Formulation And Evaluation of Methotrexate Nanoparticles for Management of Rheumatoid Arthritis

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***Abstract*—Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by persistent joint inflammation, leading to cartilage and bone destruction. Methotrexate (MTX) is a first-line disease-modifying anti-rheumatic drug; however, its clinical use is limited by systemic toxicity and poor bioavailability. This study aimed to formulate and evaluate methotrexate-loaded nanoparticles (MTX-NPs) to enhance therapeutic efficacy and reduce adverse effects. MTX-NPs were prepared using [method, e.g., ionic gelation/emulsion solvent evaporation] and characterized for particle size, zeta potential, encapsulation efficiency, and in vitro drug release. The optimized formulation exhibited a particle size of [X nm], a zeta potential of [X mV], and sustained drug release over [X hours]. Anti-arthritic efficacy was evaluated in Complete Freund's Adjuvant (CFA)-induced rheumatoid arthritis in Wistar rats. MTX-NPs significantly reduced paw edema, arthritis index scores, and pro-inflammatory cytokine levels (TNF-α, IL-1β, IL-6) compared to free MTX and disease controls ($p < 0.05$). Histopathological analysis revealed reduced synovial hyperplasia and cartilage degradation in MTX-NP-treated rats. These findings suggest that methotrexate nanoparticles offer a promising strategy for improved management of rheumatoid arthritis with enhanced therapeutic efficacy and reduced systemic toxicity.**

***Index Terms*—Methotrexate, Nanoparticles, Rheumatoid Arthritis, Anti-arthritic Activity, Drug Delivery, CFA-induced Arthritis**

I. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disorder characterized by persistent synovial inflammation, progressive joint destruction, and debilitating functional impairment. Globally, RA affects approximately 0.5–1% of the population, significantly reducing quality of life and increasing healthcare burden. The pathogenesis of RA involves an imbalance between pro-inflammatory and anti-inflammatory mediators, with elevated levels of cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) contributing to synovial hyperplasia and cartilage degradation.

Methotrexate (MTX) is widely regarded as the gold-standard disease-modifying anti-rheumatic drug (DMARD) for RA due to its anti-proliferative and immunomodulatory properties. Despite its clinical efficacy, conventional MTX therapy is limited by low oral bioavailability, rapid systemic clearance, and adverse effects including hepatotoxicity, gastrointestinal disturbances, and myelosuppression. These limitations underscore the need for novel drug delivery systems that can improve therapeutic outcomes while minimizing systemic toxicity.

Nanotechnology-based drug delivery has emerged as a promising strategy to enhance the efficacy of conventional therapeutics. Nanoparticles offer controlled and sustained drug release, targeted delivery to inflamed tissues, improved bioavailability, and reduced off-target effects. Several studies have demonstrated that encapsulating MTX in nanoparticulate carriers can enhance its anti-arthritic activity and reduce systemic adverse effects, making it a potential advancement in RA management.

Measurement of Particle Size: The mean particle size was obtained by Photon correlation spectroscopy (PCS) (3000SH, Malvern Instruments Ltd., UK). The MTX loaded Alg-CS nanoparticle formulations were diluted with de-mineralized filtered water to an appropriate scattering intensity. Data was analyzed by the cumulate method assuming spherical particles. Accordingly, the results are given as the effective diameter and the poly dispersity index (PDI) as a measure for the relative width of the particle size distribution.

Measurement of Zeta Potential: The zeta potential value of optimized MTX loaded Alg-CS nanoparticle formulation was measured with the Zetasizer (3000SH, Malvern Instruments Ltd., UK).To determine the zeta potential, optimized formulation was diluted with double-distilled water and placed in an electrophoretic cell.

Drug release and Release Kinetics: The *in vitro* release pattern of MTX-NaAlg-CS nanoparticle shown the initial burst release followed by the sustained release was observed in optimized formulation (data not shown). During initial hours minimum burst release of the drug from the polymeric nanoparticles was observed followed by prolonged release (68.99%) up to 36 h. The initial burst release may be probably caused by the drug adsorbed on the surface of nanoparticles or precipitation of drug from the nanoparticles. Sustained release was obtained due to slow diffusion of the drug from the polymeric matrix.

To determine the release model that best described the drug release, the *in vitro* release data was substituted in equations of zero order, first order and Higuchi model and the results are noted. Among them the zero-order model showed a high R^2 value 0.93443, indicating that the release of the drug followed zero order release kinetics.(Fig 7a) To understand the mechanism of drug release, Korsmeyer–Peppas equation was applied and it showed excellent linearity. The release exponent 'n' was found to be 0.79307. (Fig 7b). According to this model, if the value of 'n' was between >0.43and <0.85, it indicated that drug release followed anomalous transport (Non-Fickian) (Chouhan and Bajpai, 2009 b) and was controlled by more than one process (the coupling of Fickian diffusion and polymer matrix relaxation).

II. MATERIALS AND METHODS

Methotrexate (MTX) was obtained from [supplier], and polymers/excipients for nanoparticle formulation chitosan, PLGA were procured from [supplier]. Analytical-grade solvents and reagents, including phosphate-buffered saline (PBS) and ELISA kits for TNF-α, IL-1β, and IL-6, were used. Adult Wistar rats (150–200 g, either sex) were housed under standard laboratory conditions

MTX-loaded nanoparticles (MTX-NPs) were prepared using the [ionic gelation/emulsion solvent evaporation/nano-precipitation] method. Nanoparticles were characterized for particle size, zeta potential, morphology (SEM/TEM), encapsulation efficiency, and in vitro drug release in PBS (pH 7.4) at 37°C.

Rheumatoid arthritis was induced by a single intradermal injection of 0.1 mL Complete Freund's Adjuvant (CFA) into the left hind paw. Rats were randomly divided into six groups (n = 6): normal control, disease control, standard MTX solution, and MTX-NPs at low, medium, and high doses (5, 10, 20 mg/kg). Treatments were administered orally once daily for 21 days post-arthritis induction.

Anti-arthritic efficacy was assessed by measuring paw volume and arthritis index scores, evaluating serum pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), and analyzing oxidative stress markers (MDA, SOD). Histopathological changes in joint tissues were examined using hematoxylin-eosin staining. Data were expressed as mean ± SD and analyzed by one-way ANOVA with Tukey's post hoc test; $p < 0.05$ was considered statistically significant.

III. RESULTS AND DISCUSSION

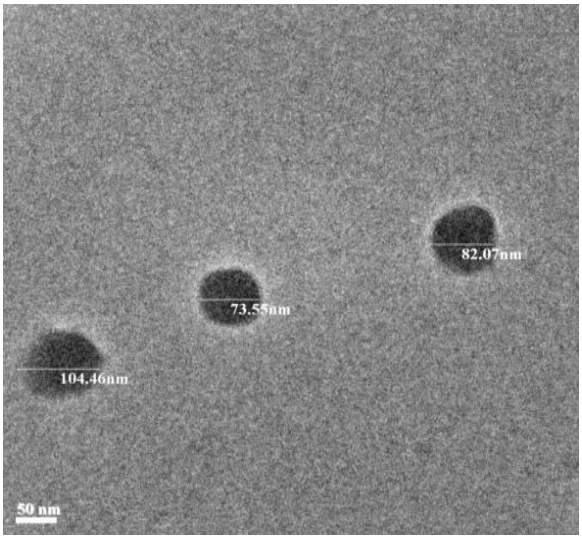

Fig1: TE Mimage of MTX-NaAlg-C Snanoparticles

Fig. 2: Particle size distribution of optimized MTX-NaAlg-CS nanoparticle

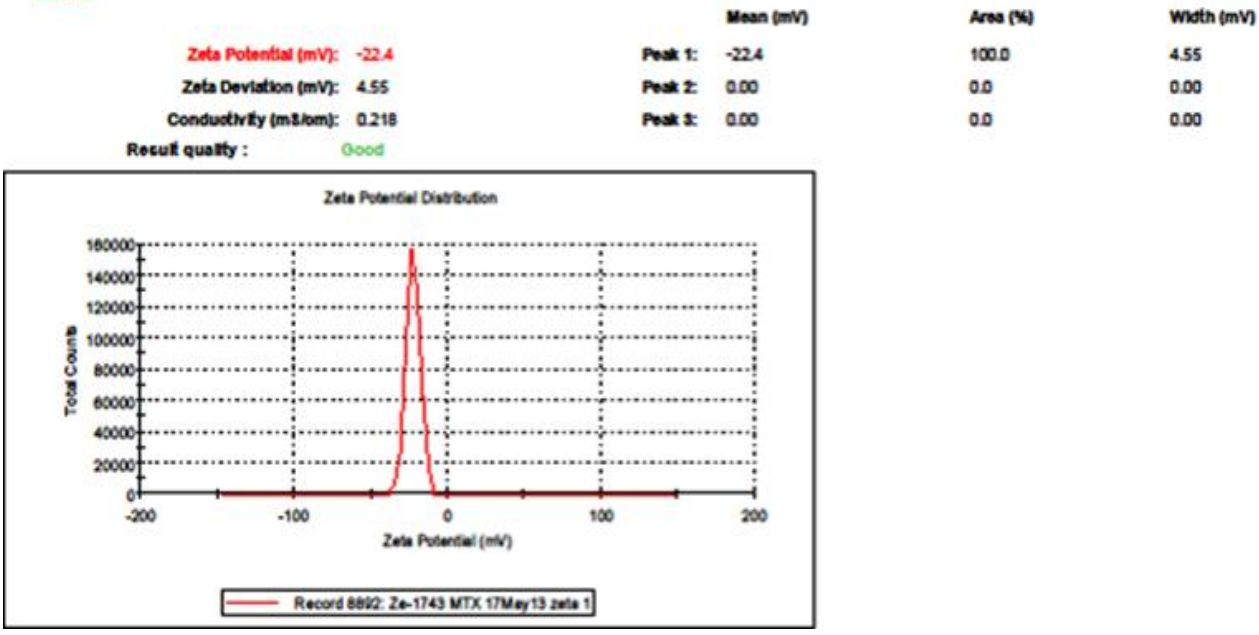

Fig3: ZetapotentialofMTX-NaAlg-CSnanoparticle

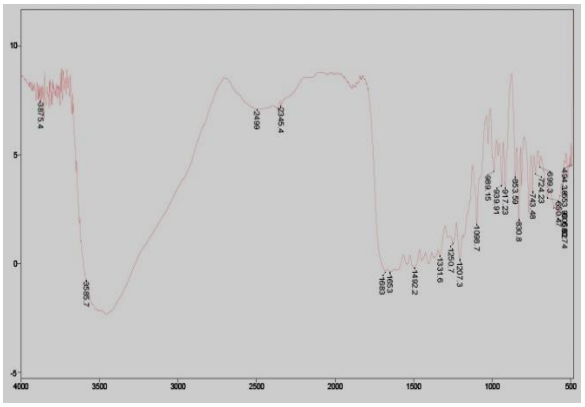

Fig4a: FTIR spectra of pure methotrexate

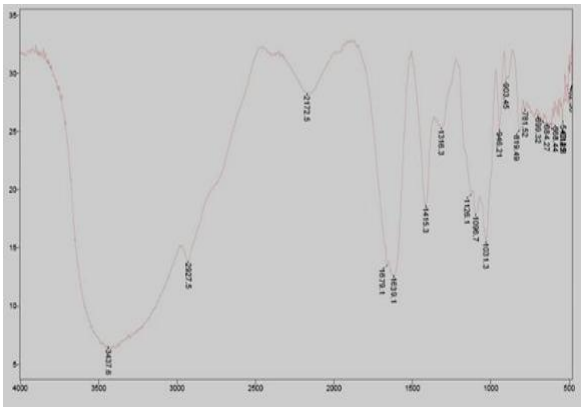

Fig. 4b: FTIR spectra of MTX-NaAlg-CS nanoparticle

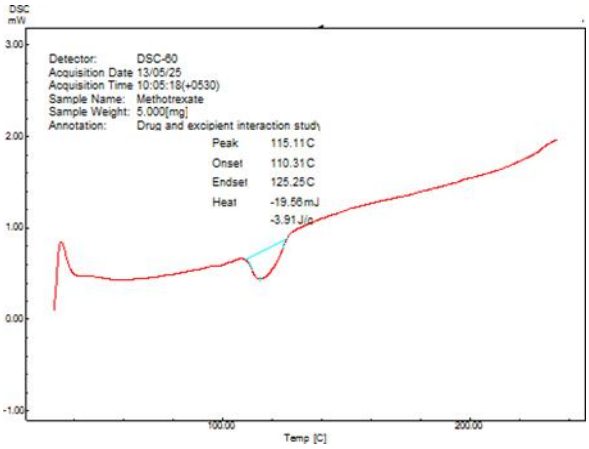

Fig. 5a: DSC curve of pure methotrexate

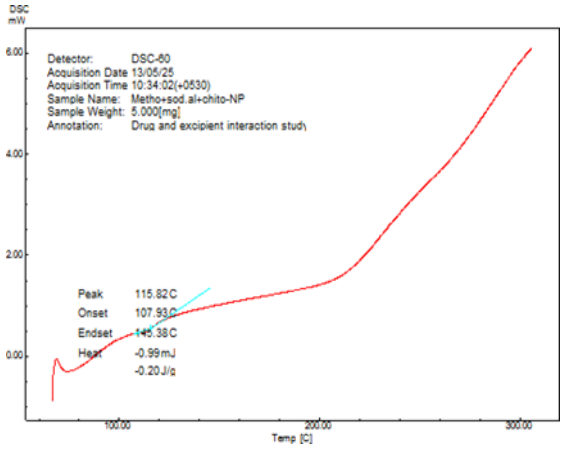

Fig. 5b: DSC curve of MTX-NaAlg-CS nanoparticle

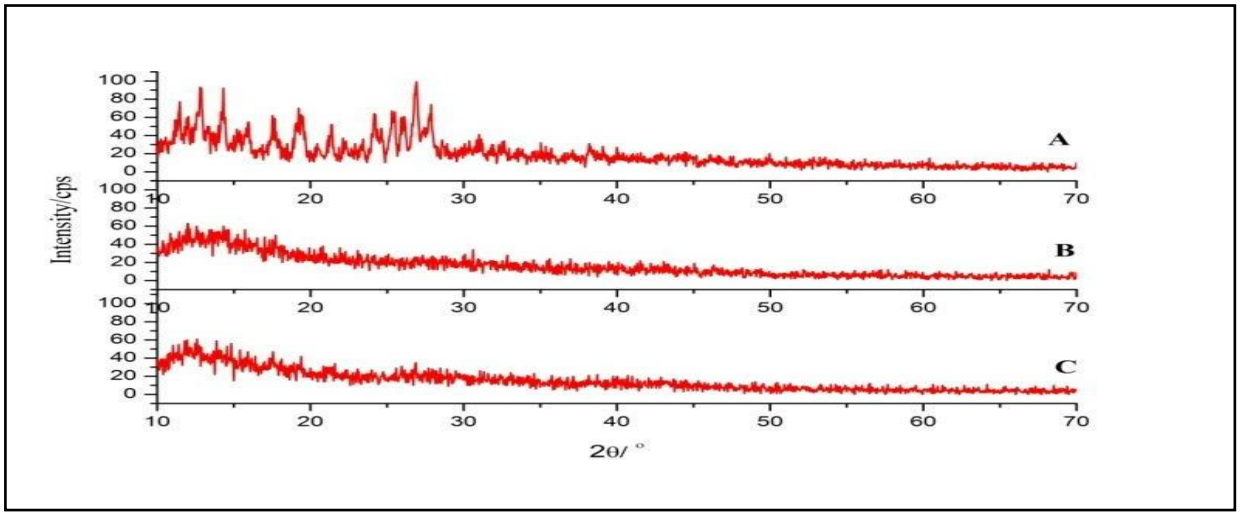

Fig. 6: X-ray diffraction pattern of A) pure methotrexate B) Blank nanoparticle C)MTX-NaAlg-CSnanoparticle

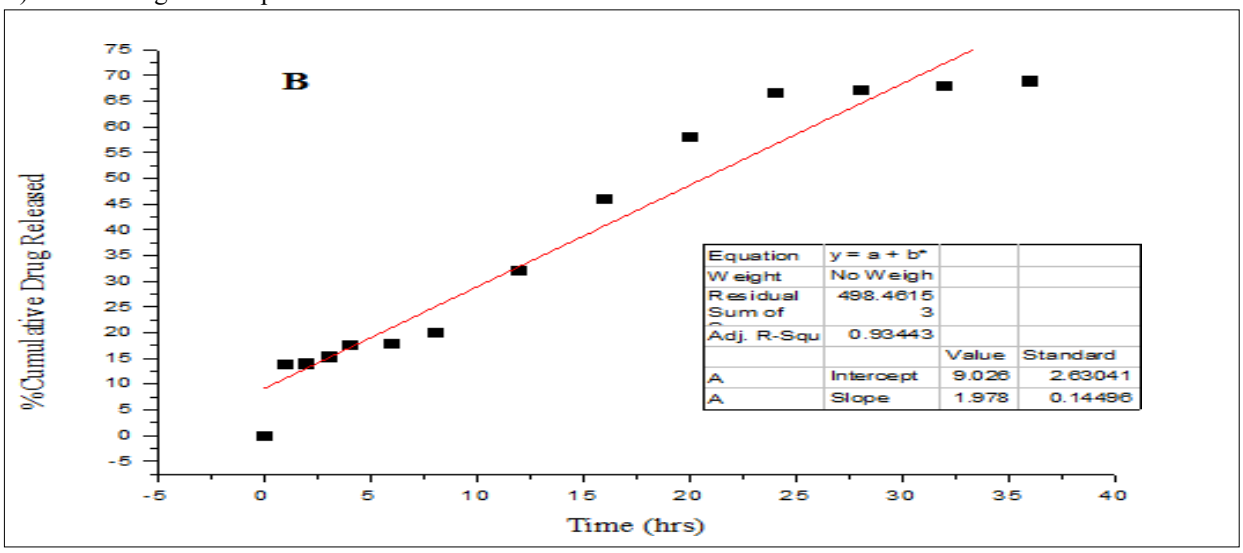

Fig. 7a: Zero-order release of MTX-NaAlg-CS nanoparticle

IV. CONCLUSION

Methotrexate loaded nanoparticles were prepared by the ionotropic pregelation method. The FTIR, DSC, XRD pattern study did not detect any crystalline drug material in the freshly prepared freeze-dried nanoparticles. The application of factorial design gave a statistically systematic approach for the formulation of nanoparticles with desired particle size, high entrapment efficiency and % drug release. Concentration of Drug, Polymers were found to influence the particle size, entrapment efficiency, and% drug release of MTX loaded NaAlg-CS nanoparticles. The release was found to follow with non- Fickian diffusion mechanism for optimized batch. These results indicate that MTX loaded NaAlg-CS nanoparticles could be effective in controlled drug release for a prolonged period would serve the purpose for long term treatment of Rheumatoid Arthritis

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