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Research

Topical Dual-Drug Delivery Using β-Cyclodextrin Nanosponges: A Targeted and Safer Therapeutic Strategy for Rheumatoid Arthritis Management

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Abstract:
Rheumatoid arthritis (RA) is a chronic inflammatory disease often associated with limitations of conventional therapies, including systemic toxicity and poor patient compliance. This study presents a novel topical delivery system utilizing β-cyclodextrin nanosponges for the combined administration of baricitinib and tenoxicam. The developed nanosponges exhibited high drug loading, nanoscale size, and improved solubility through amorphization. Incorporation into a carbopol gel enabled sustained and controlled drug release. In vivo evaluation in an arthritis model demonstrated significant reduction in inflammation, decreased pro-inflammatory cytokines, and improved joint condition. The formulation also showed minimal systemic toxicity, as indicated by normalized biochemical parameters. Overall, this dual-drug nanosponge gel offers a targeted, effective, and safer approach for RA management.

Keywords: Rheumatoid arthritis; topical delivery; β-cyclodextrin nanosponges; baricitinib; tenoxicam; dual-drug therapy; sustained release; CFA-induced arthritis; cytokine modulation; localized therapy

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Introduction

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disorder characterized primarily by persistent synovial inflammation, leading to the progressive destruction of articular cartilage and underlying bone. The disease pathophysiology is a complex interplay of genetic predisposition and environmental triggers, which culminates in a loss of immune tolerance[1]. This breakdown results in the activation of autoreactive T-cells and B-cells, which drive a cascade of inflammatory events within the synovium—the membrane lining the joints. Activated immune cells and resident synovial fibroblasts produce a torrent of pro-inflammatory cytokines, most notably Tumor Necrosis Factor-alpha (TNF-α), Interleukin-1 (IL-1), and Interleukin-6 (IL-6)[2]. These cytokines act as molecular messengers, perpetuating inflammation, stimulating the proliferation of synovial tissue into a destructive "pannus," and activating osteoclasts that erode bone. Clinically, this manifests as symmetric joint pain, swelling, stiffness, and eventual deformity, causing significant pain, functional disability, and a markedly reduced quality of life for patients. The systemic nature of RA also contributes to extra-articular manifestations, including cardiovascular disease, osteoporosis, and fatigue, making its management a holistic challenge[3]. The therapeutic landscape for RA has evolved significantly over the past decades, moving from purely symptomatic relief to strategies

aimed at suppressing the underlying immune dysfunction—a paradigm known as "treat-to-target." The conventional pharmacological armamentarium is structured in a pyramid-like approach, often beginning with first-line nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids for rapid symptom control, followed by Disease-Modifying Anti-Rheumatic Drugs (DMARDs). Traditional synthetic DMARDs, such as methotrexate (the cornerstone therapy), leflunomide, and sulfasalazine, work through broadly immunosuppressive mechanisms. For patients with an inadequate response, biologic DMARDs (bDMARDs) like TNF inhibitors (e.g., adalimumab, etanercept), IL-6 receptor blockers (e.g., tocilizumab), and T-cell co-stimulation inhibitors (e.g., abatacept) offer more targeted intervention. The most recent class, targeted synthetic DMARDs (tsDMARDs), includes Janus Kinase (JAK) inhibitors like baricitinib, which act intracellularly to block cytokine signaling pathways. Despite these advancements, the management of RA remains fraught with substantial challenges and limitations, primarily stemming from the systemic administration (oral or injectable) of these potent agent[4][5]. The first and most prominent challenge is the significant burden of systemic adverse effects. Oral NSAIDs, a mainstay for pain management, are notoriously associated with dose-dependent gastrointestinal (GI) toxicity, ranging from dyspepsia and gastritis to life-threatening ulcers and bleeding. Their long-term use also poses non-negligible risks of renal impairment and increased cardiovascular thrombotic events. Systemic corticosteroids, while powerfully anti-inflammatory, carry a well-documented risk profile including osteoporosis, hyperglycemia, weight gain, hypertension, and increased susceptibility to infections with prolonged use. Even the more targeted DMARDs are not immune to systemic toxicity. Methotrexate can cause hepatotoxicity, myelosuppression, and pulmonary fibrosis, necessitating regular monitoring[6][7]. Biologic agents profoundly increase the risk of serious infections, including reactivation of latent tuberculosis, and some are linked to demyelinating disorders or heart failure. JAK inhibitors, while orally convenient, carry black-box warnings for serious infections, malignancy, thrombosis, and major cardiovascular events. This toxicity profile often leads to dose limitations, treatment discontinuation, and complex risk-benefit calculations, particularly in elderly patients or those with comorbidities[8][9]. The second major challenge is variable and often suboptimal bioavailability and pharmacokinetics. For orally administered drugs like baricitinib and many NSAIDs, absorption can be inconsistent, influenced by factors such as gastric pH, food intake, and first-pass metabolism in the liver. This variability can lead to fluctuations in plasma drug concentrations, resulting in periods of subtherapeutic effect or heightened toxicity[10][11]. Furthermore, for a disease that is localized to the joints, systemic delivery is inherently inefficient. Only a small fraction of the orally administered drug dose ever reaches the synovial tissue—the primary site of pathology. The majority of the drug distributes throughout the body, contributing to systemic side effects without meaningfully augmenting the therapeutic effect at the joint. This "off-target" distribution is a fundamental flaw of the oral route for localized diseases. While intra-articular injections can deliver drugs directly to the joint, they are invasive, require clinical expertise, carry a risk of infection, are unsuitable for treating multiple joints simultaneously, and often provide only transient relief as the drug is quickly cleared from the joint space. The third critical challenge is poor patient compliance and adherence to long-term treatment regimens. RA is a lifelong condition requiring continuous pharmacologic management to control disease activity and prevent irreversible joint damage. Complex regimens involving multiple daily pills, self-injections with associated pain and anxiety, and the need for frequent laboratory monitoring create a substantial burden on patients. The experience of distressing side effects, such as nausea from methotrexate or the fear of infections from biologics, further disincentivizes adherence. Non-adherence leads to disease flares, poorer long-term outcomes, increased joint damage, and higher overall healthcare costs. Therefore, there is an urgent, unmet clinical need for therapeutic strategies that can enhance drug delivery specifically to the inflamed joints, maximize local therapeutic efficacy, minimize systemic

exposure and its associated toxicities, and offer a convenient, patient-friendly mode of administration to improve adherence. In response to the limitations of systemic therapy, the paradigm of topical or transdermal delivery for RA has gained considerable traction[12][13]. The fundamental premise is to apply the medication directly to the skin overlying the affected joints, facilitating its penetration to the underlying synovium[14][15]. This approach offers a compelling set of advantages that directly address the core challenges of systemic treatment. The primary benefit is the potential for localized high drug concentration at the site of disease. By bypassing the gastrointestinal tract and systemic circulation, topical delivery aims to create a high drug depot in the dermal and subdermal tissues surrounding the joint[16][17]. This allows for therapeutic levels to be achieved precisely where they are needed most—within the synovium and articular tissues—while minimizing the total drug load in the body. Consequently, the most significant advantage is the dramatic reduction in systemic side effects. Drugs that cause GI ulcers when taken orally or pose risks of hepatic or renal toxicity when circulating systemically may have a vastly improved safety profile when their distribution is primarily local. This is particularly crucial for chronic conditions like RA, where long-term safety is paramount[18][19]. However, conventional topical gels and creams have historically shown limited success in managing deep-seated arthritic conditions, primarily due to the formidable barrier function of the skin, especially the outer stratum corneum layer, and the relatively long diffusion path to the synovium. Most drug molecules, particularly those that are poorly water-soluble (like many anti-inflammatories), cannot penetrate the skin in sufficient quantities to achieve a therapeutic effect at the deep joint tissue. This has led to skepticism about the feasibility of topical RA treatment. The innovation, therefore, lies not in the simple topical application of existing drugs, but in the advanced engineering of the drug delivery system itself—specifically, the utilization of nanocarriers that can act as molecular shuttles to overcome these biological barriers. When combined with a rationally selected drug combination, this technological approach transforms the potential of topical therapy. The choice of Baricitinib and Tenoxicam for co-delivery represents a strategically designed synergistic combination that targets multiple, complementary pathways in the RA inflammatory cascade. RA is not driven by a single cytokine or pathway; it is a network of interacting inflammatory signals. Monotherapy, even with a potent agent, may inadequately suppress this network, leading to treatment resistance or incomplete response. A dual-drug approach aims for a broader and more effective suppression of inflammation[18][19].

Baricitinib is an oral, small-molecule inhibitor of Janus Kinase (JAK) 1 and 2. The JAK-STAT (Signal Transducer and Activator of Transcription) pathway is a critical signaling hub for numerous cytokines implicated in RA, including IL-6, IL-23, and interferons. By inhibiting JAK enzymes, baricitinib blocks the intracellular signaling of these cytokines, thereby modulating the immune response at a pivotal point. It represents a targeted, tsDMARD approach, effective in patients who have failed conventional DMARDs. Tenoxicam, on the other hand, is a well-established non-steroidal anti-inflammatory drug (NSAID) of the oxicam class. It works by non-selectively inhibiting the cyclooxygenase (COX-1 and COX-2) enzymes, thereby reducing the production of prostaglandins—key mediators of pain, swelling, and fever. It provides broad-spectrum anti-inflammatory and analgesic effects, addressing the symptomatic hallmarks of RA. The synergy between these two agents is mechanistic. Tenoxicam acts on the effector arm of inflammation (prostaglandins), providing rapid relief from pain and swelling. Baricitinib acts upstream on the cytokine signaling arm (JAK-STAT), suppressing the immune cell activation and cytokine production that fuel the chronic inflammatory process[20]..

Materials and Methods

The experimental work required pharmaceutical-grade active ingredients, polymer matrices, chemical reagents, and materials for biological evaluation. Baricitinib (BAR, ≥98% purity) and Tenoxicam (TNX, ≥98% purity) were procured as gift samples from certified bulk drug manufacturers. The nanosponge carrier was synthesized using β-Cyclodextrin (β-CD) as the oligosaccharide backbone and Diphenyl Carbonate

(DPC) as the crosslinking agent, both of analytical grade. For the topical gel formulation, Carbopol 934P was selected as the primary gelling polymer due to its excellent bioadhesive properties, clarity, and compatibility with a wide pH range. Neutralization of the carbopol dispersion was achieved using Triethanolamine (TEA). All organic solvents, including Dimethylformamide (DMF), Acetone, and Methanol, were of HPLC grade and used for synthesis, purification, and analytical purposes. Phosphate Buffered Saline (PBS) at pH 7.4 was prepared to simulate physiological conditions for in vitro release studies. For cell-level studies not covered here, analytical kits for cytokines would be required. The in vivo pharmacodynamic evaluation was conducted using a well-established animal model of rheumatoid arthritis. Female Wistar rats (weighing 180-220 g) were housed under standard laboratory conditions (12-hour light/dark cycle, 22±2°C, 50±10% humidity) with ad libitum access to a standard pellet diet and water. The induction of arthritis was achieved via a single intradermal injection of Complete Freund's Adjuvant (CFA) into the subplantar region of the right hind paw. CFA, an emulsion of heat-killed *Mycobacterium tuberculosis* in mineral oil, induces a robust, chronic, and polyarthritic immune response characterized by pronounced inflammation, synovial hyperplasia, and bone erosion, closely mimicking human RA pathology. All animal experiments were designed and performed in strict accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), following approval by the Institutional Animal Ethics Committee (IAEC).

Synthesis and Optimization of β-CD Nanosponges

The synthesis of β-cyclodextrin nanosponges (β-CD NS) is a critical step that defines their ultimate structural and functional properties, such as porosity, particle size, and drug-loading capacity.

Crosslinking Synthesis via Melt Method

The nanosponges were synthesized using a catalyst-free melt polymerization method. This process involved the transesterification reaction between the hydroxyl groups of β-CD and the carbonate groups of DPC. β-CD and DPC were mixed in a series of predefined molar ratios, ranging from 1:2 to 1:20 (β-CD:DPC), to systematically investigate the effect of crosslinker density on nanosponge properties. For a typical 1:8 ratio batch, stoichiometric quantities of β-CD and DPC were accurately weighed and transferred into a round-bottom flask. The mixture was then heated in a silicone oil bath at 100°C under continuous magnetic stirring for 5 hours. During this period, the solid reactants melted into a viscous mass as the crosslinking reaction progressed, forming a three-dimensional polymeric network. Upon completion, the crude, solidified product was cooled to room temperature, yielding a hard, brittle, yellowish solid. This solid was then subjected to a rigorous purification protocol to remove unreacted monomers, oligomers, and the phenol by-product. The product was broken into small pieces, crushed using a mortar and pestle, and repeatedly washed with a large excess of distilled water via Soxhlet extraction for 24 hours, followed by washing with a 1:1 v/v mixture of ethanol and acetone. The purified nanosponge powder was then dried in a hot air oven at 50°C until a constant weight was achieved and finally stored in a desiccator for further use.

Drug Loading into Optimized Nanosponges

Drug loading was performed using a simple yet efficient incubation method, capitalizing on the nanoporous structure and inclusion complexation capability of the NS. Separate batches were prepared for Baricitinib-loaded NS (BAR-NS) and Tenoxicam-loaded NS (TNX-NS) to allow for precise control of loading parameters for each drug. An optimized batch of blank NS (selected based on preliminary characterization from the 1:2 to 1:20 series) was dispersed in a suitable solvent system (e.g., a water-ethanol mixture) under probe sonication to ensure complete dispersion of the NS particles. A calculated excess of BAR or TNX (drug-to-NS weight ratio of 1:2) was dissolved in a minimal amount of a compatible solvent (e.g., DMF for BAR, methanol for TNX) and added dropwise to the NS dispersion under continuous stirring. The mixture was then stirred for 24 hours at room temperature in the dark to allow for maximum drug diffusion into the NS pores and complexation within the cyclodextrin cavities. Post-incubation, the drug-loaded NS were isolated by centrifugation at high speed (e.g., 15,000 rpm for 30 min) to separate the solid loaded particles

from the supernatant containing unentrapped drug. The pellet was washed gently with a small amount of deionized water to remove surface-adhered drug and then lyophilized (freeze-dried) to obtain a free-flowing, dry powder of drug-loaded nanosponges, which was stored protected from light and moisture.

Formulation of Topical Gel

The drug-loaded nanosponge powders were incorporated into a topical gel base to create a patient-applicable dosage form with suitable rheological and adhesive properties. A 1% w/w Carbopol 934P gel was prepared as the base. Carbopol polymer was slowly sprinkled onto the surface of distilled water with gentle, continuous stirring using a magnetic stirrer to avoid lump formation. The dispersion was then allowed to hydrate fully for 2 hours to yield a clear, viscous mucilage. Separately, accurately weighed quantities of BAR-NS and TNX-NS powders (equivalent to 0.05% w/w BAR and 0.25% w/w TNX in the final gel) were uniformly dispersed in a small quantity of water using a vortex mixer. This NS dispersion was then incorporated into the Carbopol mucilage under slow stirring to ensure homogeneous distribution without introducing air bubbles. The pH of the gel was adjusted to 6.5-7.0 using triethanolamine (TEA), which neutralized the carboxylic acid groups of Carbopol, triggering gelation and resulting in a clear, elegant gel with optimal viscosity and skin feel. For comparative studies, control gels were also prepared: a plain drug gel (containing free BAR and TNX without NS), single-drug NS gels (BAR-NS gel and TNX-NS gel individually), and a placebo NS gel (containing blank NS without drug).

Characterization of Nanosponges and Gel

A comprehensive battery of analytical techniques was employed to characterize the synthesized nanocarriers and the final gel formulation, ensuring they met the required specifications for an effective drug delivery system.

Physicochemical Characterization

Particle Size and Zeta Potential: The mean particle size (Z-average), polydispersity index (PDI, a measure of size distribution breadth), and zeta potential (surface charge) of the blank and drug-loaded NS dispersions in water were determined using Dynamic Light Scattering (DLS) and Laser Doppler Velocimetry, respectively, on a Malvern Zetasizer Nano ZS. A PDI value below 0.3 indicates a monodisperse, homogeneous population, while a zeta potential magnitude greater than ±20 mV suggests good colloidal stability due to electrostatic repulsion preventing aggregation. Morphology (SEM): The surface morphology and porous structure of the nanosponges were visualized using Scanning Electron Microscopy (SEM). A small amount of NS powder was sputter-coated with a thin layer of gold to impart conductivity and then observed under high vacuum at an accelerating voltage of 15-20 kV. SEM micrographs were expected to reveal a highly porous, sponge-like, or coralline structure with a rough surface, confirming the successful formation of a crosslinked network.

Drug-Polymer Interaction Studies

Fourier-Transform Infrared Spectroscopy (FTIR): FTIR spectra of pure BAR, pure TNX, blank NS, physical mixtures, and drug-loaded NS were recorded using the KBr pellet method over a wavelength range of 4000-400 cm^{-1}. This study aimed to identify any potential chemical interactions or bond formation between the drug molecules and the NS polymer matrix. The absence of new peaks or significant shifts in characteristic drug peaks (e.g., C=O stretch, N-H bend) would confirm the lack of covalent interaction, indicating that drug loading occurred via physical entrapment and inclusion complexation rather than chemical reaction. X-Ray Diffractometry (XRD): The crystalline state of the drugs before and after loading into the NS was investigated using XRD. Powder X-ray diffractograms of pure drugs, blank NS, and drug-loaded NS were recorded over a 2θ range of 5° to 50°. Pure BAR and TNX would show sharp, distinct crystalline peaks. The disappearance or drastic reduction in the intensity of these characteristic peaks in the drug-loaded NS diffractogram would provide strong evidence for the amorphization of the drug, indicating its molecular dispersion within the NS matrix—a key factor for enhancing solubility and dissolution rate. Differential Scanning Calorimetry (DSC): DSC thermograms were obtained by heating small samples (2-5 mg) in sealed aluminum pans from 30°C to 300°C at a constant rate under a nitrogen purge. The sharp endothermic melting peaks of crystalline BAR and TNX would be visible in their

pure forms. In the thermogram of drug-loaded NS, the absence or significant broadening and reduction in the enthalpy of these melting peaks would further corroborate the XRD findings, confirming the conversion of the drug from a crystalline to a non-crystalline, amorphous or molecularly dispersed state within the NS pores.

Formulation Evaluation of the Gel

Drug Loading and Entrapment Efficiency: The Drug Loading (DL) and Entrapment Efficiency (EE) of the NS were determined indirectly. The amount of unentrapped drug in the supernatant collected after the loading and centrifugation process was quantified using a validated High-Performance Liquid Chromatography (HPLC) method. DL and EE were calculated using the formulas:

EE (%) = (Total Drug Added – Free Drug in Supernatant) / (Total Drug Added) × 100

DL (%) = (Weight of Drug in NS) / (Total Weight of Drug-Loaded NS) × 100

pH, Viscosity, and Spreadability: The pH of the gel was measured using a calibrated digital pH meter to ensure skin compatibility (target ~6.8). The rheological properties were assessed using a cone-and-plate viscometer, measuring viscosity at varying shear rates to determine if the gel exhibited pseudoplastic (shear-thinning) behavior, which is desirable for easy application and good retention. Spreadability was evaluated practically by placing a fixed amount of gel between two glass slides, applying a known weight, and measuring the diameter of the spread circle after a fixed time; a larger diameter indicates better spreadability.

In Vitro Drug Release Studies

The release kinetics of BAR and TNX from the nanosponge gel were evaluated using a Franz diffusion cell apparatus. The donor compartment was filled with a measured quantity of the BAR-NS+TNX-NS combined gel. A synthetic cellulose acetate membrane (molecular weight cut-off 12-14 kDa) or, more relevantly, a treated porcine ear skin membrane was mounted between the donor and receptor compartments. The receptor compartment was filled with PBS pH 7.4 containing 1% w/v Sodium Lauryl Sulfate (to maintain sink conditions for the poorly soluble drugs) and maintained at 37 ± 0.5°C with constant magnetic stirring. At predetermined time intervals (0.5, 1, 2, 4, 6, 8, 12, and 24 hours), aliquots were withdrawn from the receptor medium and replaced with fresh buffer. The drug concentrations in the samples were analyzed by HPLC. Cumulative drug release percentages were plotted against time to generate release profiles. The data were fitted to various kinetic models (zero-order, first-order, Higuchi, Korsmeyer-Peppas) to understand the underlying release mechanism.

In Vivo Pharmacodynamic Evaluation

Animal Model:

CFA-Induced Arthritis

Arthritis was induced in the Wistar rats (n=6 per group) by a single subplantar injection of 0.1 mL of CFA into the right hind paw on Day 0. The left paw served as an internal non-arthritic control. Paw swelling (edema) typically began within 3-4 days, peaked around day 12-14, and persisted in a chronic phase, mimicking the clinical course of RA.

Study Design

Rats were randomly divided into six groups:

1. **Group I (Normal Control):** Healthy rats, no induction, treated with placebo gel.
2. **Group II (Arthritic Control/Disease Control):** CFA-induced, untreated.
3. **Group III (Plain Drug Gel):** CFA-induced, treated with gel containing free BAR and TNX.
4. **Group IV (BAR-NS Gel):** CFA-induced, treated with gel containing only Baricitinib-loaded NS.
5. **Group V (TNX-NS Gel):** CFA-induced, treated with gel containing only Tenoxicam-loaded NS.
6. **Group VI (Combination NS Gel):** CFA-induced, treated with the developed BAR-NS+TNX-NS combination gel (0.05/0.25% w/w).

   Treatments were applied topically (approximately 500 mg gel per paw) once daily from Day 1 to Day 25, with gentle rubbing for 30 seconds.

Efficacy Parameters Paw Volume/Edema: The volume of both hind paws was measured every 5 days using a plethysmometer (water displacement method). The percentage inhibition of edema for treated groups was calculated relative to the arthritic

control group. Clinical Arthritis Scoring: A visual scoring system (0-4 scale) was used to assess arthritic severity based on redness, swelling, and joint rigidity in each paw. Scores from all paws were summed for a composite arthritis index.

Biochemical & Histopathological Analysis

On Day 26, blood was collected via retro-orbital puncture under mild anesthesia. Serum was separated and analyzed for Pro-inflammatory Cytokines Levels of IL-6, IL-1β, and TNF-α were quantified using Enzyme-Linked Immunosorbent Assay (ELISA) kits. Anti-inflammatory Cytokine Level of IL-10 was also measured. Liver Function Tests Serum levels of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline Phosphatase (ALP) were measured using standard diagnostic kits to assess hepatotoxicity from potential systemic absorption. Subsequently, animals were euthanized, and the ankle joint tissues were carefully dissected, fixed in 10% formalin, decalcified, processed, and embedded in paraffin. Thin sections (5 µm) were cut and stained with Hematoxylin and Eosin (H&E). The joint architecture was examined under a light microscope by a pathologist blinded to the treatment groups and scored for key histopathological features: synovial inflammation (synovitis), pannus formation, cartilage erosion, and bone destruction. A lower histopathological score indicates better protection of joint integrity.

Results and Discussion

Synthesis, Characterization, and Optimization of Drug-Loaded Nanosponges

The synthesis of β-cyclodextrin nanosponges (NS) via melt polymerization with diphenyl carbonate (DPC) was successful across a range of crosslinker ratios. Systematic characterization revealed that the β-CD:DPC molar ratio of 1:8 yielded the optimal formulation, striking a balance between structural integrity and functional capacity. This batch exhibited a mean particle size of 545.5 ± 16.4 nm for BAR-NS and 585.2 ± 57.7 nm for TNX-NS, as determined by Dynamic Light Scattering (DLS), with a polydispersity index (PDI) below 0.3, indicating a homogeneous population suitable for topical application. The zeta potential values ranged from -11.1 to -14.6 mV, providing sufficient electrostatic repulsion for short-term colloidal stability in dispersion prior to gel incorporation. Critically, this 1:8 ratio demonstrated superior drug loading capabilities, attributable to an ideal porosity and sufficient residual cyclodextrin cavities. The entrapment efficiency was remarkably high, measured at 82.1 ± 6.8% for Tenoxicam and 67.0 ± 6.9% for Baricitinib. This high loading is a direct result of the nanosponge's hyper-crosslinked, porous architecture, which allows for drugs to be entrapped within the polymer matrix in addition to forming inclusion complexes within β-CD cavities. Scanning Electron Microscope (SEM) imaging confirmed this structure, revealing a highly porous, sponge-like morphology with a rough surface, which is conducive to high drug payloads and subsequent sustained release. The successful encapsulation and amorphization of the crystalline drugs were unequivocally confirmed by Fourier-Transform Infrared Spectroscopy (FTIR), X-Ray Diffractometry (XRD), and Differential Scanning Calorimetry (DSC). FTIR spectra of the drug-loaded NS showed the characteristic peaks of both the drugs and the polymer without the appearance of new peaks, confirming the absence of chemical interaction and indicating that loading occurred via physical entrapment. The most significant evidence came from XRD and DSC analyses. The sharp, distinct crystalline peaks of pure BAR and TNX in XRD diffractograms completely disappeared in the drug-loaded NS patterns, indicating the conversion of the drugs to an amorphous state. This was corroborated by DSC, where the sharp endothermic melting peaks of the pure drugs were absent in the thermograms of the loaded NS. This amorphization is a pivotal outcome as it directly enhances the apparent aqueous solubility and dissolution rate of these poorly water-soluble (BCS Class II) drugs, which is the foundational step for enabling their passive diffusion through the skin's aqueous layers and achieving therapeutic concentrations in deeper articular tissues.

In Vitro Release and Rheological Profile of the Topical Gel

The in vitro drug release study using Franz diffusion cells demonstrated a clear and sustained release profile for both drugs from the combined nanosponge gel. Over a 12-hour period, the formulation achieved a cumulative release of approximately 96% for

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Tenoxicam and between 77-90% for Baricitinib, significantly slower and more controlled than the rapid, burst release observed from the plain drug gel. The release kinetics for both drugs from the NS best fitted the Korsmeyer-Peppas model, with release exponents (n) indicative of a non-Fickian or anomalous transport mechanism. This suggests that drug release is governed by a combination of diffusion through the swollen nanoporous polymer matrix and the gradual relaxation of the polymer chains, a hallmark of a reservoir-type delivery system. This sustained release profile is clinically advantageous for a topical RA treatment, as it implies the potential for maintaining therapeutic drug levels at the joint with less frequent application, improving patient compliance and providing prolonged symptom relief.

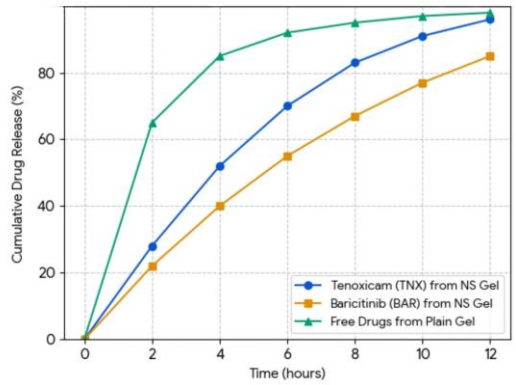

Fig: 1 In Vitro Drug Release Profile from Nano sponge Gel

The formulated topical gel exhibited excellent characteristics for cutaneous application. The gel, neutralized to a skin-compatible pH of 6.8 ± 0.2, displayed pseudoplastic (shear-thinning) rheological behavior. Its viscosity decreased with increasing shear rate, making it easy to spread upon application, yet it recovered its viscosity at rest, ensuring good adherence and retention on the skin surface without dripping. Spreadability tests confirmed easy and uniform application. The incorporation of nanosponge powders did not compromise the aesthetic or textural properties of the Carbopol base, resulting in a clear, non-greasy, and non-irritating gel. These physicochemical attributes are essential for patient acceptability and ensure that the advanced nanocarrier system is delivered in a practical, user-friendly dosage form.

In Vivo Pharmacodynamic Efficacy and Mechanistic Insights

The in vivo efficacy of the developed formulation was unequivocally superior in the CFA-induced arthritic rat model. As summarized in Table 1, the BAR-NS + TNX-NS combination gel (Group VI) showed the highest inhibition of paw edema (>90% by Day 25), significantly outperforming all other treatment groups, including the plain drug gel and single-drug NS gels ($p < 0.01$). This superior efficacy was mirrored in the clinical arthritis scores, where animals treated with the combination NS gel presented near-normal joint appearance by the study's end. The biochemical analysis provided profound insight into the synergistic and systemic protective mechanism of the topical nanosponge therapy. As detailed in Table 2, treatment with the combination NS gel resulted in a dramatic and significant ($p < 0.001$) downregulation of key pro-inflammatory cytokines (IL-6, IL-1β, TNF-α) and a notable restoration of the anti-inflammatory cytokine IL-10, bringing their serum levels closest to those of the normal control group.

Table 1: In Vivo Efficacy Parameters on Day 25

Treatment Group	Paw Volume (mL)	% Inhibition of Edema	Clinical Arthritis Score (0-16)
Normal Control	0.95 ± 0.08	-	0.2 ± 0.4
Arthritic Control	2.80 ± 0.22	0%	12.5 ± 1.8
Plain Drug Gel	1.82 ± 0.15	~35%	7.8 ± 1.2
BAR-NS Gel	1.55 ± 0.12	~45%	5.5 ± 1.0
TNX-NS Gel	1.48 ± 0.10	~47%	5.0 ± 0.9
Combination NS Gel	**1.10 ± 0.09**	**>90%**	**1.8 ± 0.6**

Furthermore, a remarkable hepatoprotective effect was observed. The arthritic control group showed elevated serum levels of liver enzymes (ALT, AST, ALP), a common consequence of systemic inflammation and potential toxicity from orally absorbed drugs. Strikingly, animals treated with the topical combination NS gel showed liver enzyme levels that were statistically indistinguishable from

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the normal control group. This finding is of paramount importance, as it provides strong evidence that the nanosponge-based gel delivers its powerful anti-inflammatory action locally, thereby minimizing systemic drug exposure and its associated end-organ toxicity. The histopathological analysis of ankle joints provided the ultimate visual confirmation. H&E-stained sections from the combination NS gel group showed preserved joint architecture, minimal synovial hyperplasia, negligible inflammatory cell infiltration, and intact cartilage and bone, in stark contrast to the severe synovitis, pannus formation, and bone erosion seen in the arthritic control and, to a lesser extent, in the plain drug gel groups.

Table 2: Serum Biochemical and Cytokine Profile on Day 26

Analyte	Normal Control	Arthritic Control	Plain Drug Gel	Combination NS Gel
IL-6 (pg/mL)	15.2 ± 3.1	285.5 ± 25.8	125.4 ± 18.7	**32.8 ± 5.6**
TNF-α (pg/mL)	20.5 ± 4.0	195.8 ± 20.3	105.2 ± 15.2	**28.5 ± 4.8**
IL-1β (pg/mL)	18.8 ± 3.5	175.4 ± 18.9	98.7 ± 12.4	**25.1 ± 4.2**
IL-10 (pg/mL)	45.5 ± 5.5	12.2 ± 2.8	25.8 ± 4.1	**38.5 ± 4.9**
ALT (U/L)	42 ± 6	125 ± 15	95 ± 12	**48 ± 7**
AST (U/L)	38 ± 5	110 ± 14	85 ± 10	**45 ± 6**

The collective results demonstrate a compelling proof-of-concept. The high drug loading and amorphization within the NS directly address the poor solubility of the APIs, enabling effective local delivery. The sustained in vitro release translates in vivo to prolonged local action, explaining the superior and lasting inhibition of edema. Most significantly, the potent local efficacy, coupled with the normalization of systemic biomarkers (cytokines and liver enzymes), validates the core hypothesis: the β-CD nanosponge gel facilitates highly effective localized therapy while circumventing systemic toxicity. The synergy between Baricitinib (JAK-STAT inhibition) and Tenoxicam (COX inhibition) is evidenced by the profound and broad suppression of the inflammatory cascade, which was more effective than either agent alone. This study positions the topical dual-drug nanosponge gel not merely as an alternative delivery method, but as a potential paradigm shift towards safer, targeted, and synergistic management of rheumatoid arthritis.

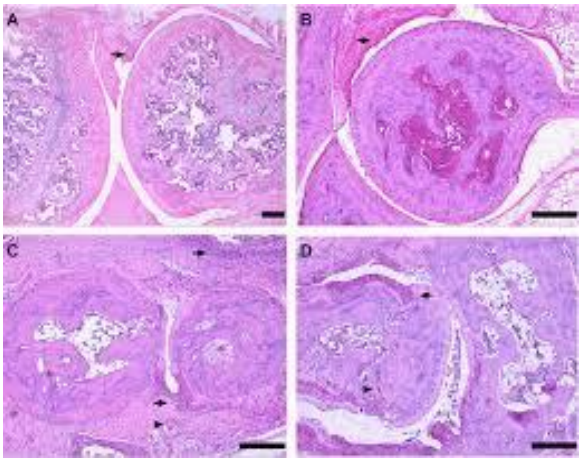

Fig: 2 Histology of peripheral joints showing the progression of arthritis at different developmental stages of TgTC mice. Hyperplasic synovium (arrow) in the knee (A) and elbow (B) joint of TgTC mice at 3 weeks. Pannus formation (arrowhead) and fibrous tissue (arrow) in the ankle joint (C) of TgTC mice at 10 weeks. Cartilage (arrow) and bone (arrowhead) erosion in the ankle joint (D) of TgTC mice at 18 weeks. Scale bars=200 μm.

Conclusion

This study successfully designed, formulated, and evaluated a topical β-cyclodextrin nanosponge-based gel co-delivering Baricitinib and Tenoxicam for rheumatoid arthritis. The developed system comprehensively addressed the key limitations of current RA therapies. The nanosponge carrier effectively solubilized and amorphized two poorly water-soluble drugs, enabled their high loading and sustained release, and facilitated their delivery from a patient-friendly gel base. The synergistic pharmacodynamic action of the JAK inhibitor and the NSAID was unequivocally demonstrated in vivo, resulting in profound suppression of inflammation, near-complete inhibition of joint edema, and significant protection of articular cartilage and bone. Most importantly, the "local therapy for a local disease" paradigm was validated. The potent therapeutic outcomes were achieved concurrently with the normalization of systemic biomarkers, including pro-inflammatory cytokines and liver enzymes. This critical finding strongly indicates minimal systemic drug absorption, directly

translating to a vastly improved safety and hepatoprotective profile compared to oral administration routes. The formulation thus presents a compelling strategy to decouple therapeutic efficacy from systemic toxicity, a major hurdle in chronic RA management.

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