RESEARCH PAPER

Quality by Design Assisted Development and Characterization of Dexibuprofen-Loaded Microspheres for Controlled Drug Release

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ABSTRACT

Background and Objective: Dexibuprofen, a nonsteroidal anti-inflammatory drug, is widely used for the management of pain and inflammation. However, its clinical use is associated with gastrointestinal adverse effects such as irritation, ulceration, and bleeding, along with the need for frequent dosing at higher concentrations. To address these limitations, dexibuprofen was formulated into microspheres to achieve controlled drug release using a Quality by Design approach.

Methods: Dexibuprofen-loaded microspheres were prepared using the emulsion solvent evaporation method. The formulation was optimized using a full factorial design, considering three factors: PLGA concentration, PVA concentration, and stirring speed, and three responses: particle size, % encapsulation efficiency, and % in vitro drug release. A total of ten formulations were generated based on the experimental design. The optimized microsphere formulation was further evaluated for drug content, zeta potential, in vitro drug release kinetics, surface morphology using scanning electron microscopy, analgesic activity, and stability studies.

Results: The microspheres were optimised numerically using the global desirability value of 0.878. The optimised dexibuprofen microspheres were found to have a particle size of 495.5 nm, entrapment efficiency of 91.65%, % in vitro drug release of 88.91%, drug content of 90.8%, and zeta potential of -26.3 mV. The release kinetics confirmed that it follows the zero-order kinetics with an R^2 value of 0.9884. The optimised microspheres showed an analgesic activity in the test group for over 12 h, confirming the controlled release of the drug. The SEM demonstrated that the produced microspheres were spherical.

Conclusion: The optimized microsphere formulation exhibited sustained drug release and effective analgesic activity, indicating its potential to enhance therapeutic efficacy while minimizing the gastrointestinal side effects of conventional dexibuprofen.

Keywords: Dexibuprofen, Full factorial design, Microspheres, Poly lactic-co-glycolic Acid, Quality by Design

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INTRODUCTION

Pain is defined as an unpleasant sensory and emotional experience connected to or resembling that connected to actual or potential tissue damage by the International Association for the Study of Pain (IASP) in 2020.[1] Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used as analgesics, antipyretics, and anti-inflammatory agents.[2] They exert their effects by inhibiting cyclooxygenase (COX-1 and COX-2) enzymes, thereby reducing the production of prostaglandins involved in pain and inflammation. While COX-2 is primarily induced at sites of inflammation, COX-1 plays a protective role in maintaining gastric integrity and platelet function. Dexibuprofen, the active enantiomer of racemic ibuprofen, selectively inhibits COX-2, leading to reduced prostaglandin synthesis in inflammatory cells.[3,4] Dexibuprofen belongs to the BCS class II. It has a half-life of 2.2-4.7 h. It is indicated for dental pain, dysmenorrhea, postoperative pain, and headache. It is available in the market as a film-coated tablet in doses of 200 mg, 300 mg, and 400 mg, administered three times a day, depending on the severity of the disease. The highest plasma concentration is attained between 2.25 and 5h after oral dosing,[5] followed by rapid elimination. The hydroxylated and carbonylated

derivatives are the main metabolites that are produced by the liver's quick metabolism and biotransformation. It is administered at high doses with high frequency, which may be associated with severe side effects, including gastric ulceration, gastrointestinal bleeding, etc, orally.[6,7]

Hence, to overcome the problems found in conventional tablets, the present research aims to develop a controlled-release microsphere of dexibuprofen. The microspheres were formulated to provide a consistent therapeutic effect over an extended period and patient compliance. In microspheres, the dose and the frequency of administration can be reduced. That can avoid the gastric irritation, gastric ulceration, and other side effects associated with the drug. Microspheres are made from free-flowing powders and spherical micro particulates made of biodegradable polymers. The optimal range for their particle size is 1 μm to 1000 μm. Drugs can be placed into microspheres and delivered under regulated conditions. The microspheres help to improve the drug's therapeutic efficacy and bioavailability while reducing its toxicity and adverse effects.[8,9] The present study aims to develop dexibuprofen-loaded microspheres that provide the controlled release of the drug, using diffusion and polymer erosion processes. This method of formulation does not just offer a controlled release of anti-inflammatory and analgesic action, but it also has minimal side effects compared to the traditional dosage form. Poly (lactic-co-glycolic acid), commonly known as PLGA, is an FDA-approved biodegradable and biocompatible polymer that has been used for the preparation of microspheres. Lactic acid (LA) and glycolic acid (GA) randomly polymerize to generate this copolymer. Because PLGA is non-toxic and has good plasticity, it is frequently utilized in medicines and medical engineering materials.[10]

The primary aim of the research was to develop dexibuprofen-loaded microspheres as an efficient substitute for traditional oral delivery. This system aims to provide controlled drug release, reduced frequent administration, reduced dosage and minimised side effects such as gastrointestinal bleeding, gastric ulceration, and other associated side effects.

MATERIALS AND METHODS

Materials

The dexibuprofen was provided as a gift sample from Mylan Laboratories Limited, Tamil Nadu, and the poly lactic co-glycolic acid (PLGA) was purchased from Nomisma Healthcare Pvt. Ltd, Gujarat, Emulsifier Polyvinyl alcohol (PVA) and Surfactant Tween 20 were procured from the SD Fine Chem Limited, Mumbai.

Methods

Quality by Design approach

The present study was designed by Quality by Design (QbD), a methodology for formulation development. According to ICH Q8 guidelines, which emphasise product and process understanding and process control. QbD provides a robust understanding of the product performance, which is essential for the Quality Target Product Profile (QTPP) framework, identifying Critical Quality Attributes (CQAs), and determining Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs).[11]

Quality Target Product Profile

The QTPP provides a detailed specification of a product that emphasises its quality characteristics to ensure product performance, safety, and efficacy.[12] The QTPP was designed for the microspheres, including the dosage strength, dosage form, drug product quality profile, route of administration, product quality attributes, drug release, and stability. Table 1 represents the QTPP for the microsphere's formulation.

Critical Quality Attributes

The CQAs are those affecting the performance of the products.[13] The CQAs for the microsphere's formulation were carefully selected based on the literature review. The drug content, particle size, encapsulation efficiency, and *in vitro* drug release were chosen. Table 2 lists the potential CQAs affecting the performance of the microspheres and their justifications.

Risk Assessment

A comprehensive risk assessment was performed to determine the factors influencing the quality of the final product. CMAs and CPPs were identified through the Ishikawa diagram, risk estimation matrix (REM), and determining Risk Priority Number (RPN). The Ishikawa diagram is a qualitative tool that helps in understanding the interactions and critical factors that impact the drug product performance,[12] as presented in Figure 1. The RPN score was calculated to get the clarity to screen out the potential factors against responses. The RPN score was studied through the Pareto charts, based on which the factors and the responses were screened. The RPN score was estimated using the QbD add-in version of JMP 18.0.2. The RPN was assessed and predicted for the selected factors against the responses. The variables are

assigned according to their risk priority, like low, medium, and high levels, with 1,3, and 9.

Risk Estimation Matrix

The FMEA tool was used effectively to calculate RPN and to select high-risk components. A REM table was constructed (Tables 3 and 4) to identify and express the relation between CQA vs QTPP and CQA vs CPP. This matrix demonstrates the potential hazards associated with material and process factors that may have a substantial influence on the product's CQAs. Each aspect's risk level was classified as low (1), medium (3), or high (9).

Design of Experiment (DOE) – Full Factorial Design

Full Factorial Design was selected for the optimization of microspheres. Based on the risk assessment study, the factors and the responses were selected. The factors were concentration of PLGA, Stirring Speed, PVA Concentration, and the responses were % Entrapment efficiency, Particle size (nm), and % *in vitro* drug release. The factors and their limits, and responses, goals, and their limits set in Full Factorial Design, are shown in Tables 5 and 6, respectively. A total of 10 runs of experiments were computed by selecting a full factorial design. All the experiments were performed and evaluated for the selected responses. The design matrix of formulation of dexibuprofen loaded microsphere has shown in Table 7. The full factorial design allowed simultaneous study of multiple factors and their interactions, offering comprehensive data for optimisation and robust statistical analysis with fewer experiments than other designs.

Preparation of the microspheres

PLGA microspheres were prepared using the oil-in-water (O/W) emulsion solvent evaporation method. The PLGA and dexibuprofen were dissolved in dichloromethane. The aqueous phase was PVA and 0.05 % of Tween 20. The oil phase, composed of DCM, was mixed and then added to 30 ml of the aqueous phase dropwise. Then the O/W emulsion was magnetically stirred at 500 to 1000 rpm and stirred for 4-6 h to evaporate the organic solvent. The dexibuprofen-loaded microspheres were collected by centrifugation at 4,000 rpm for 10 min. These microspheres were then rinsed three times with distilled water to eliminate any residual PVA.[14]

Evaluation of the CQAs

% Entrapment of efficiency (EE)

The EE of the dexibuprofen-loaded microspheres was determined using the centrifugation method. Samples were placed in centrifuge tubes and centrifuged at 1000 rpm for 10 min at room temperature to isolate the microspheres; the supernatant was collected separately. The supernatant was then diluted in ethanol and analysed for free drug content via UV spectroscopy. The % EE was calculated using the following formula.[15]

$$\%\ Entrapment\ efficiency = \frac{Total\ drug\ added\ in\ formulation - free\ drug}{Total\ drug\ added\ in\ formulation} \times 100$$

***% In vitro* drug release**

A paddle-type USP Type II dissolution test apparatus was used to conduct the *in-vitro* drug release study at 100 rpm. The dissolution media consisted of 900 ml of freshly prepared phosphate buffer of pH 6.8; the temperature was adjusted and maintained at 37 ± 0.5 °C. 5mL of each sample was taken out and replaced with an equal amount of freshly prepared medium that was kept at a precise temperature at predetermined intervals (0.5, 1, 2, 3, 4, 5, 6, 7…23, 24 h). The collected samples were filtered through No. 41 Whatman filter paper. The samples were analysed using a spectrophotometer at 222nm.[16]

Particle Size

The particle size was analyzed using the HORIBA SZ-100 (DLS); the sample was dispersed in a suitable medium, sonicated to ensure uniformity, and loaded into a clean cuvette. The measurements were performed at a fixed scattering angle of 90º and a controlled temperature of 25 ºC.[17]

Model Fit

Model fitting was the measure of the characteristics of the relationship between a response and a variable. Following the characterization of CQAs, the measured responses for each of the ten microsphere formulations were incorporated into the design and analysed to ascertain the statistical significance of the model fit. Variables with a p-value less than 0.05 were considered statistically significant.

Statistical Optimisation of Microspheres

The Dexibuprofen-loaded microspheres formulation was optimized using both numerical (Prediction profiler) and graphical methods (Counter profiler). In numerical optimisation, it showed a desirability score of 0.878, indicating a high level of prediction of the optimized factors and the corresponding responses. Further, a predicted optimized formulation was prepared and evaluated.

Characterization of Optimised Formulation

% yield of microsphere

The optimized microspheres were prepared and dried, and then weighed precisely. The formula given was then used to obtain the percentage yield below.[18]

$$\%Yield = mass\ of\ microsphere\ total\ weight\ x\ 100$$

Micromeritic Properties

The micromeritics properties of the microspheres were evaluated for flow characteristics, including bulk density, tapped density, Carr's index, Hausner's ratio, and angle of repose.[19]

Scanning Electron Microscopy

Scanning electron microscopy was studied using a ZEISS microscope. The microspheres were scanned with a focused electron beam, and the images were analyzed for surface morphology, shape, size, surface texture, and microsphere aggregation.

Particle Size

The particle size was measured using the same method mentioned above in the evaluation of the CQAs.

Zeta Potential

Zeta potential of the microspheres was measured using a Zetasizer electrophoretic light scattering. The sample was diluted with deionised water, loaded into a capillary cell, and analysed at 25 ± 1 °C. Results were recorded in millivolts (mV) to assess the surface charge and stability of microspheres.

% Entrapment Efficiency

The % Entrapment Efficiency was performed using the same method mentioned above in the evaluation of the CQAs.

Drug content

10 mg of equivalent to dexibuprofen microspheres was transferred into 100 ml of phosphate buffer 6.8, and extracted for dexibuprofen content by shaking occasionally for 1h, followed by sonication for 10 min. The samples were filtered and diluted appropriately with phosphate buffer pH 6.8, and drug content was determined by using the spectrophotometer at 222 nm.[20]

% *In vitro* drug release

The *in vitro* drug release study was performed using the same method as mentioned above in the evaluation of CQAs.

Drug Release Kinetics

To understand the drug release pattern of microspheres, different kinetic equations like zero order, first order, Higuchi Model, and Korsmeyer and Peppas were fitted to the data obtained from the *in vitro* drug release of dexibuprofen microspheres.[21] The release pattern was studied.

Pharmacodynamic Study – Analgesic activity

Eddy's hot plate method was used to assess the analgesic activity of dexibuprofen-loaded microspheres. Rats weighing 120-200 g and exhibiting a cut-off time of less than 15 seconds were chosen for testing and split into three groups of three rats each at random. Group I was given normal saline as a control; Group II was given 5 mg/kg of dexibuprofen as a standard; and Group III was given a formulation of dexibuprofen-loaded microspheres that contained the same amount of dexibuprofen as a 5 mg/kg dose. The rats were placed on a hot plate analgesiometer set to a temperature of 55 ± 1°C, where the surface was hot enough to cause discomfort without tissue damage. A cut-off time of 15 seconds was maintained to prevent paw or tissue injury. The reaction time, defined as the time in seconds until the rats began to jump or lick their paws, was measured. Reaction times for analgesic effect were recorded at 0.5, 1, 2, 4, 6, 8, 10, and 12 h. The percentage of thermal pain relief or protection against thermal pain was calculated using the formula, where Ta and Tb represent the mean reaction times of the treated and control groups, respectively.[22]

$$\%\ protection\ against\ thermal\ pain = \frac{Ta - Tb}{Tb} \times 100$$

Stability study

The optimized formulations were evaluated for their stability testing according to the ICH Q1A (R2) guidelines. The three batches of the formulations were filled into the appropriate containers, and stored at room temperature 25±2ºC. The formulation was periodically analysed for appearance and colour, drug content, % EE, and % *in vitro* drug release at intervals of 0 days, 2, 4, and 6 months. [23]

RESULTS AND DISCUSSION

Evaluation of the CQAs

% Entrapment Efficiency

Entrapment efficiency represents the proportion of drug successfully incorporated within the microspheres and ranged from 86% to 94.2% (Table 7). The results indicate that formulation and process variables had a notable influence on entrapment efficiency. An increase in PLGA concentration initially improved entrapment efficiency due to better polymeric encapsulation of the drug; however, at higher concentrations (45 mg), a slight reduction was observed, possibly due to increased viscosity leading to inefficient drug entrapment. PVA concentration also affected entrapment efficiency, where higher levels (2%) enhanced emulsion stability and reduced drug diffusion into the external phase, thereby improving drug entrapment. In contrast, an increase in stirring speed (1000 rpm) slightly decreased entrapment efficiency, likely due to enhanced shear forces causing drug loss into the continuous phase during emulsification.

% *In vitro* drug release

The *in vitro* release of the drug from PLGA microspheres is strongly influenced by formulation and process parameters, such as polymer concentration, stabiliser concentration, and stirring speed. The % *in vitro* drug release for all ten formulations ranged from 83.0 to 91.6 % in Table 7. An increase in PLGA concentration results in the formation of a thick and less porous polymeric matrix, thereby retarding drug diffusion and producing a controlled release profile, whereas lower PLGA levels promote faster release due to higher porosity. Similarly, increasing the concentration of PVA leads to the stabilisation of smaller, smoother microspheres with reduced surface-associated drug, which decreases the initial burst effect and prolongs drug release; in contrast, lower PVA levels often yield larger and more porous particles with relatively faster drug release. Stirring speed also plays a critical role, as higher agitation produces smaller microspheres with greater surface area, enhancing drug diffusion and accelerating release, while lower stirring speeds yield larger microspheres with reduced surface area, leading to slower release rates.

Particle Size

The particle size directly influences the drug release. The smaller the particle size, the higher the surface area and the more the bioavailability. The average particle size for all 10 formulations was found to be between 348.5 nm and 495.5 nm have shown in Table 7**.** Higher PLGA concentration leads to higher particle size due to the higher viscosity of the polymer. At lower PVA concentrations (1%), the stabilisation is less efficient, causing droplets to coalesce and form larger particles. When PVA concentration is increased to 2%, emulsification stabilisation improves, yielding a smaller particle size. Increasing the stirring speed generally reduces the particle size of microspheres due to higher shear forces breaking larger droplets into finer ones.

Model Fit

To verify the model fit, the data from each of the ten formulations was integrated into the design. Multiple regression models were fitted with the intercept set to zero to statistically analyze the expected data. % EE ($R^2 = 0.96$ and $p = 0.0358$), % *in vitro* Drug Release ($R^2 = 0.988$ and $p = 0.0053$), and particle Size (nm) ($R^2 = 0.96$ and $p = 0.0294$) are the R^2 and p-values derived from all responses in Figures 2, 3, and 4. The *p*-value for all 3 responses was statistically significant, which indicates goodness of fit.

Optimization of dexibuprofen-loaded microspheres

The dexibuprofen-loaded microspheres were optimized by a numerical approach using a prediction profiler showing a maximum global desirability score of 0.8788 (Figure 5), which suggests a strong prediction of optimized formulation. The optimized formulation was obtained at a PLGA concentration of 35 mg, PVA concentration of 1%, and a stirring speed of 500 rpm, achieving a desirability value of 0.878, indicating a good balance among all responses. The predicted responses, like particle size (~498.5 nm), entrapment efficiency (~92.78%), and drug release (~89.72%), demonstrate that the QbD approach effectively optimized the formulation within the design space. Overall, the profiler confirms that stirring speed is the most critical process parameter, while polymer and emulsifier concentrations play supportive roles in achieving the desired microsphere characteristics.

Characterisation of Optimised Microspheres

% Yield

The percentage yield for the prepared microspheres is 85.33%. The yield was influenced by PLGA concentration, PVA concentration, and emulsification conditions and filtration. The lower yields are attributed to losses during centrifugation, washing, and filtration of the microspheres. The results are represented in Table 8.

Micrometric Properties

The optimised formulations were evaluated for bulk density, tapped density, angle of repose, Carr's index, and Hausner's ratio, and the results were within the acceptable range, indicating excellent flow. This suggests that the microspheres have good flowability. The results are represented in Table 8.

Drug Content

The drug content of the optimised formulation was found to be 90.8 % (Table 8), which confirms the efficiency of the formulation process and the suitability of the excipients selected. The high drug content can be attributed to the optimized polymer concentration, efficient solvent evaporation, and the use of stabilizers (such as PVA in PLGA microspheres), which minimize drug leakage into the external phase.

% Entrapment Efficiency

The optimised microspheres achieved the entrapment efficiency of 91.65%. The entrapment efficiency of microspheres was affected by the concentration of PLGA, the % of PVA, and the stirring speed. PLGA provided a stable polymer matrix density to effectively encapsulate the drug, while the PVA level ensured good emulsion stability. A stirring speed of 500 rpm produced microspheres with a good particle size and better entrapment efficiency. The results are represented in Table 8.

Particle Size

The optimised formulation of microspheres showed a particle size of 495.5 nm, as illustrated in Figure 6. The decrease in the particle size increases the surface area of the microspheres. The increase in the surface area improves the drug release by enabling controlled release of the drug. Such a size range facilitates better cellular uptake and tissue penetration, making the formulation suitable for efficient drug delivery. Additionally, the particle size ensures good stability and uniformity, which are critical for reproducible therapeutic effects and scalable manufacturing. Hence, it provides better drug encapsulation, release kinetics, and biocompatibility for optimised performance in drug delivery.

Zeta Potential

A zeta potential of −26.3 mV (Figure 7) indicates that the microspheres possess a negative surface charge, providing sufficient electrostatic repulsion to reduce particle aggregation. This suggests moderate to good dispersion stability, confirming the formation of stable and well-dispersed microspheres.

Scanning Electron Microscopy

The SEM images revealed that the microspheres were predominantly spherical in shape with a relatively smooth surface, indicating the successful formation of discrete particles using the selected preparation method. The spherical morphology is advantageous for uniform packing, improved flow properties, and controlled drug release, as shown in Figure 8.

% *In vitro* Drug Release

The *in vitro* drug release of 88.91% from the microspheres indicates effective drug encapsulation and a controlled release profile. The high release suggests sustained availability of the drug over an extended period, likely governed by controlled diffusion through the PLGA matrix. It also implies minimal drug loss during formulation and good compatibility between the drug and polymer. Overall, these results confirmed the successful optimization of the microsphere formulation for controlled drug delivery. The results are presented in Table 9 and Figure 9.

Drug Release Kinetics

The optimised microsphere formulation exhibiting drug release best fitting the Zero Order Model with an R^2 value of 0.9884 in Figure 10 demonstrates a constant and predictable drug release rate over time, independent of drug concentration. This zero-order release is ideal for controlled drug delivery as it maintains steady therapeutic drug levels, minimising adverse effects. The non-Fickian diffusion mechanism indicates that drug release is governed by a combination of diffusion through the polymer matrix and polymer erosion. Such release kinetics confirm the successful optimisation of the microspheres to deliver drugs in a controlled manner, suitable for consistent dosing over extended periods.

Pharmacodynamic study- Analgesic Activity

The test group exhibited a significant increase in latency period compared to the control and standard groups (Table 10 and Figure 11). The prolonged reaction time observed in treated Wistar rats using Eddy's hot plate method confirms the analgesic activity of the microsphere formulation. These findings suggest its potential application in pain management

Stability Study

The stability studies of the microsphere's formulation were evaluated for the parameters such as appearance, % Entrapment Efficiency, drug content, % *in vitro* drug release at different time intervals, 0, 2, 4, and 6 months. The investigated parameters showed no significant changes, and no degradation in the formulation, and it was determined to be physically and chemically stable. The results are shown in Table 11.

CONCLUSION

Dexibuprofen-loaded microspheres were successfully developed and optimized using a Quality by Design (QbD) approach. The optimized microspheres, prepared by the emulsion solvent evaporation method, exhibited sustained drug release (88.91% over 24 h), following zero-order kinetics ($R^2 = 0.9884$) with a non-Fickian diffusion mechanism. Furthermore, the formulation demonstrated prolonged analgesic activity for over 12 h in Wistar rats, indicating effective controlled drug delivery. However, the study is limited to *in vitro* evaluations and animal models, lacking clinical validation in humans. Additionally, long-term stability, scalability, and reproducibility were not extensively investigated, and variability under physiological conditions, along with the safety profile of excipients, requires further evaluation. Overall, the optimized microspheres show promising potential for controlled drug delivery, with the ability to reduce dosing frequency and minimize gastrointestinal side effects associated with conventional therapy.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

API-Active Pharmaceutical Ingredient, **COX**-Cyclooxygenase, **CQA**-Critical Quality Attributes,

C**MA**-Critical Material Attributes, **CPP**-Critical Process Parameters, **DCM**-Dichloromethane, **DOE**-Design Of Experiment, **EE**-Entrapment Efficiency, **NSAIDs**-Non-Steroidal Anti-Inflammatory Drugs, **PLGA**-Poly Lactic Co Glycolic Acid, **PVA**-Poly Vinyl Alcohol, **QTPP**-Quality Target Product Profile, **QbD**-Quality by Design, **RA**-Risk Assessment, **REM**-Risk Estimation Matrix, **RPN**-Risk Priority Number, **SEM**-Scanning Electron Microscopy.

ETHICAL APPROVAL

The animal study protocol was reviewed and approved by the institutional Animal Ethics Committee (IAEC), Krupanidhi College of Pharmacy, Bangalore, Karnataka, India (Approval No: **KCP/IAEC/PCOL/QA/172/APR-2025**, dated: **05/04/2025**). All animal experiments were performed in accordance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

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Tables

Table 1: QTPP elements with their justification

QTPP elements		Target	Justification
Dosage form		Microspheres	To ensure the controlled release of the product.
Dosage strength		50mg	Reduced dose
Drug product quality profile	Appearance	White/off-white, free from clumps.	Ensures visual uniformity.
	Taste and Odour	Bland, acceptable taste	Patient compliance.
	Flow Properties	Good flow (Carr's index <20%	Ensures content uniformity and manufacturing
Route of administration		oral	Patient compliance

Product Quality attributes	Drug release rate, Particle size distribution, Encapsulation efficiency	To ensure the final quality of the product
Stability	Shelf life of ≥ 12 months	To ensure the microspheres maintain the quality attributes throughout the shelf life
Drug release	Controlled release profile over 24 h	To achieve a controlled release to maintain therapeutic levels

Table 2: CQAs elements with their justification

Critical Quality Attributes	Target	CQAs	Justification
Particle size	200 to 500 µm	Yes	Affects drug release rate, bioavailability, and stability.
Drug content	>90%	Yes	To obtain a therapeutic dose of API
Encapsulation efficiency	≥ 90%	Yes	Indicates the efficiency of the drug encapsulation process, impacting the overall yield.
In vitro drug release	Initial burst < 20%, 90-110% of QTPP.	Yes	To achieve a controlled release to maintain therapeutic levels.

Table 3: Risk estimation matrix of CQA vs QTPP

CQAs	Dosage type	Dosage Strength	Route of Administration	Cmax	Tmax	AUC	Product quality attributes	Drug release	Stability
Physical characteristics	1	3	1	1	1	1	3	1	3
Particle Size	3	9	9	9	9	9	3	9	9
%Entrapment Efficiency	1	9	1	3	3	3	9	3	3
% Yield	9	3	1	1	1	1	3	1	1
Drug content	1	9	1	3	3	3	9	3	9

% *In-vitro* Drug release	3	3	1	9	9	9	9	9	9

Table 4: Risk estimation matrix of CQA vs CPP

CQAs	Aqueous phase to Organic phase ratio	Stirring time	Polymer	Solvent	Stirring Speed	Temperature
Physical characteristics	1	3	3	1	3	3
Particle Size	9	9	9	9	9	1
% Entrapment Efficiency	9	3	9	9	9	1
% Yield	9	3	3	1	9	9
Drug content	3	3	9	1	1	1
% *In-vitro* drug release	9	1	9	3	3	3

Table 5: Factors and their limits of full factorial design

Variables	**Lower limit**	**Mid value**	**Upper limit**
PLGA concentration	35	40	45
PVA concentration	1	1.5	2
Stirring speed	500	750	1000

Table 6: Responses, goals, and their limits for Full Factorial Design

Responses	**Goal**	**Lower Limit**	**Upper Limit**
% Entrapment Efficiency	Maximize	90	100
% I*n vitro* Drug release	Maximize	85	95
Particle Size (nm)	Minimize	200	400

Table 7: Full factorial design Matrix for Formulation of Microspheres

Runs of experiment	PLGA conc.	PVA conc.	Stirring Speed	% entrapment efficiency	Particle Size(nm)	% *in vitro* drug release
1	45	1	500	89.1 ± 0.56	482.1	87.2 ± 0.63
2	35	1	500	92.1± 0.14	495.5	88.5 ± 0.49
3	45	2	1000	87.2 ± 0.61	364.2	83.9 ± 0.42
4	40	1.5	750	93 ± 0.22	405.2	91.6 ± 1.15
5	35	2	1000	94 ± 0.46	348.7	91.5 ± 0.64
6	45	2	500	86 ± 0.53	462.5	83.0 ± 0.28
7	35	1	500	92 ± 0.54	485.2	88.7 ± 0.33
8	35	1	1000	93 ± 0.28	398.7	90.5 ± 0.86
9	40	1.5	750	91 ± 0.70	406.8	88.3 ± 0.66
10	45	1	1000	88 ± 0.25	368.1	84.4 ± 0.74

Table 8: Characterisation of the Optimised Formulation

Evaluation Parameters		Average ± SD
Micromeritics properties	Tapped Density	0.526 ± 0.009
	Bulk Density	0.582 ± 0.005
	Carr's Index	9.15 ± 0.048
	Hausner Ratio	1.14 ± 0.014
	Angle of Repose	22.88 ± 0.658
Drug Content		90.8 ± 0.571
% Entrapment Efficiency		91.65 ± 0.024
% Yield		85.33± 0.471

Table 9: *In vitro* Drug Release Data of Optimised Formulation

Time in h	% drug release
0.5	4.08 ± 0.91
1	9.08 ± 0.97
2	13.26 ± 1.01
3	18.2 ± 1.10
4	25.87 ±1.11
5	34.26 ±1.01
6	40.34 ± 2.01
7	45.44 ± 1.78
23	85.68 ± 2.12
24	88.91 ± 2.01

Table 10: Eddy's Hot plate reaction time in seconds

Time in hrs	Control	Standard	Test
0.5	8.4±0.6	9.5±0.1	8.1±0.1
1	7.6±0.8	12.7±1.4	10.2±0.5
2	8.3±1.1	15.3±2.8	11.3±0.4
4	8±0.1	15.8±3.2	12.5±0.2
8	8.7±0.9	8.7±0.1	14.0±0.26
10	8.4±0.5	7.6±0.7	15.5±0.9
12	8.5±0.2	7.4±0.2	16.3±0.1

Table 11: Stability Data for the optimised Formulation

Time	Appearance	Drug content	% Entrapment efficiency	% *in vitro* drug release
0 days	Free-flowing powder	90.80	91.65	88.91
2 months	Free-flowing powder	90.59	91.30	88.84
4 months	Free-flowing powder	90.25	91.02	88.65
6 months	Free-flowing powder	90.01	90.93	88.28

FIGURES

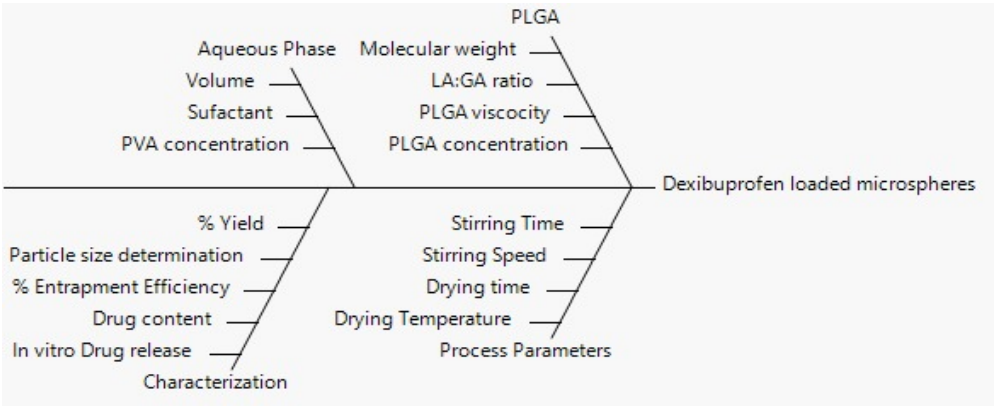

Figure 1: Ishikawa Diagram for the Dexibuprofen-Loaded Microspheres

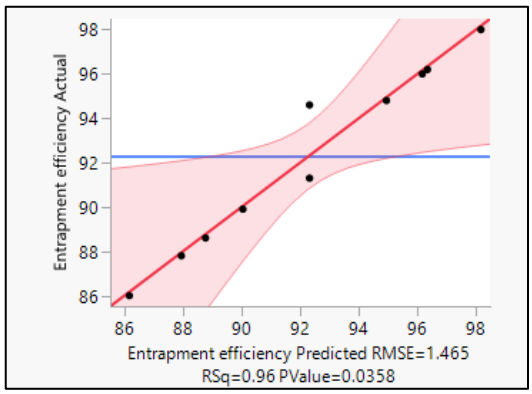

Figure 2: Actual Vs predicted plots obtained % Entrapment Efficiency

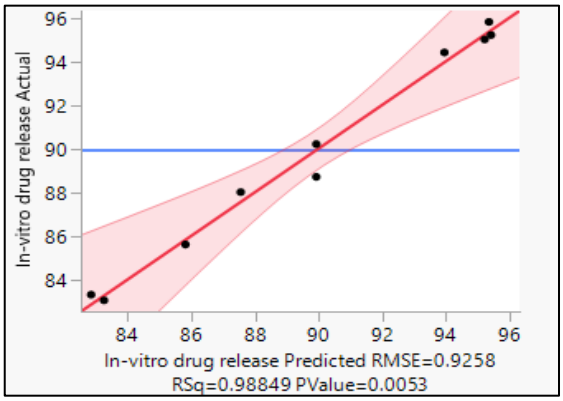

Figure 3: Actual Vs predicted plots obtained for % *in vitro* drug release

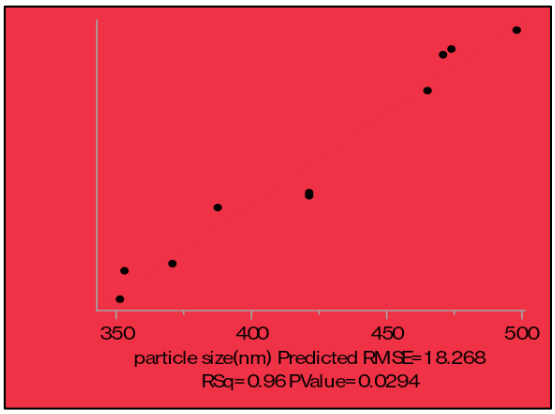

Figure 4: Actual Vs predicted plots obtained for Particle Size (nm)

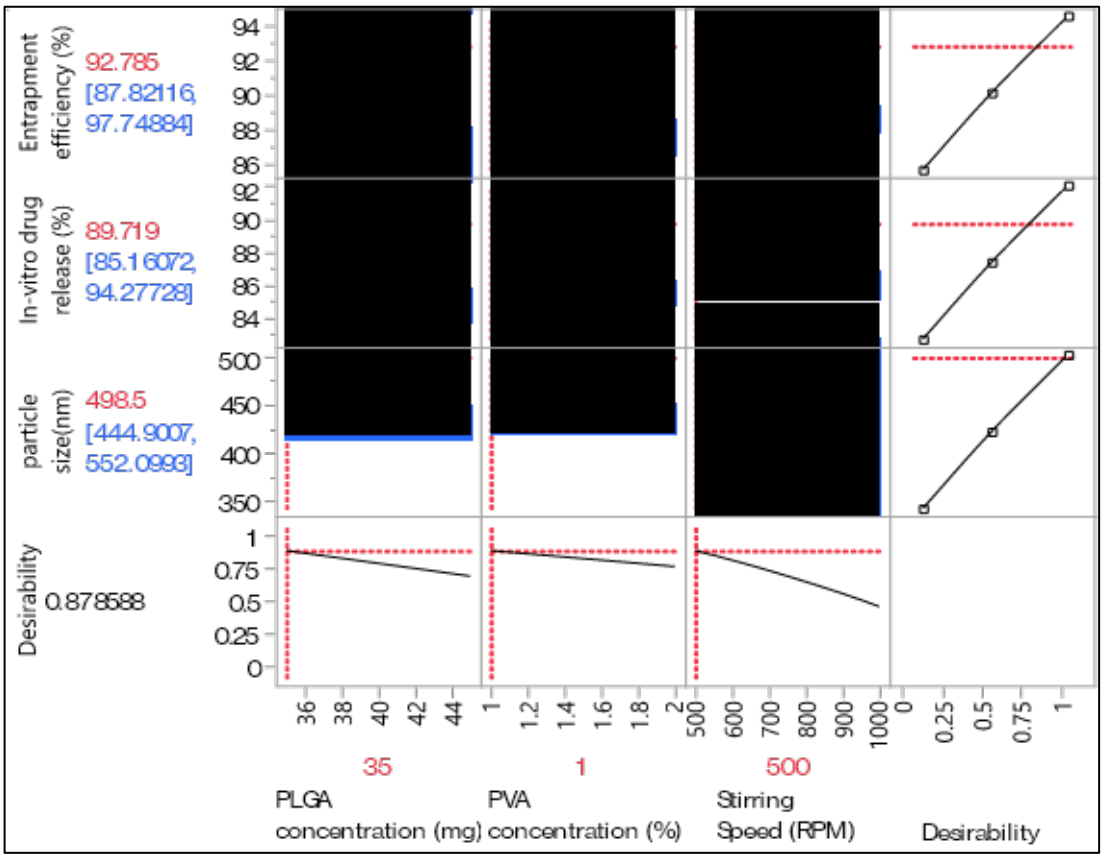

Figure 5: Optimization of Dexibuprofen-loaded microspheres using prediction profiler

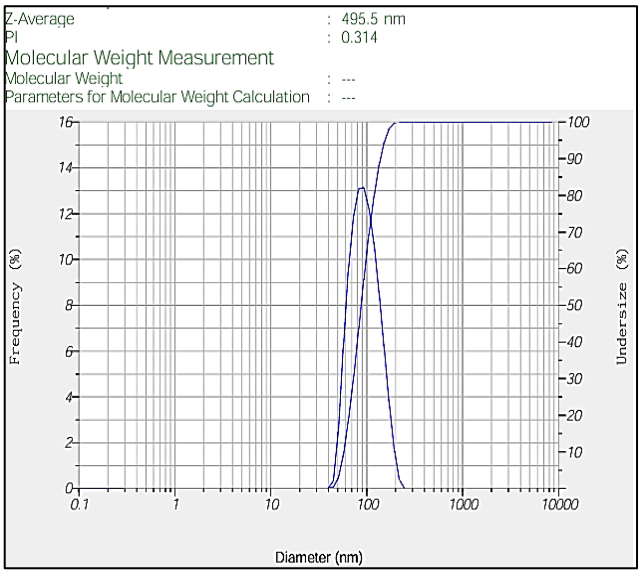

Figure 6: Particle size of the Optimised Dexibuprofen-loaded microspheres

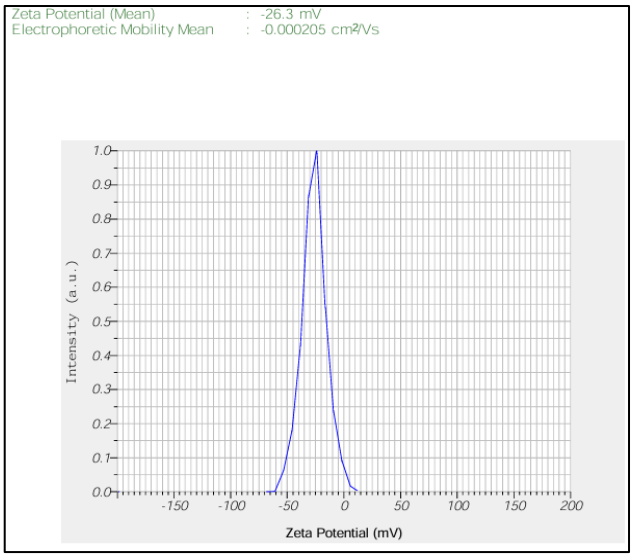

Figure 7: Zeta Potential of Optimised Dexibuprofen-loaded microspheres

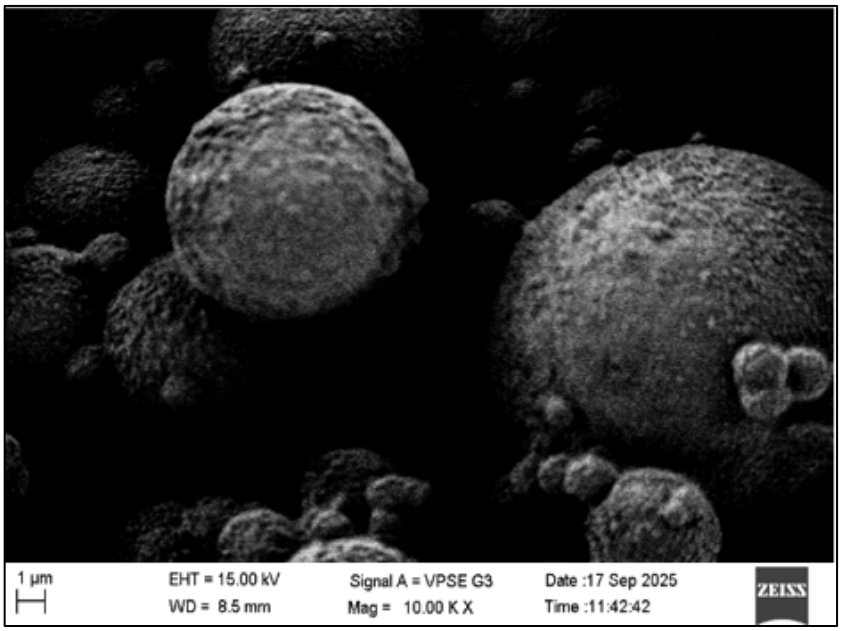

Figure 8: SEM of the Optimised Dexibuprofen-loaded microspheres

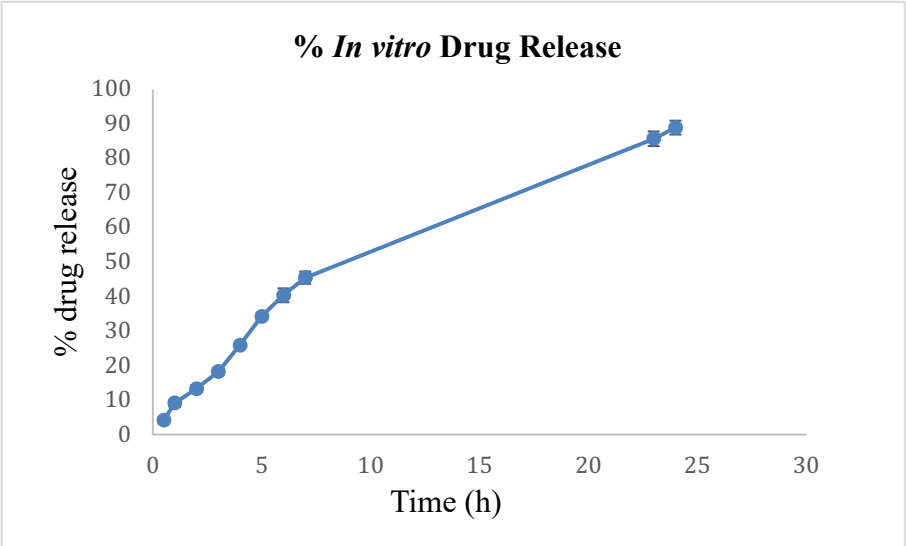

Figure 9: % *In vitro* Drug Release of optimised Formulation

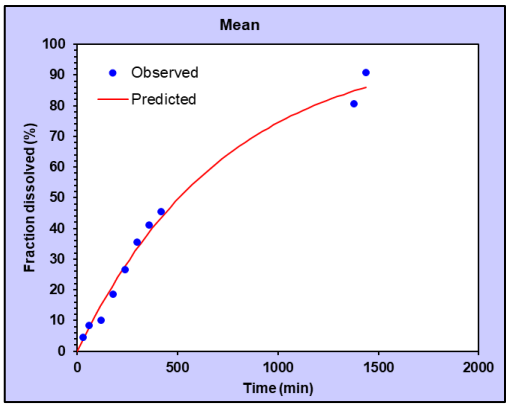

Figure 10: Zero-order release kinetics of dexibuprofen-loaded microspheres

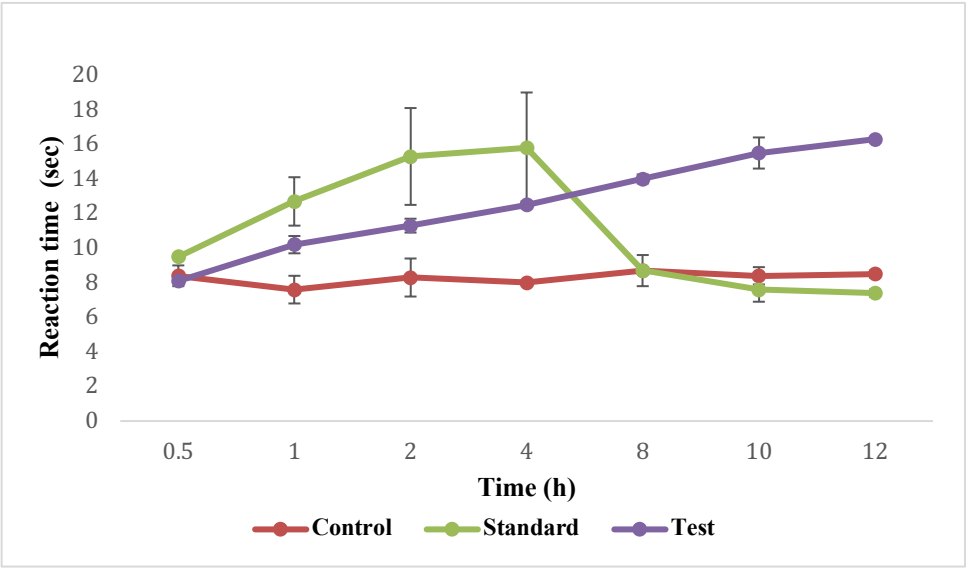

Figure 11: Graphical representation of Mean Reaction Time on Eddy's hot plate

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