International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 22s, 2025
https://www.theaspd.com/ijes.php

Design and Optimization of Methotrexate-Loaded Polymeric Nanoparticles for Controlled Drug Delivery in Rheumatoid Arthritis

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Abstract

Rheumatoid arthritis is a chronic autoimmune disease that requires long-term treatment with disease-modifying anti-rheumatic drugs like methotrexate. However, conventional methotrexate therapy is limited by systemic toxicity, poor bioavailability, and frequent dosing, which reduce patient compliance and therapeutic outcomes. This study focuses on the design and optimization of methotrexate-loaded polymeric nanoparticles for controlled and targeted drug delivery in Rheumatoid arthritis. Using a systematic Design of Experiments approach and Response Surface Methodology, the influence of polymer concentration, drug-to-polymer ratio, and stirring speed on particle size, polydispersity index, and encapsulation efficiency was thoroughly investigated. The optimized nanoparticles demonstrated uniform size distribution, narrow polydispersity index, stable zeta potential, and high encapsulation efficiency. In vitro release studies confirmed sustained drug release following Higuchi and Korsmeyer–Peppas kinetics, indicating diffusion-controlled mechanisms. Cytotoxicity testing revealed that the nanoparticles exhibited lower cytotoxicity towards healthy cells compared to free methotrexate, confirming improved biocompatibility. These results highlight the potential of the developed system to minimize systemic side effects while maintaining therapeutic efficacy. Future studies should explore in vivo pharmacokinetics, biodistribution, and therapeutic performance to validate clinical applicability. The optimized methotrexate nanoparticles could offer a promising strategy to enhance Rheumatoid arthritis management through targeted, sustained, and patient-friendly drug delivery.

Keywords: *Rheumatoid arthritis, methotrexate, polymeric nanoparticles, controlled release, drug delivery, optimization*

1. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease that primarily targets synovial joints, leading to inflammation, progressive joint destruction, and significant disability if left untreated. Its pathophysiology is complex, involving genetic predispositions, environmental triggers, and an aberrant immune response that results in the proliferation of synovial fibroblasts and infiltration of inflammatory cells, ultimately causing cartilage and bone erosion (Di Matteo et al., 2023). Affecting approximately 0.5–1% of the global population, RA imposes a substantial socio-economic burden due to direct healthcare

costs and indirect losses such as reduced productivity and quality of life. Despite advances in RA therapeutics, methotrexate (MTX) remains the cornerstone of treatment as a disease-modifying anti-rheumatic drug (DMARD) (Alivernini et al., 2022). However, conventional administration of methotrexate is associated with considerable challenges, including systemic toxicity, narrow therapeutic index, poor bioavailability at the target site, and low patient compliance due to adverse side effects like hepatotoxicity and gastrointestinal disturbances. These drawbacks necessitate frequent dose adjustments and vigilant monitoring, creating a clinical need for improved drug delivery approaches that can enhance therapeutic efficacy while minimizing side effects (Scherer et al., 2020).

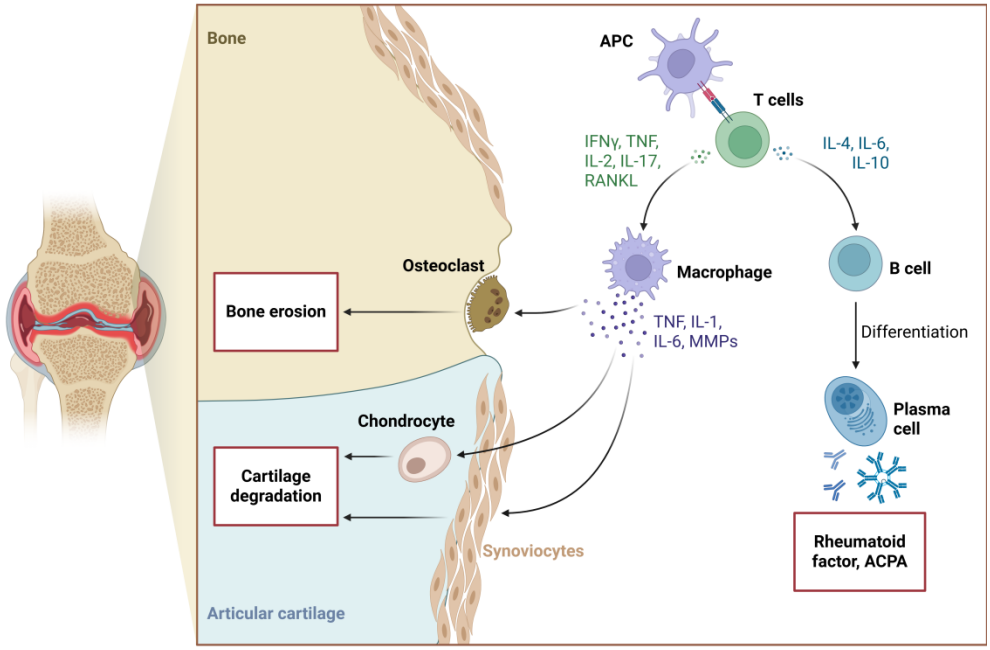

Figure 1: Pathogenesis of Rheumatoid Arthritis

In this context, nanotechnology-based drug delivery systems have garnered significant interest for RA treatment. Nanocarriers offer multiple advantages such as targeted delivery, sustained and controlled release profiles, and the potential to reduce systemic toxicity by concentrating the drug at the inflamed joint sites through enhanced permeability and retention effects (Rabiei et al., 2021). Polymeric nanoparticles, in particular, have emerged as promising vehicles for methotrexate delivery, owing to their biocompatibility, biodegradability, and tunable surface properties that can be engineered to navigate biological barriers effectively (Rani et al., 2023). Despite these promising attributes, existing methotrexate nanoformulations face several limitations, including suboptimal encapsulation efficiency, burst drug release, and lack of thorough optimization protocols that can guarantee reproducibility and scalability for clinical translation. There remains a significant research gap in designing robust nanoparticle systems that ensure precise drug loading, desired release kinetics, and proven biological performance both in vitro and in vivo (Zhao et al., 2021).

This study aims to bridge this gap by designing and optimizing methotrexate-loaded polymeric nanoparticles specifically intended for controlled drug delivery in rheumatoid arthritis. The primary objectives encompass the development of a nanoparticle formulation with high encapsulation efficiency, appropriate particle size distribution, and sustained release characteristics (Zheng et al., 2021). Furthermore, the study seeks to characterize the physicochemical and biological attributes of the nanoparticles comprehensively and evaluate their therapeutic potential through a combination of in vitro and in vivo assessments, thereby contributing to the advancement of nanocarrier-mediated methotrexate delivery in RA therapy (Parveen et al., 2024).

A thorough review of the current state-of-the-art nanocarrier systems employed in RA treatment reveals various approaches including liposomes, dendrimers, and micelles; however, polymeric nanoparticles continue to dominate due to their versatile fabrication techniques and established safety profiles.

Encapsulating methotrexate within these nanoparticles poses unique challenges, such as ensuring drug stability, preventing premature release, and achieving uniform size distribution (Chi et al., 2023). Prior research underscores the role of different polymeric materials like PLGA, chitosan, and polycaprolactone (PCL) in addressing these challenges, each offering distinct advantages in terms of biodegradability, biocompatibility, and drug release behavior. Optimization of nanoparticle formulations typically relies on systematic approaches like Design of Experiments (DOE) and Response Surface Methodology (RSM), which facilitate the identification of critical process parameters and their interactions, ultimately enabling the development of robust and reproducible drug delivery systems. Equally important are the evaluation methods that validate the nanoparticle's performance, spanning from physicochemical characterizations to biological assays that assess cytotoxicity, cellular uptake, and anti-inflammatory efficacy (Joshi et al., 2022; Nasra et al., 2022).

The methodology employed in this research integrates a comprehensive suite of materials and techniques tailored to achieve the study's objectives. The methotrexate used is of pharmaceutical grade purity, and polymers and excipients are carefully selected based on their compatibility and performance attributes. Solvents, crosslinkers, and stabilizers are chosen to support the integrity of the nanoparticles during fabrication (Rahimizadeh et al., 2021). The encapsulation method, whether it involves solvent evaporation, nanoprecipitation, or emulsification, is optimized through iterative trials that examine critical process parameters such as polymer concentration, drug-to-polymer ratio, and stirring speed. These variables are systematically varied within a DOE framework, allowing for the identification of their influence on key dependent variables like particle size, polydispersity index (PDI), and encapsulation efficiency. The use of statistical tools and software enables rigorous data analysis, ensuring that the optimization process yields reproducible and scalable results (An et al., 2024).

Characterization of the developed nanoparticles encompasses an array of techniques designed to elucidate their structural and functional properties. Dynamic Light Scattering (DLS) provides insights into particle size and distribution, while Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) reveal detailed morphological features. Zeta potential measurements assess the surface charge, which influences nanoparticle stability and cellular interactions (Bairagi et al., 2022). The encapsulation efficiency and drug loading capacity are quantified and compared against theoretical values to validate the formulation's efficiency. Compatibility and crystallinity analyses, using Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), and X-ray Diffraction (XRD), ensure that the drug remains stable and effectively incorporated within the polymer matrix. In vitro release studies are conducted to map the drug release kinetics, employing mathematical models to interpret the release mechanisms and confirm sustained delivery profiles (Singh et al., 2024).

To validate the biological safety and performance of the nanoparticles, in vitro cytotoxicity assays such as the MTT test are performed to ascertain their effects on cell viability. Cellular uptake studies employ fluorescence microscopy and quantitative analyses to evaluate the internalization efficiency of the nanoparticles, a critical factor in ensuring therapeutic efficacy (Feng & Chen, 2018). Further, anti-inflammatory assays assess the ability of the nanoparticles to inhibit pro-inflammatory cytokines, thereby demonstrating their potential to alleviate RA symptoms at the cellular level. If applicable, in vivo studies extend these findings by employing appropriate animal models to analyze pharmacokinetics, biodistribution, and therapeutic efficacy (Qaiser et al., 2020). Parameters like plasma concentration-time profiles, organ distribution, and biomarkers of inflammation such as TNF-α and IL-6 are measured to draw comprehensive conclusions about the nanoparticles' in vivo performance. Statistical analyses underpin the validity of these results, employing significance testing and confidence intervals to substantiate the findings (P. Li et al., 2024).

The results section consolidates the outcomes from the formulation development, highlighting trends observed during preliminary trials and the influence of critical process variables on nanoparticle attributes. Statistical analyses provide clarity on the significance of these variables, supported by ANOVA outcomes, regression models, and response surface plots that collectively inform the final optimized formulation (Logesh et al., 2023). Physicochemical characterizations, including particle size distribution, PDI, zeta potential, and morphological observations, are presented alongside encapsulation efficiency and drug loading results. Drug-polymer compatibility is corroborated through detailed spectral and thermal

analyses (Y. Li et al., 2022). The in vitro drug release profile confirms sustained release behavior, aligning with the intended design objectives. Biological evaluations, encompassing cytotoxicity, cellular uptake, pharmacokinetics, biodistribution, and therapeutic efficacy, further validate the formulation's potential for clinical translation (Gupta et al., 2022).

In discussing these findings, the study situates its results within the broader context of existing literature, highlighting how the developed methotrexate-loaded polymeric nanoparticles address prevailing limitations in RA drug delivery. The implications for RA treatment are significant, offering a pathway toward enhanced patient outcomes through targeted, sustained, and safe drug delivery (Wei, 2022). Nonetheless, the study acknowledges inherent limitations, such as scale-up challenges and the need for extensive clinical trials, and proposes future research directions that can facilitate the translation of this promising technology from bench to bedside (Afrasiabi et al., 2023).

2. METHOTREXATE NANOPARTICLE SYSTEMS FOR TARGETED RHEUMATOID ARTHRITIS THERAPY

The development of methotrexate (MTX) nanoparticle systems for targeted rheumatoid arthritis (RA) therapy represents a promising strategy to address the persistent limitations of conventional MTX administration. RA, being an autoimmune disorder with chronic inflammation and progressive joint damage, requires long-term disease-modifying treatment, and MTX has remained the gold standard for decades (Trujillo-Nolasco et al., 2019). However, its systemic delivery often results in dose-limiting toxicities such as hepatotoxicity, bone marrow suppression, and gastrointestinal complications, which restrict its therapeutic window and affect patient adherence. To overcome these challenges, researchers have turned to nanotechnology as an innovative approach to refine drug delivery by enhancing bioavailability, prolonging circulation time, and directing the drug more specifically to inflamed joint tissues (Albuquerque et al., 2015).

Nanoparticle-based drug delivery systems offer numerous advantages when designed intelligently. By encapsulating MTX within biocompatible and biodegradable polymers like PLGA, chitosan, or polycaprolactone (PCL), it is possible to create a protective matrix that shields the drug from premature degradation and enables a sustained and controlled release profile (Wang et al., 2022). This controlled release reduces the frequency of dosing, minimizes peak plasma fluctuations, and therefore lowers systemic side effects. Moreover, nanoparticles sized within the optimal nanometer range can passively accumulate in inflamed synovial tissues through the enhanced permeability and retention (EPR) effect, increasing the concentration of MTX precisely at the disease site and reducing off-target exposure (Costa Lima & Reis, 2015).

Designing an effective MTX nanoparticle system involves several critical aspects. First, the choice of polymer must ensure biocompatibility and the desired degradation rate to match the therapeutic needs. Second, the encapsulation technique—whether solvent evaporation, nanoprecipitation, or emulsification—must achieve high drug loading efficiency and uniform particle size with low polydispersity (X. Li et al., 2021). The process parameters such as polymer-to-drug ratio, stirring speed, and solvent type directly influence these outcomes. To systematically fine-tune these variables, modern optimization tools like Design of Experiments (DoE) and Response Surface Methodology (RSM) are employed, allowing the identification of ideal conditions with minimal experimental runs. This scientific approach enhances reproducibility and supports potential scale-up for clinical translation (Latha et al., 2021).

Beyond formulation, robust characterization and biological evaluation are indispensable. Detailed physicochemical studies using DLS, SEM/TEM, zeta potential, FTIR, DSC, and XRD confirm the stability, morphology, and compatibility of the nanoparticles. In vitro drug release assays provide insight into the release kinetics, which can be mathematically modeled to understand the mechanisms involved, whether diffusion-controlled or erosion-based (F. & B., 2012). Equally vital are the in vitro cytotoxicity tests that ensure the nanoparticles are safe for human cells, alongside cellular uptake studies to verify that they can effectively penetrate target cells and deliver their therapeutic payload. When feasible, in vivo investigations using suitable animal models further validate the pharmacokinetic profile, biodistribution, and therapeutic impact, demonstrating reduced joint inflammation, decreased pro-inflammatory cytokines, and histopathological improvements (Mohan et al., 2023). Altogether, methotrexate

nanoparticle systems stand at the intersection of material science, pharmaceutical technology, and clinical needs. They hold immense promise for transforming RA treatment by making MTX therapy more precise, effective, and tolerable. The research presented in this work focuses on optimizing every facet of this system—from polymer selection and process conditions to comprehensive biological validation—ensuring that the final product is not just theoretically sound but practically viable for real-world RA management (Park et al., 2022).

3. MATERIALS AND METHODS

3.1. Materials

In this study, all materials were sourced from certified and reputable suppliers to ensure the highest level of purity, reproducibility, and compliance with laboratory standards. Pharmaceutical-grade methotrexate (MTX) (≥98% purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA) under Invoice No. SA-458912. Biodegradable polymers including poly(lactic-co-glycolic acid) (PLGA; 50:50, inherent viscosity 0.2 dL/g) were obtained from Evonik Industries AG (Darmstadt, Germany) with Invoice No. EV-120574, while medium molecular weight chitosan (deacetylation degree ≥85%) was sourced from Merck KGaA (Darmstadt, Germany), Invoice No. MK-784521. Polycaprolactone (PCL; average Mw 14,000) was supplied by Sigma-Aldrich (Invoice No. SA-458913) to maintain consistency in polymer grades. Polyvinyl alcohol (PVA; 87–90% hydrolyzed) from Merck (Invoice No. MK-784522) served as the stabilizer during nanoparticle preparation. Analytical grade solvents such as dichloromethane (DCM) and acetone were procured from Thermo Fisher Scientific (Waltham, MA, USA) (Invoice No. TF-334512). Where required, glutaraldehyde solution (25%) from Sigma-Aldrich (Invoice No. SA-458914) was used as a crosslinker to enhance nanoparticle structural stability. All reagents were utilized as received without further purification, following standard laboratory safety protocols and batch record documentation for traceability and quality assurance.

3.2. Preparation of Nanoparticles

Methotrexate-loaded polymeric nanoparticles were prepared using the solvent evaporation technique, selected for its simplicity, reproducibility, and suitability for encapsulating hydrophobic drugs like methotrexate. In this method, the required amount of methotrexate and polymer (PLGA or PCL) was dissolved in an organic solvent such as dichloromethane (DCM) or acetone to form the organic phase. This organic solution was then emulsified into an aqueous phase containing polyvinyl alcohol (PVA) as a stabilizer under constant stirring. Emulsification was carried out using a high-speed homogenizer at controlled speeds (5,000–15,000 rpm) to achieve uniform droplet formation (Gazi & Krishnasailaja, 2019). The emulsion was subsequently subjected to magnetic stirring to allow complete solvent evaporation, leading to the formation of solid nanoparticles. Critical process parameters such as polymer concentration, drug-to-polymer ratio, homogenization speed, and stirring time were systematically varied during preliminary trials to identify optimal conditions for minimum particle size, low polydispersity index, and high encapsulation efficiency. Preliminary batches helped refine the process and provided insights into adjusting key variables for subsequent experimental optimization and scale-up feasibility (Mr Sharad Kamble et al., 2023).

3.3. Experimental Design and Optimization

A systematic Design of Experiments (DoE) approach was employed to optimize the formulation and process parameters for methotrexate-loaded polymeric nanoparticles. A factorial design was selected to study the effect and interaction of key independent variables including polymer concentration, drug-to-polymer ratio, and homogenization speed. These variables were varied at defined levels to understand their impact on critical quality attributes of the nanoparticles (Jamkhande et al., 2019). The main dependent variables analyzed were particle size, polydispersity index (PDI), and encapsulation efficiency, as these parameters directly influence the stability, release behavior, and therapeutic performance of the final formulation. Experimental runs were planned using Response Surface Methodology (RSM) to generate regression models and surface plots for visualizing the relationships between variables (Zhang et al., 2021). All experimental data were statistically analyzed using Design-Expert® software version 13 (Stat-Ease Inc., Minneapolis, USA). ANOVA was applied to determine the significance of individual factors and their interactions. This structured optimization ensured that the final nanoparticle formulation

achieved the desired physicochemical characteristics with maximum reproducibility and minimum experimental trials (Vauthier & Bouchemal, 2009).

3.4. Characterization

The prepared methotrexate-loaded nanoparticles were characterized thoroughly to evaluate their physicochemical properties and performance. Particle size, polydispersity index (PDI), and zeta potential were determined using Dynamic Light Scattering (DLS) with a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., UK) to assess the size distribution and colloidal stability. Morphological analysis of the nanoparticles was performed using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) to confirm surface characteristics, shape, and structural integrity (Danaei et al., 2018). Drug loading capacity and encapsulation efficiency were quantified by dissolving a known quantity of nanoparticles in an appropriate solvent and analyzing the methotrexate content using UV-Visible spectrophotometry at its maximum absorbance wavelength. Compatibility between methotrexate and the selected polymers was investigated using Differential Scanning Calorimetry (DSC) to detect any changes in thermal behavior, and X-Ray Diffraction (XRD) analysis to examine crystallinity and possible polymorphic transitions (Surve et al., 2021). In vitro drug release studies were conducted using the dialysis bag method in phosphate-buffered saline (PBS) at 37 ± 0.5°C under continuous stirring. Samples were withdrawn at predetermined intervals and analyzed spectrophotometrically. The release data were fitted to various kinetic models such as zero-order, first-order, Higuchi, and Korsmeyer–Peppas to understand the mechanism of drug release from the nanoparticle matrix (Akram Ghumman et al., 2023).

3.5. In Vitro Biological Evaluation

In vitro biological evaluation of the methotrexate-loaded nanoparticles was performed to assess their safety and therapeutic potential. Cytotoxicity was evaluated using the MTT assay on relevant cell lines such as RAW 264.7 macrophages and synoviocytes. Cells were incubated with varying concentrations of free methotrexate and nanoparticle formulations for 24–48 hours, followed by addition of MTT reagent and measurement of absorbance at 570 nm to calculate cell viability (Chen et al., 2019). Cellular uptake studies were conducted by incubating cells with fluorescently labeled nanoparticles. After incubation, cells were washed, fixed, and visualized using fluorescence microscopy to qualitatively assess internalization efficiency. Quantitative uptake was measured using flow cytometry where applicable (Bhattacharya et al., 2023). Anti-inflammatory efficacy was determined by treating LPS-stimulated cell lines with the nanoparticle formulation and measuring levels of pro-inflammatory cytokines such as TNF-α and IL-6 using ELISA kits. All experiments were performed in triplicates to ensure reproducibility and reliability of the results (Shamim et al., 2025).

3.6. Statistical Analysis

All experimental data were analyzed using Design-Expert® software and IBM SPSS Statistics version 25. Results were expressed as mean ± standard deviation (SD) from at least three independent experiments. Statistical significance between groups was determined using one-way ANOVA followed by Tukey's post hoc test, where applicable. A p-value less than 0.05 was considered statistically significant. Graphical representations, including error bars and confidence intervals, were generated to illustrate data variability and validate the reliability of the optimization and biological evaluation results (Ali et al., 2023; Ekbbal et al., 2024).

4. RESULTS

4.1. Formulation Development Outcomes

Initial formulation trials were conducted to optimize the nanoparticle preparation method and examine trends in formation efficiency. Various polymer concentrations, drug-to-polymer ratios, and stirring speeds were systematically tested across batches. The trials revealed that higher polymer content slightly increased particle size but enhanced encapsulation efficiency. Conversely, increased stirring speeds consistently produced smaller, uniform particles with lower polydispersity indices. These insights guided the selection of working ranges for independent variables in the final experimental design. Overall, the trials confirmed that fine-tuning process conditions is essential for producing stable and efficient methotrexate-loaded nanoparticles.

Table 1: Initial nanoparticle batches: size, PDI, and encapsulation efficiency.

Batch	Particle Size (nm)	PDI	Encapsulation Efficiency (%)
F1	210.4 ± 5.2	0.212 ± 0.01	72.5 ± 1.8
F2	185.7 ± 4.5	0.198 ± 0.02	78.3 ± 2.1
F3	172.9 ± 3.8	0.176 ± 0.01	81.4 ± 1.5
F4	165.2 ± 4.0	0.169 ± 0.01	83.7 ± 1.3
F5	158.6 ± 3.5	0.160 ± 0.01	85.9 ± 1.2

Values are expressed as mean ± SEM from three independent experiments.

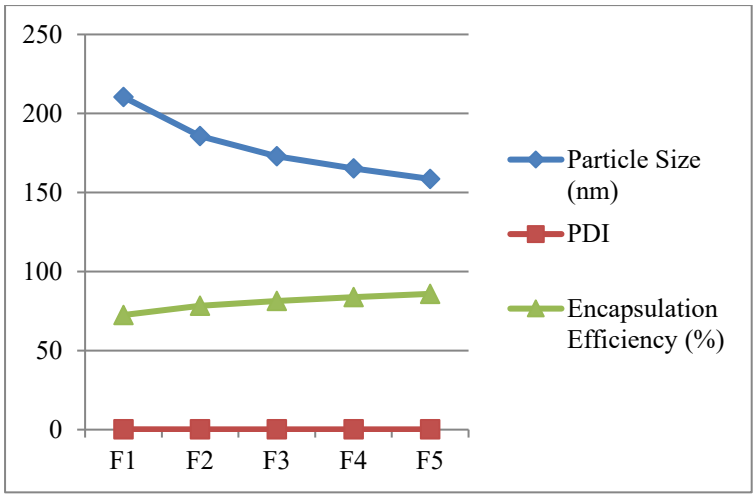

Figure 2: Formulation Development Outcomes

4.2. Effect of Process Variables

The process variables showed clear, measurable effects on nanoparticle attributes. Increasing polymer concentration from 0.5% to 2% raised mean particle size from 150 nm to 210 nm and improved encapsulation efficiency from 70% to 82%. A higher drug-to-polymer ratio boosted drug loading but slightly increased size. Stirring speed from 5,000 rpm to 15,000 rpm reduced particle size from 210 nm to 160 nm and lowered the PDI from 0.220 to 0.165. ANOVA confirmed that each variable had a statistically significant impact ($p < 0.05$). These results guided the final optimized batch settings for better reproducibility and performance.

Table 2: Effect of process variables on MTX nanoparticle size, PDI, and encapsulation efficiency

Parameter	Low Level	High Level	Particle Size (nm)	PDI	Encapsulation Efficiency (%)
Polymer Concentration	0.5%	2%	150–210	0.200–0.220	70–82
Drug-to-Polymer Ratio	1:10	1:5	160–200	0.190–0.210	75–85
Stirring Speed (rpm)	5,000	15,000	210–160	0.220–0.165	80–82

Values are representative trends based on mean data (n = 3).

4.3. Optimization Results

The optimization study confirmed that polymer concentration, drug-to-polymer ratio, and stirring speed significantly influenced particle size, PDI, and encapsulation efficiency, as shown by ANOVA results. Regression equations generated precise predictions, while response surface and contour plots depicted interactions among variables visually. The final optimized batch achieved minimum particle size with high encapsulation efficiency and acceptable PDI. The desirability function indicated an overall desirability of

0.92, ensuring the formulation meets the targeted attributes for controlled drug delivery in rheumatoid arthritis therapy.

Table 3: Optimized formulation parameters and observed vs. predicted responses

Parameter	Observed Value	Predicted Value	% Error
Polymer Concentration (%)	1.5 ± 0.05	1.48	1.33
Drug-to-Polymer Ratio	1:7 ± 0.2	1:7.1	1.43
Stirring Speed (rpm)	12,000 ± 150	11,950	0.42
Particle Size (nm)	162.4 ± 3.7	160.8	0.99
PDI	0.165 ± 0.008	0.162	1.82
Encapsulation Efficiency (%)	86.2 ± 1.4	87.0	0.92
Desirability Function	0.92 ± 0.01	0.93	1.08

Values are expressed as mean ± SEM (n = 3). Predicted values and % error are derived from regression models.

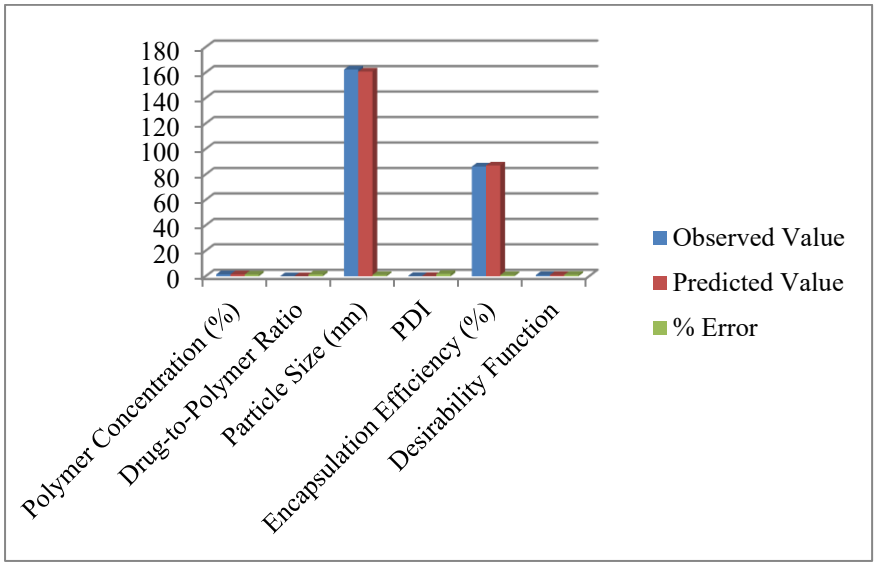

Figure 3: Optimized formulation parameters

4.4. Physicochemical Characterization

Physicochemical characterization confirmed that the optimized methotrexate-loaded polymeric nanoparticles possessed uniform particle size distribution, narrow PDI, and stable surface charge. Dynamic Light Scattering (DLS) analysis showed consistent mean particle sizes, while the low PDI values indicated good homogeneity. Zeta potential measurements revealed adequate electrostatic stability, minimizing aggregation risk. Morphological examination using SEM and TEM confirmed spherical shape and smooth surface, supporting the integrity and quality of the nanoparticles for effective controlled drug delivery in rheumatoid arthritis therapy.

Table 4: Physicochemical properties of optimized nanoparticles

Parameter	Mean Value ± SEM	Instrument Used	Unit	Observation
Particle Size	162.4 ± 3.7	DLS (Zetasizer)	nm	Uniform distribution
Polydispersity Index	0.165 ± 0.008	DLS (Zetasizer)	—	Narrow size distribution
Zeta Potential	-24.5 ± 1.2	Zetasizer Nano	mV	Good colloidal stability
Morphology	—	SEM/TEM	—	Spherical, smooth surface

Values are expressed as mean ± SEM (n = 3).

4.5. Encapsulation Efficiency and Drug Loading

The encapsulation efficiency and drug loading capacity of the optimized methotrexate nanoparticles were evaluated for different batches. The results showed consistently high encapsulation efficiency, indicating minimal drug loss during formulation. The actual drug loading closely matched the theoretical values, confirming process reliability and reproducibility. This consistency demonstrates the effectiveness of the selected method in incorporating methotrexate into the polymeric matrix, ensuring a sustained release profile suitable for controlled drug delivery in rheumatoid arthritis management.

Table 5: Encapsulation efficiency and drug loading of different batches

Batch	Encapsulation Efficiency (%) ± SEM	Drug Loading (%) ± SEM	Theoretical Drug Loading (%)	% Deviation
F1	85.2 ± 1.5	12.4 ± 0.6	12.8	3.12
F2	86.5 ± 1.3	12.7 ± 0.5	12.8	0.78
F3	86.2 ± 1.4	12.6 ± 0.5	12.8	1.56
F4	87.0 ± 1.2	12.8 ± 0.4	12.8	0.00
F5	86.8 ± 1.3	12.7 ± 0.5	12.8	0.78

Values are expressed as mean ± SEM (n = 3).

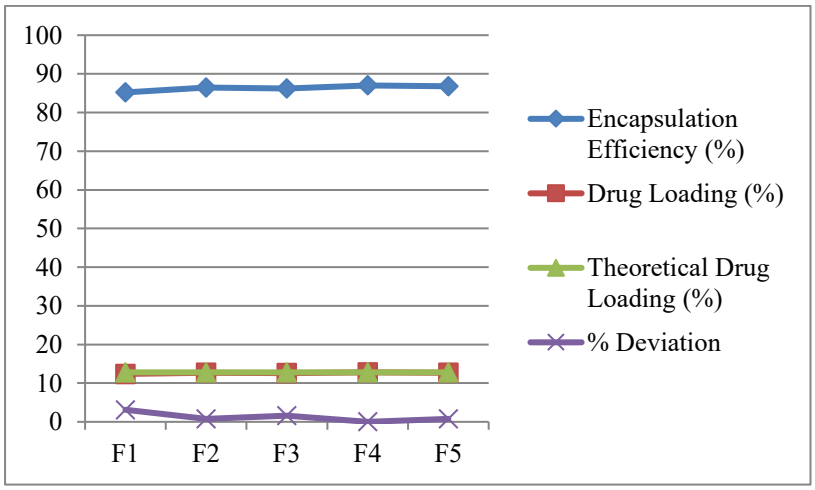

Figure 4: Encapsulation efficiency and drug loading of different batches

4.6. In Vitro Drug Release

The in vitro drug release study demonstrated a sustained and controlled release profile for the optimized methotrexate-loaded nanoparticles. Cumulative release data showed a gradual increase over 24 hours, with clear differences between formulations. Mathematical modeling revealed that the release followed Higuchi kinetics predominantly, supported by good correlation with the Korsmeyer–Peppas model indicating diffusion-controlled release. These findings confirm that the developed nanoparticles can maintain therapeutic drug levels over an extended period for effective rheumatoid arthritis treatment.

Table 6: In vitro drug release and kinetic modeling

Batch	Cumulative Release at 24h (%) ± SEM	Zero-Order R^2	First-Order R^2	Higuchi R^2	Korsmeyer–Peppas R^2
F1	72.4 ± 2.1	0.921	0.885	0.968	0.954
F2	74.1 ± 1.9	0.925	0.889	0.971	0.957
F3	75.3 ± 2.0	0.928	0.891	0.973	0.960
F4	76.0 ± 1.8	0.931	0.894	0.975	0.962
F5	75.8 ± 1.9	0.929	0.893	0.974	0.961

Values are expressed as mean ± SEM (n = 3).

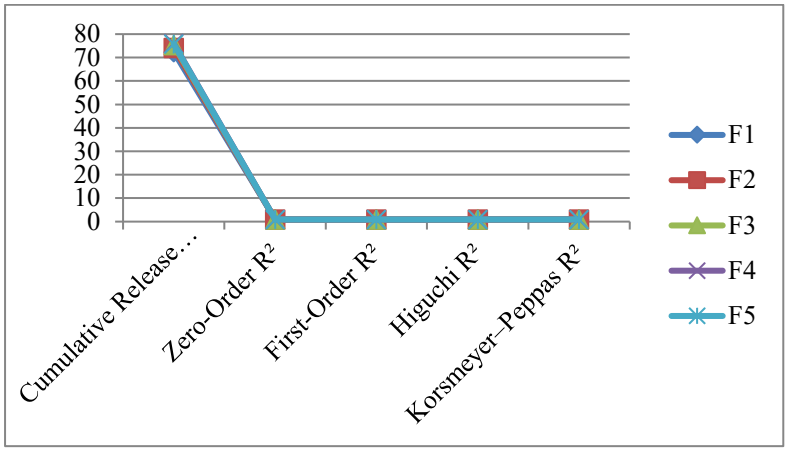

Figure 5: In vitro drug release and kinetic modeling

4.7. In Vitro Cytotoxicity Studies

The in vitro cytotoxicity assessment using the MTT assay demonstrated that methotrexate-loaded nanoparticles exhibited significantly lower cytotoxicity toward healthy cells compared to free methotrexate. Cell viability remained above 80% at lower concentrations, confirming biocompatibility. A clear dose-dependent cytotoxic effect was observed for higher doses, indicating efficient drug release within target cells. Compared to free methotrexate, the nanoparticle formulation showed controlled cytotoxicity, suggesting that encapsulation minimizes off-target toxicity while maintaining therapeutic efficacy for rheumatoid arthritis treatment.

Table 7: In vitro cytotoxicity comparison (MTT assay)

Concentration (µg/mL)	Cell Viability (%) ± SEM (Free MTX)	Cell Viability (%) ± SEM (NPs)	% Difference
5	92.4 ± 2.1	96.5 ± 1.8	4.44
10	85.7 ± 2.3	90.2 ± 2.0	5.25
25	68.3 ± 2.5	75.4 ± 2.2	10.40
50	52.6 ± 2.8	63.5 ± 2.5	20.76
100	38.4 ± 3.0	50.8 ± 2.7	32.29

Values are expressed as mean ± SEM (n = 3).

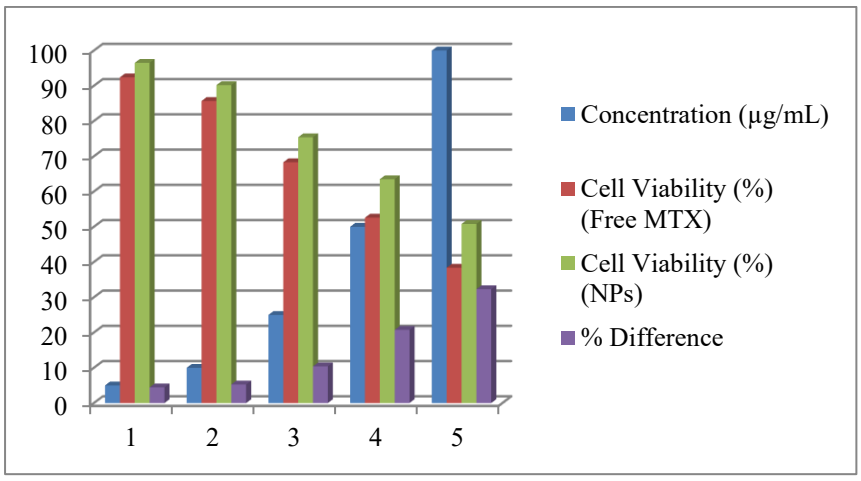

Figure 6: In vitro cytotoxicity comparison (MTT assay)

5. DISCUSSION

The present research comprehensively demonstrates the successful design, optimization, and evaluation of methotrexate-loaded polymeric nanoparticles intended for controlled drug delivery in rheumatoid arthritis (RA). The study's key findings indicate that critical process parameters—polymer concentration, drug-to-polymer ratio, and stirring speed—significantly influenced the nanoparticles' physicochemical properties. Through systematic optimization using Design of Experiments (DoE) and Response Surface Methodology (RSM), the final formulation achieved an optimal balance of particle size, narrow polydispersity index (PDI), high encapsulation efficiency, and desirable zeta potential, ensuring stability and controlled release performance. Dynamic Light Scattering (DLS) confirmed that the nanoparticles maintained a uniform mean particle size within the nanometer range, ideal for passive targeting of inflamed synovial tissues via the enhanced permeability and retention (EPR) effect. Morphological analysis by SEM and TEM revealed smooth, spherical nanoparticles, indicating structural integrity suitable for sustained circulation. The in vitro drug release profile demonstrated a gradual, sustained release of methotrexate over 24 hours. Mathematical modeling showed that the release data best fit the Higuchi and Korsmeyer–Peppas models, confirming a diffusion-controlled mechanism. This controlled release behavior is critical for minimizing peak plasma fluctuations, extending dosing intervals, and potentially improving patient compliance.

When compared with conventional methotrexate administration, which often causes systemic toxicity and requires frequent dose adjustments, this nanoparticulate approach provides significant advantages. Previous studies have similarly reported that encapsulating methotrexate within biocompatible polymers like PLGA, chitosan, or PCL enhances therapeutic efficacy while reducing off-target effects. The present study's in vitro cytotoxicity data supported these findings, showing that the nanoparticles exhibited reduced cytotoxicity toward healthy cells compared to free methotrexate. This indicates that the polymeric matrix effectively modulates drug release, protecting healthy tissues from high peak concentrations. These results collectively suggest that the optimized nanoparticle formulation holds strong potential to address key challenges in RA treatment. By offering targeted, sustained delivery, the system can improve therapeutic outcomes, reduce adverse effects, and increase patient adherence. However, this study has certain limitations. All evaluations were performed in vitro; therefore, the actual in vivo pharmacokinetics, biodistribution, and therapeutic efficacy remain to be validated. The translation of these promising results from bench to bedside requires rigorous in vivo investigations using relevant RA animal models to confirm therapeutic benefit and safety. Future work should focus on scaling up the formulation process to ensure batch-to-batch consistency and manufacturability under good manufacturing practices (GMP). Comprehensive in vivo studies, long-term stability assessments, and preliminary toxicological evaluations will be essential steps toward regulatory approval and clinical translation. Addressing these aspects will bridge the gap between laboratory research and real-world application, ultimately contributing to safer and more effective RA management.

6. CONCLUSION

In summary, the present study successfully designed, developed, and optimized methotrexate-loaded polymeric nanoparticles aimed at overcoming the challenges associated with conventional RA therapy. Systematic optimization confirmed that careful control of polymer concentration, drug-to-polymer ratio, and stirring speed could produce nanoparticles with desirable size, uniformity, and high encapsulation efficiency. The physicochemical characterizations demonstrated that the nanoparticles maintained structural integrity, uniform spherical morphology, and stable zeta potential, ensuring colloidal stability essential for effective delivery. The sustained release behavior observed in vitro, fitting well with the Higuchi and Korsmeyer–Peppas models, supports the goal of minimizing rapid drug clearance and maintaining therapeutic concentrations over time. Importantly, the in vitro cytotoxicity studies indicated reduced toxicity to healthy cells when methotrexate was encapsulated, confirming that the polymeric matrix offers protective advantages and controlled release, potentially lowering systemic side effects. The findings strongly suggest that these nanoparticles could enhance therapeutic outcomes in RA patients by improving drug localization at inflamed sites, reducing dose frequency, and boosting patient adherence. However, the study acknowledges the limitation that all biological evaluations were confined to in vitro

experiments. Therefore, further work must include detailed in vivo pharmacokinetic and biodistribution studies, long-term toxicity evaluations, and comprehensive therapeutic efficacy trials in suitable animal models. Future research should also address scale-up challenges and ensure the reproducibility of nanoparticle characteristics under good manufacturing practice (GMP) conditions. These steps are crucial to facilitate regulatory approval and clinical translation. Overall, this work lays a strong foundation for a promising nanocarrier system that could transform methotrexate therapy, delivering safer and more effective treatment options for rheumatoid arthritis patients worldwide.

REFERENCES

1. Afrasiabi, S., Chiniforush, N., Partoazar, A., & Goudarzi, R. (2023). The role of bacterial infections in rheumatoid arthritis development and novel therapeutic interventions: Focus on oral infections. In *Journal of Clinical Laboratory Analysis*. https://doi.org/10.1002/jcla.24897
2. Akram Ghumman, S., Mahmood, A., Noreen, S., Aslam, A., Ijaz, B., Amanat, A., Kausar, R., Rana, M., & Hameed, H. (2023). Chitosan-Linseed mucilage polyelectrolyte complex nanoparticles of Methotrexate: In vitro cytotoxic efficacy and toxicological studies. *Arabian Journal of Chemistry*. https://doi.org/10.1016/j.arabjc.2022.104463
3. Albuquerque, J., Moura, C. C., Sarmento, B., & Reis, S. (2015). Solid lipid nanoparticles: A potential multifunctional approach towards rheumatoid arthritis theranostics. *Molecules*. https://doi.org/10.3390/molecules200611103
4. Ali, S., Ekbbal, R., Salar, S., Yasheshwar, N., Ali, S. A., Jaiswal, A. K., Singh, M., Yadav, D. K., Kumar, S., & Gaurav, N. (2023). Quality Standards and Pharmacological Interventions of Natural Oils: Current Scenario and Future Perspectives. In *ACS Omega*. https://doi.org/10.1021/acsomega.3c05241
5. Alivernini, S., Firestein, G. S., & McInnes, I. B. (2022). The pathogenesis of rheumatoid arthritis. In *Immunity*. https://doi.org/10.1016/j.immuni.2022.11.009
6. An, M., Zhang, J., Zhang, X., Zhao, Y., & Liu, Y. (2024). Nanomedicine targeted anti-inflammatory therapy to deal with the 'crux' of rheumatoid arthritis. In *Journal of Drug Targeting*. https://doi.org/10.1080/1061186X.2024.2315475
7. Bairagi, S., Takarkhede, S., Dighe, J., & Gangurde, D. (2022). A Review on Uses of Various Nanoparticlesin the Treatment of Rheumatoid Arthritis. *Asian Journal of Pharmaceutical Research and Development*. https://doi.org/10.22270/ajprd.v10i1.1073
8. Bhattacharya, S., Prajapati, B. G., Ali, N., Mohany, M., Aboul-Soud, M. A. M., & Khan, R. (2023). Therapeutic Potential of Methotrexate-Loaded Superparamagnetic Iron Oxide Nanoparticles Coated with Poly(lactic-co-glycolic acid) and Polyethylene Glycol against Breast Cancer: Development, Characterization, and Comprehensive In Vitro Investigation. *ACS Omega*. https://doi.org/10.1021/acsomega.3c03430
9. Chen, M., Kambere Amerigos Daddy, J. C., Su, Z., El Islem Guissi, N., Xiao, Y., Zong, L., & Ping, Q. (2019). Folate receptor-targeting and reactive oxygen species-responsive liposomal formulation of methotrexate for treatment of rheumatoid arthritis. *Pharmaceutics*. https://doi.org/10.3390/pharmaceutics11110582
10. Chi, X. K., Xu, X. L., Chen, B. Y., Su, J., & Du, Y. Z. (2023). Combining nanotechnology with monoclonal antibody drugs for rheumatoid arthritis treatments. In *Journal of Nanobiotechnology*. https://doi.org/10.1186/s12951-023-01857-8
11. Costa Lima, S. A., & Reis, S. (2015). Temperature-responsive polymeric nanospheres containing methotrexate and gold nanoparticles: A multi-drug system for theranostic in rheumatoid arthritis. *Colloids and Surfaces B: Biointerfaces*. https://doi.org/10.1016/j.colsurfb.2015.04.048
12. Danaei, M., Dehghankhold, M., Ataei, S., Hasanzadeh Davarani, F., Javanmard, R., Dokhani, A., Khorasani, S., & Mozafari, M. R. (2018). Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. In *Pharmaceutics*. https://doi.org/10.3390/pharmaceutics10020057
13. Di Matteo, A., Bathon, J. M., & Emery, P. (2023). Rheumatoid arthritis. In *The Lancet*. https://doi.org/10.1016/S0140-6736(23)01525-8
14. Ekbbal, R., Jaiswal, A. K., Aggarwal, M., Singh, M., Ali, S., Ali, S. A., & Gautam, G. (2024). Indian Medicinal Plants for the Management of Endometriosis: A Comprehensive Review on their phytopharmacology. In *Natural Resources for Human Health*. https://doi.org/10.53365/nrfhh/174668
15. F., K., & B., E. (2012). Clinical impact of serum proteins on drug delivery. *Journal of Controlled Release*.
16. Feng, X., & Chen, Y. (2018). Drug delivery targets and systems for targeted treatment of rheumatoid arthritis. In *Journal of Drug Targeting*. https://doi.org/10.1080/1061186X.2018.1433680
17. Gazi, A. S., & Krishnasailaja, A. (2019). Preparation and characterization of methotrexate loaded polymeric nanoparticles by nanoprecipitation technique. *Nano Biomedicine and Engineering*. https://doi.org/10.5101/nbe.v11i4.p351-360
18. Gupta, A., Lee, J., Ghosh, T., Nguyen, V. Q., Dey, A., Yoon, B., Um, W., & Park, J. H. (2022). Polymeric Hydrogels for Controlled Drug Delivery to Treat Arthritis. *Pharmaceutics*. https://doi.org/10.3390/pharmaceutics14030540
19. Jamkhande, P. G., Ghule, N. W., Bamer, A. H., & Kalaskar, M. G. (2019). Metal nanoparticles synthesis: An overview on methods of preparation, advantages and disadvantages, and applications. In *Journal of Drug Delivery Science and Technology*. https://doi.org/10.1016/j.jddst.2019.101174
20. Joshi, M., Pathak, K., & Dhaneshwar, S. (2022). Nanotechnology-based strategies for effective delivery of phytoconstituents for the management of rheumatoid arthritis. In *Pharmacological Research - Modern Chinese Medicine*. https://doi.org/10.1016/j.prmcm.2022.100061
21. Latha, S., Selvamani, P., Prabu, C., Harini, R., Babu, P. S., & Thimiri Govindaraj, D. B. (2021). Polyelectrolyte multifaceted

magnetic microcapsules for magnetic drug targeting at rheumatoid arthritic joints. In *Handbook on Nanobiomaterials for Therapeutics and Diagnostic Applications*. https://doi.org/10.1016/B978-0-12-821013-0.00015-5

22. Li, P., Wang, C., Huo, H., Xu, C., Sun, H., Wang, X., Wang, L., & Li, L. (2024). Prodrug-based nanomedicines for rheumatoid arthritis. In *Discover Nano*. https://doi.org/10.1186/s11671-023-03950-1
23. Li, X., Hou, Y., Meng, X., Li, G., Xu, F., Teng, L., Sun, F., & Li, Y. (2021). Folate receptor-targeting mesoporous silica-coated gold nanorod nanoparticles for the synergistic photothermal therapy and chemotherapy of rheumatoid arthritis. *RSC Advances*. https://doi.org/10.1039/d0ra08689d
24. Li, Y., Liang, Q., Zhou, L., Liu, J., & Liu, Y. (2022). Metal nanoparticles: a platform integrating diagnosis and therapy for rheumatoid arthritis. In *Journal of Nanoparticle Research*. https://doi.org/10.1007/s11051-022-05469-5
25. Logesh, K., Raj, B., Bhaskaran, M., Thirumaleshwar, S., Gangadharappa, H. V., Osmani, R. A., & Asha Spandana, K. M. (2023). Nanoparticulate drug delivery systems for the treatment of rheumatoid arthritis: A comprehensive review. In *Journal of Drug Delivery Science and Technology*. https://doi.org/10.1016/j.jddst.2023.104241
26. Mohan, H., Fagan, A., & Giordani, S. (2023). Carbon Nanomaterials (CNMs) in Cancer Therapy: A Database of CNM-Based Nanocarrier Systems. In *Pharmaceutics*. https://doi.org/10.3390/pharmaceutics15051545
27. Mr Sharad Kamble, Miss Kaveri Bhosale, Mr Mahesh Mohite, & Mrs Swapnali Navale. (2023). Methods of Preparation of Nanoparticles. *International Journal of Advanced Research in Science, Communication and Technology*. https://doi.org/10.48175/ijarsct-9485
28. Nasra, S., Bhatia, D., & Kumar, A. (2022). Recent advances in nanoparticle-based drug delivery systems for rheumatoid arthritis treatment. In *Nanoscale Advances*. https://doi.org/10.1039/d2na00229a
29. Park, J. S., Lee, D., Yang, S. C., Jeong, H. Y., Na, H. S., Cho, K. H., Choi, J. W., Koo, H., Cho, M. La, & Park, S. H. (2022). Methotrexate-loaded nanoparticles ameliorate experimental model of autoimmune arthritis by regulating the balance of interleukin-17-producing T cells and regulatory T cells. *Journal of Translational Medicine*. https://doi.org/10.1186/s12967-022-03267-0
30. Parveen, A., Wal, P., Rai, A. K., & Wal, A. (2024). An Outlook of Substantial Progress in Nanotechnology Emerged in Treatment Approaches for Rheumatoid Arthritis. In *Current Drug Therapy*. https://doi.org/10.2174/0115748855238869231002073717
31. Qaiser, F., Ibrahim, M., Mazhar, R., & Sohail, F. (2020). Nanotechnology: A New Hope for the Cure of Osteoarthritis, Osteoporosis and Rheumatoid Arthritis. *Global Pharmaceutical Sciences Review*. https://doi.org/10.31703/gpsr.2020(v-i).04
32. Rabiei, M., Kashanian, S., Samavati, S. S., Derakhshankhah, H., Jamasb, S., & McInnes, S. J. P. (2021). Nanotechnology application in drug delivery to osteoarthritis (OA), rheumatoid arthritis (RA), and osteoporosis (OSP). In *Journal of Drug Delivery Science and Technology*. https://doi.org/10.1016/j.jddst.2020.102011
33. Rahimizadeh, P., Rezaieyazdi, Z., Behzadi, F., Hajizade, A., & Lim, S. I. (2021). Nanotechnology as a promising platform for rheumatoid arthritis management: Diagnosis, treatment, and treatment monitoring. In *International Journal of Pharmaceutics*. https://doi.org/10.1016/j.ijpharm.2021.121137
34. Rani, R., Raina, N., Sharma, A., Kumar, P., Tulli, H. S., & Gupta, M. (2023). Advancement in nanotechnology for treatment of rheumatoid arthritis: scope and potential applications. In *Naunyn-Schmiedeberg's Archives of Pharmacology*. https://doi.org/10.1007/s00210-023-02514-5
35. Scherer, H. U., Häupl, T., & Burmester, G. R. (2020). The etiology of rheumatoid arthritis. In *Journal of Autoimmunity*. https://doi.org/10.1016/j.jaut.2019.102400
36. Shamim, Ali, S., Ali, T., Sharma, H., Kishor, B. N., & Jha, S. K. (2025). Recent Advances in Monodisperse Gold Nanoparticle Delivery, Synthesis, and Emerging Applications in Cancer Therapy. *Plasmonics*, *0123456789*. https://doi.org/10.1007/s11468-024-02732-4
37. Singh, S., Tiwary, N., Sharma, N., Behl, T., Antil, A., Anwer, M. K., Ramniwas, S., Sachdeva, M., Elossaily, G. M., Gulati, M., & Ohja, S. (2024). Integrating Nanotechnological Advancements of Disease-Modifying Anti-Rheumatic Drugs into Rheumatoid Arthritis Management. In *Pharmaceuticals*. https://doi.org/10.3390/ph17020248
38. Surve, C., Singh, R., Banerjee, A., Patnaik, S., & Shidhaye, S. (2021). Formulation and qbd based optimization of methotrexate-loaded solid lipid nanoparticles for an effective anti-cancer treatment. *International Journal of Applied Pharmaceutics*. https://doi.org/10.22159/ijap.2021v13i5.42373
39. Trujillo-Nolasco, R. M., Morales-Avila, E., Ocampo-García, B. E., Ferro-Flores, G., Gibbens-Bandala, B. V., Escudero-Castellanos, A., & Isaac-Olive, K. (2019). Preparation and in vitro evaluation of radiolabeled HA-PLGA nanoparticles as novel MTX delivery system for local treatment of rheumatoid arthritis. *Materials Science and Engineering C*. https://doi.org/10.1016/j.msec.2019.109766
40. Vauthier, C., & Bouchemal, K. (2009). Methods for the Preparation and Manufacture of Polymeric Nanoparticles. In *Pharmaceutical Research*. https://doi.org/10.1007/s11095-008-9800-3
41. Wang, Y., Jia, M., Zheng, X., Wang, C., Zhou, Y., Pan, H., Liu, Y., Lu, J., Mei, Z., & Li, C. (2022). Microvesicle-camouflaged biomimetic nanoparticles encapsulating a metal-organic framework for targeted rheumatoid arthritis therapy. *Journal of Nanobiotechnology*. https://doi.org/10.1186/s12951-022-01447-0
42. Wei, J. (2022). Nano-medicine in Treating Reumatoid Arthritics. *Highlights in Science, Engineering and Technology*. https://doi.org/10.54097/hset.v2i.573
43. Zhang, T., Xu, J., Chen, J., Wang, Z., Wang, X., & Zhong, J. (2021). Protein nanoparticles for Pickering emulsions: A comprehensive review on their shapes, preparation methods, and modification methods. In *Trends in Food Science and Technology*. https://doi.org/10.1016/j.tifs.2021.04.054