Article

Investigating the Feasibility of Mefenamic Acid Nanosuspension for Pediatric Delivery: Preparation, Characterization, and Role of Excipients

Nikhat Perween¹, Sultan Alshehri² , T. S. Easwari¹, Vivek Verma¹, Md. Faiyazuddin^{3,4}, Abdullah Alanazi² and Faiyaz Shakeel^{2,*}

¹ Department of Pharmaceutics, Faculty of Pharmacy, IIMT Colleges of Medical Sciences, Meerut 250001, Uttar Pradesh, India; nikhatperween86@gmail.com (N.P.); dreaswari@rediffmail.com (T.S.E.); verma125ifm@gmail.com (V.V.)

² Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia; salshehri1@ksu.edu.sa (S.A.); alanazylab@gmail.com (A.A.)

³ School of Pharmacy, Alkarim University, Katihar 854106, Bihar, India; md.faiyazuddin@gmail.com

⁴ Nano Drug Delivery®, Raleigh-Durham, NC 27705, USA

* Correspondence: fsahmad@ksu.edu.sa or faiyazs@fastmail.fm

Citation: Perween, N.; Alshehri, S.; Easwari, T.S.; Verma, V.; Faiyazuddin, M.; Alanazi, A.; Shakeel, F. Investigating the Feasibility of Mefenamic Acid Nanosuspension for Pediatric Delivery: Preparation, Characterization, and Role of Excipients. *Processes* **2021**, *9*, 574. <https://doi.org/10.3390/pr9040574>

Academic Editors: Carla Vitorino, Sandra Nunes and Tânia Cova

Received: 23 February 2021

Accepted: 23 March 2021

Published: 25 March 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: Molecules with poor aqueous solubility are difficult to formulate using conventional approaches and are associated with many formulation delivery issues. To overcome these obstacles, nanosuspension technology can be one of the promising approaches. Hence, in this study, the feasibility of mefenamic acid (MA) oral nanosuspension was investigated for pediatric delivery by studying the role of excipients and optimizing the techniques. Nanosuspensions of MA were prepared by adopting an antisolvent precipitation method, followed by ultrasonication with varying concentrations of polymers, surfactants, and microfluidics. The prepared nanosuspensions were evaluated for particle size, morphology, and rheological measures. Hydroxypropyl methylcellulose (HPMC) with varying concentrations and different stabilizers including Tween[®] 80 and sodium dodecyl sulfate (SLS) were used to restrain the particle size growth of the developed nanosuspension. The optimized nanosuspension formula was stable for more than 3 weeks and showed a reduced particle size of 510 nm with a polydispersity index of 0.329. It was observed that the type and ratio of polymer stabilizers were responsive on the particle contour and dimension and stability. We have developed a biologically compatible oral nanoformulation for a first-in-class drug beautifully designed for pediatric delivery that will be progressed toward further in vivo enabling studies. Finally, the nanosuspension could be considered a promising carrier for pediatric delivery of MA through the oral route with enhanced biological impact.

Keywords: drug release; excipients; mefenamic acid; nanosuspension; pediatric delivery; stability

1. Introduction

Mefenamic acid (MA) (N-[(2, 3-dimethyl phenyl) amino] benzoic acid; Figure 1) is a potent nonsteroidal anti-inflammatory drug (NSAID) that has low oral bioavailability due to poor aqueous solubility and insufficient dissolution [1,2]. The solubility of MA is one of the critical aspects in formulation and development [2]. At present, 40% of new drugs are poorly water soluble and less permeable [3,4]. Pharmaceutically, MA appears as microcrystalline powder with low aqueous solubility and high permeability [biopharmaceutical classification system (BCS) class II drug], which is a major challenge in the clinical application for its pediatric delivery [5].

Figure 1. Molecular structure of mefenamic acid (MA): (A) 2D structure and (B) 3D structure [1].

This may suggest that drug dissolution rate plays a critical role in MA bioavailability and its therapeutic efficacy. In one of the studies, MA demonstrated significantly better antipyretic activity as compared with paracetamol ($p < 0.05$) over the entire period of observation and ibuprofen ($p < 0.05$) in the 2 to 4 h range [6]. According to a comparative clinical study on 124 pediatric patients, MA (4 mg/kg) was found to be more effective for treating fever and equally tolerable as paracetamol (15 mg/kg) [7]. However, poorly water-soluble compounds are difficult to develop as drug products, especially as liquid products, and conventional formulation techniques are frequently abandoned early in the discovery. Therefore, the enhancement of MA solubility must be achieved in its development. Presently, there is no efficient nanosuspension for MA except the premix. Many conventional approaches have been used to increase drug solubility, including cosolvents, micronization, and complexation [8,9]. Nevertheless, bioavailability issues remain as such in several cases, as micronization is not capable of producing enough surface area in order to increase the dissolution rate of poorly soluble compounds. Consequently, the trend has moved forward from microtechnology to nanotechnology in the production of novel pharmaceuticals [10–15].

In order to enhance the solubility of poorly water soluble compounds, knowledge of solubility, absorption, and dissolution is most important [14–16]. In recent days, the production of nanopharmaceuticals has revolutionized the pharmaceutical industry by improving the performance of poorly soluble compounds. Nanoapproaches are known to enhance solubility/dissolution rate and to improve bioavailability/oral absorption [16–19].

MA shows preferential inhibition of cyclooxygenase-II, thereby inhibiting the synthesis of prostaglandins [1]. It is used in the treatment of wounds, sport injuries, nonarticular rheumatism, osteoarthritis, periodontitis, rheumatoid arthritis, and other painful musculoskeletal illnesses [20]. Nanosuspensions can be produced by utilizing two different techniques: (i) bottom-up process (controlled precipitation/crystallization) and (ii) top-down process (reduction of larger particles to smaller ones) [21]. In this paper, we have presented a systematic investigation on the formulation strategy and role of excipients and pediatric tolerability of oral MA nanosuspension formulation. Finally, the MA formulations were successfully prepared by utilizing a nanoprecipitation technique with varying concentrations of polymers and surfactants, followed by an ultrasonication method, an approach that combines bottom-up and the top-down techniques to enhance the solubility and bioavailability and drug delivery impact.

2. Materials and Methods

2.1. Materials

MA (purity > 99.5%) was received as a kind gift sample from Orchid Pharmaceutical Ltd. (Roorkee, India). Hydroxypropyl methylcellulose-K4M (HPMC-K4M), sodium dodecyl sulfate (SLS), polysorbate 80 (Tween[®] 80), hydrochloric acid (HCl), methanol (MeOH), dimethyl sulfoxide (DMSO), sodium hydroxide (NaOH), potassium dihydrogen phosphate (KH₂PO₄), sodium chloride (NaCl), ethanol (C₂H₅OH), potassium chloride (KCl), disodium hydrogen phosphate dodecahydrate (Na₂HPO₄·12H₂O), N,N-dimethylformamide (DMF), and acetonitrile (CH₃CN) were commercially obtained from Central Drug House Ltd. (New Delhi, India). The water utilized for all experiments was high-purity/deionized water, which was obtained using a Milli-Q[®] water purification unit (Milli-Q Co., Lyon, France). All other materials used were of analytical/pharmaceutical grade.

2.2. Solubility Study

The MA solubility was determined using the mechanical shaker method [22]. The concentrated suspension of MA was obtained by placing its excess amount in water and optimized MA nanosuspension formulation. Each sample was placed in a mechanical shaker for 72 h at 25 °C to attain the equilibrium. After equilibrium/saturation, the samples were taken out and filtered through Whatman filter paper (0.45 µm) and quantified for MA content in each sample using UV spectrophotometry after suitable dilution at 240 nm [20].

2.3. Partition Coefficient

The partition coefficient of the MA was determined by using the shake-flask method and expressed as the ratio of concentration of drug in organic phase to the concentration of drug in aqueous phase [23]. Equal quantities of 1-octanol and phosphate buffer at pH 7.4 were taken into a separating funnel, and the drug sample was transferred into phosphate buffer–octanol mixture. The resulting mixture was then kept in a mechanical shaker at 25 °C for 30 min and stored overnight. After 24 h, the aqueous phase was separated from the separating funnel and quantified by UV spectrophotometry at 240 nm [20]. The partition coefficient (P) was determined using Equation (1):

$$P = \frac{\text{Conc. of drug in organic phase}}{\text{Conc. of drug in aqueous phase}} \quad (1)$$

2.4. Formulation Development

MA nanosuspension was prepared via antisolvent precipitation method using water as an antisolvent. In brief, the MA was dissolved in suitable organic solvent (acetone) in which the drug was soluble. The resulting solution was slowly injected with a syringe into the antisolvent (water) containing a growth inhibitor or stabilizer (HPMC-K4M or Tween 80) and kept at a low temperature on a bath sonicator [24]. An organic solvent was left to evaporate off under a slow magnetic stirring of the nanosuspension at room temperature for 1 h. To achieve the scalability of the process, the batch size of 100 mL was produced using optimum formulation and processing conditions determined from 25 mL of small-scale nanosuspension batches. The nanosuspensions were collected into glass vials, labeled, and used for subsequent tests. The compositions of different MA nanosuspensions are listed in Table 1.

Table 1. Formulation compositions of medicated mefenamic acid (MA) nanosuspension.

Ingredients	Formulation Matrices							
	F1	F2	F3	F4	F5	F6	F7	F8
MA (mg) (200 mg/20 mL)	200	200	200	200	200	200	200	200
HPMC-K4M (mg)	10	20	30	40	10	20	30	40
SLS (% w/v)	0.1	0.2	0.3	0.4	-	-	-	-
Tween 80 (% w/v) (0.1–0.4%)	-	-	-	-	0.02	0.04	0.06	0.08
Acetone (mL)	1	1	1	1	1	1	1	1
Water (mL)	20	20	20	20	20	20	20	20

2.5. Nanosuspension Characterization

2.5.1. Determination of Particle Size, Polydispersity Index, and Zeta Potential

The particle size and polydispersity index (PDI) of MA nanosuspensions were determined using the Malvern Zetasizer HAS3600 (Malvern Instruments, Malvern, UK) at 25 °C at a scattering angle of 90°. The zeta potential of MA nanosuspension was determined by utilizing disposable zeta cells for using the electrophoretic mobility technique (Zetasizer ZS3600, Malvern Instruments, Malvern, UK). The samples of MA nanosuspensions were sufficiently diluted with deionized water for the determination of particle size, PDI, and zeta potential.

2.5.2. Surface Morphology

The surface morphology of an optimized nanosuspension of MA was evaluated using scanning electron microscopy (SEM) (JSM-5200, JEOL Inc., Tokyo, Japan). A very small drop of concentrated aqueous suspension of MA was placed on a glass slide and then quickly dried under vacuum conditions. The sample was then fixed on a SEM stub utilizing double-sided adhesive tape and coated with gold at 20 mA for 2 min using an Auto-fine Coater (Ion sputter JFC 1600, Tokyo, Japan). After coating, photomicrographs of the sample were taken using SEM with a secondary electron detector with an accelerating voltage of 10 kV. The sample was shadowed in a cathodic evaporator with a gold layer of 20 nm thickness [25].

2.5.3. Drug Content

The prepared nanosuspensions were analyzed for drug content by UV spectroscopic method at 240 nm [20]. Different batches of nanosuspension equivalent to 10 mg of MA were weighed accurately and dissolved in 10 mL ethanol. The stock solutions were diluted with methanol and analyzed by UV spectroscopy at 240 nm.

2.5.4. Sedimentation Rate

For the determination of sedimentation rate for MA nanosuspensions, the original height (H_0) of 25 mL MA nanosuspension was measured after shaking in a 50 mL graduated cylinder. The height of sedimentation (H) was then measured in a 50 mL graduated cylinder after 3 h of standing. The sedimentation rate for each formulation was obtained using Equation (2):

$$F = \frac{H}{H_0} \quad (2)$$

2.5.5. Redispersibility

For the determination of redispersibility/redispersion time for MA nanosuspensions, 25 mL of each formulation was stored in a 50 mL graduated amber-colored bottle for about 3 months to produce the settling of the particles, followed by the shaking of each formulation under a magnetic shaker rotating at 20 rev/min. The redispersion time for each formulation was recorded in order to evaluate redispersibility.

2.5.6. pH Value

The pH value of MA nanosuspensions was evaluated using a Hydriion® pH indicator paper (MicroEssential Laboratory, Mumbai, India) according to the instructions.

2.6. In Vitro Drug Release

In vitro release of MA from different nanosuspensions was studied in phosphate-buffered saline at pH 7.4 (PBS) using a cell membrane diffusion method. Assembly of diffusion cell for an in vitro study was designed as per the dimension given for the oral diffusion cell [26]. The diffusion cell was placed on a magnetic stirrer. A receptor compartment was filled with PBS. Then, the prepared egg membrane was mounted on the cell carefully so as to avoid the entrapment of air bubbles under the membrane. Intimate contact of the egg membrane with receptor fluid was ensured by placing it tightly with a clamp. Each MA nanosuspension was placed on a donor compartment of the diffusion cell. The speed of the stirring was kept constant throughout the experiment. Around 1 mL of aliquots was taken from a receiver compartment at fixed time intervals, and the same volume of drug-free PBS was taken to maintain the persistent volume. After filtration, the MA contents in samples were determined using a UV spectrophotometer at 240 nm [20].

Kinetic Release Study

The release data of various formulations were put in various kinetic models (i.e., zero-order, first-order, Higuchi model, Hixson–Crowell model, and Korsmeyer–Peppas model) [27]. The graph was plotted and R^2 was calculated.

2.7. Stability Study

Stability studies were conducted on optimized MA nanosuspension, which showed satisfactory in vitro performance for various evaluation parameters. These formulations were subjected to accelerated stability and normal stability studies as per the International Council for Harmonisation (ICH) Q1A (R2) guidelines at long-term and accelerated conditions for a period of 3 months. The MA optimized nanosuspension was examined for various physicochemical parameters, like particle size, PDI, and in vitro release [24].

2.8. Statistical Analysis

All the data are expressed as mean $\pm$ SD of at least three independent experiments. The statistical evaluation was performed through one-way analysis of variance (ANOVA) using the SPSS 11.0 program for windows (SPSS Co., San Diego, CA, USA). Significant difference and extremely significant differences were considered at p -values of 0.05 and 0.01, respectively.

3. Results and Discussion

3.1. Solubility Data and Partition Coefficient of MA

The equilibrium solubility of MA in pure water at 25 °C was estimated to be 10.82 ± 0.02 µg/mL. However, the equilibrium solubility of MA in optimized nanosuspension at 25 °C was recorded to be 5122.28 ± 3.18 µg/mL. The solubility of MA was significantly enhanced in an optimized nanosuspension formulation compared with its aqueous solubility ($p < 0.01$). The enhancement of MA solubility in an optimized nanosuspension was 473.40-fold compared with that of water. The enhancement of MA solubility in an optimized nanosuspension could be possible due to nanosized particles and the presence of a surfactant (SLS) in an optimized formulation. These results were in accordance with those reported for the solubility enhancement of cyadox via nanosuspension formulations [28]. The equilibrium solubility of MA in pure water at 25 °C has been reported to be 10.00 µg/mL [29]. The recorded solubility of MA (10.82 µg/mL) in the present work was found to be in good agreement with its literature value [29].

The logarithmic partition coefficient ($\log P$) value of MA at 25 °C was estimated to be 5.30 ± 0.05 . The equilibrium $\log p$ value of MA at 25 °C has been reported to be 5.12 [30].

The recorded log P of MA (5.30) in the present work was found to be in good agreement with its literature value [30].

3.2. Nanosuspension Characterization

The medicated nanosuspensions were characterized for the various physicochemical parameters, which are summarized in Table 2 and described as follows.

Table 2. Physicochemical parameters of different nanosuspension formulations of MA (mean $\pm$ SD; $n = 3$); MA: mefenamic acid, PDI: polydispersity index, ZP: zeta potential, F: sedimentation rate, SD: standard deviation.

Code	Particle Size (nm) $\pm$ SD	PDI	ZP (mV) $\pm$ SD	Drug Content (%) $\pm$ SD	pH $\pm$ SD	F $\pm$ SD	Redispersion Time (sec) $\pm$ SD
F1	672 $\pm$ 6.84	0.762	−26 $\pm$ 1.21	42.21 $\pm$ 0.68	6.80 $\pm$ 0.15	1.12 $\pm$ 0.01	52 $\pm$ 1.85
F2	656 $\pm$ 6.58	0.563	−28 $\pm$ 1.26	64.32 $\pm$ 1.20	6.90 $\pm$ 0.18	1.10 $\pm$ 0.02	48 $\pm$ 1.74
F3	554 $\pm$ 6.04	0.474	−30 $\pm$ 1.19	70.10 $\pm$ 2.42	7.30 $\pm$ 0.28	0.96 $\pm$ 0.03	60 $\pm$ 2.12
F4	510 $\pm$ 5.92	0.329	−31 $\pm$ 1.34	82.41 $\pm$ 1.94	7.10 $\pm$ 0.21	1.04 $\pm$ 0.00	42 $\pm$ 1.42
F5	862 $\pm$ 8.14	0.536	−17 $\pm$ 1.12	54.10 $\pm$ 2.51	7.50 $\pm$ 0.31	0.94 $\pm$ 0.04	62 $\pm$ 2.14
F6	732 $\pm$ 7.18	0.764	−19 $\pm$ 0.98	32.34 $\pm$ 0.87	6.20 $\pm$ 0.12	0.93 $\pm$ 0.01	64 $\pm$ 2.17
F7	612 $\pm$ 7.91	0.575	−20 $\pm$ 0.82	38.21 $\pm$ 0.96	6.40 $\pm$ 0.11	0.95 $\pm$ 0.05	65 $\pm$ 2.19
F8	534 $\pm$ 5.48	0.847	−21 $\pm$ 1.28	42.21 $\pm$ 1.21	6.10 $\pm$ 0.14	0.90 $\pm$ 0.04	68 $\pm$ 2.32

3.2.1. Particle Size, PDI, and Zeta Potential

The mean particle size, PDI, and zeta potential of all nanosuspension formulations were determined using a Zetasizer. The resulting data for all formulations are given in Table 2. The particle sizes of medicated nanosuspensions (F1–F8) were recorded to be 510–862 nm. The results indicated that the particle size of a formulation was reduced significantly with an increase in the concentration of SLS (when Tween 80 was absent), as observed in the formulations F1–F4 ($p < 0.05$). Similarly, the particle size of a formulation was found to be reduced significantly with an increase in the concentration of Tween 80 (when SLS was absent), as observed in the formulations F5–F8 ($p < 0.05$). Overall, the lowest and highest particle sizes were recorded in the formulations F4 (510 nm) and F5 (862 nm), respectively. The PDI values of the formulations F1–F8 were estimated to be 0.329–0.847. The minimum PDI was recorded in the formulation F4 (0.329), suggesting the maximum uniformity in particle size distribution compared with other formulations studied. The minimum PDI of the formulation F4 was possible due to its lowest particle size and the presence of high concentrations of SLS and HPMC. The results of the physicochemical parameters were found in accordance with those reported in the literature for nanosuspensions of different drugs [31,32]. The zeta potentials of the formulations F1–F8 were found to be −17 to −30 mV. The zeta potential value at a magnitude of ± 30 mV suggested the maximum stability of nanoformulation [33]. Hence, the formulation F4 was found to be maximally stable compared with the other formulations studied.

3.2.2. Drug Content and pH

The resulting data of drug content and pH for all formulations are summarized in Table 2. The drug contents of medicated nanosuspensions (F1–F8) were found to be 32.34–82.41%. The maximum drug content was also recorded in the formulation F4 (82.41%), which was significant compared with the other formulations investigated ($p < 0.05$). The maximum drug content in the formulation F4 was possible due to its lowest particle size, lowest PDI, and the presence of high concentrations of SLS and HPMC. The pH values of the formulations F1–F8 were determined to be 6.10–7.10. The pH of most of the studied formulations was found to be close to intestinal pH (6.8), suggesting the suitability of the formulations for oral drug delivery systems. These results were found to be in good agreement with those reported for oral nanosuspensions in the literature [28].

3.2.3. Sedimentation Rate and Redispersibility

The results for sedimentation rate (F) and redispersibility (redispersion time) for all formulations are summarized in Table 2. The F values of the medicated nanosuspensions (F1–F8) were found to be 0.90–1.12. An F value of 1.0 indicated the maximum stability of the nanosuspension formulations [31]. The F value for all the formulations was found to be close to 1.0, suggesting the stability of the prepared nanosuspensions. The redispersion times for the formulations F1–F8 were found to be 42–68 s. The lowest redispersion time was recorded in the formulation F4 (42 s). The recorded results for sedimentation rate and redispersion time were acceptable and in good agreement with those reported for cyadox nanosuspensions in the literature [28].

3.2.4. Surface Morphology

The surface morphology of the optimized nanosuspension F4 was studied using SEM, and results are presented in Figure 2. The morphology of the optimized formulation F4 was found to be spherical with a smooth surface. The size of the nanosuspension was also in nanometer range and were in good agreement with those recorded using the Malvern Zetasizer. These results were found in accordance with those reported in the literature [28].

Figure 2. Scanning electron microscopy (SEM) photograph of the optimized nanosuspension F4 showing the surface morphology and particle size recorded at an accelerated voltage of 10 kV ($\times 20,000$).

3.3. In Vitro Drug Release Study

The in vitro drug release profile of MA from all the nanoformulations was carried out using diffusion cell. The release profile of MA from the SLS nanosuspensions (F1–F4) is presented in Figure 3.

The release rate of MA from the SLS formulations was found to be increased significantly with an increase in SLS concentration in the formulation ($p < 0.05$). Hence, the maximum release was observed in the formulation F4 (79.76%), and the minimum one was found in the formulation F1 (30.98%). The maximum drug release profile of MA from the formulation F4 was possible due to the lowest particle size of F4 compared with the other formulations studied. The release profile of MA from the Tween 80 nanosuspensions F5–F8 is presented in Figure 4.

Figure 3. In vitro drug release of MA from the sodium lauryl sulfate (SLS) nanosuspensions F1–F4 in phosphate-buffered saline (PBS) (mean $\pm$ SD, $n = 3.0$).

Figure 4. In vitro drug release of MA from the Tween 80 nanosuspensions F5–F8 in PBS (mean $\pm$ SD, $n = 3.0$).

The release rate of MA from the Tween 80 formulations was also found to be increased significantly with an increase in Tween 80 concentration in the formulation ($p < 0.05$). Therefore, the maximum release was observed in the formulation F8 (26.67%), and the minimum one was found in the formulation F5 (12.65%). The maximum drug release profile of MA from the formulation F8 was possible due to the lowest particle size of F8 compared with the other formulations investigated. The comparative drug release profile of MA from all the formulations (F1–F8) is presented in Figure 5. Overall, the maximum release of MA was obtained from the formulation F4, and the minimum one was obtained from the formulation F5. In general, the release rate of MA was increased with an

increase in the concentrations of the surfactants (SLS and Tween 80) and polymer (HPMC) and a reduction in the particle size in the formulations. Both of the surfactants (SLS and Tween 80) and the polymer were found to be suitable for enhancing the drug release rate of MA from the medicated nanosuspensions. However, SLS was more effective than Tween 80 in enhancing the drug release rate of MA compared with Tween 80. This observation suggests that SLS had great solubilization potential for MA compared with Tween 80, which could finally result in an enhanced drug release rate of MA. Although the particle sizes of the formulations F4 and F8 were recorded to be at a similar magnitude, their drug release profiles were significantly different ($p < 0.05$). It was due to the presence of SLS in the formulation F4 compared with the formulation F8. The results of drug release studies were found to be in good agreement with those reported for drug release profiles of cyadox and aprepitant from nanosuspensions [28,31]. Based on these results, the formulation F4 was selected as an optimized medicated nanosuspension for oral drug delivery of MA.

Figure 5. In vitro release of MA from all the nanoformulations (F1–F8) in PBS (mean $\pm$ SD, $n = 3.0$).

3.4. Kinetic Release

The in vitro release profiles of all the nanoformulations (F1–F8) were fitted in different kinetic release models (i.e., zero-order, first-order, Higuchi, Hixson–Crowell, and Korsmeyer–Peppas model) [27]. The determination coefficient (R^2) values of all the models were calculated, which are summarized in Table 3. The formulations F1, F2, F6, and F7 followed zero-order kinetics, as R^2 values were maximum for the zero-order kinetics model. However, other formulations, including F3, F4, F5, and F8, followed the Higuchi model of drug release, as R^2 values were maximum for the Higuchi model. The optimized formulation F4 followed the Higuchi model of drug release. The release mechanism of drugs from nanosuspensions depends on the physicochemical properties of the matrix used for their preparation [34]. Different mechanisms of drug release from nanosuspensions have been reported in the literature [34,35]. The Higuchi model release of MA from the optimized formulation F4 could be due to the presence of the HPMC matrix.

Table 3. Kinetic data of the various models for release study.

Code	Zero-Order	First-Order	Higuchi	Hixson–Crowell	Korsmeyer–Peppas		Best Fit Model
	R^2	R^2	R^2	R^2	R^2	n	
F1	0.977	0.978	0.991	0.983	0.976	0.798	Zero-order
F2	0.985	0.963	0.976	0.972	0.978	0.701	Zero-order
F3	0.711	0.929	0.977	0.944	0.952	0.744	Higuchi
F4	0.980	0.909	0.986	0.940	0.964	0.775	Higuchi
F5	0.964	0.964	0.989	0.966	0.931	0.392	Higuchi
F6	0.997	0.996	0.979	0.993	0.993	0.568	Zero-order
F7	0.9997	0.9994	0.9927	0.9972	0.9893	0.4975	Zero-order
F8	0.9879	0.9832	0.9936	0.9932	0.9496	0.4445	Higuchi

3.5. Stability Study

Stability studies were performed on the optimized formulation F4 as per ICH guidelines. The samples were placed in the stability chamber. The particle size, PDI, and % release were determined at 1, 2, and 3 months. The results of the stability studies are summarized in Table 4. The results showed no significant changes in particle size, PDI, and % release of the optimized formulation F4, suggesting that the medicated optimized nanosuspension of MA was sufficiently stable. The stability of nanosuspensions has already been proved previously in the literature [28,31–35]. Several drugs, such as cyadox, apreptant, and azoxystrobin, were found to be stable when formulated into nanosuspension formulations [28,31,35]. Hence, the results of the stability evaluation were in accordance with the literature.

Table 4. Particle size, PDI, and release data for the optimized formulation F4 for a 3-month stability study as per ICH guidelines; Temp: temperature, RH: relative humidity.

Time (Month)	Temp/RH ($25 \pm 2^\circ\text{C}/60 \pm 5\%$)			Temp/RH ($40 \pm 2^\circ\text{C}/75 \pm 5\%$)		
	Particle Size (nm)	PDI	Release (%)	Particle Size (nm)	PDI	Release (%)
1	542.23	0.323	79.23	540.23	0.321	79.59
2	544.24	0.327	80.43	541.23	0.326	79.56
3	543.53	0.329	79.45	538.23	0.331	78.56

4. Conclusions

The present study was satisfactorily attempted to formulate a medicated nanosuspension of MA using polymer, such as HPMC. Prepared formulations were characterized well for different physicochemical parameters. From the experimental results, it was found that the increased concentration of the surfactant (SLS or Tween 80) resulted in a reduction in particle size and an increase in the drug release profile proportionally. The medicated nanosuspensions F1, F2, F6, and F7 followed zero-order kinetics. However, the other formulations, such as F3, F4, F5, and F8, followed the Higuchi model of drug release. The stability studies suggest that the optimized formulation F4 was sufficiently stable. These results indicate the feasibility of medicated nanosuspensions for oral delivery of MA for pediatric patients. However, further pharmacodynamic and pharmacokinetic investigations are required to explore the complete potential of medicated nanosuspension for pediatric delivery.

Author Contributions: Conceptualization, F.S. and M.F.; methodology, N.P., A.A., V.V., and T.S.E.; software, N.P.; validation, F.S., M.F., and S.A.; formal analysis, A.A.; investigation, N.P., M.F., and V.V.; resources, S.A.; data curation, A.A.; writing—original draft preparation, N.P.; writing—review and editing, F.S., M.F., S.A., and T.S.E.; visualization, F.S.; supervision, F.S.; project administration, F.S.; funding acquisition, S.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Deanship of Scientific Research (DSR) at King Saud University, Riyadh, Saudi Arabia, through research group project number RGP-1438-013, and the APC was funded by DSR.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: This study did not report any data.

Acknowledgments: The authors extend their sincere appreciation to the Deanship of Scientific Research at King Saud University, Riyadh, Saudi Arabia, for funding this work through research group project number RGP-1438-013.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Alshehri, S.; Shakeel, F.; Ibrahim, M.; Elzayat, E.; Altamimi, M.; Shazly, G.; Mohsin, K.; Alkholief, M.; Alsulays, B.; Alshetaili, A.; et al. Influence of microwave technology on solid dispersions of mefenamic acid and flufenamic acid. *PLoS ONE* **2017**, *12*, e01822011. [\[CrossRef\]](#)
2. Alshehri, S.; Shakeel, F.; Elzayat, E.; Almeanazel, O.; Altamimi, M.; Shazly, G.; Kazi, M.; Almutaity, B.; Alsulays, B.; Alshetaili, A.; et al. Rat palatability, pharmacodynamics effects and bioavailability of mefenamic acid formulations utilizing hot-melt extrusion technology. *Drug Dev. Ind. Pharm.* **2019**, *45*, 1610–1616. [\[CrossRef\]](#)
3. Sharma, P.; Denny, W.A.; Garg, S. Effect of wet milling process on the solid state of indomethacin and simvastatin. *Int. J. Pharm.* **2009**, *380*, 40–48. [\[CrossRef\]](#)
4. Kakran, M.; Sahoo, N.G.; Li, L.; Judeh, Z.; Wang, Y.; Chong, K.; Loh, L. Fabrication of drug nanoparticles by evaporative precipitation of nanosuspension. *Int. J. Pharm.* **2010**, *383*, 285–292. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Rao, K.S.; Nagabhushanam, M.V.; Chowdary, K.P. In vitro dissolution studies on solid dispersions of mefenamic acid. *Indian J. Pharm. Sci.* **2011**, *73*, 243–247.
6. Khubchandani, R.P.; Ghatikar, K.N.; Keny, S.; Usgaonkar, N.G. Choice of antipyretic in children. *J. Assoc. Physicians India* **1995**, *43*, 614–616. [\[PubMed\]](#)
7. Aishwarya, S.; Chavva, A.K. Evaluation of efficacy and tolerability of acetaminophen (paracetamol) and mefenamic acid as antipyretic in pediatric patients with febrile illness: A comparative study. *Int. J. Med. Res. Health Sci.* **2013**, *2*, 23–29.
8. Alshehri, S.; Shakeel, F. Solubility determination, various solubility parameters and solution thermodynamics of sunitinib malate in some cosolvents, water and various (Transcutol + water) mixtures. *J. Mol. Liq.* **2020**, *307*, 112970. [\[CrossRef\]](#)
9. Kale, A.R.; Kakade, S.; Bhosale, A. A review on: Solubility enhancement techniques. *Curr. Pharm. Res.* **2020**, *10*, 3630–3647.
10. Farokhzad, O.C.; Langer, R. Impact of nanotechnology on drug delivery. *ACS Nano* **2009**, *3*, 16–20. [\[CrossRef\]](#)
11. Fernandes, G.J.; Kumar, L.; Sharma, K.; Tunge, R.; Rathnanand, M. A review on solubility enhancement of carvedilol—A BCS class II drug. *J. Pharm. Innov.* **2018**, *13*, 197–212. [\[CrossRef\]](#)
12. Hu, J.; Johnston, K.P.; Williams, R.O., III. Nanoparticle engineering processes for enhancing the dissolution rates of poorly water soluble drugs. *Drug Dev. Ind. Pharm.* **2004**, *30*, 233–245. [\[CrossRef\]](#)
13. Lawrence, X.Y.; Amidon, G.L.; Polli, J.E.; Zhao, H.; Mehta, M.U.; Conner, D.P.; Shah, V.P.; Lesko, L.J.; Chen, M.L.; Lee, V.H.; et al. Biopharmaceutics classification system: The scientific basis for biowaiver extensions. *Pharm. Res.* **2002**, *19*, 921–925.
14. Rabinow, B.E. Nanosuspensions in drug delivery. *Nat. Rev. Drug Discov.* **2004**, *3*, 785–796. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Shakeel, F.; Ramadan, W.; Faisal, M.S.; Rizwan, M.; Faiyazuddin, M.; Mustafa, G.; Shafiq, S. Transdermal and topical delivery of anti-inflammatory agents using nanoemulsion/microemulsion: An updated review. *Curr. Nanosci.* **2010**, *6*, 184–198. [\[CrossRef\]](#)
16. Qadir, A.; Faiyazuddin, M.; Hussain, M.T.; Alshammari, T.M.; Shakeel, F. Critical steps and energetics involved in a successful development of a stable nanoemulsion. *J. Mol. Liq.* **2016**, *214*, 7–18. [\[CrossRef\]](#)
17. Faiyazuddin, M.; Ahmad, N.; Khar, R.K.; Bhatnagar, A.; Ahmad, F.J. Stabilized terbutaline submicron drug aerosol for deep lungs deposition: Drug assay, pulmonokinetics and biodistribution by UHPLC/ESI-q-TOF-MS method. *Int. J. Pharm.* **2012**, *434*, 59–69. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Ahmad, M.Z.; Rizwanullah, M.; Ahmad, J.; Alasmery, M.Y.; Akhter, M.D.; Abdel-Wahab, B.A.; Warsi, M.H.; Haque, A. Progress in nanomedicine-based drug delivery in designing of chitosan nanoparticles for cancer therapy. *Int. J. Polym. Mater. Polym. Biomater.* **2021**. [\[CrossRef\]](#)
19. Hafez, D.A.; Elkhodairy, K.A.; Taleb, M.; Elzoghby, O.A. Nanomedicine-based approaches for improved delivery of phytotherapeutics for cancer therapy. *Expert Opin. Drug Deliv.* **2020**, *17*, 279–285. [\[CrossRef\]](#)
20. Alarfaj, N.A.; Altamimi, S.A.; Almarshady, L.Z. Spectrophotometric determination of mefenamic acid in pharmaceutical preparations. *Asian J. Chem.* **2009**, *21*, 217–226.
21. Jacob, S.; Nair, A.B.; Shah, J. Emerging role of nanosuspensions in drug delivery systems. *Biomater. Res.* **2020**, *24*, 3. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Higuchi, T.; Connors, K.A. Phase-solubility techniques. *Adv. Anal. Chem. Instr.* **1965**, *4*, 117–122.

23. Wong, J.J.; Yu, H.; Hadinoto, K. Examining practical feasibility of amorphous curcumin-chitosan nanoparticle complex as solubility enhancement strategy of curcumin: Scaled-up production, dry powder transformation, and long-term physical stability. *Coll. Surf. A* **2018**, *537*, 36–43. [[CrossRef](#)]
24. Shid, R.L.; Dhole, S.N.; Kulkarni, N.; Shid, S.L. Formulation and evaluation of nanosuspension delivery system for simvastatin. *Int. J. Pharm. Sci. Nanotechnol.* **2014**, *7*, 2459–2476. [[CrossRef](#)]
25. Amsa, P.; Tamizharasi, S.; Jagadeeswaran, M.; Sivakumar, T. Preparation and solid state characterization of simvastatin nanosuspensions for enhanced solubility and dissolution. *Int. J. Pharm. Pharm. Sci.* **2014**, *6*, 265–269.
26. Shah, K.; Shrivastava, S.K.; Mishra, P. Formulation and evaluation of suspensions: Mefenamic acid prodrugs. *Pak. J. Pharm. Sci.* **2014**, *27*, 917–923.
27. Costa, P.; Lobo, J.M.S. Modeling and comparison of dissolution profiles. *Eur. J. Pharm. Sci.* **2001**, *15*, 123–133. [[CrossRef](#)]
28. Sattar, A.; Chen, D.; Jiang, L.; Pan, Y.; Tao, Y.; Huang, L.; Liu, Z.; Xie, S.; Yuan, Z. Preparation, characterization and pharmacokinetics of cyadox nanosuspension. *Sci. Rep.* **2017**, *7*, 2289. [[CrossRef](#)]
29. Sriamornsak, P.; Limmatvapirat, S.; Piriyaprasath, S.; Mansukmanee, P.; Huang, Z. A new self-emulsifying formulation of mefenamic acid with enhanced drug dissolution. *Asian J. Pharm. Sci.* **2015**, *10*, 121–127. [[CrossRef](#)]
30. Pyka-Pajak, A.; Parys, W.; Dolowy, M. Comparison of the utility of RP-TLC technique and different computational methods to assess the lipophilicity of selected antiparasitic, antihypertensive, and anti-inflammatory drugs. *Molecules* **2019**, *24*, 3187. [[CrossRef](#)] [[PubMed](#)]
31. Kalvakuntla, S.; Deshpande, M.; Attari, Z.; Koteswara, K.B. Preparation and characterization of nanosuspension of apripitant by H96 process. *Adv. Pharm. Bull.* **2016**, *6*, 83–90. [[CrossRef](#)] [[PubMed](#)]
32. Attari, Z.; Kalvakuntla, S.; Reddy, M.R.; Deshpande, M.; Rao, C.M.; Koteswara, K.B. Formulation and characterization of nanosuspension of BCS class II and IV drugs by combinative method. *J. Exp. Nanosci.* **2016**, *11*, 276–288. [[CrossRef](#)]
33. Shakeel, F.; Shazly, G.A.; Raish, M.; Ahmad, A.; Kalam, M.A.; Ali, N.; Ansari, M.A.; Elosaily, G.M. Biological investigation of supersaturated self-nanoemulsifying drug delivery system of Piper cubeba essential oil. *RSC Adv.* **2015**, *5*, 105206–105217. [[CrossRef](#)]
34. Mircioiu, C.; Voicu, V.; Anuta, V.; Tudose, A.; Celia, C.; Paolino, D.; Fresta, M.; Sandulovici, R.; Mircioiu, I. Mathematical modeling of release kinetics from supramolecular drug delivery systems. *Pharmaceutics* **2019**, *11*, 140. [[CrossRef](#)]
35. Yao, J.; Cui, B.; Zhao, X.; Wang, Y.; Zeng, Z.; Sun, C.; Yang, D.; Liu, G.; Gao, J.; Cui, H. Preparation, characterization, and evaluation of azoxystrobin nanosuspension produced by wet media milling. *Appl. Nanosci.* **2018**, *8*, 297–307. [[CrossRef](#)]