Pharmaceutical Sciences 2024: Navigating the Future of Drug Discovery and Development
November 2024

Exploring Albumin Microspheres for Enhanced Delivery of Ketoprofen: Formulation And Assessment

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ABSTRACT

The aim of this study was to develop and evaluate albumin-based microspheres loaded with ketoprofen (KP), a nonsteroidal anti-inflammatory drug (NSAID), to achieve sustained drug release and reduce dosing frequency. KP, an NSAID with a short half-life, requires frequent administration, which can be addressed by formulating a sustained-release delivery system. Albumin microspheres of KP were prepared using the solvent evaporation method with bovine serum albumin (BSA) as the polymer. Six formulations were developed with varying BSA proportions and evaluated for multiple parameters, including FTIR, SEM, particle size, yield, drug content, entrapment efficiency, in vitro dissolution, release kinetics, DSC, and XRD. FTIR analysis confirmed no interaction between KP and BSA, while SEM imaging verified the spherical shape of the KP microspheres with a normal size distribution. The optimized formulation achieved a maximum drug entrapment efficiency of 96.50%. In vitro dissolution tests demonstrated sustained KP release, influenced by BSA concentration. Release kinetics followed zero-order kinetics, with diffusion-controlled release as per the Higuchi model, while Korsmeyer-Peppas model indicated a non-Fickian release mechanism. The DSC and XRD analysis showed a reduction in KP crystallinity within the microspheres, suggesting effective drug encapsulation. This study highlights the potential of albumin microspheres in achieving a sustained release of NSAIDs, with applications for enhanced therapeutic efficacy and patient compliance.

Keywords: Sustained release, albumin microspheres, ketoprofen, drug encapsulation.

I. INTRODUCTION

Microspheres are defined as small, solid, spherical particles ranging from 1 to 1000 μm in diameter, made from a variety of polymeric, waxy, or protective materials. These materials may include synthetic biodegradable polymers, such as polylactic acid and polyglycolic acid, and modified natural products, including starches, gums, proteins, fats, and waxes. Natural polymers, like albumin and gelatin, offer advantages as they are derived from living organisms, are readily available, cost-effective, eco-friendly, and can undergo various chemical modifications. Microspheres possess a high surface-to-volume ratio, making them ideal for applications in drug delivery systems due to

their colloidal properties and enhanced interfacial activity. In recent years, microspheres have gained significant attention for their potential to improve drug stability, enhance handling, and deliver drugs in a controlled, sustained-release manner.

The pharmaceutical industry has explored microspheres for diverse applications such as converting oils and liquids into solids, taste masking, improving drug stability, and enhancing flow properties of powders. Moreover, microspheres allow for safe handling of toxic substances and offer improved solubility for water-insoluble drugs. Specifically, sustained-release microsphere formulations reduce dose dumping compared to traditional drug delivery systems, making them highly suitable for delivering nonsteroidal anti-inflammatory drugs (NSAIDs) such as ketoprofen (KP). KP, known for its anti-inflammatory properties, has a short half-life, necessitating frequent administration, which can lead to gastrointestinal (GI) side effects. A sustained-release form, particularly using albumin microspheres, has the potential to alleviate these issues, improve patient compliance, and reduce adverse effects.

Natural polymers, especially albumin, are widely researched for drug delivery due to their non-toxic, biodegradable, and non-antigenic properties. Albumin, which constitutes a significant portion of plasma proteins, has shown promising results in sustained-release systems and as a carrier for tumor-targeted therapies due to its selective accumulation in solid tumors. Research has highlighted its utility in targeting drugs to inflamed joints in rheumatoid arthritis, as albumin binds to the synovium similarly to tumor cells, thereby increasing drug circulation half-life and minimizing distribution to non-target tissues. This specificity reduces adverse effects and improves treatment efficiency, demonstrating albumin’s versatility in biomedical applications.

Various techniques for preparing albumin microspheres include solvent evaporation, spray drying, wax coating, coacervation, and chemical cross-linking. Each technique offers distinct benefits based on parameters such as particle size, drug encapsulation efficiency, and desired release profile. For example, solvent evaporation is suitable for both aqueous and non-aqueous systems, making it a flexible option, while spray drying provides a rapid and single-step approach for heat-sensitive materials. Each method is adaptable based on the required particle size, drug loading, and release duration, making albumin microspheres an attractive option for sustained and controlled drug release.

Despite their advantages, there are some limitations associated with NSAID-loaded albumin microspheres. For instance, modified release can vary based on gut transit time, food intake, and the integrity of the dosage form. Additionally, the larger size of sustained-release formulations may pose ingestion difficulties, and prolonged release may lead to GI toxicity. Nevertheless, the therapeutic benefits of albumin microspheres in delivering NSAIDs outweigh these challenges, positioning them as a promising technology for sustained drug delivery with potential for enhanced patient outcomes and compliance.

II. LITRETURE REVIEW

Murali Mohan Babu GV et al. formulated a controlled-release version of Diclofenac Sodium using a gum karaya-chitosan complex via the coacervation method. In vivo studies revealed a sustained blood level pattern for the microcapsules, comparable to that of a commercial controlled-release formulation, highlighting the potential for extended drug action through natural polymer complexes.

Thakkar H et al. prepared Celecoxib-loaded albumin microspheres using BSA through an emulsification chemical cross-linking method. In vitro release studies indicated that the microspheres could sustain drug release for approximately six days. Blood kinetic studies further revealed that Celecoxib-loaded albumin microspheres provided prolonged circulation compared to a standard Celecoxib solution.

Tabassi SAS et al. developed albumin microspheres encapsulating Propranolol Hydrochloride using an emulsion-internal phase stabilization technique. The resulting BSA microspheres had diameters ranging from 1 to 25 μm, and drug release studies indicated that approximately 70% of Propranolol Hydrochloride was released after 12 hours, suggesting a controlled release mechanism.

Rajamanickam D et al. formulated albumin microspheres containing Aceclofenac aimed at providing sustained release. Egg albumin was employed as a release-retarding agent, resulting in discrete, spherical microspheres with an average particle size of 99.6 μm. The optimized formulation exhibited significant analgesic and anti-inflammatory activity, underlining the utility of albumin microspheres as effective release-retarding agents.

Deore BV et al. designed sustained-release microspheres of Ketoprofen (KP) via a quasi-emulsion solvent diffusion method. The KP microspheres incorporated aerosol as an inert dispersing carrier to

enhance dissolution rate and Eudragit RS as a retarding agent to control release rate. The particle size was within the 104-108 μm range, and drug content was between 62-96%. The study concluded that an increase in Eudragit concentration led to a decreased release rate of KP, affirming its effectiveness in sustaining drug release.

III. RESEARCH METHODOLOGY

Bovine serum albumin microspheres were prepared using a solvent evaporation method, adapted from Tabassi et al. with slight modifications. A 1% w/v solution of BSA was prepared in distilled water, and Ketoprofen (KP) was dispersed into this solution. This mixture was then dispersed in 100 ml of sunflower oil containing 0.5 ml of Tween 80 in a 200 ml beaker, and stirred at 600 rpm for 30 minutes. After stirring, the microspheres were centrifuged, washed several times with petroleum ether and acetone, then dried at 50°C, and stored in a desiccator.

Evaluation of Ketoprofen-Loaded Albumin Microspheres

1. Drug-Polymer Interaction (FTIR) Study

FTIR spectroscopy was performed on a Fourier transform infrared spectrophotometer (IR Affinity-1, Shimadzu, Japan). Drug and potassium bromide pellets were prepared by compressing the powders at 20 psi for 10 minutes on a KBr press, and spectra were scanned in the wave number range of 4000-600 cm^{-1}. FTIR analysis was conducted on KP, a physical mixture of KP and polymer, KP microspheres, and blank microspheres.

2. Surface Morphology (SEM)

Scanning electron microscopy (SEM) was used to determine particle size distribution, surface topography, and morphology. SEM analysis was conducted using a JEOL JSM T-330A scanning microscope (Japan). Dry KP microspheres were placed on a brass stub, coated with an ion sputter, and imaged by random scanning.

3. Frequency Distribution Analysis

The average particle size of KP microspheres was measured using optical microscopy with a stage micrometer. A small amount of KP microspheres was spread on a clean glass slide, and the size of 300 microspheres was recorded for each batch. A histogram was created to present the particle size distribution.

4. Percentage Yield

The percentage yield was calculated to evaluate the efficiency of the production method, using the formula:

Percentage Yield=(Practical YieldTheoretical Yield)×100\text{Percentage Yield} = \left(\frac{\text{Practical Yield}}{\text{Theoretical Yield}} \right) \times 100Percentage Yield=(Theoretical YieldPractical Yield)×100

5. Percentage Drug Entrapment Efficiency (PDE)

The drug entrapment efficiency was calculated using the formula:

PDE=(Practical Drug ContentTheoretical Drug Content)×100\text{PDE} = \left(\frac{\text{Practical Drug Content}}{\text{Theoretical Drug Content}} \right) \times 100PDE=(Theoretical Drug ContentPractical Drug Content)×100

6. Calibration Curve of Ketoprofen in Phosphate Buffer (pH 7.0)

A standard stock solution of Ketoprofen was prepared by dissolving 10 mg of KP in phosphate buffer (pH 7.0) in a 100 ml volumetric flask. Aliquots were taken to create drug concentrations ranging from 2.0 to 10.0 µg/ml, and absorbances were measured at 258 nm to validate the calibration curve.

7. Practical Drug Content Analysis

KP microspheres equivalent to 100 mg of KP were dissolved in 100 ml of phosphate buffer (pH 7.0) and allowed to dissolve completely overnight. After filtering and diluting to a concentration of 10 µg/ml, absorbance was measured at 258 nm, and the percentage of KP was calculated.

In Vitro Dissolution Studies

Calibration Curve of Ketoprofen

The calibration curve of Ketoprofen in phosphate buffer (pH 7.0) was constructed as previously described.

Dissolution Studies

Dissolution studies were conducted using a USP XXIII dissolution apparatus in phosphate buffer (pH 7) for 12 hours, with a maintained temperature of 37 ± 0.5°C and a basket rotation speed of 50 rpm. Samples were withdrawn at 1-hour intervals and replaced with fresh dissolution media. The amount of KP released was measured by UV absorption at 258 nm.

Kinetics of Drug Release

The in vitro release data were fitted to various kinetics models:

- Cumulative Percentage Drug Release vs. Time (Zero Order)
- Log Cumulative Percentage Drug Retained vs. Time (First Order)

- Cumulative Percentage Drug Release vs. √T (Higuchi's Model)
- Log of Cumulative Percentage Drug Release vs. Log Time (Peppas Model)

Differential Scanning Calorimetry (DSC)

The physical state of KP in the microspheres was analyzed by Differential Scanning Calorimetry (Mettler-Toledo star 822e system, Switzerland), with thermograms recorded at a scanning rate of 10°C/min over a temperature range of 25–300°C.

X-Ray Power Diffractometry (XRD) Study

XRD analysis of KP, the KP-polymer physical mixture, and KP microspheres was performed using a Joel JDX-8030 diffractometer with graphite crystal monochromator (Cu-Kα) radiation to determine the physical state of KP in the microspheres.

IV. RESULT AND DISCUSSIONS

The present study reports a novel attempt to prepare microspheres of the non-steroidal anti-inflammatory drug (NSAID) Ketoprofen (KP) using a natural polymer, Bovine Serum Albumin (BSA), as a carrier for improved treatment of rheumatoid arthritis, pain, inflammation, and related conditions. The microspheres of KP were prepared by the solvent evaporation method utilizing BSA. Various evaluation parameters were assessed with the aim of obtaining a sustained release of KP.

In this work, a total of six formulations were prepared, with detailed compositions shown in Table 4.3. The prepared KP microspheres were subjected to Fourier Transform Infrared (FTIR) spectroscopy, Scanning Electron Microscopy (SEM), particle size and size distribution analysis, percentage yield, drug content, drug entrapment efficiency, in vitro dissolution studies, release kinetics analysis, Differential Scanning Calorimetry (DSC), and X-Ray Powder Diffractometry (XRD).

Preformulation Studies

The solubility of KP in 10 mg/10 ml of solvent was assessed, revealing that KP is freely soluble in ethanol, chloroform, acetone, and ether, and soluble in benzene and strong alkali, but practically insoluble in water at 20°C. The melting point of KP was found to be 94°C, complying with IP standards, thus indicating the purity of the obtained drug sample. A solution of KP with a concentration of 10 µg/ml was prepared in ethanol, and the UV spectrum was taken using a

Shimadzu (UV-1800) double beam spectrophotometer, scanning between 200 to 400 nm. The maxima obtained in the graph were considered as λmax for the drug KP.

Evaluation of Ketoprofen Microspheres

Drug-Polymer Interaction (FTIR) Study

FTIR spectra were obtained for KP, the physical mixture of KP and polymer, KP microspheres, and blank microspheres (Fig. 5.1 to 5.4). The characteristic peaks of KP were compared with those of the physical mixture of KP and polymer. The findings are summarized in Table 5.1. The characteristic peaks found in KP, the physical mixture, and the formulations suggest that there was no chemical interaction between KP and the polymer, indicating that the characteristic bands of KP were not affected after successful loading.

Surface Morphology of Ketoprofen Microspheres (SEM)

The surface morphology of the KP microspheres was studied using SEM. The SEM photographs of various formulations are shown in Fig. 5.5. The surface smoothness of the KP microspheres increased with higher polymer concentration, confirmed by SEM. At lower polymer concentration (1:1), a rough, wrinkled surface of KP microspheres was observed (Fig. 5.5 KP1), whereas at a higher polymer concentration (1:6), smooth KP microspheres were obtained (Fig. 5.5 KP6).

Frequency Distribution Analysis

As the KP-to-polymer ratio increased, the mean particle size of KP microspheres also increased (Table 5.2 and Fig. 5.7). This significant increase may be attributed to the higher viscosity of the droplets, which could be due to the increased concentration of the polymer solution. The KP microspheres exhibited a size range of 10 to 240 µm (Table 5.3 and Fig. 5.8) with a normal frequency distribution.

Percentage Yield

The percentage yield for KP microspheres was calculated as follows:

- **Formulation KP1:** 50.91%
- **Formulation KP2:** 66.46%
- **Formulation KP3:** 79.10%
- **Formulation KP4:** 83.86%
- **Formulation KP5:** 90.40%
- **Formulation KP6:** 96.99%

These results are presented in Table 5.5.

Percentage Drug Entrapment Efficiency

Entrapment efficiency was observed to increase with higher polymer concentration. The results indicate a proper distribution of KP in the microspheres, with deviations within acceptable limits. The percentage of drug content in the formulations ranged from 12.32% to 20.42%, while the percentage entrapment efficiency ranged from 26.00% to 96.50%. The results are detailed in Table 5.5 and represented in histograms in Fig. 5.9. A maximum entrapment efficiency of 96.50% was achieved in KP microspheres prepared using BSA, further confirming that drug entrapment was proportional to the KP: polymer ratio and the size of the microspheres.

In Vitro Dissolution Studies

The in vitro performance of KP microspheres demonstrated prolonged and sustained release of KP. The results of the dissolution studies for formulations KP1 to KP6 are shown in Table 5.6 and Fig. 5.10. The findings indicated that the amount of drug released decreased with an increase in polymer concentration. Formulation KP1 showed a maximum cumulative drug release of 90.54%, while formulation KP6 exhibited a minimum release of 59.18%.

Release Kinetics of Ketoprofen Microspheres

The plots of cumulative percentage drug release versus time, cumulative percent drug retained versus root time, log cumulative percent drug retained versus time, and log cumulative percent drug release versus log time were drawn and are graphically represented in Fig. 5.11 to 5.14 and Tables 5.7 to 5.10. The slopes and the regression coefficients of determination (r^2) are listed in Table 5.11. The coefficients of determination indicated that the release data were best fitted with zero-order kinetics. The Higuchi equation explains the diffusion-controlled release mechanism. The diffusion exponent 'n' values of the Korsemeyer-Peppas model were found to be in the range of 0.5 to 1 for the KP microspheres prepared with BSA, indicating a non-Fickian drug release mechanism.

Differential Scanning Calorimetry (DSC)

To confirm the physical state of KP in the microspheres, DSC of KP, the physical mixture of KP and polymer, KP microspheres, and blank microspheres were performed (Fig. 5.15 to 5.18). The DSC trace of KP showed a sharp endothermic peak at 94.96°C, its melting point. The physical mixture of KP and polymer, as well as blank microspheres, displayed similar thermal behavior at 93.04°C, indicating no interaction between KP and the polymer in the solid state. The absence of the endothermic peak of KP at 94.96°C in the DSC of KP microspheres suggests that KP exists in an amorphous or disordered crystalline phase as a molecular dispersion in the polymeric matrix.

X-Ray Powder Diffractometry (XRD)

To confirm the physical state of KP in the microspheres, powder X-ray diffraction studies of KP, the physical mixture of KP and polymer, and KP microspheres were performed. The X-ray diffractograms (Fig. 5.19 to 5.21) indicated that KP is still present in its lattice structure in the physical mixture, whereas it appears completely amorphous within the KP microspheres. This may be due to the conditions used to prepare the KP microspheres, which led to the complete drug amorphization.

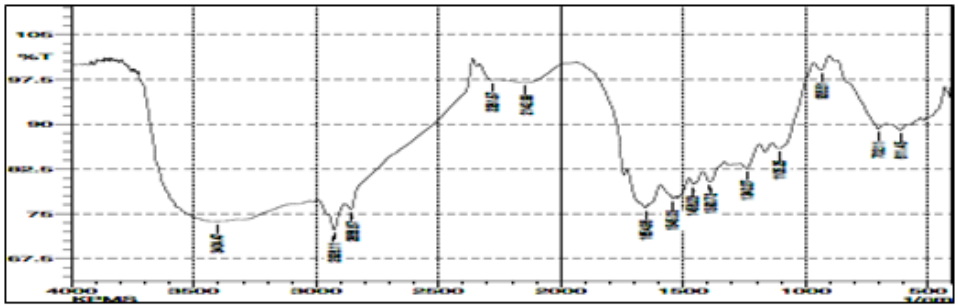

Figure 1: FTIR Spectrum of Ketoprofen microspheres using Bovine serum albumin of the optimized formulation

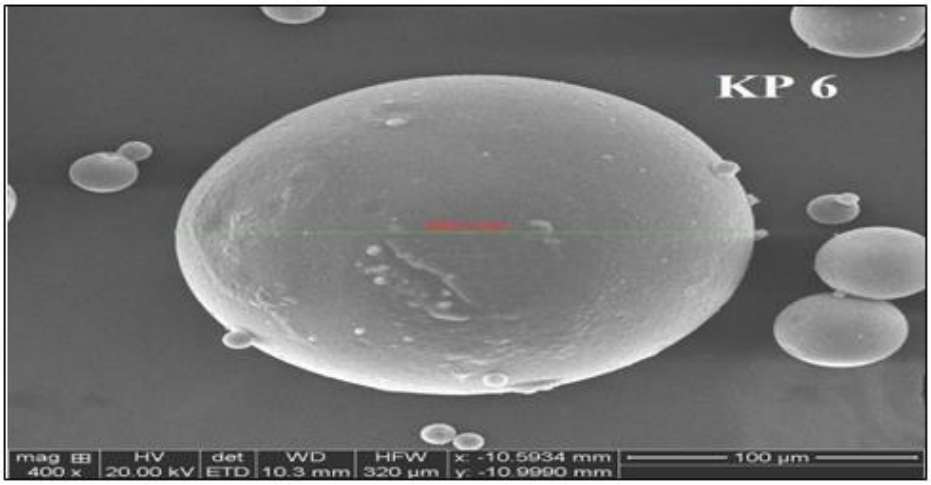

Figure 2: SEM of Ketoprofen microspheres using Bovine serum albumin of the optimized formulation

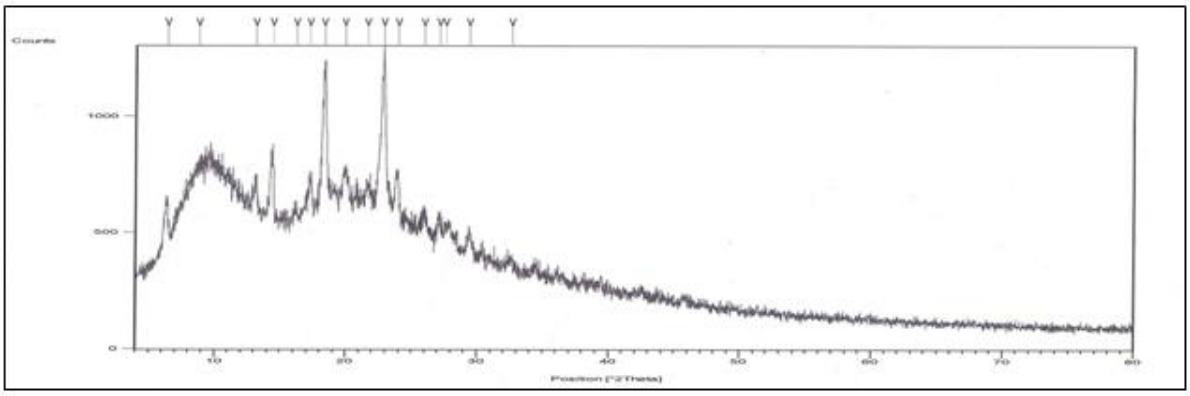

Figure 3: XRD of Ketoprofen microspheres using Bovine serum albumin of the optimized formulation

Formulation	Zeroorder	Firstorder	HiguchiMatrix	Peppasplot	
				r^2value	'n'value
KP1	0.9927	0.9227	0.9758	0.9881	0.7082
KP2	0.9930	0.9512	0.9741	0.9868	0.7084
KP3	0.9959	0.9671	0.9764	0.9928	0.7634
KP4	0.9971	0.9773	0.9764	0.9937	0.7952
KP5	0.9990	0.9820	0.9761	0.9978	0.8705
KP6	0.9472	0.8867	0.8587	0.9162	0.8992

Table 1 : Regression co-efficient (r^2) values of different kinetic models and diffusion exponent(n)of Peppas model for Ketoprofen microspheres

V. CONCLUSION

Preformulation studies, including solubility, melting point, and UV analysis of ketoprofen (KP), complied with IP standards. The FTIR spectra revealed no interaction between the polymers and KP, indicating compatibility of all the polymers used with KP. Surface smoothness of the KP microspheres increased with higher polymer concentration, as confirmed by SEM analysis. Additionally, as the drug-to-polymer ratio increased, the mean particle size of the KP microspheres also increased, resulting in a normal frequency distribution. Entrapment efficiency improved with an

increase in polymer concentration. The results suggest a proper distribution of KP within the microspheres, with deviations falling within acceptable limits. The study further indicated that the amount of drug release decreased with increasing polymer concentration, demonstrating that the in vitro performance of KP microspheres showed prolonged and sustained release of the drug. The coefficient of determination indicated that the release data were best fitted with zero-order kinetics. The Higuchi equation explains the diffusion-controlled release mechanism, while the diffusion exponent 'n' values from the Korsmeyer-Peppas model ranged from 0.5 to 1, indicating Non-Fickian drug transport through the KP microspheres prepared with BSA. DSC and XRD data indicated that KP remained in its lattice structure in the physical mixture, while it was completely amorphous inside the KP microspheres. This amorphization may be attributed to the conditions used in preparing the KP microspheres. The melting points of KP, estimated by open capillaries, were consistent with the DSC data. Overall, this study demonstrates that promising sustained-release microspheres of KP can be developed using the solvent evaporation technique with natural polymers such as BSA.

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