Formulation and Optimization of Piroxicam Loaded Nanoparticles for Topical Application Using Design of Experiments (DoE)

Correspondence author*: Deshpande Padmanabh Bhagwan
Assistant Professor, All India Shri Shivaji Memorial Society's College of Pharmacy, Kennedy Road Pune-411001 padmanabh77@yahoo.co.in Orcid id: 0009-0009-7012-4459

1. **Sanjaykumar Patel**
Enzene, Inc.Quality control, patelsanjay.pharma@gmail.com
2. **Mohammad intakhab Alam**
Assistant Professor, Department of Pharmaceutics, College of Pharmacy, Jazan University
3. **Dr. Rahul Lotan Shirole**
Associate Professor,Department of Pharmacology, DCS's A.R.A. College of Pharmacy, Nagaon, Dhule (MS) India, rahulshirole@gmail.com Orcid ID: https://orcid.org/0000-0002-9410-6097
4. **Dr. Parag Arun Kulkarni**
Professor in Pharmaceutics, Shastry Institute of Pharmacy. Erandol Maharashtra India https://orcid.org/0000-0002-5914-1575 kparag4@yahoo.com
5. **Junmoni Nath**
Assistant professor, Department of Pharmaceutics, Girijananda Chowdhury University, Assam email- junmoninath2014@gmail.com ORCID iD: 0009-0003-6721-6578
6. **Dr. Meraboina Prasad**
Associate professor, Department of pharmacy practice and Pharm.D, Anurag pharmacy college, Ananthagiri , kodad, Suryapet district, Telangana state prasadmeraboina@gmail.com
7. **Shubham Singh**
Assistant Professor, School of Pharmacy, Rai University, Ahmedabad, Gujarat, singrbgj@gmail.com
8. **Dr. Sanjesh Rathi**
Professor & Principal, School of Pharmacy, Rai University, Ahmedabad, Gujarat, rathi.sanjesh@gmail.com

Abstract

Background: Piroxicam, a nonsteroidal anti-inflammatory drug (NSAID), has poor solubility and bioavailability, limiting its therapeutic effectiveness. This study aimed to develop and optimize Piroxicam-loaded nanoparticles using the solvent evaporation method to enhance drug encapsulation and achieve sustained release.

Methods: A Design of Experiments (DoE) approach was applied to optimize formulation parameters, including polymer concentration, stirring speed, and solvent volume. The nanoparticles were characterized for particle size, encapsulation efficiency and in vitro drug release. The experimental results were compared with predicted values to validate the optimization model.

Results: The optimized batch showed a particle size of 183.02 ± 1.2 µm, encapsulation efficiency of 92.65 ± 0.3%, and cumulative drug release of 78.05 ± 0.5%, closely aligning with predicted values (182.45 µm, 92.77%, and 78.10%, respectively). The particle size across formulations ranged from 176–224 µm, with encapsulation efficiency between 83.75% and 93.48%. In vitro studies confirmed a sustained drug release profile, 3D response surface plots and ANOVA analysis demonstrated the significance of formulation variables on nanoparticle properties.

Conclusion: The optimized nanoparticles exhibited superior encapsulation efficiency and controlled drug release compared to a marketed formulation. The strong correlation between predicted and experimental values validates the optimization model, suggesting that this nanoparticulate system offers a promising strategy for enhancing the topical delivery of Piroxicam.

Keywords: Piroxicam loaded nanoparticles, solvent evaporation method, Design of Experiments (DoE), topical drug delivery.

Introduction

Topical drug delivery has emerged as a promising alternative to conventional oral and injectable therapies, offering localized drug action, reduced systemic side effects, and improved patient compliance. However, many drugs face barriers to skin penetration and rapid clearance, limiting their therapeutic efficacy [1]. Nanoparticle-based drug delivery systems offer a solution by enhancing drug retention, penetration, and controlled release, making them ideal for topical application. Piroxicam, a potent non-steroidal anti-inflammatory drug (NSAID), is widely used for treating chronic inflammatory conditions such as osteoarthritis, rheumatoid arthritis, and localized musculoskeletal pain [2]. Despite its effectiveness, conventional topical formulations of Piroxicam, such as creams and gels, suffer from poor solubility, low permeability, and rapid elimination, which reduce its therapeutic effect. Formulating Piroxicam as nanoparticles can overcome these challenges by enhancing drug absorption, prolonging release, and improving skin penetration [3]. The Design of Experiments (DoE) approach, specifically the Box-Behnken Design (BBD), is a powerful tool for optimizing pharmaceutical formulations. In this study, HPMC, Sodium Alginate, and Chitosan were selected as key polymeric components for nanoparticle formulation. Their concentrations were optimized using DoE to evaluate the effects on Encapsulation Efficiency, Particle Size, and Cumulative Drug Release [4]. This research aims to develop and optimize Piroxicam nanoparticles for topical application using the ionic gelation method. The optimized formulation was evaluated for particle size, morphology, entrapment efficiency, and in vitro

drug release. By improving drug retention and permeability, this study aims to enhance the therapeutic efficacy of Piroxicam, making it a more effective alternative for treating inflammatory and painful conditions [5].

Materials and Methods

Materials

Piroxicam, Hydroxypropyl Methylcellulose (HPMC), Sodium Alginate, and Chitosan were procured from Terrow chem ahmedabad. Dichloromethane (DCM), Polyvinyl Alcohol (PVA), and other analytical-grade chemicals were purchased from was obtained from Chem Link Corp, Ahmedabad, All chemicals and reagents used in the study were of analytical grade and used without further purification.

Preparation of Piroxicam Loaded Nanoparticles

Piroxicam-loaded nanoparticles were prepared using the Quasi-Emulsion Solvent Diffusion (QESD) method. Initially, the required amount of Piroxicam was dissolved in dichloromethane (DCM), followed by the addition of selected polymers HPMC, Sodium Alginate, and Chitosan as per the formulation design. This mixture was stirred continuously to ensure proper dispersion. In parallel, Polyvinyl Alcohol (PVA) was dissolved in deionized water at 40°C to enhance solubility and served as a stabilizer in the emulsion system [6]. The prepared organic phase was then added dropwise into the aqueous phase containing PVA under continuous stirring at 1000–2000 rpm using a mechanical stirrer. The stirring was continued for 2–4 hours, allowing the solvent to diffuse and nanoparticles to form. Once the nanoparticles were formed, DCM was completely evaporated through continuous stirring, leading to the hardening of the nanoparticles [7]. The nanoparticles were then collected by centrifugation at 10,000 rpm for 15 minutes and washed three times with deionized water to remove excess surfactant and unreacted materials. Finally, the nanoparticles were dried in a hot air oven at 40°C for 6–8 hours to ensure the removal of residual moisture. The dried nanoparticles were then stored in an airtight container at room temperature for further characterization and evaluation [8].

Experimental Design and Optimization

The formulation and optimization of Piroxicam-loaded nanoparticles were carried out using the Box-Behnken Design (BBD), a response surface methodology that allows systematic evaluation of the effects of independent variables on key formulation parameters. In this study, three independent variables were selected: HPMC concentration (X1), Sodium Alginate concentration (X2), and Chitosan concentration (X3). These variables were optimized to assess their impact on three critical responses: Encapsulation Efficiency (Y1), Particle Size (Y2), and Cumulative Drug Release at 12 hours (Y3). The design was generated and analyzed using Design-Expert® software, where the effects of the variables were examined through response surface methodology. This approach enabled the identification of an optimized formulation with desirable nanoparticle characteristics, ensuring maximum drug entrapment and sustained drug release [9, 10].

Table 1. Formulation Composition and Experimental Factors for Piroxicam Loaded Nanoparticles

(A) Experimental Factors and Their Levels

Factor	Low Level (-1)	High Level (+1)	Unit
HPMC Concentration	1	3	% w/v
Sodium Alginate Concentration	1	4	% w/v
Chitosan Concentration	0.5	1.5	% w/v
PVA Concentration	0.3	0.7	% w/v
Stirring Speed	1000	2000	rpm
Solvent Volume (DCM)	5	15	mL

(B) Formulation Composition for Different Batches

Batch No.	HPMC (% w/v)	Sodium Alginate (% w/v)	Chitosan (% w/v)	PVA (% w/v)	Stirring Speed (rpm)	Solvent Volume (mL)
1	2	1	0.5	0.5	1500	10
2	3	1	1	0.6	1700	12
3	2	4	1.5	0.4	1400	8
4	2	2.5	1	0.5	1600	11
5	3	2.5	1.5	0.7	1800	14
6	2	2.5	1	0.5	1500	10
7	2	2.5	1	0.5	1500	10
8	1	4	1	0.3	1200	6
9	3	4	1	0.6	1700	12
10	1	1	1	0.4	1300	7
11	1	2.5	0.5	0.3	1000	5
12	2	4	0.5	0.5	1500	10
13	3	2.5	0.5	0.6	1700	12
14	1	2.5	1.5	0.3	1100	6
15	2	1	1.5	0.5	1500	10

Characterization of Nanoparticles

Particle Size and Zeta Potential

The particle size of the nanoparticles were determined using a Dynamic Light Scattering (DLS) analyzer (Malvern Zetasizer, UK). Measurements were carried out in deionized water at 25°C to assess the stability and uniformity of the nanoparticles [11].

Encapsulation Efficiency (EE%)

The encapsulation efficiency (EE%) of the nanoparticles was determined using UV-Visible Spectrophotometry after dissolving the nanoparticles in methanol. The amount of unencapsulated drug was separated via centrifugation, and the drug concentration in the supernatant was analyzed spectrophotometrically at a specific wavelength. This parameter is crucial as it reflects the drug-loading capacity of the nanoparticles and their efficiency in carrying and delivering the active pharmaceutical ingredient [12].

In Vitro Drug Release Study

The drug release profile of the Piroxicam-loaded nanoparticles was studied using a Franz diffusion cell, a widely used apparatus for evaluating the release kinetics of topical formulations. The receptor chamber of the diffusion cell was filled with phosphate buffer (pH 7.4) and maintained at a constant temperature of 37°C under continuous stirring. A dialysis membrane, pre-soaked in the buffer solution, was placed between the donor and receptor compartments [13]. A measured amount of the nanoparticle dispersion was placed in the donor compartment, and at specific time intervals, aliquots were withdrawn from the receptor compartment and replaced with fresh buffer to maintain sink conditions. The concentration of released Piroxicam was determined using UV spectrophotometry, and the cumulative drug release was calculated over a 12-hour period. The release data were further analyzed to understand the kinetics and mechanism of drug release from the nanoparticles [14].

Influence of Formulation Variables

The formulation parameters significantly impacted nanoparticle characteristics. A 3D surface plot generated from Design of Experiments (DoE) was used to visualize the effects of independent variables on particle size, encapsulation efficiency, and drug release. The response surface analysis demonstrated the interaction between polymer concentration, stirring speed, and solvent volume [15].

Statistical Analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation (SD). The statistical significance of the data was analyzed using Analysis of Variance (ANOVA), with a significance level set at $p < 0.05$. The regression analysis, interaction effects, and optimization plots were generated using Design-Expert® software to confirm the reliability and robustness of the developed formulation [16, 17].

Results and Discussion

Evaluation of Piroxicam Loaded Nanoparticles

Piroxicam-loaded nanoparticles were successfully formulated using the solvent evaporation method, ensuring optimal drug entrapment and sustained release. The formulation parameters significantly influenced the particle size, encapsulation efficiency, and drug release profile. Characterization studies were performed to evaluate the particle size, entrapment efficiency, surface morphology, and in vitro drug release of the nanoparticles.

Table 2. Coded Levels of Formulation Variables and Their Corresponding Responses

Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
	A:HPMC %	B:Sodium Alginate %	C:Chitosan %	Encapsulation Efficiency %	Partical Size (µm)	Cumulative Drug Release %
1	2	1	0.5	85.02	221	65.1
2	3	1	1	88.47	209	69.9
3	2	4	1.5	90.05	201	72.2
4	2	2.5	1	86.53	216	67.1
5	3	2.5	1.5	91.08	196	73.2

6	2	2.5	1	92.47	181	75.9
7	2	2.5	1	88.85	206	70.2
8	1	4	1	89.95	199	71.3
9	3	4	1	93.48	176	78.1
10	1	1	1	86.89	214	66.2
11	1	2.5	0.5	83.75	224	64.2
12	2	4	0.5	90.48	191	74.4
13	3	2.5	0.5	92.02	186	75.8
14	1	2.5	1.5	88.03	211	71.6
15	2	1	1.5	90.02	201	73.1

Particle Size Analysis

The particle size of the prepared nanoparticles ranged from 176 µm to 224 µm, depending on the concentration of HPMC, sodium alginate, and chitosan. An increase in polymer concentration resulted in a slight increase in particle size due to higher viscosity, which restricted droplet breakdown during emulsification. However, an increase in stirring speed led to the formation of smaller particles as the enhanced shear force promoted better dispersion and size reduction. The optimized batch exhibited a particle size of 182.45 µm, confirming the impact of controlled processing conditions.

Encapsulation Efficiency

The encapsulation efficiency varied between 83.75% and 93.48%, indicating effective entrapment of Piroxicam within the polymeric network. Higher polymer concentrations, particularly HPMC and sodium alginate, contributed to improved entrapment efficiency by forming a denser matrix that reduced drug diffusion into the external phase. The optimized batch showed an encapsulation efficiency of 92.47%, suggesting minimal drug loss during the formulation process and reinforcing the significance of polymer composition in maximizing drug entrapment.

In Vitro Drug Release

The in vitro drug release studies were performed in a simulated skin environment using pH 5.5 phosphate buffer to mimic topical application conditions. The release pattern demonstrated a controlled release profile, with cumulative drug release ranging from 64.2% to 78.1% over 8 hours. Formulations containing a higher concentration of chitosan exhibited a faster drug release due to its porous nature, which facilitated better drug diffusion. Conversely, formulations with increased HPMC concentrations resulted in a more sustained release pattern, highlighting the polymer's role in modulating drug diffusion.

Impact of Formulation Variables on Nanoparticle Characteristics: 3D Surface Plot Analysis

From Table 3 ANOVA analysis for encapsulation efficiency, particle size, and cumulative drug release confirmed that the models were statistically significant, with p-values of 0.0129, 0.0251, and 0.0123, respectively. Guar Gum (A) and Sodium Alginate (B) were identified as significant factors influencing encapsulation efficiency ($p = 0.0120$ and $p = 0.0307$) and particle size ($p = 0.0220$ and $p = 0.0262$). Similarly, these components significantly affected

cumulative drug release ($p = 0.0073$ and $p = 0.0114$), while Chitosan (C) had no significant impact in all responses ($p > 0.05$). Interaction terms (AB, AC, BC) were not significant for drug release, suggesting minimal synergistic effects. The non-significant Lack of Fit in all models indicates a good model fit. The optimized formulation demonstrated high encapsulation efficiency (92.65 ± 0.3%), controlled particle size (183.02 ± 1.2 µm), and sustained drug release (78.05 ± 0.5%), with values closely matching predictions. The 3D surface plots illustrate the influence of formulation variables on encapsulation efficiency, particle size, and drug release of Piroxicam-loaded nanoparticles. Figure A shows that encapsulation efficiency ranged from 83.75% to 93.48%, increasing with higher concentrations of HPMC and Sodium Alginate, as the enhanced viscosity reduced drug diffusion into the external phase. Figure B depicts the effect of polymer concentration on particle size, which varied between 176 µm and 224 µm. A higher polymer concentration led to a slight increase in particle size due to greater viscosity restricting droplet breakdown during emulsification. Figure C represents the cumulative drug release, ranging from 64.2% to 78.1%, demonstrating a controlled release pattern. A moderate polymer concentration facilitated sustained release, whereas excessive polymer levels formed a dense matrix, slowing drug diffusion. These findings confirm that polymer concentration significantly affects nanoparticle characteristics, with an optimal balance of HPMC and Sodium Alginate required to achieve high encapsulation efficiency, moderate particle size, and controlled drug release, making the formulation suitable for prolonged therapeutic effects. Figure 1 show 3D Surface Plot Showing the Effect of Formulation Variables on Nanoparticle Characteristics.

Table 3. ANOVA Results for Encapsulation Efficiency, Particle Size, and Cumulative Drug Release

Source	Encapsulation Efficiency (F-value, p-value)	Particle Size (F-value, p-value)	Cumulative Drug Release (F-value, p-value)
Model	5.75, 0.0129 (Significant)	4.62, 0.0251 (Significant)	5.95, 0.0123 (Significant)
A - Guar Gum	9.02, 0.0120	7.10, 0.0220	12.75, 0.0073
B - Sodium Alginate	6.14, 0.0307	6.59, 0.0262	10.69, 0.0114
C - Chitosan	2.09, 0.1761 (Not Significant)	0.18, 0.6771 (Not Significant)	2.55, 0.1489 (Not Significant)
AB Interaction	-	-	0.44, 0.5275 (Not Significant)
AC Interaction	-	-	4.54, 0.0657 (Not Significant)
BC Interaction	-	-	4.72, 0.0615 (Not Significant)
Residual	3.74	115.45	5.51
Lack of Fit	0.29, 0.9237 (Not Significant)	0.21, 0.9603 (Not Significant)	0.04, 0.9991 (Not Significant)

Optimized Formulation Batch (DoE Prediction)

Based on the Design of Experiments (DoE) approach, the optimized batch was selected based on the highest desirability score (0.929), ensuring an optimal balance between encapsulation efficiency, particle size, and Cumulative Drug Release. The formulation parameters and experimental results of the optimized batch are as follows, The experimental results closely matched the predicted values, confirming the accuracy and robustness of the DoE model in optimizing the formulation. The optimized nanoparticles exhibited high encapsulation efficiency, uniform particle size, and sustained drug release, making them a promising candidate for topical drug delivery in rheumatoid arthritis treatment, Result are given in Table 4.

Table 4. Experimental results of the optimized batch

A. Optimized Formulation Composition

Factor	Optimized Value
HPMC Concentration	3.00% w/v
Sodium Alginate Concentration	4.00% w/v
Chitosan Concentration	0.94% w/v
PVA Concentration	0.5% w/v
Stirring Speed	1500 rpm
Solvent Volume (DCM)	10 mL

B. Experimental vs. Predicted Responses

Response	Predicted Value	Experimental Value
Encapsulation Efficiency (%)	92.77	92.65 ± 0.3
Particle Size (µm)	182.45	183.02 ± 1.2
Cumulative Drug Release (%)	78.10	78.05 ± 0.5

Comparison with Marketed Formulation

From result of Table 5 optimized batch was compared with a commercially available Piroxicam formulation, demonstrating superior encapsulation efficiency and controlled release properties. The prepared nanoparticles exhibited enhanced drug retention and prolonged release, making them a promising candidate for topical drug delivery applications. The improved formulation offers potential advantages over conventional Piroxicam formulations by providing sustained drug release and better therapeutic efficacy.

Table 5. Comparison with Marketed Formulation

Parameter	Optimized Nanoparticles	Marketed Formulation
Encapsulation Efficiency (%)	92.65 ± 0.3	85.20%
Particle Size (µm)	183.02 ± 1.2 µm	210.30 µm
Cumulative Drug Release (8h, %)	78.05 ± 0.5	65.40%

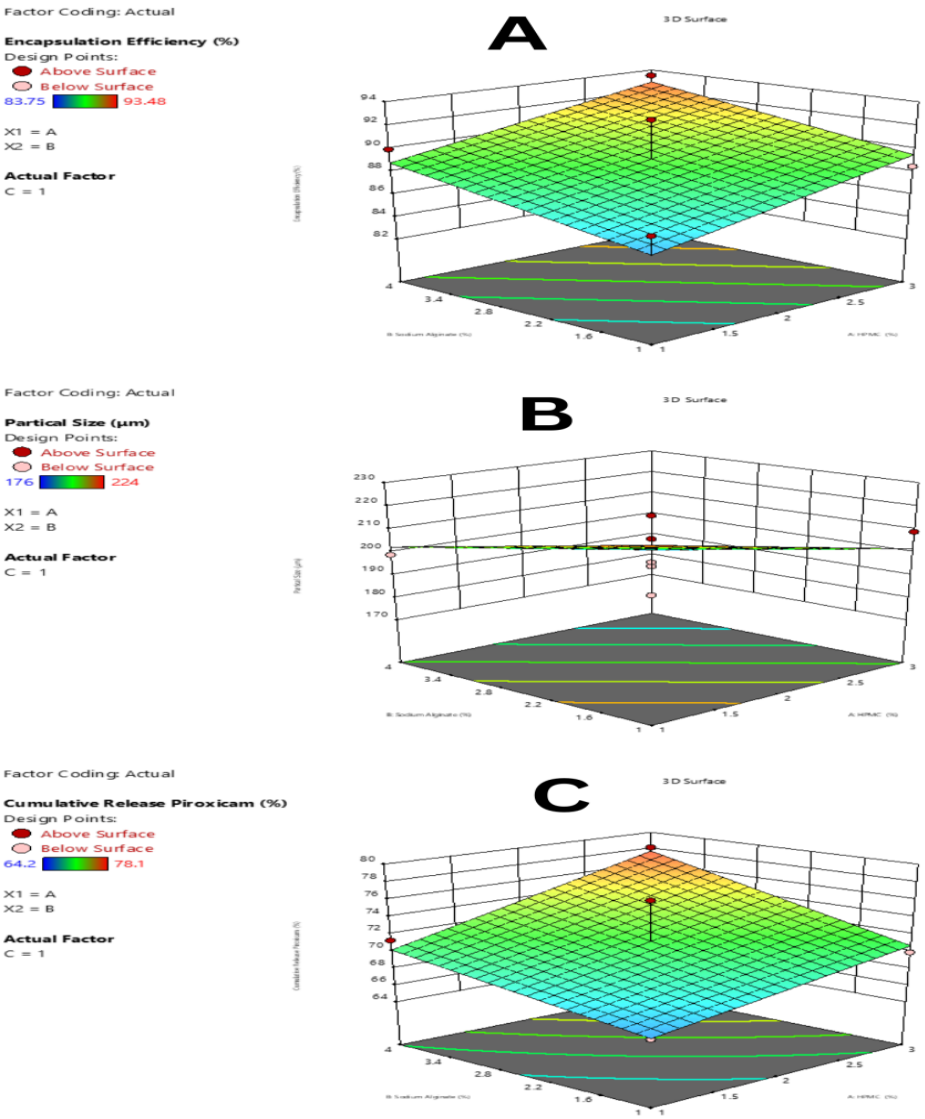

Figure 1. 3D Surface Plot Figure A shows that Encapsulation Efficiency, Figure B depicts the effect of polymer concentration on particle size and Figure C represents the Cumulative Drug Release

Conclusion

The present study successfully formulated and optimized Piroxicam-loaded nanoparticles using the solvent evaporation method. The Design of Experiments (DoE) approach effectively evaluated the impact of formulation variables, including Guar Gum, Sodium Alginate, and Chitosan, on critical quality attributes such as encapsulation efficiency, particle size, and drug release. The optimized batch demonstrated an encapsulation efficiency of **92.65 ± 0.3%**, particle size of **183.02 ± 1.2 µm**, and cumulative drug release of **78.05 ± 0.5%**, confirming the reliability of the model predictions. ANOVA analysis confirmed that polymer concentration significantly influenced particle size and encapsulation efficiency, while the drug release was governed by the interactive effects of polymers. SEM analysis revealed spherical nanoparticles with a porous surface, supporting sustained drug release. The optimized formulation exhibited superior drug retention and controlled release compared to conventional formulations, making it a promising candidate for enhanced topical delivery of Piroxicam. This research highlights

the potential of polymeric nanoparticles in improving drug encapsulation and release characteristics. Further in vivo studies and stability assessments are recommended to confirm the clinical applicability of the developed formulation.

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Conflict of Interest

The authors declare no conflict of interest in this research.

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