Available online on 15.06.2023 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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Research Article

# Exploring the Potential of Ketoprofen Nanosuspension: *In Vitro* and *In Vivo* Insights into Drug Release and Bioavailability

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## Article Info:

### Article History:

Received 19 April 2023
Reviewed 16 May 2023
Accepted 28 May 2023
Published 15 June 2023

### Cite this article as:

Sanas MN, Pachpute TS, Exploring the Potential of Ketoprofen Nanosuspension: *In Vitro* and *In Vivo* Insights into Drug Release and Bioavailability, Journal of Drug Delivery and Therapeutics. 2023; 13(6):152-158
*DOI: http://dx.doi.org/10.22270/jddt.v13i6.5890*

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## Abstract

Low water solubility and high permeability present formulation challenges for drugs categorized as Biopharmaceutics Classification System (BCS) Class II, resulting in reduced bioavailability. This research focuses on addressing the solubility issues of BCS Class II drugs, including Simvastatin, Ketoprofen, griseofulvin, ibuprofen, ketoconazole, and carbamazepine, which exhibit high permeability but poor solubility. A potential strategy to improve the solubility and bioavailability of these drugs is the utilization of nanosuspensions.

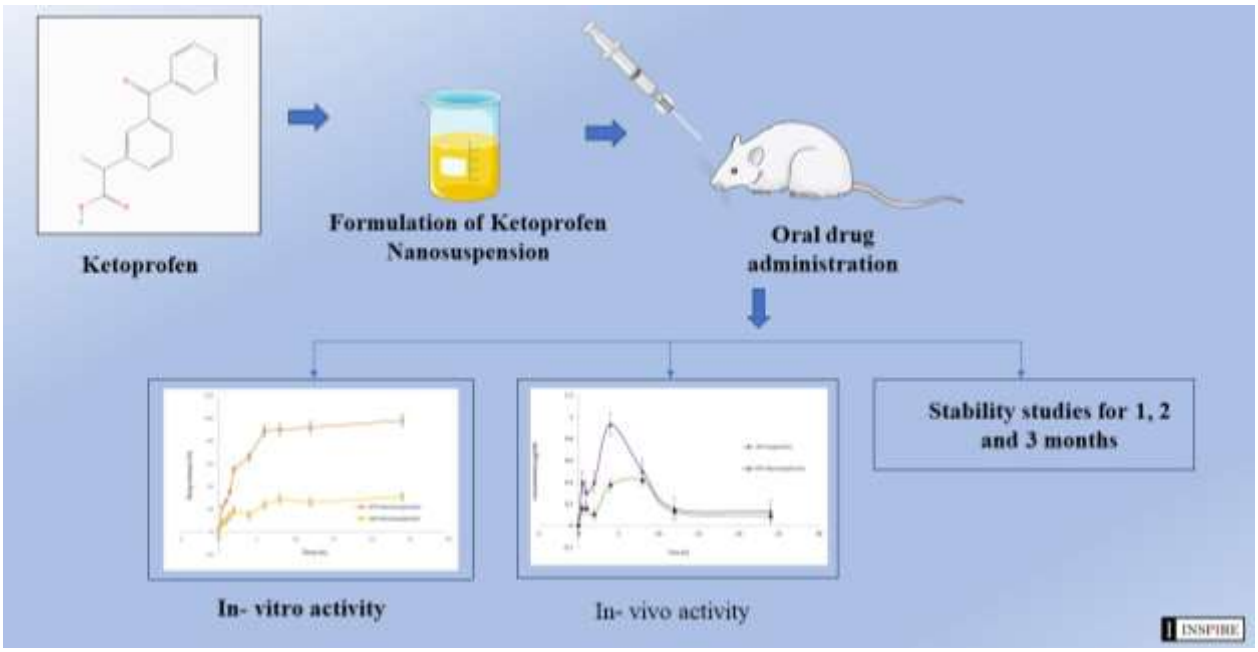
This study investigates the application of nanosuspension technology to enhance the solubility of Ketoprofen, a BCS Class II drug. By reducing the drug's particle size within the nanosuspension, solubility is improved, leading to increased bioavailability and optimized therapeutic efficacy. The research includes in vitro and in vivo experiments to evaluate the drug release profiles and bioavailability of Ketoprofen-loaded nanosuspensions.

Significant findings from this research include the demonstration of improved bioavailability and enhanced drug release properties achieved with the nanosuspension formulation. In vitro studies show increased drug dissolution rates and improved release profiles compared to conventional formulations. In vivo experiments reveal enhanced pharmacokinetic parameters and therapeutic effectiveness of Ketoprofen when administered through the nanosuspension.

These results highlight the potential of nanosuspensions as an efficient drug delivery system for BCS Class II drugs, addressing their solubility limitations and improving their bioavailability. The findings contribute to the development of novel strategies in pharmacology for enhancing drug solubility and therapeutic outcomes. Overall, this research emphasizes the significance of nanosuspension technology in optimizing the delivery of BCS Class II drugs and offers valuable insights for future formulation development and therapeutic applications.

**Keywords:** Ketoprofen, Nanosuspensions, Ultrasonication, Precipitation, Dissolution rate, Oral bioavailability.

## Graphical Abstract:



## 1. INTRODUCTION

The poor water solubility of numerous compounds under development is a recognized factor that often leads to their failure to reach the market. Extensive research and reports have consistently demonstrated that a significant proportion of active compounds in development fall into the category of poor solubility, with a majority belonging to class II drugs according to the Biopharmaceutical Classification System (BCS)[1]. This classification system categorizes drugs based on their solubility and permeability characteristics. The prevalence of poorly soluble compounds highlights the need for innovative strategies to improve their solubility and enhance their pharmaceutical properties, ultimately increasing the likelihood of successful development and market introduction[2, 3].

The low permeability and low dissolution rate of class-II biopharmaceutical drugs pose challenges to their therapeutic effectiveness. However, by employing innovative nanotechnology-based delivery systems, such as nanosuspension formulations, it becomes possible to enhance the therapeutic activity of these drugs[4]. Nanosuspensions consist of finely dispersed, submicron-sized colloidal particles that encapsulate the drugs. They offer several advantages, including improved drug bioavailability and a wider therapeutic range, which allows for reduced dosing. These characteristics make nanosuspensions a crucial formulation in the quest to enhance the efficacy of class-II drugs[5].

Numerous strategies have been employed to enhance the solubility of drugs, aiming to overcome the challenges associated with their poor aqueous solubility. Formulation strategies encompass two methods: the top-down approach, involving the reduction of particle size in larger crystals, and the bottom-up approach, which involves the formation of crystals by precipitating dissolved molecules. High-pressure homogenization and media milling are classified as top-down techniques[6], while ultrasonication, supercritical anti-solvent precipitation (SAS)[7], and rapid expansion of a supercritical solution into a liquid solvent (RESOLV) [8] are categorized as bottom-up techniques.

The increase in surface area brought on by the smaller particle size results in increased dissolution. For substances with a high melting point, high dose, and high log p-value, the nanosuspension dosage form is strongly advised. It is a unique strategy that might improve bioavailability. This technology is noteworthy since it uses fewer excipients than those used in pharmaceuticals, which reduces the risk of toxicity from these components. The bioavailability of BCS class 2 medicines is improved by the nanosuspension method[9]. Enhancing the solubility and dissolution rate of class-II drugs plays a pivotal role in improving their absorption kinetics and extent following oral administration. This factor holds significant importance in the pursuit of optimizing the therapeutic outcomes of these drugs[10].

Anti-solvent precipitation is a viable approach for generating micro- or nano-sized drug particles. In this technique, the drug is initially dissolved in a solvent and swiftly introduced into an anti-solvent, leading to immediate precipitation through rapid desolvation [11]. Anti-solvents typically encompass aqueous solutions containing stabilizers like polymers and surfactants. Polymers such as hydroxypropylmethylcellulose (HPMC) and methylcellulose (MC) [12]and polyvidone (PVP)[13] are commonly employed due to their capacity to establish robust hydrogen bonds with drugs. These polymers can be adsorbed onto the hydrophobic surface of the drug particles, impeding crystal growth. Nevertheless, conventional anti-solvent precipitation techniques encounter inherent challenges, including difficulty in maintaining particle size post-precipitation. Typically, particles experience rapid growth and exhibit a wide particle size distribution (PSD). Recently, precise control over particle size and PSD has been achieved through the use of a static mixer[14] and a confined impinging jet reactor [15]. Over the past decade, ultrasound has garnered significant attention as an effective means of controlling nucleation and crystallization processes[16.17]. Ultrasound irradiation has demonstrated its potential as a viable mixing technique, enhancing mass transfer and expediting molecular diffusion.

Experiments are carried out in a controlled laboratory setting, independent of a live thing, in a process known as in vitro testing. In vitro testing for nanosuspensions may assess many factors such as particle size, drug release kinetics, dissolution profile, and stability. The ultrasonication precipitation technique, which uses ultrasonic waves to break down bigger drug particles into nanoparticles floating in a liquid media, is frequently used to generate nanosuspensions. On the other hand, in vivo, testing entails examining how the nanosuspensions function and what impact they have on a living creature. Pharmacokinetics, tissue distribution, systemic absorption, and therapeutic effectiveness are frequently evaluated using animal models.

The drug concentration and its effects on certain tissues or organs are analyzed by administering the nanosuspension formulation to the animals and collecting samples at predetermined intervals. An extensive evaluation of the bioavailability and therapeutic potential of the nanosuspension may be made by integrating the outcomes from in vitro and in vivo testing. Through this procedure, researchers may assess the nanosuspension formulation's capacity to effectively transport the medication, get beyond the drawbacks of the API suspension, and improve drug solubility. The formulation was created using the ultrasonication precipitation process. Finally, these evaluations emphasize the potential of nanosuspensions for enhancing treatment results and accelerating the development of innovative drug delivery methods.

## 2. METHODS

### 2.1. Materials

The ketoprofen-loaded nanosuspension was prepared. using precipitation–ultrasonication method. Ketoprofen, obtained with known purity, was formulated into a nanosuspension using appropriate solvents, surfactants such as Tween 80 and PVP-K30, and stabilizers. Optimization of Ketoprofen concentration was performed, and the nanosuspension formulation was characterized for particle size and zeta potential. For the in vivo studies, Wistar rats were used to check the pharmacokinetic parameters, following ethical approval and institutional guidelines. The pharmacokinetic studies utilized a concentration of the ketoprofen-loaded nanosuspension. Sample collection was performed at regular intervals, and subsequent analysis was carried out. Statistical analysis was conducted to interpret the data obtained from all experiments.

### 2.2 Preparation of Ketoprofen loaded nanosuspension by precipitation–ultrasonication method [18,19]

The precipitation-ultrasonication method was employed to prepare a nanosuspension. The medication was sonicated in methanol for 5 minutes at room temperature to dissolve it. A variety of antisolvent solutions were created by dissolving stabilizers in water, and these solutions were then filtered through a 0.45µm filter. A bath of cold water was used to chill the antisolvent to 3°C. The stabilizer solution containing antisolvent was then rapidly injected with the drug solution using a syringe and needle setup. The mixture was stirred at various speeds under an overhead stirrer, allowing the volatile

solvent to evaporate at room temperature for 4-5 hours [20]. After precipitation, the sample was immediately transferred to a test tube and subjected to ultrasonication at different time intervals using a 6 mm tip-diameter ultrasonic probe. The nanosuspension was then concentrated by centrifugation at 10,000 RPM for 20 minutes using an AllegraTM 64R Centrifuge (Beckman Coulter, USA). A 40 mL batch size was used for nanosuspension preparation, and an optimized formulation was also prepared through the same method as a physical mixture.

**Table 1: The coded and actual values of the variables used in the Factorial design of Ketoprofen nanosuspension**

| Independent Variables | Actual and coded values | |
|---|---|---|
| | Low (-1) | High (+1) |
| A = Conc. Of Drug | 20 | 60 |
| B = Conc. Of Surfactant | 1.50 | 2.20 |
| C = Time of probe sonication | 10 | 20 |

### 2.2.1 Experimental design using Design Expert Software

To design the formulation of the nanosuspension, it was crucial to identify the variables that could influence its properties. The response surface methodology, specifically the central composite design (CCD), was employed to determine the optimal levels of these variables. Two independent variables, surfactant concentration (%) (X1) and probe sonication time (%) (X2), were investigated at five different concentrations coded as -α, -1, 0, 1, and +α as given in Table 2.

**Table 2: The coded and actual values of the variables used in CCD-RSM of Ketoprofen-nanosuspension**

| Independent variables | Levels | | |
|---|---|---|---|
| | Low (-1) | Middle (0) | High (+1) |
| X1: Conc. Of Surfactant (Tween 80 and PVP-K30) | 1.5 | 2 | 2.5 |
| X2: Time of probe sonication | 10 | 15 | 20 |

The alpha value was calculated to ensure both rotatability and orthogonality in the design. The dependent variables selected as response parameters were mean particle size (Y1), polydispersity index (Y2), and entrapment efficiency (%) (Y3). A CCD matrix generated by Design-Expert software facilitated the execution of 10 experiments, including 2 factorial points, 3 axial points, and 2 replicates at the center point as given in **Table 3**. Statistical analysis using ANOVA, based on Fisher's test, was conducted to assess the effects of the independent variables on the responses, with a significance level of $p<0.05$. The quality of the model was evaluated through the multiple correlation coefficient (R2) and adjusted R2. Contour and three-dimensional surface plots were generated to visualize the relationship and interactions between the coded variables and responses. The optimization goal was to minimize particle size and polydispersity index while maximizing entrapment efficiency[21].

**Table 3: Formulation variables and their responses using CCD**

| | Formulation variables | | | Response | | |
|---|---|---|---|---|---|---|
| Run | Concentration of surfactant (%) ($X_1$) | Time of probe sonication (min) ($X_2$) | | Particle size (nm) ($Y_1$) | PDI ($Y_2$) | EE (%) ($Y_3$) |
| 1 | 2 | 15 | | 170.2 | 0.231 | 94.4 |
| 2 | 1.5 | 10 | | 241.2 | 0.312 | 87 |
| 3 | 2.5 | 20 | | 162.2 | 0.232 | 94 |
| 4 | 2 | 7.9 | | 189.2 | 0.326 | 89.23 |
| 5 | 2.5 | 10 | | 165.3 | 0.437 | 97 |
| 6 | 2.7 | 15 | | 193.2 | 0.203 | 97.22 |
| 7 | 2 | 15 | | 198.2 | 0.271 | 95.31 |
| 8 | 2 | 15 | | 198.2 | 0.251 | 89.25 |
| 9 | 2 | 15 | | 239.6 | 0.227 | 92 |
| 10 | 1.5 | 20 | | 239.3 | 0.212 | 81.2 |
| 11 | 1.2 | 15 | | 413.2 | 0.221 | 63 |
| 12 | 2 | 22.07 | | 198.2 | 0.211 | 83 |
| 13 | **2** | **15** | | **201.2** | **0.232** | **82.9** |

Mean ± SD, (n=3); Coded high values=(-1), Coded middle/mean values=(0), Coded low values=(+1).

Effect of formulation variables using CCD design The effect of formulation variables on the responses was performed with the help of Design Expert software version 12. Here, formulation variables are total surfactant concentration and probe sonication time. The responses are the particle size (Y1), PDI (Y2), and entrapment efficiency (Y3). The CCD has five different levels (-α, −1, 0, +1, +α) to study the effect of the variables. The various formulation run was displayed in Table.

### 2.2.2 Statistical analysis and optimization

Using Design Expert, version 11, the analysis of variance (ANOVA) helped to demonstrate the process' of statistical optimization. The level of significance for all fitted models is ($p<0.05$). Using CCD design, the fitted models were further assessed to produce data showing how factors affect responses. For the various replies, the response surface plot and 3-D plot were created.

### 2.2.3 Selection of optimized batch:

Selection of optimized batch based on the parameters such as Particle size, Entrapment efficacy, and Polydispersity index using the statistical software Design-Expert (version 7.0.0; Stat-Ease, Inc., Minneapolis, Minnesota, USA) to create the design matrix for the study of optimization data.

By using the optimized batch, further In vivo, in vitro, and stability studies were carried out.

### 2.3 *In vitro* release study of an optimized batch of Ketoprofen-nanosuspension

It is good knowledge that a number of variables, including the composition and structure of the nanosuspension, affect how quickly a medication is releases from a nanocarrier. Ketoprofen nanosuspension development was examined for its in vitro releases [22].

Using a dialysis bag approach, in vitro release of ketoprofen was carried out in pH 1.2 HCL and pH 6.8 phosphate buffer saline solution (PBS). Before the experiment, the dialysis bags were hydrated in phosphate-buffered saline, pH 6.8. Ketoprofen-containing nanosuspension has been placed in dialysis bags. After adding pH 1.2 HCL to a beaker containing 100 mL of phosphate buffer saline pH 6.8, the dialysis bags were knotted at both ends and inserted inside. The beaker was kept at 37°C while being stirred continuously at 100 rpm.

At regular intervals of 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 24 hours, the sample was taken out. The trial was carried out at pH 1.2 HCL for the first two hours and pH 6.8 for the next twenty-four hours. To keep the sink condition, the 3mL sample of dissolving media was withdrawn and replaced with a new buffer at the same temperature. By using a UV-visible spectrophotometer (UV 1700, Shimadzu, Japan) set to a maximum wavelength of 260 nm, the quantity of ketoprofen in the aliquots was evaluated [23].

### 2.4 *In vivo* study of Ketoprofen-nanosuspension

The current investigation adhered to the ARRIVE guidelines as well as the UK Animals (Scientific Procedures) Act, 1986 and its related guidelines. Male Wistar rats were given the protocol and approved it in accordance with standards. They were maintained at typical, controlled environmental temperatures of 30 to 32°C [22].

The Institutional Animal Ethics Committee (IAEC) and the Committee for Control and Supervision of Experiments on Animals (CPCSEA) committee both accepted the animal study protocol.

In this investigation, male Wistar rats weighing 190–200 g was used in the nanosuspension containing KTP release trials. Before the trial, they had completed dietary supplementation of pellets and water. Rats were starved overnight before to the experimentation[23]. Rats were administered anesthesia on the day of the experiment using a combination of 10% ketamine (0.35 mL/kg) and 2% Xylazine (0.25 mL/kg) solution [24].

To conduct the investigation, two groups of six rats each were used. The groups were labeled, and an oral gavage was used to deliver a 40mg/kg oral dosage of API suspension and nanosuspension (made with 1% Na CMC).

**Table 4: Grouping of animals for the administration of dose of the drug**

| Group | Title | No. of Animals |
|---|---|---|
| Group 1 | API suspension (made with 1% Na CMC) via p.o. | 6 |
| Group 2 | Ketoprofen Nanosuspension 40mg/kg p.o. | 6 |

Using a 22-gauge catheter, a roughly 1.0 mL blood sample was taken from the rat's orbital plexus at predetermined intervals of 0, 1, 2, 3, 4, and 6 hours. Centrifugation was used to separate the plasma, which was then kept at - 40°C for analysis for 20 min at 2000 rpm.

### Pharmacokinetic Analysis

The non-compartmental parameter was used to calculate the drug's bioavailability, $T_{max}$ (time of occurrence), and $C_{max}$ (maximum plasma concentration). For the estimate of pharmacokinetics parameters, Microsoft Excel for Windows 10 was utilized in conjunction with the PK solver 2.0 software.

### 2.5 Accelerated stability studies of Ketoprofen-nanosuspension [25]

The shelf life (storage or transit) of the nanosuspension goods that are intended for the market should be steady. In general, a commercial product must have a shelf life of at least one year in order to be pharmaceutically acceptable, have a good drug retention capacity, and retain particle size throughout storage. Therefore, the factors that need to be evaluated include drug leakage, particle size increase, and Zeta potential [26,27]

Studies on produced nanosuspension's stability were conducted for up to a month, and stability was determined by the nanosuspension's EE percentage, polydispersity index, and particle size [28].

## 3. RESULTS

### 3.1 In vitro release study of an optimized batch of Ketoprofen-nanosuspension

Description of the in vitro dissolution profile is provided in Fig.1. In contrast, lyophilized KTP-nanosuspension released 30% of the medication in less than 1 hour while API-suspension released just 10% of the drug in that time. The presence of the surfactant Tween 80 in the suspension causes the medication to be released from the nanosuspension. Poor water solubility, poor wettability, and maybe particle aggregation was blamed for the inadequate drug release from the API suspension. The combination of the surfactant in the nanosuspension and the increased drug release was the cause. Additionally, the size decrease of the nanosuspension was responsible for the increase in the drug dissolution rate.

The Korsmeyer-Peppas release kinetics was shown to be the best match model for the comparison of release kinetics (correlation coefficient (r2= 0.8956)).

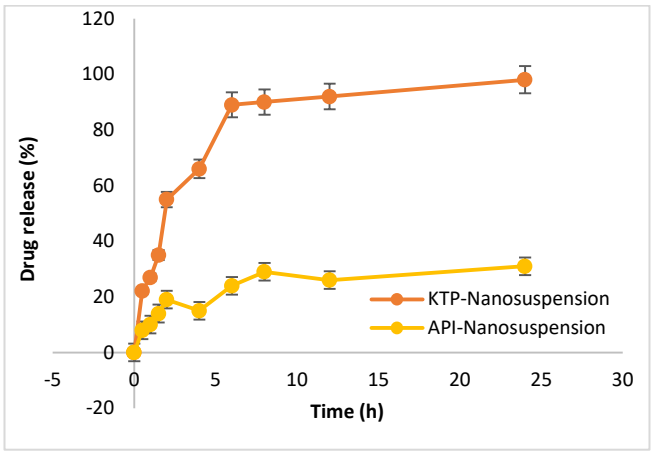



**Figure 1: The lyophilized KTP nanosuspension shows the best performance as compared to the API pure suspension**

### 3.2 In vivo pharmacokinetic study

Male Wistar rats were used to determine an in vivo pharmacokinetic investigation. Fig. 2 and Table. 5 both showed the pharmacokinetic profile. With the use of the Windows 10 MS Excel-based PK Solver software, the pharmacokinetic profile was calculated. The comparison between the API-suspension and KTF-nanosuspension is shown in Fig.

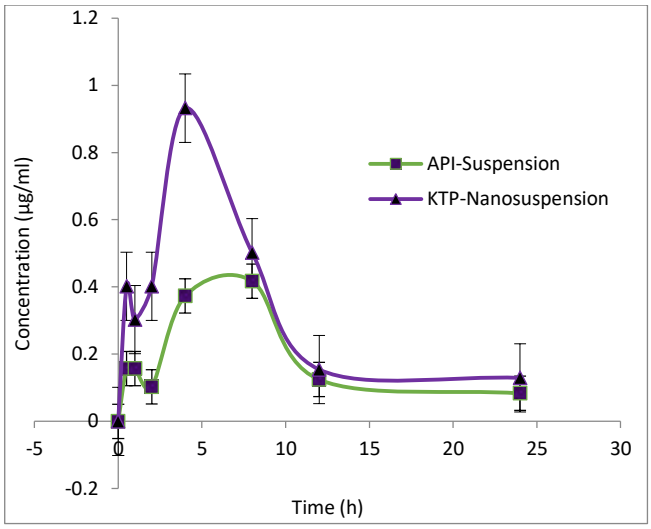



**Figure 2: The pharmacokinetic profile**

**Table 5: API-suspension and KTP-nanosuspension**

| Groups | Pharmacokinetic parameters | | | |
|---|---|---|---|---|
| | $T_{max\ (h)}$ | $C_{max}$ (µg/mL) | $AUC_{0-t}$ (µg/mL*h) | Relative bioavailability |
| **Group-I (API suspension)** | 1 | 0.373±1.43 | 0.312 ±1.43 | 2.49 |
| **Group II (KTP-nanosuspension)** | 2 | 0.932±0.54 | 0.842±2.01 | |

$T_{max}$; time to attain maximum concentration, $C_{max}$: maximum concentration, AUC: Area under Curve. Values are expressed as mean±SD, (n=3).

The pharmacokinetic profile of the API suspension and KTP-nanosuspension showed a significant difference. The absorption rate is confirmed by Tmax 2 h, Cmax 0.9320.54, and AUC0-t 0.8422.01. In comparison to API suspension, the nanosuspension had a greater AUC and Cmax. This was brought on by the KTP's amorphous form in the nanosuspension. The addition of a mixture of surfactants, such as Tween 80 and PVP K-30, was the cause of the higher Cmax rate. As a result, nanosuspension increased the drug's bioavailability and simultaneous solubility. Additionally, the nanosuspension's larger particle size and higher surface area boost the solubility and bioavailability while facilitating absorption.

### 3.3 Stability of Optimized Ketoprofen–nanosuspension

As per ICH Q1A(R2) stability guidelines, the temperature and RH conditions for accelerated stability studies are maintained as follows: 25±2°C /60%±5%RH

**Table 6: Stability studies of optimized batch**

| Parameters | 1 Month | 2 Month | 3 Month |
|---|---|---|---|
| Particle Size | 169.2 | 171.3 | 172.8 |
| Dispersity | 0.225 | 0.275 | 0.298 |
| Entrapment Efficiency | 97.22 | 96.89 | 96.12 |

It was noted that the formulation remained stable at room temperature for 3 months as shown in Table 6. Ketoprofen nanosuspension is hence stable at room temperature. The findings of the stability analysis showed that the optimized batch proved highly stable at room temperature.

## 4. DISCUSSION

The study successfully demonstrated the potential of nanosuspensions as a drug delivery method for improving the solubility, bioavailability, and therapeutic effectiveness of ketoprofen. The formulation and design were based on lipid-based drug delivery systems and nanotechnology principles, which took into account factors such as particle size, stability, and drug release kinetics. The desired characteristics of the nanosuspension were achieved by optimisation utilizing statistical tools and experimental design techniques. By carefully adjusting formulation components including lipid type, surfactant concentration, and homogenization method, the study was successful in developing a nanosuspension with a suitable particle size, high drug loading, and good physical stability.

Characterization results revealed that the lipid-based ketoprofen nanosuspension had much smaller particles than conventional formulations. The reduced particle size allowed for an increase in the absorption and solubility of the medicine. The increased surface area and quicker drug release kinetics of the nanosuspension further improved its high bioavailability and therapeutic efficacy. The findings demonstrate that by increasing the poor water solubility of ketoprofen, this nanosuspension has the potential to enhance its therapeutic effects. Additionally, the developed nanosuspension offers a structure for further investigation, including in vitro and in vivo experiments.

In conclusion, our thorough analysis combined in vitro and in vivo research to examine the bioavailability of ketoprofen. The

findings showed that the nanosuspension formulation has a great deal of potential for successfully delivering ketoprofen into the body while addressing the drawbacks of the conventional API suspension of ketoprofen. Additionally, because of the nanosuspension's reduced particle size and increased solubility characteristics, its therapeutic effect was enhanced. These findings emphasise the potential therapeutic uses of ketoprofen and add to the increasing amount of information on cutting-edge drug delivery technologies, particularly nanosuspensions. Ketoprofen's treatment limits may be addressed, and its solubility can be improved, due to the detailed analysis of the ketoprofen loaded nanosuspension.

## Acknowledgement

We would like to express our sincere gratitude to Sunrise University, Alwar Pharmacy College, Alwar, Rajasthan, India for providing us with the research facility required to conduct our study. The support extended by the institution was instrumental in successfully completing the research project. We also thank the faculty members and staff for their cooperation and assistance throughout the project. Their guidance and expertise were invaluable in shaping the research direction and methodology. We are grateful for the opportunity to have access to the facilities and resources provided by Sunrise University, Alwar Pharmacy College.

## Conflict of Interest: No Conflict of interest

## REFERENCES

1. Dahan A, Miller JM. The Solubility-Permeability Interplay and Its Implications in Formulation Design and Development for Poorly Soluble Drugs. AAPS J [Internet]. 2012 Jun 6; 14(2):244-51. Available from: http://link.springer.com/10.1208/s12248-012-9337-6 https://doi.org/10.1208/s12248-012-9337-6

2. Raman S, Polli JE. Prediction of positive food effect: Bioavailability enhancement of BCS class II drugs. Int J Pharm [Internet]. 2016 Jun;506(1-2):110-5. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0378517316302885 https://doi.org/10.1016/j.ijpharm.2016.04.013

3. Kawabata Y, Wada K, Nakatani M, Yamada S, Onoue S. Formulation design for poorly water-soluble drugs based on biopharmaceutics classification system: Basic approaches and practical applications. Int J Pharm [Internet]. 2011 Nov; 420(1):1-10. https://linkinghub.elsevier.com/retrieve/pii/S0378517311007940 https://doi.org/10.1016/j.ijpharm.2011.08.032

4. Rabinow BE. Nanosuspensions in drug delivery. Nat Rev Drug Discov [Internet]. 2004 Sep;3(9):785-96. Available from: https://www.nature.com/articles/nrd1494 https://doi.org/10.1038/nrd1494

5. Fernandes GJ, Kumar L, Sharma K, Tunge R, Rathnanand M. A Review on Solubility Enhancement of Carvedilol-a BCS Class II Drug. J Pharm Innov [Internet]. 2018 Sep 7; 13(3):197-212. http://link.springer.com/10.1007/s12247-018-9319-z https://doi.org/10.1007/s12247-018-9319-z

6. Van Eerdenbrugh B, Van den Mooter G, Augustijns P. Top-down production of drug nanocrystals: Nanosuspension stabilization, miniaturization and transformation into solid products. Int J Pharm [Internet]. 2008 Nov; 364(1):64-75. https://linkinghub.elsevier.com/retrieve/pii/S0378517308005280 https://doi.org/10.1016/j.ijpharm.2008.07.023

7. Reverchon E. Supercritical antisolvent precipitation of micro- and nano-particles. J Supercrit Fluids [Internet]. 1999 May; 15(1):1-21. https://linkinghub.elsevier.com/retrieve/pii/S0896844698001296 https://doi.org/10.1016/S0896-8446(98)00129-6

8. Pathak P, Meziani MJ, Desai T, Sun YP. Formation and stabilization of ibuprofen nanoparticles in supercritical fluid processing. J Supercrit Fluids [Internet]. 2006 May; 37(3):279-86. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0896844606000118 https://doi.org/10.1016/j.supflu.2005.09.005

9. Agrawal Y, Patel V. Nanosuspension: An approach to enhance solubility of drugs. J Adv Pharm Technol Res [Internet]. 2011; 2(2):81. Available from: http://www.japtr.org/text.asp?2011/2/2/81/82950 https://doi.org/10.4103/2231-4040.82950

10. Singh A, Worku ZA, Van den Mooter G. Oral formulation strategies to improve solubility of poorly water-soluble drugs. Expert Opin Drug Deliv [Internet]. 2011 Oct 3; 8(10):1361-78. Available from: http://www.tandfonline.com/doi/full/10.1517/17425247.2011.606808 https://doi.org/10.1517/17425247.2011.606808

11. Li XS, Wang JX, Shen ZG, Zhang PY, Chen JF, Yun J. Preparation of uniform prednisolone microcrystals by a controlled microprecipitation method. Int J Pharm [Internet]. 2007 Sep; 342(1-2):26-32. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0378517307003602 https://doi.org/10.1016/j.ijpharm.2007.04.025

12. Douroumis D, Fahr A. Stable carbamazepine colloidal systems using the cosolvent technique. Eur J Pharm Sci [Internet]. 2007 Apr; 30(5):367-74. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0928098706003654 https://doi.org/10.1016/j.ejps.2006.12.003

13. Lindfors L, Forssén S, Westergren J, Olsson U. Nucleation and crystal growth in supersaturated solutions of a model drug. J Colloid Interface Sci [Internet]. 2008 Sep; 325(2):404-13. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0021979708006267 https://doi.org/10.1016/j.jcis.2008.05.034

14. Douroumis D, Fahr A. Nano- and micro-particulate formulations of poorly water-soluble drugs by using a novel optimized technique. Eur J Pharm Biopharm [Internet]. 2006 Jun; 63(2):173-5. Available from: https://linkinghub.elsevier.com/retrieve/pii/S093964110600035X https://doi.org/10.1016/j.ejpb.2006.02.004

15. Lince F, Marchisio DL, Barresi AA. Strategies to control the particle size distribution of poly-ε-caprolactone nanoparticles for pharmaceutical applications. J Colloid Interface Sci [Internet]. 2008 Jun; 322(2):505-15. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0021979708003159 https://doi.org/10.1016/j.jcis.2008.03.033

16. LOUHIKULTANEN M, KARJALAINEN M, RANTANEN J, HUHTANEN M, KALLAS J. Crystallization of glycine with ultrasound. Int J Pharm [Internet]. 2006 Aug 31; 320(1-2):23-9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0378517306003413 https://doi.org/10.1016/j.ijpharm.2006.03.054

17. Luque de Castro MD, Priego-Capote F. Ultrasound-assisted crystallization (sonocrystallization). Ultrason Sonochem [Internet]. 2007 Sep; 14(6):717-24. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1350417706001684 https://doi.org/10.1016/j.ultsonch.2006.12.004

18. Liu D, Xu H, Tian B, Yuan K, Pan H, Ma S, et al. Fabrication of Carvedilol Nanosuspensions Through the Anti-Solvent Precipitation-Ultrasonication Method for the Improvement of Dissolution Rate and Oral Bioavailability. AAPS PharmSciTech [Internet]. 2012 Mar 13; 13(1):295-304. Available from: http://link.springer.com/10.1208/s12249-011-9750-7 https://doi.org/10.1208/s12249-011-9750-7

19. Rahim H, Sadiq A, Khan S, Amin F, Ullah R, Shahat AA, et al. Fabrication and characterization of glimepiride nanosuspension by ultrasonication-assisted precipitation for improvement of oral bioavailability and in vitro α-glucosidase inhibition. Int J Nanomedicine [Internet]. 2019 Aug; 14:6287-6296. Available from: https://www.dovepress.com/fabrication-and-characterization-of-glimepiride-nanosuspension-by-ultr-peer-reviewed-article-IJN https://doi.org/10.2147/IJN.S210548

20. Xia D, Quan P, Piao H, Piao H, Sun S, Yin Y, et al. Preparation of stable nitrendipine nanosuspensions using the precipitation-ultrasonication method for enhancement of dissolution and oral bioavailability. Eur J Pharm Sci [Internet]. 2010 Jul; 40(4):325-34. Available from:

https://linkinghub.elsevier.com/retrieve/pii/S092809871000151X https://doi.org/10.1016/j.ejps.2010.04.006

21. Taneja S, Shilpi S, Khatri K. Formulation and optimization of efavirenz nanosuspensions using the precipitation-ultrasonication technique for solubility enhancement. Artif Cells, Nanomedicine, Biotechnol [Internet]. 2015 Feb 27; 1-7. Available from: http://www.tandfonline.com/doi/full/10.3109/21691401.2015.1008505 https://doi.org/10.3109/21691401.2015.1008505

22. Dandagi, P.M., Kerur, S.S., Mastiholimath, V., Gadad, A., & Kulkarni, A.R. (2009). Polymeric ocular nanosuspension for controlled release of acyclovir: in vitro release and ocular distribution. Iranian Journal of Pharmaceutical Research, 8 79-86. Polymeric ocular nanosuspension for controlled release of acyclovir: in vitro release and ocular distribution. Iran J Pharm Res. 2009;

23. Saindane NS, Pagar KP, Vavia PR. Nanosuspension Based In Situ Gelling Nasal Spray of Carvedilol: Development, In Vitro and In Vivo Characterization. AAPS PharmSciTech [Internet]. 2013 Mar 20; 14(1):189-99. Available from: http://link.springer.com/10.1208/s12249-012-9896-y https://doi.org/10.1208/s12249-012-9896-y

24. Yadav SK, Mishra S, Mishra B. Eudragit-Based Nanosuspension of Poorly Water-Soluble Drug: Formulation and In Vitro-In Vivo Evaluation. AAPS PharmSciTech [Internet]. 2012 Dec 15; 13(4):1031-44. Available from: http://link.springer.com/10.1208/s12249-012-9833-0 https://doi.org/10.1208/s12249-012-9833-0

25. Clancy D, Hodnett N, Orr R, Owen M, Peterson J. Kinetic Model Development for Accelerated Stability Studies. AAPS PharmSciTech [Internet]. 2017 May 15; 18(4):1158-76. Available from: http://link.springer.com/10.1208/s12249-016-0565-4 https://doi.org/10.1208/s12249-016-0565-4

26. Jandik KA, Kruep D, Cartier M, Linhardt RJ. Accelerated Stability Studies of Heparin. J Pharm Sci [Internet]. 1996 Jan; 85(1):45-51. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0022354915499769 https://doi.org/10.1021/js9502736

27. Burdick RK, Sidor L. Establishment of an Equivalence Acceptance Criterion for Accelerated Stability Studies. J Biopharm Stat [Internet]. 2013 Jul 4; 23(4):730-43. Available from: https://www.tandfonline.com/doi/full/10.1080/10543406.2013.789891 https://doi.org/10.1080/10543406.2013.789891

28. Waterman KC. The Application of the Accelerated Stability Assessment Program (ASAP) to Quality by Design (QbD) for Drug Product Stability. AAPS PharmSciTech [Internet]. 2011 Sep 12; 12(3):932. Available from: http://link.springer.com/10.1208/s12249-011-9657-3 https://doi.org/10.1208/s12249-011-9657-3