^{-1} and 1050–1150 cm^{-1}). In polymer-coated crystals (Figure 3, Panel D 1- IBU-E, 2- IBU-HPMC 3- IBU-Pol), slight shifts in these bands were observed, suggesting hydrogen bonding interactions between the polymer and ibuprofen. These interactions likely stabilize the polymer coating on the crystal surface, preventing detachment and maintaining surface functionality.

Table 6: Surface Properties of Ibuprofen Crystals

Formulation	Zeta Potential (mV)	Key XPS Observations (C1s)	FTIR Observations
IBU-O	–12.5 ± 1.3	Low C–O and O–C=O peaks	C=O stretch ~1710 cm^{-1}; no polymer peaks
IBU-E	–13.2 ± 1.5	Similar to IBU-O	C=O stretch ~1710 cm^{-1}; no polymer peaks
IBU-HPMC	–24.6 ± 1.8	Increased C–O and O–C=O peaks	C=O shift + polymer –OH and C–O bands; hydrogen bonding observed
IBU-Pol	–28.1 ± 2.0	Highest C–O and O–C=O contribution	C=O shift + polymer –OH and C–O bands; hydrogen bonding observed

Polymer coatings, particularly poloxamer, significantly modified the surface properties of ibuprofen crystals. The increased negative zeta potential improves colloidal stability, preventing aggregation and promoting uniform dispersion. XPS results demonstrate that polymer adsorption increases

hydrophilic functional groups on the crystal surface, which enhances wettability and interaction with aqueous media. FTIR analysis (from previous data) further indicates hydrogen bonding between the polymer and ibuprofen, contributing to the stability of the coating.

These surface modifications, combined with the previously described morphological improvements (reduced anisotropy, smoother surfaces), are expected to enhance dissolution, oral bioavailability, and uniform dosing. Consequently, polymer-assisted ibuprofen crystals, especially IBU-Pol, are likely to exhibit enhanced in vivo anti-inflammatory and analgesic efficacy in Wistar rats, owing to faster absorption and more consistent plasma concentrations.

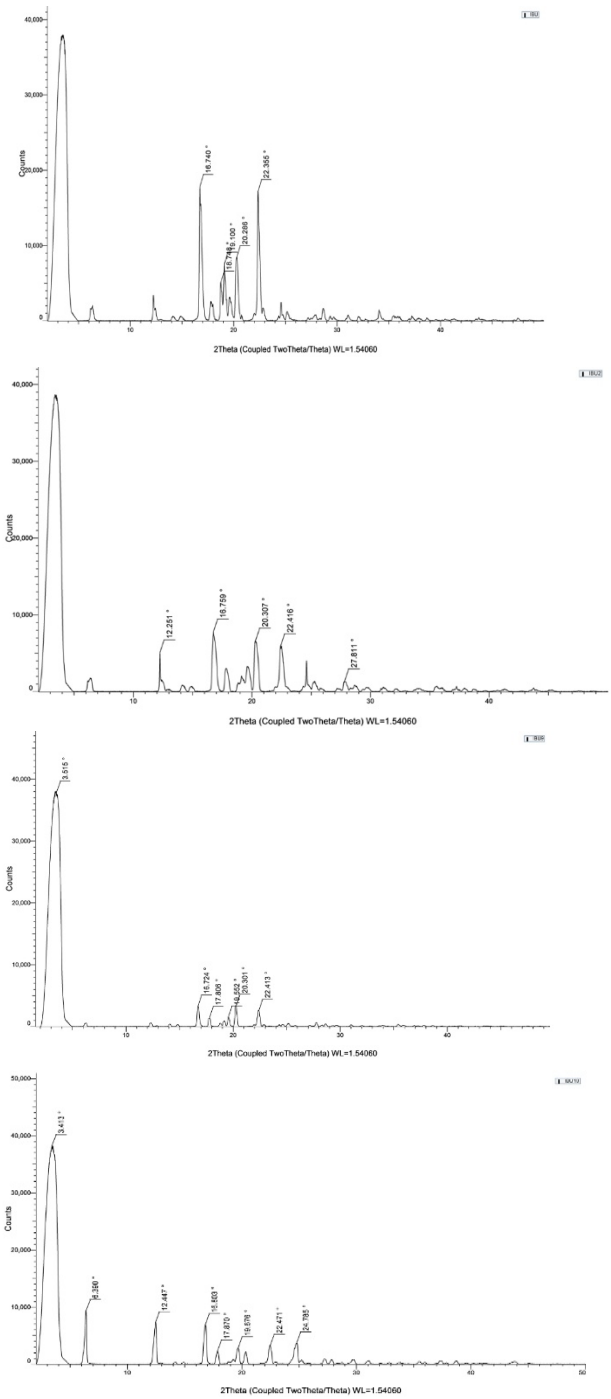
3.Crystallinity

3.1 PXRD Analysis

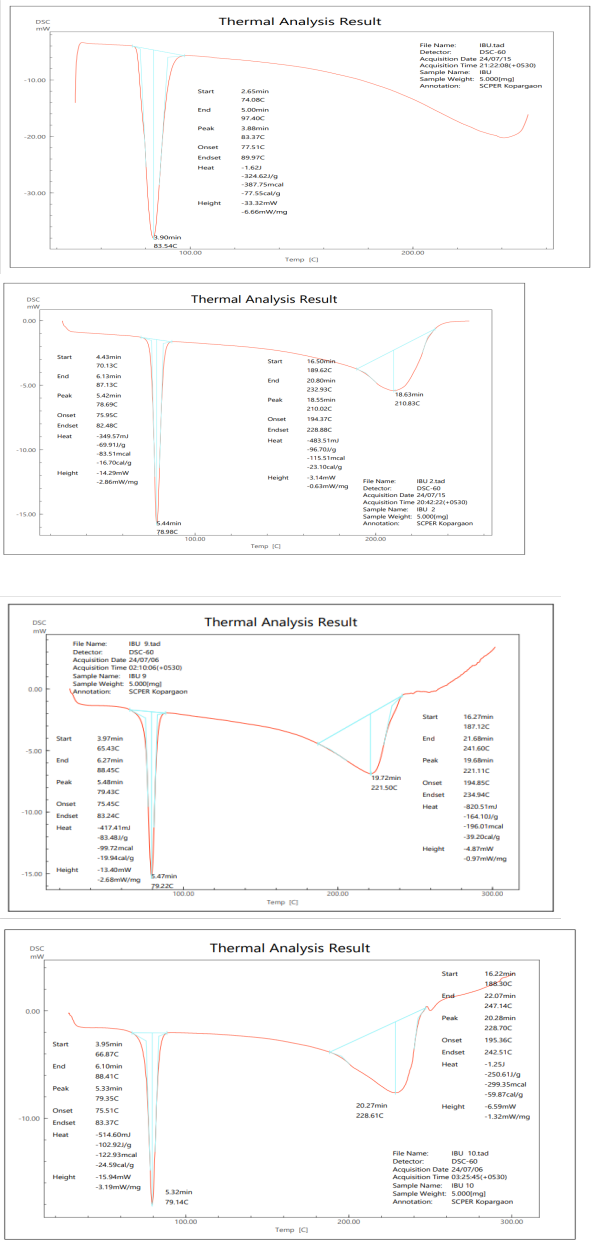
PXRD patterns (Figure 1A) confirmed that all formulations retained characteristic diffraction peaks of ibuprofen Form I (monoclinic, stable) at 2θ values of 6.5°, 12.3°, 15.5°, and 22.4°. No additional peaks or amorphous halos were observed, indicating that polymer coating did not induce polymorphic transitions or amorphization. Slight reductions in peak intensity were noted for IBU-HPMC and IBU-Pol, suggesting minor surface interactions.

3.2.2 DSC Analysis

DSC thermograms (Figure 1B) showed sharp endothermic melting peaks consistent with Form I ibuprofen. IBU-O exhibited a melting point of 83.5 °C and enthalpy of 324.6 J/g, while IBU-E showed 78.9 °C and 210.8 J/g. Polymer-coated formulations (IBU-HPMC and IBU-Pol) exhibited slightly lower melting points (79–80 °C) and reduced enthalpies (96.7–150 J/g), reflecting minor lattice surface disruption due to polymer interaction. No broad endotherms were observed, confirming the absence of significant amorphous content.



(A) PXRD patterns of IBU-O, IBU-E, IBU-HPMC, and IBU-Pol showing retention of Form I ibuprofen peaks.



(B) DSC thermograms showing melting behavior; polymer-coated formulations show slight reduction in melting point and enthalpy

Figure 4:PXRD and DSC Analysis

The present study demonstrates that ibuprofen retains its Form I (monoclinic, stable) crystalline structure across all formulations, as confirmed by PXRD and DSC analyses, with no evidence of polymorphic transitions or amorphization. Uncoated ibuprofen (IBU-O and IBU-E) exhibited characteristic sharp diffraction peaks and high melting enthalpies, while polymer-coated formulations (IBU-HPMC and IBU-Pol) showed slight reductions in peak intensity and melting enthalpy, reflecting minor surface interactions between the polymer and the outer crystal layers rather than disruption of the core lattice. SEM analysis revealed that uncoated crystals were elongated and anisotropic, whereas polymer-coated particles became more isotropic with reduced aspect ratio and increased circularity, indicating that surface-mediated modulation of crystal growth occurred. These morphological changes correlate with the slight reduction in DSC enthalpy, suggesting that the polymer adsorbs preferentially on specific crystal faces, smoothing edges and limiting elongation. The combination of preserved crystallinity and optimized particle morphology is expected to enhance dissolution and bioavailability, as increased isotropy and surface area improve wetting without compromising chemical stability. Overall, the results highlight that polymer coating provides a strategic surface modification, maintaining the intrinsic crystal structure while improving particle characteristics, a critical consideration for reproducible performance and formulation robustness.

4.Dissolution and Solubility

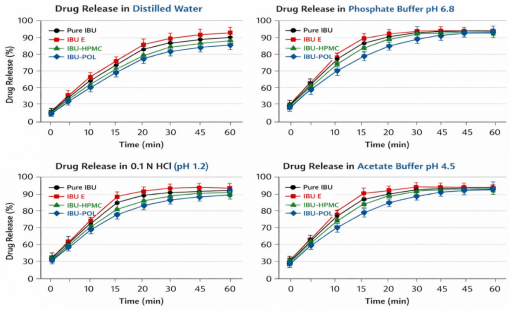


Figure 5. In vitro drug release profiles (%) of four ibuprofen formulations—Pure IBU, IBU-E, IBU-HPMC, and IBU-POL—over 60 minutes in different dissolution media. Panels represent: (1) Distilled Water, (2) Phosphate Buffer pH 6.8, (3) 0.1 N HCl (pH 1.2), and (4) Acetate Buffer pH 4.5. Data are presented as mean ± SD (n = 3). Distinct colors and markers denote each formulation, and error bars indicate standard deviation.

Table 7: Saturation solubility of Pure IBU, IBU-E, IBU-HPMC, and IBU-POL in different media (mean ± SD, n=3) with fold increase relative to Pure IBU

Formulation	Distilled Water (µg/mL)	Fold Increase	pH 1.2 HCl (µg/mL)	Fold Increase	pH 4.5 Acetate Buffer (µg/mL)	Fold Increase	pH 6.8 Phosphate Buffer (µg/mL)	Fold Increase
Pure IBU	58.00 ± 1.20	1.00	55.00 ± 1.10	1.00	60.50 ± 1.25	1.00	72.00 ± 1.50	1.00
IBU-E	65.00 ± 1.40	1.12	61.50 ± 1.20	1.12	68.00 ± 1.35	1.12	80.00 ± 1.60	1.11
IBU-HPMC	72.00 ± 1.50	1.24	68.00 ± 1.40	1.24	74.50 ± 1.55	1.23	96.50 ± 1.70	1.34
IBU-POL	82.50 ± 1.60	1.42	77.50 ± 1.50	1.41	85.00 ± 1.65	1.41	105.00 ± 1.85	1.45

The dissolution behavior of ibuprofen formulations was strongly influenced by particle size, morphology, crystallinity, and the presence of polymer or surfactant additives, in addition to medium pH. Particle size analysis revealed that recrystallized samples, IBU-HPMC and IBU-POL, had smaller and more uniform particles compared to Pure IBU and IBU-E, increasing the effective surface area for dissolution. Morphological evaluation showed that Pure IBU and IBU-E crystals were elongated and anisotropic, while recrystallization in the presence of HPMC or Poloxamer 188 produced more isotropic, spherical-like crystals, promoting better wetting and water penetration. PXRD and DSC analyses confirmed that all formulations retained the crystalline form of ibuprofen, although minor reductions in crystallinity were observed for polymer-coated samples, potentially enhancing solubility by reducing lattice energy.[31]

In distilled water, dissolution increased gradually for all formulations, with Pure IBU achieving the highest cumulative release at 60 min, followed by IBU-POL, IBU-E, and IBU-HPMC. The slightly slower initial release of IBU-HPMC is consistent with a thin polymer layer partially impeding water access. In phosphate buffer (pH 6.8), dissolution rates were higher for all samples due to increased ionization above the ibuprofen pKa (~4.5). Near-complete release was observed by 60 min, with IBU-POL showing the fastest release, likely due to enhanced wettability from Poloxamer 188 and reduced particle anisotropy. In acidic medium (0.1 N HCl, pH 1.2), Pure IBU and IBU-E showed slower early dissolution, while IBU-HPMC and IBU-POL released more rapidly, reflecting the effect of surface modification and polymer/surfactant coating in promoting early-stage solubility. At pH 4.5 (acetate buffer), dissolution was intermediate; IBU-HPMC exhibited slightly slower release compared to IBU-POL and IBU-E, attributable to HPMC's gel-forming behavior acting as a minor diffusion barrier.

These trends are clearly illustrated in Figure 5, which shows the cumulative percent drug release of all four formulations over 60 minutes with error bars representing standard deviation. Overall, the results indicate that particle size reduction, isotropic morphology, and polymer or surfactant coating synergistically enhance dissolution, particularly under conditions of limited solubility, highlighting the importance of formulation strategy in achieving optimized oral bioavailability of ibuprofen.[32,33]

5.Animal Study

In Vivo Pharmacological Evaluation of Polymer-Assisted Ibuprofen Crystals

The anti-inflammatory and analgesic potential of polymer-assisted ibuprofen formulations was evaluated using four established pharmacological models in Wistar rats: carrageenan-induced paw edema, hot plate test, acetic acid-induced writhing, and xylene-induced ear edema. These models allowed assessment of both central and peripheral analgesia as well as systemic and topical anti-inflammatory activity.

5.1 Carrageenan-Induced Paw Edema Model

Carrageenan injection produced a time-dependent increase in paw thickness in the control group, peaking at 180 minutes (Table 8, Figure 6). Oral administration of all ibuprofen formulations significantly reduced paw edema compared with control ($p < 0.05$). Among the tested formulations, IBU–Poloxamer 188 showed the highest inhibition (40.85%), followed by IBU–HPMC (35.51%) and IBU–Ethanol (33.15%), while Original IBU exhibited 40.60% inhibition. Polymer-assisted formulations demonstrated an earlier onset of edema reduction, greater intensity of inhibition, and a sustained effect over 180 minutes, indicative of prolonged duration of action. These improvements are attributed to enhanced dissolution and gastrointestinal absorption resulting from polymer-assisted recrystallization.

Table 8: Mean paw edema (mm) ± SD and % inhibition in Wistar rats after oral administration of original and polymer-assisted ibuprofen (n = 6). Statistical significance vs. control: *P < 0.05, **P < 0.01, ***P < 0.001.*

Sr No.	Groups	Mean Paw edema at different time intervals					% edema inhibition in different time intervals			
		0 min	30 min	60 min	120 min	180 min	30 min	60 min	120 min	180 min
1.	Control	4.16 ± 0.025*	8.09 ± 0.087**	7.89 ± 0.032**	7.76 ± 0.048**	7.66 ± 0.085**	-	-	-	-
2.	Original IBU	5.01 ± 0.089*	7.91 ± 0.087**	7.44 ± 0.154**	6.31 ± 0.056**	4.55 ± 0.086**	2.22 %	5.70 %	18.68 %	40.60 %
3.	IBU–Ethanol	5.13 ± 0.056*	7.94 ± 0.096***	7.17 ± 0.251	6.48 ± 0.089	5.12 ± 0.065***	1.85 %	9.12 %	16.49 %	33.15 %
	IBU–HP (HPMC)	4.94 ± 0.125*	8.78 ± 0.087***	7.96 ± 0.089***	7.485 ± 0.086***	5.43 ± 0.17***	%0.56	8.82 %	12.92 %	35.51 %
	IBU–Poloxamer 188	4.57 ± 0.056*	8.80 ± 0.76***	7.61 ± 0.63***	6.425 ± 0.185***	4.98 ± 0.048***	0.90 %	12.82 %	25.26 %	40.85 %

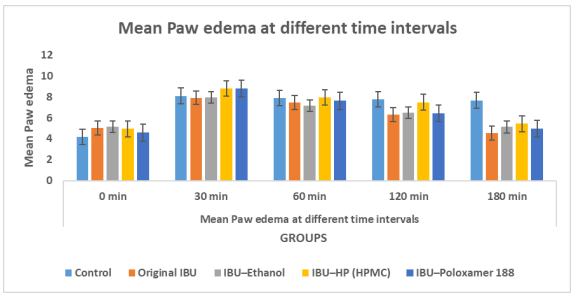


Figure 6: Mean Paw edema at different time intervals

5.2 Hot Plate Test (Central Analgesia):

Central analgesic activity was assessed using the hot plate method at 55 ± 1 °C following oral administration of 100 mg/kg ibuprofen or polymer-assisted formulations (Table 9, Figure 7). Baseline

reaction latencies (~9 s) were comparable across groups. Polymer-assisted formulations significantly increased latency at all time points ($p < 0.05$), with peak effect at 1 hour. IBU–Poloxamer 188 achieved the highest latency (15.4 ± 1.2 s), followed by IBU–HPMC (14.8 ± 1.3 s) and IBU–Ethanol (14.0 ± 1.1 s), while Original IBU reached 13.0 ± 1.3 s. The faster attainment of peak latency indicates improved onset, higher latency reflects enhanced intensity, and maintained effect up to 4 hours demonstrates prolonged duration, suggesting greater systemic exposure and enhanced oral bioavailability.

Table 9: Hot Plate Test Reaction latency (seconds, mean ± SD) after oral administration of 100 mg/kg of test substances (n = 06 per group).

Time (hr)	Control	Original IBU	IBU–Ethanol	IBU–HPMC (HP)	IBU–Poloxamer 188
0.0	9.5 ± 0.8	9.3 ± 0.9	9.4 ± 0.7	9.2 ± 1.0	9.3 ± 0.9
0.5	10.0 ± 0.9	11.2 ± 1.0*	11.8 ± 0.8*	12.5 ± 1.2*	13.0 ± 1.0*
1.0	10.1 ± 1.0	13.0 ± 1.3**	14.0 ± 1.1**	14.8 ± 1.3**	15.4 ± 1.2**
2.0	10.0 ± 0.9	12.1 ± 1.0***	13.0 ± 1.2***	13.5 ± 1.0***	14.4 ± 1.3***
3.0	9.8 ± 1.0	11.0 ± 0.9***	12.0 ± 1.1***	12.5 ± 1.1***	13.2 ± 1.1***
4.0	9.6 ± 0.8	10.9 ± 1.0***	11.5 ± 0.8***	11.8 ± 1.0***	12.5 ± 1.1***

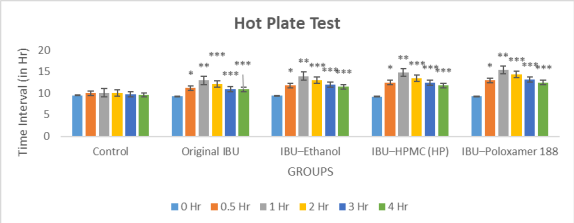


Figure 7. Hot plate test: reaction latency (seconds) over 4 hours, Data represent mean ± SD (n = 6); *p < 0.05, **p < 0.01, ***p < 0.001 vs. control."

5.3 Acetic Acid-Induced Writhing Test (Peripheral Analgesia):

Peripheral analgesic activity was evaluated via intraperitoneal administration of 0.6% acetic acid and counting abdominal constrictions (writhes) over 15 minutes (Table 10, Figure 8). The control group exhibited 55.2 ± 3.0 writhes, while Original IBU reduced writhing to 30.3 ± 2.4 ($p < 0.01$). Recrystallized formulations further reduced writhes: IBU–Ethanol (25.0 ± 2.1, $p < 0.001$), IBU–HPMC (18.1 ± 1.7, $p < 0.001$), and IBU–Poloxamer 188 (15.0 ± 1.5, $p < 0.001$). Polymer-assisted crystals exhibited earlier reduction in writhes, greater intensity of analgesic effect, and sustained reduction throughout the observation period, consistent with faster absorption, higher systemic exposure, and enhanced bioavailability.

Table 10: Acetic Acid-Induced Writhing Test

Group	No. of Writhes
Control	55.2 ± 3.0
Original IBU	30.3 ± 2.4**
IBU–Ethanol	25.0 ± 2.1***
IBU–HP (HPMC)	18.1 ± 1.7***
IBU–Poloxamer 188	15.0 ± 1.5***

Significant vs. control; **p < 0.01, ***p < 0.001 (one-way ANOVA, Dunnett's test)

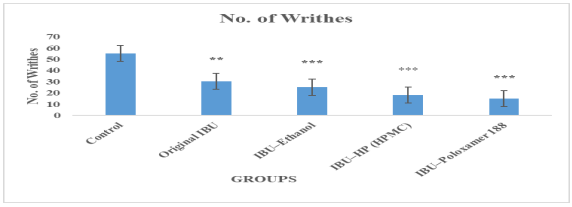


Figure. 8 Acetic Acid-Induced Writhing Test Number of Writhes in 15 Minutes

5.4 The xylene-induced ear edema mode

Topical anti-inflammatory activity was assessed by measuring ear swelling after xylene application (Table 10, Figure 9). The control group exhibited maximum ear edema (25.0 ± 1.5 mg), whereas Original IBU reduced swelling to 15.0 ± 1.2 mg ($p < 0.01$). Recrystallized formulations further reduced edema: IBU–Ethanol (12.0 ± 1.0 mg, $p < 0.001$), IBU–HPMC (10.0 ± 0.9 mg, $p < 0.001$), and IBU–Poloxamer 188 (8.0 ± 0.8 mg, $p < 0.001$). These results demonstrate faster onset of edema inhibition, greater intensity, and prolonged duration for polymer-assisted formulations, indicative of enhanced tissue penetration and improved local bioavailability.

Table 10. Effect of polymer-assisted ibuprofen crystals on xylene-induced ear edema (mg, mean ± SD, n = 6)

Group	Ear swelling (mg)
Control	25.0 ± 1.5
Original IBU	15.0 ± 1.2**
IBU–Ethanol	12.0 ± 1.0***
IBU–HPMC (HP)	10.0 ± 0.9***
IBU–Poloxamer 188	8.0 ± 0.8***

Notes: Statistical significance: **p < 0.01, ***p < 0.001 compared with control.

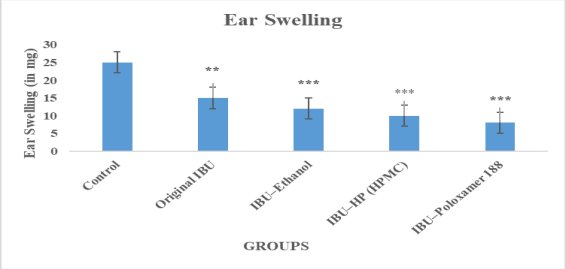


Figure. 9 Ear swelling (mg)

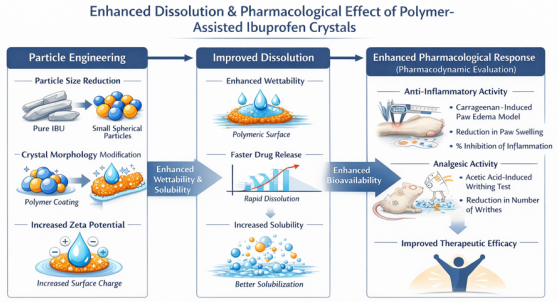


Figure 10. Proposed mechanism showing how polymer-assisted crystallization improves ibuprofen performance

Overall Interpretation:

Across all four models, polymer-assisted ibuprofen crystals consistently exhibited enhanced onset, intensity, and duration of anti-inflammatory and analgesic effects compared with unmodified ibuprofen (Figure 10 Proposed mechanism showing how polymer-assisted crystallization improves ibuprofen performance). These pharmacodynamic improvements provide strong indirect evidence of improved oral and systemic bioavailability, likely mediated by enhanced dissolution, faster gastrointestinal absorption, and optimized crystal habit. While the pharmacological models demonstrate functional bioavailability improvements, future pharmacokinetic studies (C_max, T_max, AUC) are recommended to directly quantify systemic exposure.

Limitations

The improved pharmacological response observed for polymer-assisted ibuprofen crystals may be attributed to enhanced dissolution and improved systemic availability. Although pharmacokinetic parameters such as Cmax, Tmax, and AUC were not evaluated in the present study, the improved pharmacological responses together with enhanced in vitro dissolution suggest improved systemic availability of ibuprofen. Therefore, the enhanced bioavailability is inferred indirectly from dissolution behavior and pharmacodynamic outcomes. Modification of crystal habit reduced particle anisotropy and increased surface wettability, facilitating faster drug release and absorption.

However, certain limitations should be acknowledged. The pharmacological evaluation in the present study was restricted to acute models, and long-term therapeutic efficacy and safety were not investigated. In addition, pharmacokinetic studies to directly confirm enhanced systemic exposure were not performed. Future studies focusing on chronic dosing models and pharmacokinetic evaluation would further strengthen the clinical relevance of the developed crystal forms.

4. Conclusion

The present study demonstrated that polymer-assisted recrystallization significantly improves the pharmacological performance of ibuprofen in vivo. Formulations prepared with Poloxamer 188 and HPMC showed enhanced anti-inflammatory and analgesic activities compared with original ibuprofen. The improved efficacy may be attributed to enhanced solubility, wettability, and systemic absorption of the drug. These findings highlight the potential of polymer-assisted crystallization as an effective strategy to improve the therapeutic performance of poorly soluble drugs.

The results demonstrate that polymer-assisted crystallization improves the pharmacological performance of ibuprofen. Enhanced anti-inflammatory and analgesic activity observed in vivo suggests improved systemic availability of the drug due to modified crystal habit and improved dissolution behavior.

The pharmacological models used in this study indirectly reflect the systemic availability of ibuprofen following oral administration. Ibuprofen is a BCS Class II drug, where dissolution is the rate-limiting step for absorption. Modification of crystal habit through polymer-assisted crystallization can enhance surface wettability, reduce particle anisotropy, and improve dissolution behavior.

Improved dissolution facilitates faster drug release in gastrointestinal fluids, leading to enhanced absorption and increased systemic drug concentration. As a result, higher systemic availability of ibuprofen may

produce a stronger anti-inflammatory and analgesic response in pharmacological models.

The carrageenan-induced paw edema and xylene-induced ear edema models reflect the anti-inflammatory activity mediated by inhibition of prostaglandin synthesis, which depends on adequate systemic drug levels. Similarly, the acetic acid–induced writhing test evaluates peripheral analgesic activity, while the hot plate test assesses central nociceptive response. Enhanced inhibition of inflammation and pain responses observed with polymer-assisted ibuprofen crystals therefore suggests improved pharmacological efficacy, which is likely associated with increased systemic availability of the drug.

Thus, the improved pharmacological responses observed in these models indirectly indicate enhanced bioavailability resulting from improved dissolution and absorption of the modified ibuprofen crystals.

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