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Original Article

IBUPROFEN INJECTABLE MICROEMULSION PREPARATION COATED BY CHITOSAN: FORMULATION, CHARACTERIZATION, *IN VITRO* PERFORMANCE, ANTI-INFLAMMATION ACTIVITY, AND HEMATOLOGY ASSESSMENT

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ABSTRACT

Objective: This study aimed to develop, characterize, and conduct stability evaluations to ensure compliance with intravenous administration for microemulsion ibuprofen injection. In addition, hematology assessment and profile of drug release kinetics were analyzed.

Methods: The formulation process commenced by introducing various chitosan concentrations into microemulsion ibuprofen injection, following a method established in a previous study. Formulation parameters studied include particle size, polydispersity index (PDI), zeta potential, kinetic of drug release, anti-inflammation activity using the 1% carrageenin induction method, and hematology assessment.

Results: The results showed that the addition of 1% chitosan solution allowed for the development of the ideal microemulsion formula, with droplet size, zeta potential, and PDI of 19.37±0.32 nm,-1.53±0.12 mV, and 0.38±0.02, respectively. Kinetics of chitosan-coated ibuprofen microemulsion (MK) were governed by the squared root of time paradigm, suggesting that drug release proceeded by diffusion and was influenced by the carrier. Compared to the other groups, the paw injected with MK indicated a strong anti-inflammatory effect and did not differ significantly from the control group ($p>0.05$). However, Hematology analysis showed no statistically significant variations in leukocyte and erythrocyte profiles between the treatment and control groups ($p>0.05$).

Conclusion: MK met the criteria as an intravenous preparation based on the characteristics and safety.

Keywords: Chitosan-coated ibuprofen microemulsion, Kinetic of drug release, Hematology assessment, Anti-inflammation

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INTRODUCTION

Ibuprofen is one of the most used NSAID for treating mild to moderate inflammation. This drug is safer than other NSAID groups and works by inhibiting cyclooxygenase, the enzyme responsible for prostaglandin production. The inhibition reduces prostaglandin levels and inflammatory processes [1, 2]. However, a drawback of ibuprofen in inflammation therapy is the frequent occurrence of abdominal pain as a side effect. Most formulations available in the market are in oral and topical forms compared to injections and are unsuitable for emergency treatment. Several studies on the intravenous (IV) use of ibuprofen with morphine showed that administering this combination reduced pain in postoperative patients compared to those given a placebo or morphine [3]. In laparoscopic sleeve gastrectomy, IV administration of ibuprofen has a more favorable effect than paracetamol, reducing opioid usage in the first 24 h post-surgery [4]. IV ibuprofen has shown rapid efficacy compared to ketorolac in alleviating pain associated with renal colic and enhances the sleep quality of individuals suffering from non-specific musculoskeletal pain. The drug is more effective and safe for acute postoperative pain, but only two types of formulations are currently available with a short shelf life [5]. These formulations include ibuprofen+lysine and freeze-dried parenteral ibuprofen, developed with expensive technology [6–9]. The limited availability is due to the low water solubility, high lipophilicity, and the need for frequent dosing intervals, reaching 4-6 times a day [10]. A previous study also stated that many side effects are associated with IV ibuprofen in patients [12]. Furthermore, microemulsion is optically transparent with a diameter ranging from 10-100 nm, and formed after mixing with oil, water, and an emulsifying agent [14]. It is an excellent carrier for hydrophilic and hydrophobic drugs in parenteral formulations due to the superior stability compared to nanoemulsions and those with a size below 50 nm [11, 15]. This compound can be used as carrier for parenteral vaccines to enhance the cellular immune response to viruses [16]. Compared to other colloidal carriers, microemulsion can effectively serve as drug delivery system for hydrophobic drugs due to the unique characteristics, including thermodynamic stability, tiny droplet size, and ease of fabrication attributed to the role of the oil component [17]. Microemulsion improves the residence time of drugs, thereby increasing bioavailability, which reduces the volume of dosing and frequency of administration, providing comfort to patients [18]. A low-toxicity carrier polymer for enhancing stability in nanoparticle formulations is needed to achieve specific therapeutic objectives. Chitosan is a biodegradable, biocompatible polymer that is safe for use and applied as a carrier for oral and parenteral applications [19]. Studies related to microemulsion injections with chitosan showed that this polymer could enhance the penetration of active substances in nasal formulations after being mixed in the dispersed phase [20]. Chitosan also improves the mechanical properties of microemulsion for topical use. The procedure is achieved by increasing the adhesive properties of the formula on the mucosa, thereby prolonging the residence time on the mucosa [21]. This study aimed to investigate chitosan role in enhancing microemulsion stability. Additionally, the release profile and blood response following the intravenous injection of ibuprofen microemulsion was analyzed. The efficacy of the results was then tested for anti-inflammatory effects using the 1% carrageenan induction method.

MATERIALS AND METHODS

Material

The materials used included ibuprofen 99% B. P. and Virgin coconut oil (VCO) purchased from (P. T. Dwilab Mandiri Scientific and CV As Sohwah), low molecular weight (Mw) chitosan (Mw average ≤2000 Da) from (Xi'an *Best Bio-Tech, Co., LTD*), olive oil, MCT oil, Tween 80 food-grade, propylene glycol USP pharma-grade from (Emas biru), and

Original Infusan RL from (CV Syifa Herbal Alami), MCTMAX, Raja Kimia, Emas Biru, and Sanbe, respectively. Aquadest (aqua distillates), methanol, HPLC for Chromatography, and acetate acid glacial (100%) were purchased from Merck, while carrageenan Pro Analyst (Sigma Aldrich) was bought from Asian Bahan Farmasi, CMC Na Food Grade.

Methods

Formulation of chitosan-coated ibuprofen microemulsion (MK) at various chitosan concentrations

A preliminary study yielded an optimal formula of 9% oil (MCT: olive oil 1:1), 45.5% Smix (Tween 80+propylene glycol), and 45.5% water. Subsequently, chitosan was added to the optimal formula at concentrations of 0.2%, 0.4%, 0.6%, 0.8%, and 1% in a 1% acetic acid solution, followed by homogenization using a vortex for 5 min [22].

Stability evaluation of chitosan-coated ibuprofen microemulsion (MK) preparation

The stability test of ibuprofen microemulsion was evaluated using centrifugation, heat stress, and differences in storage temperature.

a. Centrifugation Method. Microemulsion was centrifuged at 1000, 2500, and 3500 rpm for 15 min to accelerate possible instability phenomena, and stability parameter used % transmitting.

b. Heat Resistance Method. Microemulsion was packaged in pre-sterilized multidose vials and heated in a water bath at 40 °C to 80 °C. The temperature was increased by 5 °C every 30 min, and stability parameter used visual observation.

c. Storage Temperature. The selected microemulsion was prepared in triplicate, and samples were stored at 25±2 °C and 40±2 °C. The tests were performed 1, 30, 60, 90, and 120 d after preparation, and the analytical measurements of droplet Size, zeta potential, and polydispersity index (PDI) were conducted using the Horiba Scientific SZ 100 tool with the Dynamic Light Scattering test method in purified water 100 times. Subsequently, the solution was transferred to a disposable cuvette, and the results were the average of 3 measurements. The software used was SZ-100 for Windows, supplied by the manufacturer (HORIBA Instruments Ltd).

Determination of encapsulation efficiency

About 5 ml of oil phase mixture, including oil, surfactant, and co-surfactant, was used to dissolve ibuprofen, measuring 750-1000 mg. Vortexing was carried out for 1 min for dissolution, followed by sonication for 35 min, and incubation for 30 min at 45 °C until a transparent oil phase system was obtained. Weighing was stopped when oil phase system became cloudy, indicating saturation. The stable oil phase system was then stored at 25 °C for 2 d to determine stability. Subsequently, the water phase was mixed in a specific ratio of 1:5, followed by analysis using HPLC to determine Encapsulation Efficiency (EE). The mobile phase in this study used 4 g of chloroacetic acid, then dissolved with 400 ml distilled water, and pH was adjusted to 3 with NH4OH. About 600 ml acetonitrile was added and filtered with a 0.45 µm ultrafilter. The column used in the experiment was a Purospher® STAR RP-18 end-capped column with dimensions of 250-4.6 mm. The injection volume was 5 µl, and the flow rate was 2 ml/minute. A UV detector set at a wavelength of 254 nm was used and EE was calculated based on the method described by [23]:

$$EE\% = \frac{\text{Quantity of ibuprofen entrapped}}{\text{total quantity of ibuprofen added}} \times 100$$

Microemulsion sterilization process

The microemulsion was subjected to aseptic filtration through an ultrafilter Chromafil xtra with 0.22 µm pore size. Following each sterilization procedure, visual inspection was carried out for phase separation, and the percentage transmittance was determined using a Shimadzu Ultraviolet (UV) 1800 spectrophotometer at a wavelength of 650 nm, while aquadest served as the blank [24].

Determination of osmolarity, pH, and viscosity

Osmolality testing was carried out on ibuprofen microemulsion formulation at a concentration of 4 mg/ml, following the stipulated standard. The KNAUER K-7400S Semi-micro osmometer was used for this analysis. A volume of 150 µl was examined alongside standards with concentrations of 450 mOsmol/kg and 850 mOsmol/kg, each amounting to 150 µl.

Transmission electronic microscopy

To ascertain morphological properties and validate data acquired through Dynamic Light Scattering regarding droplet size and PDI, Transmission Electron Microscopy (TEM) was used. TEM analysis was conducted using Jeol Jem-1400, Tokyo, Japan, operating at 120 kV. The microemulsion was deposited on a carbon-coated copper grid for TEM sample preparation and negatively stained with 1% (w/v) uranyl acetate. This method facilitated detailed observation and analysis.

APIs release kinetics evaluation

Ibuprofen release tests were conducted with a modified Erweka DT 700 US Pharmacopeia apparatus type II utilizing Spectrapor 10 kDa (22 mm) dialyzer tubing sourced from Arthur H. Thomas CO., Philadelphia, PA, USA., and HPLC ALLIANCE WATER e 2695. A 2 ml sample containing 8 mg/ml of ibuprofen was placed into the dialysis tube, sealed, attached to the paddle apparatus, and immersed in 900 ml pH 7.4 phosphate buffer (34 °C, 50 rpm). Sampling intervals were set at 5, 10, 15, 30, 60, 90, 120, 180, and 240 min, at each interval, 2 ml samples were withdrawn and replaced with fresh dissolution media. After the experiment, samples, including the donor compartment were analyzed using HPLC with a minimum of three replications. HPLC analysis was performed by preparing the mobile phase using acetonitrile and a 0.025 M phosphate buffer at pH 3.5. A standard curve was established at 0, 2, 4, 6, 8, 10, and 12 ppm concentrations, and an injection volume of 2 µL was used with a run time of 12 min. The data were plotted using zero-order ($Q = Q0 + k \times t$), first-order ($\ln Q = \ln Q0 + k \times t$), and time square root ($Q = k \times \sqrt{t}$) models, where Q (mg), Q0, and k represented the cumulative amount of drug released at time t (h), the initial amount of drug at t = 0, and the release constant [23].

***In vivo* studies**

All procedures related to blood tests and rats follow the Ethical Approval by the Animal Ethics Committee Faculty of Veterinary Medicine, IPB University (No. 049/KEH/SKE/XI/2021).

Anti-inflammatory activity

In the anti-inflammatory activity test, male Wistar strain rats weighing 190–220 g were used, totaling 45 divided into nine treatment groups, as shown in table 1. Rats were maintained under standard conditions at the Experimental Animal Facility of Tropical Biopharmaka Research Center, IPB. Rats were subjected to a 7-day acclimatization period, fasted 1 d before the test [25] and the dose of ibuprofen used was 7.2 mg/200 g and 14.4 mg/200 g BW.

The anti-inflammatory test procedure was initiated with the measurement of the right leg volume of rats before treatment to establish the baseline value (V_0) using a plethysmometer in the respective test animal group. The test formulation was administered 1 h before the subplantar injection of 1% carrageenan into the right leg at a volume of 0.1 ml, and the resulting volume was recorded as the 0-minute edema volume (Vt_0). The edema volume was measured every 30 min for 6 h.

The calculation of anti-inflammatory potency followed the method of Ikawati *et al.* [26] with slight modifications, where anti-inflammatory potency was determined by the volume of edema (Ve) obtained from the formula Ve = Vt–Vo. The area under the curve (AUC) represents the amount of inflammation obtained from Ve versus time. AUC was obtained from the formula:

$$AUC_{tn-1}^{tn} = \frac{(Vt_{n-1} + Vt_n)}{2} \times \Delta t$$

Vt_{n-1}: average of edema volume in t_{n-1} Δt: time interval of paw measurement

Vt_n: average of edema volume in t_n

Table 1: Test animal treatment group

Groups	Treatment
Normal	No treatment
Oral Placebo	Five rats received oral CMC Na 5%
MO Placebo	Five rats with microemulsion without ibuprofen 1 ml intravenously
MK Placebo	Five rats with chitosan-coated microemulsion without ibuprofen 1 ml intravenously
Oral Ibuprofen	Five rats received oral ibuprofen at a dose of 14.4 mg/200 g BW dissolved in CMC Na 5% up to 5 ml
MO with dosage 7.2 mg	Five rats received ibuprofen microemulsion at a dose of 7.2 mg/200 g BW rats intravenously
MO with dosage 14.4 mg	Five rats received ibuprofen microemulsion at a dose of 14.4 mg/200 g BW rats intravenously
MK with dosage 7.2 mg	Five rats received chitosan-coated ibuprofen microemulsion at a dose of 7.2 mg/200 g BW rats intravenously
MK with dosage 14.4 mg	Five rats received chitosan-coated ibuprofen microemulsion at a dose of 14.4 mg/200 g BW rats intravenously

MO: chitosan-uncoated ibuprofen microemulsion; MK: chitosan-coated ibuprofen microemulsion

Anti-inflammatory potency was represented as percent inhibition, where AUC_c and AUC_t indicated the mean of placebo and treatment groups, respectively. Placebo oral was used for the positive control group and microemulsion for ibuprofen microemulsion group. Anti-inflammatory potency was calculated using the formula:

$$\% \text{ Inhibition} = \frac{\text{AUC Placebo} - \text{AUC Treatment}}{\text{AUC Placebo}} \times 100\%$$

Hematology assessment

Blood for hematology analysis was collected from the same group of rats used in the anti-inflammatory test. Blood samples were collected from three rats in each treatment group, either 15 min after administering the test formulation or 45 min before the induction of 1% carrageenan, through the lateral tail vein into EDTA tubes.

Total leukocyte and erythrocyte profile examination

The total leukocyte and erythrocyte profiles were assessed based on established procedures at the UKHP Biopharma IPB. About 0.5 ml of blood samples were collected in EDTA tubes, then homogenization was performed to prevent clotting and measurements were carried out using a hematology analyzer (Mindray BC-2800vet).

Determination of leukocyte differential count

Leukocyte differential count was conducted by preparing a blood smear and a tiny droplet was placed about 2-3 mm from the edge of a glass slide. A spreader slide was used at a 30-45° angle in front of the droplet. Blood spread to the edge of the slide and was pushed firmly to create a smear approximately 3-4 cm long. The smear was left to air dry before being placed on two staining dishes. Subsequently, it was fixed with absolute methanol for 2-3 min, stained with 5% Giemsa stain, and left for 20-30 min. Rinsing was carried out with a slow and more substantial stream of water to remove excess stain and left to dry. A microscope with a 10x magnification was used to examine the evenly spread erythrocytes found on the stained and dried blood smear. Meanwhile, the objective lens was switched to 40x, and 100x magnification to classify and record 100 consecutive nucleated cells in the region of interest before calculating the percentage of each cell type [27].

Data analysis

Statistical analysis was conducted using SPSS and the results were reported as mean±SD. Normal distribution was assessed using the Shapiro-Wilk Normality Test, while comparisons among multiple groups were performed with one-way ANOVA and followed by Tukey post hoc analysis. For a non-normal distribution, a comparison was conducted using the Kruskal-Wallis and Mann-Whitney U tests to determine significant differences between the groups and P value of<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Chitosan-coated microemulsion and stability at various concentrations

Centrifugation, heat resistance, and storage temperature were used to assess stability of microemulsion at different concentrations of chitosan solution. The optimal formulation was selected based on stability considerations. The original formulation demonstrated stability across chitosan concentration series of 0.2%-1% in the centrifugation test. However, the higher chitosan concentration of 1% showed the highest stability in the heat stress test. The designated chitosan concentration was 1%, resulting in the most stable microemulsion, as indicated by stability tests in table 2. In this study, chitosan played a crucial role in enhancing stability and activity of ibuprofen microemulsion. This was supported by a previous study stating that chitosan contributed significantly to augmenting stability and activity of microemulsion [28]. Stability was intricately influenced by cohesive and adhesive forces regulated by factors such as molecular weight, polarity, and the polymer structure of chitosan. Chitosan not only elevated microemulsion stability but also reduced the particle size of the original formula. In addition, the Newtonian flow properties and low viscosity further facilitated the formulation process [29].

Table 2: Results of stability testing through centrifugation and heat resistance

Sample	Centrifugation (% transmittance)	Heat resistance (Formation)
MK 1%	98.90	stable
MK 0.8%	98.85	Crystal
MK 0.4%	98.05	Crystal
MK 0.2%	98.70	Crystal

MK-chitosan-coated ibuprofen microemulsion

The addition of 1% chitosan increased stability of microemulsion for up to 365 d at room temperature, as shown in table 3. The increase was attributed to the ability of chitosan to disperse effectively on the surface of microemulsion, thereby reinforcing stability. Microemulsion characteristics were assessed based on droplet size, zeta potential value, and PDI. The globule sizes remained below 20 nm from day 0 to 365, with a zeta potential varying from-23.57 to-1.3 mV and a low PDI close to 0. Although zeta potential did not approach±30 mV, stability was still achieved due to the tiny globules, which minimized gravitational forces and prevented precipitation. As stated by [30], stability was also influenced by the choice of nonionic surfactants. For example,

Tween 80 stabilized the system with a steric effect by forming a highly hydrated film around the droplets. It also significantly influenced PDI size close to zero, ensuring uniform particle size distribution.

Table 3: Characterization results of 1% MK based on storage time

Parameter	Days 30	Days 90	Days 120	Days 365
Transmitting (%)	98.5±0,10	98,3±0.10	98.2±0.40	-
Droplet size (nm)	13.13±0.11	14.77±0.15	16.47±0.57	19.37±0.32
Zeta potential (mV)	-13.67±0.25	-23.57±0.23	-9.73±0.46	-1.53±0.12
Polydiversity index	0.15±0.00	0.15±0.27	0,264±0.03	0,383±0.02

All values are expressed in mean±RSD, n=3. MK-chitosan-coated ibuprofen microemulsion

Encapsulation efficiency and sterilization

In this study, microemulsion oil phase contained 166.7 mg/ml ibuprofen. However, to make the dose determination easier, the system was set at a concentration of 150 mg/ml. EE was carried out using HPLC after mixing with the water phase (1:5) to obtain values as shown in table 4. In this experiment, microemulsions coated with and without chitosan were compared.

The sterilization method used in this study was filtration, selected for the simplicity and the absence of hot steam [31]. A 0.22 µm ultrafilter was used to ensure that microemulsion globules could pass through the filter while obstructing the passage of bacteria and pyrogens [32]. Stability observations were essential to assess whether microemulsion remained stable post-filtration and transmittance measurements served as an initial indicator to evaluate stability. As shown in table 3, the transmittance values of microemulsion after the filtering process were 97.0% and 98.5%, respectively. The results indicated that microemulsion remained close to 100%, signifying stability after sterilization, as shown in table 4 [33]. Additionally, sterilization methods at high temperatures, such as steam or dry heat, could lead to the hydrolysis of some lipids, resulting in the release of free fatty acids, which could decrease emulsion stability. This effect was particularly pronounced in nanoemulsion systems coated with chitosan, as cross-linking between chitosan chains could occur due to the high temperature related to amino groups [34].

Several intermolecular activities of the components in microemulsion influenced drug loading in microemulsion. Among these were π-π stacking and electrostatic activities originating from the molecular hollow structure and nanometer-scale size [35]. Chitosan with low molecular weight enhanced entrapment efficiency on microemulsion globules due to the ability to reduce interfacial tension, resulting in smaller microemulsion globule sizes (table 4). Conversely, high molecular weight chitosan tended to increase the size of microemulsion globules and trigger the depletion effect [36].

Osmolarity, pH, and viscosity

Ibuprofen microemulsion was designed for intravenous administration, suggesting the need to meet specific standards, such as the osmolarity value. Freezing point osmometer method was used in this study, resulting in values of 240±20.22 m Osmol, pH 4-5, 27 Cpas. The osmolarity requirements for injection preparations fall in the range of 200-300 m Osmol. A high osmolarity level can potentially lead to pain and burning sensations during injection [16] and the specific value depends on the number of moles in the chemical compound. Based on the results, MK fell in the acceptable range for injection preparation requirements. As an additional advantage, simple reconstitution with WFI without the inclusion of isotonicity substances has achieved isotonicity parameters, simplifying the application in medical settings.

Table 4: EE with HPLC analysis and transmittance for sterilization

Sample	Concentration (mg/ml)	EE (%)	Transmittance (%)
MO	30.61±1.52	102.04±5.04	97.00±0.00
MK	32.06±0.92	106.87±0.92	98.50±0.10

All values are expressed in mean±RSD; MO: chitosan-uncoated ibuprofen microemulsion; MK, MCT: chitosan-coated ibuprofen microemulsion, n=3.

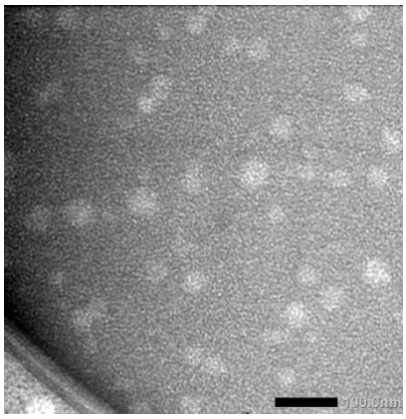

Fig. 1: Morphology of 1% MK, magnification at 40000x

Transmission electronic microscopy

TEM analysis showed that the droplet was spherical (fig. 1) and the size scale was similar to the dynamic light scattering results measurement. Based on the measurement results using the Dynamic Light Scattering method and the morphological observation with TEM, coated microemulsion globules had a size below 30 nm. This phenomenon may lead to an increased bioavailability of ibuprofen due to the resistance against the reticuloendothelial system (RES), consequently extending the drug retention time in the bloodstream [35].

***In vitro*, APIs release kinetics evaluation**

The release of MO and MK followed the squared root of the time model, as described in table 5. MK experienced faster release in the early minutes than MO (fig. 2). This was attributed to MK having a more extensive interface area that affected smaller microemulsion droplets, resulting in a finer dispersion of ibuprofen in microdroplets. The homogeneous dispersion of the active substance in the excipients also prevented recrystallization during dissolution, enhancing stability of MK [23]. However, after 60 min, due to the presence of a polar protic solvent (acetic acid), the release of MK reduced, causing the effect to last longer

compared to MO [37]. In line with the results by Chirio *et al.* [38], chitosan also showed a slow-release effect attributed to the hydrophilic effect of the coating, which minimized opsonization, thereby prolonging the systemic circulation.

Anti inflammation activity

Ibuprofen, an anti-inflammatory and analgesic agent, functions by non-selectively inhibiting the synthesis of cyclooxygenase 1 and 2 enzymes that produce prostaglandins [39]. The formulated microemulsion produced anti-inflammatory effects in this study, as illustrated in fig. 3.

Table 5: Release of MK and MO based on a mathematical model of zero-order, first-order, and squared root of time release

Sample	Zero-order		First-Order		√t	
	r^2	$k(mgh^{-1})$	r^2	$k(h^{-1})$	r^2	$k(mgh^{-1/2})$
MO	0,8932	0,0252	0,5642	0,0181	0,9741	0,4648
MK	0,9182	0,0223	0,7974	0,0095	0,9742	0,4054

MO: chitosan-uncoated ibuprofen microemulsion; MK: chitosan-coated ibuprofen microemulsion

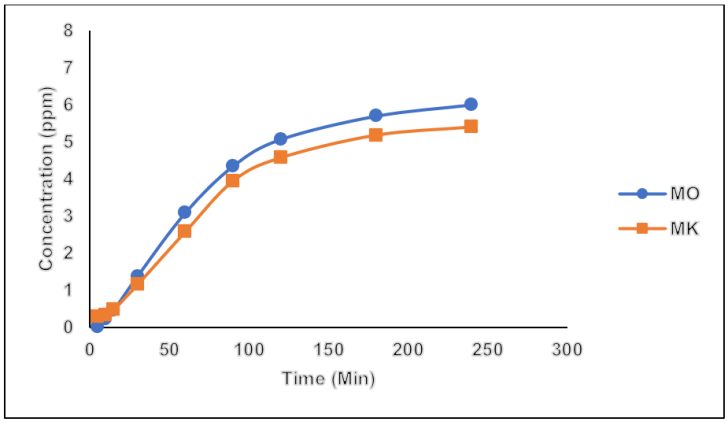

Fig. 2: Ibuprofen release vs time

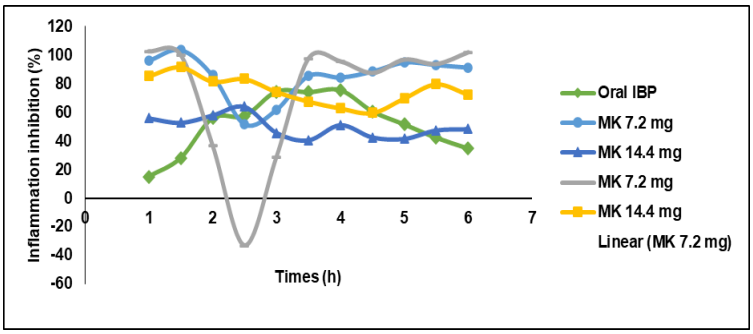

Fig. 3: Percent inhibition vs time, all values shown in the graph are measured as mean±SD, n=5. Error bars are omitted. MK-chitosan-coated ibuprofen microemulsion, IBP-Ibuprofen

At a dose of 14.14 mg, microemulsion, whether coated with chitosan or uncoated, had a higher edema volume than the 7.4 mg dose (fig. 4 and 5). This difference could be attributed to the larger injection volume (2-6 ml) used for the 14.4 mg dose, potentially increasing the edema volume. In contrast, the standard intravenous administration volume for test animals, such as rats, is typically only 0.5 ml. Injection volume in this study also impacted the observed edema volume in the body of rats. At the injection dose of 7.2 mg for MK, edema ceased after 6 h, compared to other groups. This suggests that the formulation has the potential to achieve the same optimal effect as the standard control while requiring a lower administered dose, providing a faster anti-inflammatory effect [40].

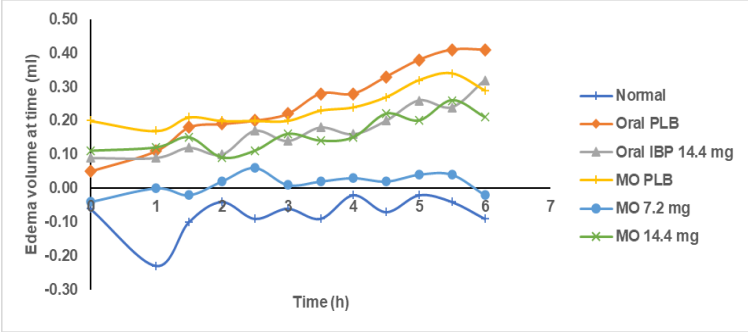

Fig. 4: Edema volume of injectable MO vs oral ibuprofen, all values shown in the graph are measured as mean±SD, n=5. Error bars are omitted. MK-chitosan-coated ibuprofen microemulsion, IBP-Ibuprofen, PLB–Placebo

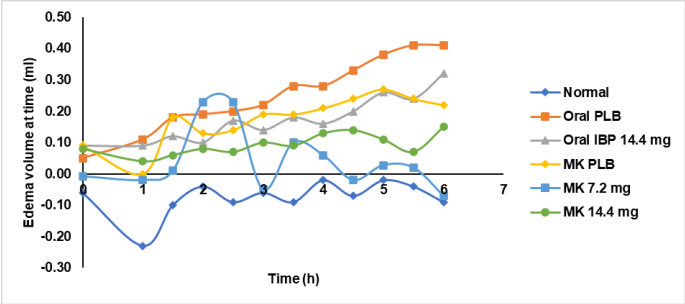

Fig. 5: Edema volume of injectable MK vs oral ibuprofen, all values shown in the graph are measured as mean±SD, n=5. Error bars are omitted. MK-chitosan-coated ibuprofen microemulsion, IBP-Ibuprofen, PLB–Placebo

The drug effect was higher in MK formulation than in the regular type due to the ability of chitosan to extend the systemic circulation of ibuprofen by minimizing the opsonization [38]. Based on statistical analysis using One-Way ANOVA and Kruskal-Wallis, the group injected with MK at 7.2 mg and 14.4 mg did not show significant differences in paw edema compared to the standard group. Therefore, it was concluded that ibuprofen formulated in the form of MK had a better effect than MO, demonstrating a significant difference in the volume of the paw edema compared to the standard group.

Hematology profile of test rats after administration of formulation

The differential leukocyte and erythrocyte profile in blood was examined to assess the health status of the test animals following MK and MO injections. These examinations were used to determine the effects of the test formulation on the physiological properties of blood.

Acute inflammation can elevate leukocyte levels, with fluctuations serving as indicators of underlying diseases [41]. The rise in neutrophil count in MK placebo group was attributed to the smaller size of the microemulsion produced, measuring below 15 nm, as shown in table 6. Tiny globules led to a higher concentration of microemulsion in blood, recognized as foreign bodies. Each leukocyte component plays a role in the body defense system, for example, neutrophils act as the initial defense in allergic reactions or inflammation [42]. Other components, such as eosinophils, constitute a small population of total leukocytes. Accumulation of eosinophils may signal malignancy, infection, or disruptions of homeostasis in the body [43]. Excessive monocyte activation is an early sign of inflammation, and a decrease in lymphocyte count indicates a compromised immune system [41].

A comparison using One-Way ANOVA and Kruskal-Wallis for normally and non-normally distributed data groups (table 6) was conducted with a confidence level of 95%. Based on the results, when evaluated against the standard group, no significant increase was observed in lymphocytes ($p<0.05$), neutrophils ($p<0.05$), eosinophils ($p<0.05$), and monocytes ($p<0.05$). This lack of significant variation indicated that ibuprofen injection preparations, whether MK or MO, did not induce acute inflammatory reactions in rats.

Table 6: Differential leukocyte profile of rats after administration of the test formulation

Groups	Leukocytes $10^3/\mu l$	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocytes (%)
Normal	8.84±1.45	27.40±8.82	66.60±7.76	1.60±1.95	1.20±0.45
MO placebo	6.47±1.79	43.00±16.98	54.20±18.85	0.4±0.55	0.40±0.89
MK placebo	7.40±1.92	51.00±18.07	47.67±17.69	0.50±0.84	0.50±0.84
MO 7.2 mg	5.33±1.69	34.67±10.79	65.00±11.35	0.33±0.58	0.00±0.00
MO 14.4 mg	7.77±0.78	23.75±6.50	73.75±3.77	0.50±1.00	1.00±1.41
MK 7.2 mg	6.63±1.90	37.67±25.01	62.34±25.01	0.00±0.00	0.00±0.00
MK 14.4 mg	6.00±1.39	40.00±13.00	56.00±8.72	1.34±0.58	1.67±2.88

*P>0.05: significantly different from standard; all values are expressed in mean±RSD (n=3); MO: chitosan-uncoated ibuprofen microemulsion; MK: chitosan-coated ibuprofen microemulsion

Erythrocytes were examined to detect hemorrhage, particularly in terms of hemoglobin levels, hematocrit, and platelet count [44]. As shown in table 7, the differences in the values of each erythrocyte profile did not show significant variations based on the statistical analysis using One-Way ANOVA and Kruskal-Wallis. The statistical analysis values implied that erythrocyte profile values between the standard and treatment groups were similar ($p>0.05$), except for the hemoglobin level between the standard and MK placebo ($p<0.05$). Hence, none of the test formulations impacted the erythrocyte profile of rats.

Table 7: Erythrocyte profile of rats after administration of test formulation

Groups	Erythro-cytes (RBC)	Hemoglobin (Hgb)	Hematocrit (Hct)	MCV	MCH	MCHC	PLT
	10^6/µl	g/dl	%	fl	pg	g/dl	10^3/µl
Normal	7.49±0.93	13.54±0.73	39.68±2.77	53.42±3.76	18.16±1.44	34.10±0.64	815±410.76
MO Placebo	8.22±0.43	16.23±0.76	48.17±2.48	58.73±4.09	19.70±0.78	33.67±1.11	1320.67±322.18
MK Placebo	9.51±1.25	17.63±2.44*	51.00±6.97	53.63±1.00	18.47±0.35	34.53±0.06	1173±421.66
MO 7.2 mg	9.27±2.30	14.60±1.82	51.87±14.06	55.77±1.35	16.57±5.35	29.93±10.08	1689±417.26
MO 14.4 mg	7.65±0.87	14.47±1.36	41.30±3.92	54.20±1.25	18.90±0.44	34.97±0.25	1273.67±241.98
MK 7.2 mg	8.66±0.58	16.43±0.93	46.43±2.57	53.70±0.79	35.37±0.25	35.37±0.25	1214±322.01
MK 14.4 mg	7.79±1.04	14.93±1.99	43.27±5.94	55.63±0.81	19.13±0.40	34.47±0.32	1162.33±280.60

*P>0.05: significantly different from standard; all values are expressed in mean±RSD (n=3); MO: chitosan-uncoated ibuprofen microemulsion, MK: chitosan-coated ibuprofen microemulsion

CONCLUSION

In conclusion, the successful preparation of MK was achieved with a polymer content of 1%. The incorporation of chitosan polymer significantly enhanced stability of microemulsion, producing a threefold increase. Microemulsion met the essential criteria for intravenous preparations, maintaining standards for osmolarity (240±20.22 mOsmol), pH (4-5), viscosity (27 Cpas), and globule size (19.37±0.32 nm). The release test demonstrated that MK followed the square root of the time model, indicating controlled drug release through diffusion. The anti-inflammatory effect observed in the paw injected with MK surpassed that of other groups, particularly in the 400 mg (7.2 mg in rat dose) injection group. The paw volumes showed no significant differences compared to the standard group. Moreover, Erythrocyte and leukocyte profile tests on rats showed no significant differences between the treatment and standard groups. The results suggested the potential development of MK into an intravenous dosage form.

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AUTHORS CONTRIBUTIONS

Purwantiningsih Sugita and Mohammad Khotib, who also assisted with the analysis of research materials and edited the report, were the ones who conceptualized the design investigation. Aulia Ul Hafizah conducted the experiment and authored, revised, and rewrote the paper. Umi Cahyaningsih and Siti Sadiah oversaw the *in vivo* and *in vitro* investigations and edited the content. The final manuscript has been reviewed by all authors and approved.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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