

**FORMULATION AND EVALUATION OF MESOPOROUS
SILICA NANOPARTICLES LOADED ANTIARTHRITIC
GEL AS A TARGETED DRUG DELIVERY SYSTEM**

**फार्मूलेशन एंड इवैल्यूएशन ऑफ़ मेसोपोरोस सिलिका नैनोपार्टिकल्स लोडेड
एंटीअर्थरिटिक जेल एज अ टारगेटेड ड्रग डिलीवरी सिस्टम**

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Thesis**

**Submitted for the Award of the Ph.D. degree of
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RESEARCH UNIVERSITY**

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2024

DECLARATION

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Signature of the Candidate

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It gives me immense pleasure in certifying that the thesis entitled **“FORMULATION AND EVALUATION OF MESOPOROUS SILICA NANOPARTICLES LOADED ANTIARTHRITIC GEL AS A TARGETED DRUG DELIVERY SYSTEM”** and submitted by **DINESH DAYARAMJI CHAKOLE** is based on the work research carried out under my guidance. He has completed the following requirements as per Ph.D. regulations of the University;

- i. Course work as per University rules.
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- iii. Regularly presented Half Yearly Progress Report as prescribed by the University.
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Place: Udaipur

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DINESH DAYARAMJI CHAKOLE

ABSTRACT

The thesis entitled “**Formulation and Evaluation of mesoporous silica nanoparticles loaded antiarthritic gel as a targeted drug delivery system**” contains the research work carried out for Ph.D. Degree, at Pacific University of Higher Education and Research, Rajasthan, India under the supervision of **Dr. Rakte Amol Sharanappa** and under Co-Supervision of **Dr. Vishal Vijay Pande**.

This system aims to improve the solubility, permeability, and bioavailability of poorly soluble drugs and enhance the targeted delivery to arthritic sites, thereby maximizing therapeutic efficacy while minimizing systemic side effects.

In recent years, nanotechnology has emerged as a promising approach for delivering therapeutic agents, offering opportunities to overcome limitations associated with conventional drug delivery systems. Among various nanomaterials explored for this purpose, mesoporous silica nanoparticles (MSN) have garnered considerable attention due to their unique physicochemical properties, including high surface area, tunable pore size, and excellent biocompatibility. These characteristics make MSN ideal candidates for encapsulating and delivering a wide range of therapeutic compounds, including poorly soluble drugs. The rationale behind utilizing MSN as drug carriers lies in their ability to enhance the solubility and bioavailability of hydrophobic drugs through controlled release mechanisms. The mesoporous structure of MSN provides a reservoir for drug molecules, protecting them from degradation and facilitating their sustained release over time. Furthermore, the surface of MSN can be functionalized to tailor drug loading and release properties, allowing precise control over drug delivery kinetics.

In this study, we focus on two therapeutic agents, methotrexate and tofacitinib citrate. Methotrexate is commonly used in the treatment of various cancers and autoimmune diseases also it is considered as first choice for treatment of rheumatoid arthritis, while tofacitinib citrate is indicated for the management of rheumatoid arthritis and ulcerative colitis. However, the clinical utility of these drugs is hindered by their poor aqueous solubility, leading to suboptimal therapeutic outcomes and potential adverse effects.

Methotrexate and Tofacitinib Citrate, when incorporated into the MSN-based gel, can potentially offer significant improvements in drug delivery. The high surface area and controlled release properties of MSNs allow for targeted delivery of these drugs to the affected joints, enhancing their therapeutic efficacy while reducing systemic side effects. This approach not only addresses the limitations associated with oral drug administration but also provides a more convenient and effective treatment option for patients suffering from arthritis

The whole thesis was divided into seven chapters as follows:

Chapter 1: Introduction

In this chapter gives information about Arthritis and its different aspects and treatments, Mesoporous silica Nanoparticles (MSNs) as a effective drug carrier, Use of Nanogel for Targeted drug delivery system.

Arthritis, a group of inflammatory joint disorders, affects millions worldwide. Effective pain management of arthritis often requires prolonged treatment with antiarthritic medications. Two commonly used drugs in the treatment of arthritis are Methotrexate and Tofacitinib Citrate. Methotrexate is a disease-modifying anti-rheumatic drug (DMARD) that inhibits cellular metabolism and reduces inflammation, making it a cornerstone in the treatment of rheumatoid arthritis. Tofacitinib Citrate, is a Janus kinase (JAK) inhibitor that interferes with specific intracellular signaling pathways to diminish the inflammatory response.

Mesoporous silica nanoparticles (MSNs) have emerged as highly effective drug carriers due to their unique properties. MSNs are characterized by their high surface area, tunable pore size, and ability to provide controlled release of encapsulated drugs. These attributes make MSNs ideal candidates for drug delivery systems that require targeted and sustained release.

Topical gels are a promising method for localized drug delivery in arthritis treatments. Arthritis is often characterized by inflammation and pain in the joints, making it important to deliver medications directly to the affected area. Gels provide an ideal medium for this because of their semi-solid nature and ease of application. They spread easily over the skin, forming a thin film, which ensures better contact between the drug and the skin surface.

Chapter 2: Review of literature

In this chapter important literatures were done to understand the effectiveness of MSNs as drug carrier and systemic limitations of conventional drug delivery of Methotrexate and Tofacitinib citrate in the treatment of arthritis. To design the research work in detail.

Methotrexate and Tofacitinib Citrate, when incorporated into the MSN-based gel, can potentially offer significant improvements in drug delivery. The high surface area and controlled release properties of MSNs allow for targeted delivery of these drugs to the affected joints, enhancing their therapeutic efficacy while reducing systemic side effects. This approach not only addresses the limitations associated with oral drug administration but also provides a more convenient and effective treatment option for patients suffering from arthritis.

Chapter 3: Aim and Objectives

The aim of this research is to formulate and evaluate a mesoporous silica nanoparticle (MSN)-based antiarthritic gel as a targeted drug delivery system- to improve the solubility, permeability, and bioavailability of poorly soluble drugs and enhance the targeted delivery to arthritic sites, thereby maximizing therapeutic efficacy while minimizing systemic side effects.

Objectives:

1. **Synthesis, Surface modification and Characterization of Mesoporous Silica Nanoparticles (MSNs):** MSNs will act as the drug carriers due to their high surface area, tunable pore sizes, and excellent biocompatibility.

Characterization of MSNs: using techniques such as

- **Fourier-Transform Infrared Spectroscopy (FTIR):** (presence of characteristic peaks for organic functional groups indicates successful surface modification.)
- **Differential Scanning Calorimetry (DSC)** (for thermal properties),
- **Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM)** (to study nanoparticle morphology),

- **Brunauer-Emmett-Teller (BET) analysis** (to measure the surface area of MSNs).
 - **pXRD** (to understand structural integrity and crystallinity)
2. **Loading of Antiarthritic Drugs into surface modified MSNs:** This step will focus on improving the drug's solubility and dissolution rate of poorly soluble drugs to ensure efficient drug delivery.
 3. **Formulation of MSN-Loaded Nanogel:** This objective focuses on the formulation of a topical gel incorporating MSN-loaded antiarthritic drugs to create a targeted drug delivery system to ensure the drug is released at the desired site of action (arthritic joints), enhancing local bioavailability while minimizing systemic exposure.
 4. **Characterization of the MSN-Loaded Nanogel:** using advanced techniques such as:
 - Viscosity
 - Spreadability
 - Texture analysis
 - Particle size and Particle size distribution
 - Zeta potential
 - Drug release profile
 5. **Stability of the antiarthritic Nanogel**

To establish the good shelf life and to provide flexibility in storage options.
 - 6 **Dermatokinetics Study:** The final objective is to perform dermatokinetic of the formulated MSN-loaded nanogel. This will provide insight into how well the formulation delivers the drug to the targeted site and its overall therapeutic efficacy.

Chapter 4: Plan of work

This chapter details the plan of work-

- 1 Selection of drug and excipient (Tofacitinib Citrate and Methotrexate)
- 2 Synthesis of mesoporous silica i.e. SBA-15

- 3 Characterization of synthesized SBA-15
- 4 Amine functionalization of SBA-15
- 5 Characterization of surface modified Mesoporous Silica
- 6 Loading of antiarthritic drugs in Surface Modified MSNs
- 7 Characterization of antiarthritic drugs loaded MSNs
- 8 Incorporation of MSN's in to gel base to prepare Nanogel
- 9 Characterization of the MSN-Loaded Nanogel
- 10 Stability study
- 11 Dermatokinetic evaluation of Antiarthritic gel

Chapter 5: Materials and methods

This chapter provided the detailed information on the materials and instruments used for manufacturing and evaluation of MSNs and Formulation Nanogel.

Detailed information on Synthesis, Surface modification and Characterization of MSNs was given. Formulation development deals with gel base manufacturing and then incorporation of antiarthritic drug loaded MSN in to gel base. Characterization of gel formulation provided the information on suitability of gel formulation for arthritic treatment for the topical purpose.

Chapter 6: Results and discussion

In this chapter results obtained for Synthesis and characterization of MSNs confirms the desired MSN platform was obtained when evaluated using sophisticated instruments like FTIR, DSC, SEM, TEM, BET, pXRD etc. A honeycomb like regular structure uniform pore sizes, typically in the range of 2 to 50 nm, distributed throughout the silica matrix with suitable surface morphology properties.

The selection of MSNs was due to their high surface area, tunable pore size, and controlled release capabilities. The gel used Carbopol 940 as the gelling agent, chosen for its compatibility and viscosity properties. The formulation process involved dispersing Carbopol 940 in distilled water, hydrating, and adjusting the pH before incorporating the drug-loaded MSNs. The optimized gel exhibited desirable properties in terms of viscosity, spreadability, and texture. Particle size analysis showed a narrow distribution, and zeta potential

measurements confirmed the stability of the gel. *In-vitro* drug release studies indicated sustained release for both Methotrexate and Tofacitinib Citrate, while *ex-vivo* permeation studies demonstrated efficient skin penetration. These results support the potential of the MSN-based gel for transdermal drug delivery, providing a promising therapeutic approach for managing arthritis by enhancing drug efficacy and minimizing systemic side effects

Gel formulation remains stable for all formulation parameters at accelerated conditions, room temperatures and at lower temperatures providing flexibility in storage options.

Chapter 7: Summary and Conclusion

This research presents a novel approach for enhancing the therapeutic efficacy of antiarthritic drugs through advanced nanotechnology. The study comprehensively investigates the structural, morphological, and textural properties of MSNs using various analytical techniques including FTIR spectroscopy, particle size analysis, TEM, SEM, DSC, and BET analysis. These characterizations confirm the successful functionalization and high surface area of MSNs, making them an ideal candidate for targeted drug delivery systems.

The antiarthritic drugs Methotrexate and Tofacitinib Citrate were effectively loaded into the surface-modified MSNs, as confirmed by the combination of FTIR and pXRD analyses, which demonstrated the structural integrity of the MSNs post-drug loading.

The loaded MSNs exhibited high drug loading efficiency and a sustained release profile, which are crucial for improving drug solubility, stability, and targeted delivery, ultimately enhancing therapeutic outcomes for arthritis patients. The *in-vitro* and *ex-vivo* evaluations demonstrated that the MSNs-based formulation could achieve controlled and sustained drug release, highlighting its potential as a robust drug delivery platform.

In conclusion, the formulated MSNs-loaded antiarthritic gel offers a promising approach for the targeted transdermal delivery of antiarthritic drugs, providing sustained and controlled drug release that could significantly enhance therapeutic outcomes for arthritis patients. The study's findings highlight the potential of mesoporous silica nanoparticles in developing advanced drug delivery systems that improve drug solubility, stability, and targeted delivery.

Key words:

Mesoporous silica nanoparticles, antiarthritic gel, controlled release, transdermal delivery, Methotrexate, Tofacitinib Citrate

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ABBREVIATIONS

MSNs	: Mesoporous Silica Nanoparticles
DMARDs	: Disease-Modifying Antirheumatic Drugs
JAK	: Janus Kinase
NSAIDs	: Nonsteroidal Anti-Inflammatory Drugs
COX	: Cyclooxygenase
AUC	: Area under the Curve
MTX	: Methotrexate
TC	: Tofacitinib Citrate
TEM	: Transmission Electron Microscopy
SEM	: Scanning Electron Microscopy
BET	: Brunauer-Emmett-Teller
CAS	: Chemical Abstracts Service
BP	: British Pharmacopoeia
TEA	: Triethanolamine
API	: Active Pharmaceutical Ingredient
GRAS	: Generally Recognized as Safe
EDTA	: Ethylenediaminetetraacetic Acid
PG	: Propylene Glycol
DMSO	: Dimethyl Sulfoxide
KBr	: Potassium Bromide
TEOS	: Tetraethyl Orthosilicate
FTIR	: Fourier Transform Infrared Spectroscopy
DLS	: Dynamic Light Scattering
APTES	: 3-Aminopropyltriethoxysilane
PBS	: Phosphate-Buffered Saline
pXRD	: Powder X-ray Diffraction

C _{max}	:	Maximum Concentration
T _{max}	:	Time to Reach Maximum Concentration
PDI	:	Polydispersity Index
APTES	:	3-Aminopropyltriethoxysilane
K _a	:	Absorption Rate Constant
K _e	:	Elimination Rate Constant
t _{1/2}	:	Half-Life
CFU	:	Colony-Forming Unit



INTRODUCTION



CHAPTER 1

INTRODUCTION

1.1 Background

1.1.1 Introduction to Arthritis

The word "arthritis" refers to a collection of diseases that impact the surrounding tissues and joints. Greek words "arthro," which means joint, and "itis," which means inflammation, are the roots of the English word arthritis. Therefore, inflammation of the bones is the literal definition of arthritis. It is one of the most prevalent causes of disability globally and is a general term that encompasses over 100 distinct kinds of joint illnesses and disorders.

Individuals from every age, sexual orientation, and cultural origins are affected by arthritis. It is typified by joint pain, swelling, rigidity, and reduced range of motion. These symptoms may be intermittent or persistent, varying in severity, and ongoing. Severe cases of arthritis can result in irreversible damage to joints and disability, which would greatly lower a person's standard of living [1].

Historical Perspective of Arthritis

The human condition known as arthritis has been around for thousands of years; it is not a recent development. The bone fragments from medieval Egyptian mummies and the preserved bones of ancient creatures have been shown to contain evidence of arthritis. Various societies have recognized and managed arthritis in different ways throughout history. For instance, rheumatoid arthritis symptoms were first described by ancient Greek doctors like Hippocrates, and traditional Chinese medicine has long treated joint pain with acupuncture and herbal remedies [2-3].

Prevalence of Arthritis

Arthritis is a common ailment that impacts millions of individuals globally. The World Health Organization (WHO) estimates that over 350 million people globally have arthritis. The Centers for Disease Control and Prevention (CDC) project that 54 million adults in the US alone suffer from arthritis, and by 2040, that figure is predicted to increase to 78 million.

Arthritis is more common in women than in men, and its prevalence rises with age. But it can impact people of all ages, even young ones. One kind of arthritis that affects kids younger than 16 is called juvenile arthritis [4].

1.1.2 Types of Arthritis

There are over 100 different types of arthritis, but the most common types include:

Osteoarthritis (OA)

Osteoarthritis, the most common form of arthritis, impacts millions of individuals worldwide. Arthritis is commonly termed "worn-out" arthritis due to the degeneration of the cartilage layer that cushions the ends of the bones over time. This results in friction between the bones, leading to pain, swelling, and stiffness. Osteoarthritis predominantly impacts the spine, hands, knees, and hips.

Factors contributing to osteoarthritis (OA) include aging, obesity, genetics, joint injuries, and repetitive joint stress. Despite OA being a chronic condition that may deteriorate over time, appropriate care and lifestyle adjustments can mitigate its progression.

Rheumatoid Arthritis (RA)

Rheumatoid arthritis is an autoimmune disorder in which the immune system mistakenly attacks the synovium, the membrane lining the joints. This results in inflammation, which may damage the bones and cartilage within the joint. While rheumatoid arthritis (RA) can impact the skin, eyes, lungs, heart, and blood vessels, it predominantly affects the small joints of the hands and feet.

Rheumatoid arthritis typically manifests in individuals aged 40 to 60 and is more prevalent in women than in men. Rheumatoid arthritis is induced by an abnormal immune response, whereas osteoarthritis is primarily caused by degeneration. The exact etiology of RA remains unidentified, but a combination of environmental and genetic factors is believed to contribute.

Psoriatic Arthritis (PsA)

Individuals with psoriasis, characterized by red, scaly lesions, may develop psoriatic arthritis, a form of inflammatory arthritis. Psoriatic arthritis (PsA) can induce joint pain,

stiffness, and swelling, which are indicative of rheumatoid arthritis and can impact any joint in the body. Psoriatic arthritis (PsA) can cause alterations in the nails and inflammation in various body regions, including the eyes, alongside joint-related symptoms.

PsA is a chronic condition characterized by varying degrees of severity. Some individuals may experience only mild joint symptoms, while others may endure significant joint damage and become profoundly disabled. Early diagnosis and treatment are essential for managing PsA and preventing joint damage.

Gout

Gout is a form of arthritis characterized by the accumulation of uric acid crystals in the joints, resulting in abrupt and intense episodes of pain, swelling, and erythema. The hallux is the most frequently impacted, although gout may also involve other joints, including the ankles, knees, elbows, wrists, and fingers.

Gout is more prevalent in men than in women and frequently manifests in individuals with elevated uric acid levels in their bloodstream. Contributors to gout encompass a diet rich in purines (present in red meat, shellfish, and alcohol), obesity, specific medications, and a familial predisposition to gout.

Ankylosing Spondylitis (AS)

Ankylosing spondylitis is a form of arthritis that predominantly impacts the spine, resulting in inflammation of the vertebrae, which may cause significant, chronic pain and discomfort. In advanced instances, inflammation may lead to the fusion of spinal bones, resulting in diminished flexibility and a stooped posture. AS may also impact additional joints, including the hips, shoulders, and ribs.

AS is more prevalent in men than in women and generally commences in early adulthood. The precise etiology of AS remains unidentified; however, it is thought to result from an interplay of genetic and environmental influences.

Lupus

Lupus, or systemic lupus erythematosus (SLE), is an autoimmune disorder that can impact the joints and various organs, including the skin, kidneys, heart, and lungs. In lupus, the immune system assaults healthy tissues, resulting in inflammation and damage. Arthralgia and edema are prevalent manifestations of lupus, which may also induce fatigue, dermal eruptions, and various systemic symptoms.

Lupus is more prevalent in women than in men and usually manifests between the ages of 15 and 44. The precise etiology of lupus remains unidentified; however, it is thought to encompass a confluence of genetic, hormonal, and environmental influences [5-10].

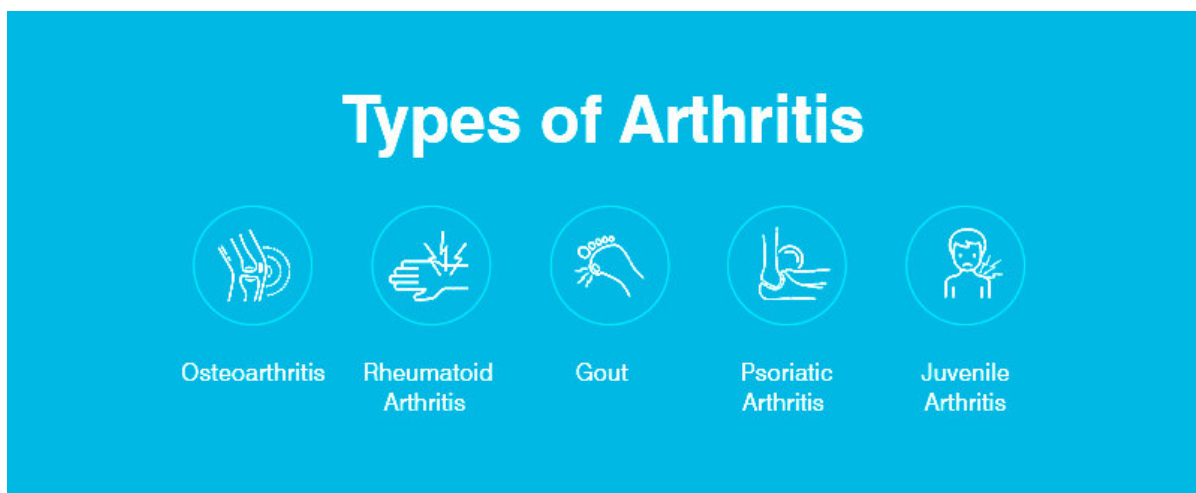


Figure 1.1: Types of Arthritis

1.1.3 Socio-Economic Impact of Arthritis

Arthritis has a significant socio-economic impact, both on individuals and society as a whole. The condition not only affects the physical health of those who suffer from it, but it also has a profound impact on their quality of life, ability to work, and financial stability.

Impact on Quality of Life

The quality of life for an individual afflicted with arthritis can be significantly diminished. Walking, dressing, and cooking can be arduous daily activities due to the pain, stiffness, and fatigue induced by the condition. This may lead to feelings of loneliness and depression due to a loss of independence and reduced ability for social interaction.

The chronic nature of arthritis results in many individuals experiencing symptoms for years, if not decades. The persistent discomfort and limitations may induce anxiety, depression, and a reduced sense of wellbeing, all of which can adversely affect mental health.

Impact on Employment

Arthritis is a predominant cause of global disability, significantly affecting employment. A significant number of individuals with arthritis are incapacitated from work or compelled to diminish their hours due to the pain and physical constraints imposed by the condition. This may result in diminished income and financial instability, which can further intensify the difficulties associated with living with arthritis. Arthritis exerts both a direct influence on employment and an indirect effect on the economy. Arthritis-related disability and absenteeism result in billions of dollars annually in lost wages and diminished economic productivity.

Healthcare Costs

Arthritis management incurs significant costs. The direct costs of arthritis encompass medical expenses such as prescription medications, physician consultations, physical therapy, and surgical procedures. Indirect costs associated with the illness also exist, including lost income and diminished productivity.

Approximately \$300 billion is expended annually on medical expenses and lost income attributable to arthritis in the United States. Consequently, the management of arthritis ranks among the most expensive chronic conditions.

Impact on Families and Caregivers

Arthritis affects not only the individuals afflicted by the condition but also significantly impacts their families and caregivers. Numerous individuals with arthritis necessitate support for daily activities, imposing a considerable strain on family members and caregivers. This may result in stress, burnout, and financial hardship, particularly if the caregiver must decrease their work hours or resign from their position to offer care [11-16].

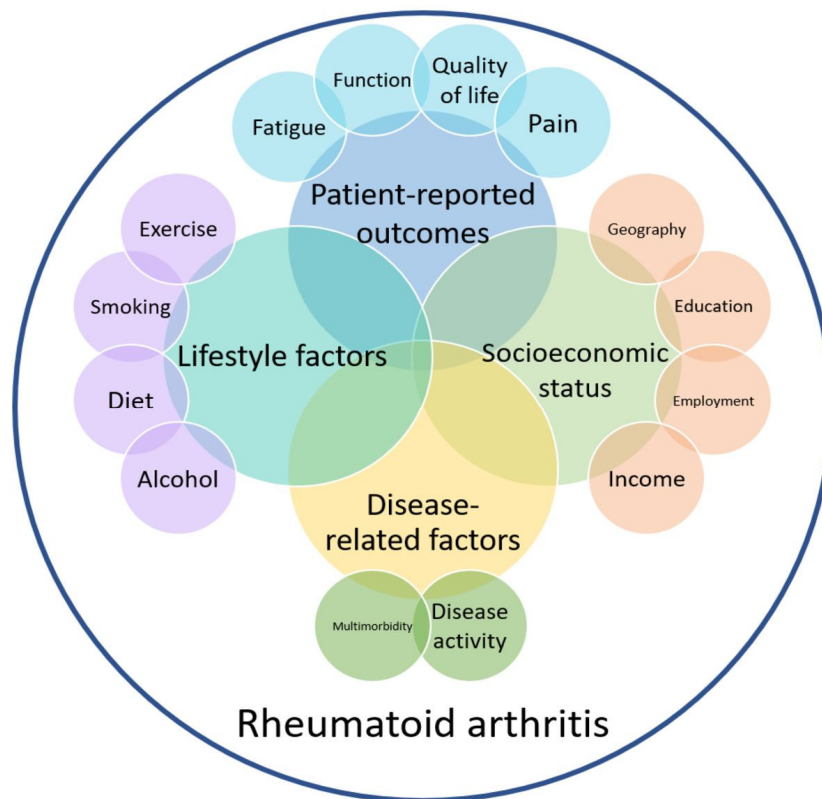


Figure 1.2: Socio-Economic factors of arthritis

1.1.4 Conventional Treatments for Arthritis

The principal objective of arthritis treatment is to alleviate symptoms such as pain and inflammation, enhance joint functionality, and avert additional joint deterioration. Traditional therapies for arthritis encompass various medications and treatments that have been utilized for many years. The treatments are primarily classified into three principal categories: Nonsteroidal Anti-Inflammatory Drugs (NSAIDs), Corticosteroids, and Disease-Modifying Antirheumatic Drugs (DMARDs). Each class of these medications is essential for arthritis management; however, they possess considerable limitations that impact their long-term efficacy and safety.

Nonsteroidal Anti-Inflammatory Drugs (NSAIDs): NSAIDs are among the most commonly prescribed medications for arthritis. They work by blocking the enzyme cyclooxygenase (COX), which is responsible for producing prostaglandins, the chemicals in the body that cause inflammation, pain, and fever. By inhibiting these enzymes, NSAIDs reduce inflammation and pain.

- **Common NSAIDs:**
 - Ibuprofen (Advil, Motrin)
 - Naproxen (Aleve)
 - Diclofenac (Voltaren)
 - Celecoxib (Celebrex) – a selective COX-2 inhibitor
- **Mechanism of Action:**
 - NSAIDs inhibit both COX-1 and COX-2 enzymes, but it's the inhibition of COX-2 that primarily reduces inflammation and pain. However, COX-1 inhibition can lead to gastrointestinal (GI) side effects, as COX-1 is also involved in protecting the stomach lining.
- **Benefits:**
 - NSAIDs are effective in reducing pain and swelling in both osteoarthritis (OA) and rheumatoid arthritis (RA).
 - They can be used for short-term pain relief during arthritis flare-ups.
- **Limitations and Side Effects:**
 - **Gastrointestinal Issues:** Long-term use of non-selective NSAIDs can lead to stomach ulcers, bleeding, and gastritis due to COX-1 inhibition. Even COX-2 selective inhibitors, while safer for the stomach, may still cause GI issues.
 - **Cardiovascular Risks:** NSAIDs, particularly COX-2 inhibitors, have been associated with an increased risk of heart attack and stroke. This risk limits their use in patients with cardiovascular disease.
 - **Kidney Damage:** Prolonged use of NSAIDs can lead to kidney damage, especially in people with pre-existing kidney conditions or those who are dehydrated.
 - **Limited Efficacy in Disease Progression:** While NSAIDs effectively relieve symptoms, they do not slow down the progression of arthritis. They primarily address pain and inflammation without targeting the underlying disease process.

- **Why NSAIDs Alone Aren't Enough:**

- Despite their effectiveness in managing symptoms, NSAIDs do not alter the course of the disease. For chronic conditions like rheumatoid arthritis, where joint destruction can continue silently, NSAIDs are not sufficient as a standalone therapy. Long-term use also increases the risk of serious side effects, making them unsuitable for ongoing management in many patients.

Corticosteroids: Corticosteroids, also known as steroids, are potent anti-inflammatory drugs that can be used to treat a wide range of inflammatory conditions, including arthritis. These drugs mimic the effects of cortisol, a hormone naturally produced by the adrenal glands that helps regulate inflammation in the body.

- **Common Corticosteroids:**

- Prednisone
- Methylprednisolone (Medrol)
- Dexamethasone
- Hydrocortisone

- **Mechanism of Action:**

- Corticosteroids work by suppressing the immune system and reducing the production of inflammatory chemicals such as prostaglandins, cytokines, and interleukins. They inhibit multiple pathways involved in inflammation, making them highly effective in reducing joint swelling, pain, and other symptoms of arthritis.

- **Benefits:**

- **Rapid Relief:** Corticosteroids provide fast and powerful relief from inflammation, which can be particularly useful during severe arthritis flare-ups.
- **Versatile Administration:** They can be taken orally, injected directly into the affected joint, or applied topically. Intra-articular injections of corticosteroids can provide localized relief without the systemic side effects of oral medications.

- **Useful for Multiple Forms of Arthritis:** Corticosteroids are used to treat various types of arthritis, including RA, PsA, and gout.
- **Limitations and Side Effects:**
 - **Bone Loss (Osteoporosis):** Long-term use of corticosteroids can lead to a loss of bone density, increasing the risk of fractures, particularly in older adults.
 - **Increased Risk of Infections:** Corticosteroids suppress the immune system, making patients more susceptible to infections. This is a significant concern for people with chronic arthritis, who may already have compromised immune function.
 - **Weight Gain and Fluid Retention:** These drugs can cause significant weight gain, fluid retention, and changes in fat distribution (e.g., moon face, buffalo hump), which can negatively affect patients' quality of life.
 - **Insulin Resistance and Diabetes:** Prolonged corticosteroid use can lead to insulin resistance, raising blood sugar levels and potentially resulting in diabetes.
 - **Adrenal Suppression:** Long-term corticosteroid use can suppress the adrenal glands' ability to produce cortisol, leading to adrenal insufficiency. This can make it difficult for the body to cope with stress or trauma, such as surgery or infection.
- **Why Corticosteroids Aren't a Long-Term Solution:**
 - Although corticosteroids are highly effective in reducing inflammation, their severe side effects limit their use as a long-term therapy. They are typically reserved for short-term use during severe flare-ups or as a bridge therapy until slower-acting drugs (like DMARDs) take effect. Patients on long-term corticosteroids must be closely monitored for potential side effects, and efforts should be made to taper off the medication when possible.

Disease-Modifying Antirheumatic Drugs (DMARDs): DMARDs are a class of medications specifically designed to slow down the progression of rheumatoid arthritis and other autoimmune forms of arthritis. Unlike NSAIDs and corticosteroids, which primarily address symptoms, DMARDs target the underlying disease process.

- **Traditional DMARDs:**
 - Methotrexate (MTX)
 - Sulfasalazine
 - Hydroxychloroquine (Plaquenil)
 - Leflunomide (Arava)
- **Biologic DMARDs:**
 - Tumor necrosis factor (TNF) inhibitors (e.g., etanercept, adalimumab)
 - Interleukin-6 (IL-6) inhibitors (e.g., tocilizumab)
 - B-cell inhibitors (e.g., rituximab)
 - T-cell co-stimulation inhibitors (e.g., abatacept)
- **Mechanism of Action:**
 - **Traditional DMARDs:** These drugs work by suppressing the immune system, though their exact mechanisms can vary. For example, methotrexate, the most commonly used DMARD, inhibits folate metabolism, which in turn reduces the production of DNA and RNA in rapidly dividing cells, including immune cells that contribute to inflammation.
 - **Biologic DMARDs:** Biologics are engineered proteins that specifically target molecules involved in the immune response. For example, TNF inhibitors block the activity of TNF, a cytokine that plays a key role in inflammation. By targeting specific pathways in the immune system, biologics offer a more targeted approach to treatment compared to traditional DMARDs.
- **Benefits:**
 - **Disease Control:** DMARDs, particularly methotrexate and biologics, can significantly slow down or even halt the progression of rheumatoid arthritis and other inflammatory forms of arthritis. This can prevent joint damage and preserve function.
 - **Combination Therapy:** DMARDs are often used in combination with other medications, such as NSAIDs or corticosteroids, to provide comprehensive management of arthritis.

- **Improvement in Quality of Life:** By controlling the disease process, DMARDs can reduce pain, improve physical function, and enhance overall quality of life for patients with chronic arthritis.
- **Limitations and Side Effects:**
- **Slow Onset of Action:** DMARDs do not provide immediate relief. It can take weeks or even months for these medications to take full effect, which is why they are often combined with faster-acting drugs in the initial stages of treatment.
- **Immune Suppression:** Like corticosteroids, DMARDs suppress the immune system, increasing the risk of infections. Biologics, in particular, are associated with a higher risk of serious infections, such as tuberculosis.
- **Liver Toxicity:** Methotrexate and other DMARDs can cause liver damage, requiring regular monitoring of liver function. Alcohol consumption is usually discouraged for patients on methotrexate due to the increased risk of liver toxicity.
- **Blood Disorders:** DMARDs can cause blood cell abnormalities, including anemia, leukopenia (low white blood cell count), and thrombocytopenia (low platelet count). Regular blood tests are needed to monitor these potential side effects.
- **Injection and Infusion Reactions:** Biologic DMARDs are often administered by injection or infusion, which can cause local reactions at the injection site or systemic infusion reactions, including allergic responses.
- **Why DMARDs Aren't Always the Perfect Solution:**

Although DMARDs are effective in managing disease progression, they have inherent limitations. Their immunosuppressive effects render patients more susceptible to infections, and their delayed onset of action necessitates the use of supplementary medications to manage symptoms during the initial phases of treatment. Furthermore, biologics are costly, and patient responses to them vary significantly. For certain patients, the potential for side effects surpasses the advantages, requiring a meticulous evaluation of treatment alternatives.

Janus Kinase (JAK) Inhibitors:

Janus kinase inhibitors (JAK inhibitors) are a newer class of targeted synthetic disease-modifying antirheumatic drugs (DMARDs) that specifically block the activity of Janus kinases, enzymes that play a crucial role in the immune response by transmitting signals

related to inflammation. By inhibiting these enzymes, JAK inhibitors help reduce the inflammation that leads to joint damage in rheumatoid arthritis and other autoimmune forms of arthritis.

- **Common JAK Inhibitors:**

- Tofacitinib (Xeljanz)
- Baricitinib (Olumiant)
- Upadacitinib (Rinvoq)

- **Mechanism of Action:** JAK inhibitors work by blocking the JAK-STAT signaling pathway, which is involved in the production of cytokines responsible for immune-mediated inflammation. By interrupting this pathway, JAK inhibitors help prevent the overactive immune response that leads to joint damage in inflammatory arthritis.

- **Benefits:**

- **Targeted Approach:** JAK inhibitors provide a more focused method of reducing inflammation by specifically targeting the JAK-STAT pathway, unlike traditional DMARDs that broadly suppress the immune system.
- **Oral Administration:** Unlike biologics, which often require injections or infusions, JAK inhibitors are available in oral form, offering convenience for patients.
- **Efficacy in Multiple Forms of Arthritis:** JAK inhibitors have shown effectiveness in treating rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis.

- **Limitations and Side Effects:**

- **Infection Risk:** Like other immunosuppressive therapies, JAK inhibitors increase the risk of serious infections, including tuberculosis and opportunistic infections.
- **Blood Clots and Cardiovascular Risks:** JAK inhibitors, particularly at higher doses, have been associated with an increased risk of blood clots, cardiovascular events, and cancer, prompting caution in certain populations.
- **Elevated Cholesterol Levels:** Some JAK inhibitors can raise cholesterol levels, necessitating regular monitoring of lipid profiles.

- **Liver Function and Blood Cell Abnormalities:** JAK inhibitors may cause liver enzyme elevation and changes in blood counts, requiring periodic blood tests.
- **Why JAK Inhibitors Aren't for Everyone:** Despite their effectiveness, JAK inhibitors are not suitable for all patients due to the risks associated with immunosuppression and cardiovascular issues. Close monitoring is essential for patients on these medications, and they are often used when patients do not respond adequately to traditional DMARDs or biologic therapies.

Combination Therapy: Given the limitations of each drug class, arthritis treatment often involves a combination of therapies to optimize outcomes. For example, a patient may be prescribed a DMARD to control disease progression while also taking NSAIDs for pain relief and corticosteroids for flare-ups. The goal of combination therapy is to manage symptoms effectively while minimizing side effects and preventing disease progression [17-26].

1.1.5 Need for Improved Drug Delivery

Effective management of arthritis, including osteoarthritis, rheumatoid arthritis, and other variants, necessitates the resolution of various therapeutic challenges. Notwithstanding the availability of numerous medications, many patients persist in experiencing insufficient relief, disease progression, and considerable adverse effects. A primary reason for this is the intrinsic constraints of traditional drug delivery systems, which frequently do not transport the therapeutic agent to the target location in a controlled and efficient manner. This section will examine the principal challenges related to contemporary therapies, including low bioavailability, systemic side effects, and insufficient targeted drug delivery.

1.1.5.1 Low Bioavailability:

Bioavailability denotes the fraction of a drug that reaches systemic circulation upon administration and exerts a pharmacological effect. It is the proportion of a given dose that enters the bloodstream in its active state. For a drug to be efficacious, it must be absorbed in adequate amounts, arrive at the designated target site, and persist there for a sufficient duration to produce its therapeutic effects.

Challenges with Bioavailability in Arthritis Treatment: In the context of arthritis, many drugs, particularly those administered orally, suffer from low bioavailability. Several factors contribute to this problem:

- **Poor Solubility:** Many anti-arthritic drugs have low solubility in water, making it difficult for them to be absorbed through the gastrointestinal (GI) tract when taken orally. Drugs like methotrexate, a common DMARD, face challenges with solubility, which limits the amount of the drug that can be absorbed into the bloodstream.
- **First-Pass Metabolism:** After oral administration, drugs pass through the liver before reaching systemic circulation. This process, known as first-pass metabolism, can significantly reduce the concentration of the drug that reaches the bloodstream. For instance, a significant portion of orally administered corticosteroids may be metabolized in the liver, reducing their effectiveness.
- **Variable Absorption:** The absorption of drugs can be affected by various factors such as food intake, gastric pH, and the presence of other medications. This variability can lead to inconsistent therapeutic outcomes in patients with arthritis. NSAIDs, for example, may have different absorption rates depending on whether they are taken with or without food.
- **Short Half-Life:** Some drugs have a short half-life, meaning they are quickly eliminated from the body, requiring frequent dosing. This not only affects patient compliance but also limits the drug's ability to maintain therapeutic levels in the body over time. For example, the frequent dosing required for NSAIDs can lead to poor patient adherence and fluctuating pain levels.

Impact of Low Bioavailability: The low bioavailability of many arthritis medications leads to several problems:

- **Inadequate Therapeutic Effect:** Because only a small fraction of the drug reaches the target site, the therapeutic effect may be insufficient to control the symptoms of arthritis. Patients may continue to experience pain, inflammation, and joint damage despite being on medication.
- **Higher Doses Required:** To compensate for low bioavailability, higher doses of the drug are often required, which can increase the risk of side effects and toxicity. This

is particularly concerning for drugs like NSAIDs and corticosteroids, where higher doses are associated with significant adverse effects.

- **Increased Costs:** The need for higher doses and frequent administration increases the cost of treatment, both in terms of medication expenses and the need for ongoing medical monitoring.

1.1.5.2 Systemic Side Effects:

Systemic side effects refer to adverse effects that occur throughout the body, rather than being localized to the site of drug action. These side effects are often a result of the drug affecting organs and tissues other than the intended target. In arthritis treatment, systemic side effects are a major concern due to the chronic nature of the disease, which often requires long-term medication use.

Causes of Systemic Side Effects in Arthritis Therapies: Several factors contribute to the systemic side effects of arthritis medications:

- **Non-Selective Drug Action:** Many drugs used to treat arthritis, such as NSAIDs and corticosteroids, are non-selective, meaning they affect multiple systems in the body. For example, NSAIDs inhibit both COX-1 and COX-2 enzymes, which reduces inflammation but also impairs the protective functions of COX-1 in the stomach lining, leading to gastrointestinal issues.
- **Cumulative Toxicity:** Long-term use of medications, particularly in chronic diseases like arthritis, can lead to cumulative toxicity. This means that the harmful effects of the drug build up over time, increasing the risk of serious health problems. For instance, prolonged use of corticosteroids can lead to bone loss (osteoporosis), muscle weakness, and increased susceptibility to infections.
- **Widespread Distribution:** Many drugs do not specifically target the affected joints but instead circulate throughout the body. This widespread distribution increases the likelihood of side effects in other organs and systems. Methotrexate, for example, affects rapidly dividing cells throughout the body, which can lead to liver toxicity, bone marrow suppression, and gastrointestinal side effects.

Common Systemic Side Effects in Arthritis Treatment:

- **Gastrointestinal Issues:** NSAIDs are notorious for causing gastrointestinal problems, including stomach ulcers, bleeding, and gastritis. These side effects result

from the inhibition of COX-1, which plays a protective role in the stomach lining. Even with COX-2 selective inhibitors like celecoxib, GI issues can still occur.

- **Cardiovascular Risks:** Both NSAIDs and corticosteroids have been associated with increased cardiovascular risks, including heart attack and stroke. The use of COX-2 inhibitors, while reducing gastrointestinal side effects, has been linked to higher rates of cardiovascular events, making them unsuitable for some patients.
- **Bone Loss and Osteoporosis:** Long-term corticosteroid use can lead to significant bone loss, increasing the risk of fractures. This is particularly concerning for older adults with arthritis, who may already be at risk for osteoporosis.
- **Increased Risk of Infections:** Immunosuppressive drugs, such as corticosteroids and DMARDs, weaken the immune system, making patients more susceptible to infections. This is a serious concern, especially for patients on biologic DMARDs, which specifically target immune system components.
- **Hepatotoxicity:** Drugs like methotrexate can cause liver toxicity, necessitating regular liver function tests to monitor for potential damage. Patients must be cautious with alcohol consumption and other liver-toxic substances while on these medications.
- **Kidney Damage:** Prolonged use of NSAIDs can lead to kidney damage, particularly in patients with pre-existing kidney conditions. This is due to the reduction in blood flow to the kidneys, which can impair their function over time.

Impact of Systemic Side Effects: Systemic side effects can significantly impact a patient's quality of life and adherence to treatment:

- **Reduced Compliance:** Patients experiencing severe side effects may be less likely to adhere to their medication regimen, leading to suboptimal disease control and increased risk of flare-ups.
- **Need for Additional Medications:** Managing side effects often requires additional medications, which can lead to polypharmacy and increase the risk of drug interactions. For example, patients on NSAIDs may need to take proton pump inhibitors (PPIs) to protect against stomach ulcers, adding complexity to their treatment plan.

- **Increased Healthcare Costs:** The need for additional monitoring, treatments, and hospitalizations due to side effects can significantly increase healthcare costs, both for the patient and the healthcare system.

1.1.5.3 Lack of Targeted Drug Delivery:

Targeted drug delivery refers to the ability to direct a therapeutic agent specifically to the site of disease, minimizing its effects on healthy tissues. In the context of arthritis, targeted delivery would ideally focus the medication directly on the affected joints, reducing inflammation and pain without impacting other parts of the body.

Challenges with Conventional Drug Delivery Systems: Most conventional arthritis treatments lack specificity, meaning that the drug circulates throughout the body, affecting both healthy and diseased tissues. This lack of targeting results in several issues:

- **Non-Specific Action:** Traditional medications, such as oral NSAIDs, spread throughout the bloodstream and affect the entire body, leading to systemic side effects. This non-specific action limits the ability to deliver high doses directly to the affected joints without causing harm to other organs.
- **Inefficient Drug Delivery to Joints:** The delivery of drugs to the joints is often inefficient, as the blood supply to the joints is limited compared to other tissues. This makes it difficult for drugs to accumulate in sufficient concentrations at the site of inflammation, reducing their effectiveness.
- **Short Duration of Action:** Because conventional drugs are rapidly cleared from the bloodstream, their effects are often short-lived. This necessitates frequent dosing, which can be inconvenient for patients and increases the risk of side effects.
- **Variable Distribution:** The distribution of drugs can vary depending on factors such as blood flow, tissue permeability, and the presence of transport proteins. This variability can lead to inconsistent therapeutic outcomes, with some patients experiencing better relief than others.

Need for Targeted Drug Delivery Systems: Targeted drug delivery systems offer several potential advantages over conventional therapies:

- **Enhanced Efficacy:** By concentrating the drug at the site of disease, targeted delivery systems can achieve higher therapeutic concentrations in the affected joints, improving symptom relief and disease control.
- **Reduced Side Effects:** Targeted delivery minimizes exposure to healthy tissues, reducing the risk of systemic side effects. For example, a targeted NSAID delivery system could provide pain relief in the joints without causing gastrointestinal or cardiovascular issues.
- **Sustained Drug Release:** Many targeted delivery systems are designed to release the drug slowly over time, maintaining therapeutic levels in the joints for longer periods. This reduces the need for frequent dosing and improves patient compliance.
- **Personalized Medicine:** Targeted drug delivery systems can be tailored to individual patients based on factors such as disease severity, genetics, and response to treatment. This personalized approach could lead to better outcomes and fewer side effects.

Examples of Targeted Drug Delivery in Arthritis:

- **Liposomal Drug Delivery:** Liposomes are spherical vesicles that can encapsulate drugs and deliver them directly to the affected joints. This technology has been used to improve the delivery of corticosteroids and other anti-inflammatory drugs in arthritis.
- **Nanoparticles:** Nanoparticles, such as mesoporous silica nanoparticles (MSNs), offer a promising approach to targeted drug delivery in arthritis. These tiny particles can be engineered to carry drugs directly to the inflamed joints, enhancing bioavailability and reducing side effects.
- **Biodegradable Polymers:** Biodegradable polymers can be used to create drug delivery systems that release the drug slowly over time. These systems can be injected directly into the joints, providing sustained relief from arthritis symptoms.
- **Monoclonal Antibodies:** Biologic DMARDs, such as monoclonal antibodies, are designed to target specific molecules involved in the inflammatory process. By focusing on these specific targets, biologics can reduce inflammation with fewer side effects than traditional immunosuppressive drugs [27-32].

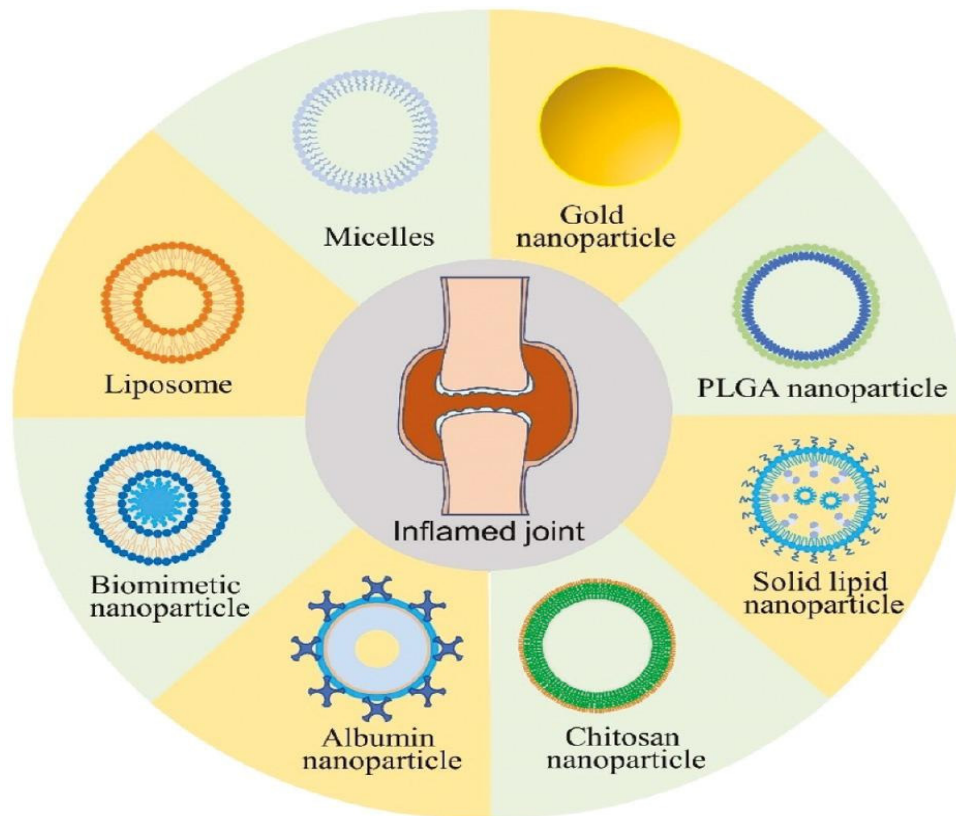


Figure 1.3: Nanoparticles used in arthritis

1.1.6 Nanotechnology in Medicine

Nanotechnology, a discipline that manipulates matter at the atomic and molecular level, has transformed various industries, particularly medicine. In recent years, nanotechnology has demonstrated significant potential in improving drug delivery systems, particularly for chronic illnesses such as cancer, cardiovascular diseases, and arthritis. This section will examine the principles of nanotechnology, its application in drug delivery systems, and its particular advantages for managing chronic diseases such as arthritis.

1.1.6.1 Understanding Nanotechnology:

Nanotechnology encompasses the design, synthesis, characterization, and application of materials and devices measuring between 1 and 100 nanometers. At this scale, materials demonstrate distinct physical, chemical, and biological properties that diverge from those of their bulk equivalents. These properties can be utilized to develop innovative drug delivery systems that enhance the efficacy and safety of therapeutic agents.

Nanotechnology facilitates meticulous regulation of the dimensions, morphology, surface characteristics, and functionality of materials. This accuracy enables scientists to engineer nanoparticles that can surmount numerous limitations inherent in traditional drug delivery systems. Nanoparticles can be designed to enhance drug solubility, safeguard drugs from degradation, improve drug absorption, and facilitate targeted drug delivery, thereby minimizing systemic side effects.

Types of Nanomaterials Used in Medicine:

- **Lipid-Based Nanoparticles:** These include liposomes and solid lipid nanoparticles (SLNs). Lipid-based nanoparticles are biocompatible and can encapsulate both hydrophilic and hydrophobic drugs, improving their solubility and stability.
- **Polymeric Nanoparticles:** These nanoparticles are made from biodegradable polymers such as poly(lactic-co-glycolic acid) (PLGA) and are used for controlled drug release. They can be engineered to release drugs over extended periods, reducing the need for frequent dosing.
- **Inorganic Nanoparticles:** Inorganic nanoparticles, such as gold nanoparticles, silica nanoparticles, and quantum dots, offer unique optical and electronic properties that can be used for imaging, diagnostics, and therapy. Mesoporous silica nanoparticles (MSNs) are particularly promising for drug delivery due to their high surface area, tunable pore size, and biocompatibility.
- **Dendrimers:** These are highly branched, tree-like molecules with multiple functional groups on their surface, allowing for the attachment of drugs, targeting ligands, and imaging agents. Dendrimers can enhance drug solubility and target specific cells or tissues.
- **Carbon-Based Nanomaterials:** These include carbon nanotubes and graphene, which have unique mechanical and electrical properties. While still in the experimental stage, these materials hold potential for drug delivery and tissue engineering applications [33-40].

1.1.6.2 Nanotechnology in Arthritis Treatment:

Challenges in Treating Arthritis: Osteoarthritis (OA) and rheumatoid arthritis (RA) in particular are chronic inflammatory diseases that cause swelling, stiffness, and pain in the joints. NSAIDs, corticosteroids, and DMARDs are examples of conventional treatments for arthritis that frequently have drawbacks like low bioavailability, systemic side effects, and lack of targeted delivery. Improving patients' quality of life and achieving long-term disease control are challenging due to these issues.

How Nanotechnology Can Address These Challenges: Nanotechnology offers several advantages in the treatment of arthritis:

- **Targeted Delivery to Inflamed Joints:** Anti-inflammatory medications can be delivered straight to the site of inflammation by using specially designed nanoparticles that are intended to target inflammatory joints. This minimizes side effects and lessens the requirement for high systemic doses. To guarantee that they accumulate in the inflammatory joints, MSNs, for instance, can be functionalized with targeting ligands and loaded with anti-inflammatory medications.
- **Improved Drug Retention in Joints:** The quick clearance of medications from the joints, which requires frequent dosing, is one of the difficulties in treating arthritis. Because they cling to joint tissues and release the drug gradually, nanoparticles can enhance drug retention in the joints. This lessens the need for repeated injections and offers long-lasting symptom relief.
- **Reduced Systemic Exposure:** By targeting the drug directly to the affected joints, nanotechnology can reduce systemic exposure to the drug, minimizing the risk of side effects. This is particularly important for drugs like corticosteroids, which can cause significant systemic toxicity when used long-term.
- **Combination Therapy:** Nanoparticles can be designed to carry multiple drugs, allowing for combination therapy in a single formulation. For example, a nanoparticle could deliver both an anti-inflammatory drug and a disease-modifying agent, providing comprehensive treatment for arthritis.

Examples of Nanotechnology in Arthritis Treatment:

- **Liposomal Delivery of Methotrexate:** Methotrexate is a commonly used DMARD for the treatment of RA, but its systemic side effects limit its long-term use.

Liposomal formulations of methotrexate have been developed to target the drug directly to the inflamed joints, reducing its toxicity and improving its efficacy.

- **Mesoporous Silica Nanoparticles (MSNs):** MSNs are particularly promising for arthritis treatment due to their high surface area, tunable pore size, and biocompatibility. MSNs can be loaded with anti-inflammatory drugs and functionalized with targeting ligands to ensure they accumulate in the inflamed joints. This targeted delivery improves drug bioavailability and reduces the risk of systemic side effects.
- **Gold Nanoparticles for RA:** Gold nanoparticles have been studied for their potential in treating RA due to their anti-inflammatory properties. These nanoparticles can be functionalized with drugs and targeting ligands, allowing for targeted delivery to the inflamed joints. Gold nanoparticles also have the potential to be used for imaging, providing a theranostic approach to RA treatment.

1.1.6.3 Future Directions in Nanotechnology-Based Drug Delivery:

Personalized Medicine: Because it makes it possible to create medication delivery systems that are specifically suited to each patient's needs, nanotechnology holds the promise of revolutionizing personalized medicine. Nanoparticles can be tailored to deliver the right medication at the right dose to the right patient by taking into account variables like genetics, the severity of the disease, and the patient's response to treatment. This individualized approach may result in fewer side effects and improved treatment outcomes.

Smart Drug Delivery Systems: The "smart" nanoparticles of the next generation of drug delivery systems will probably be able to react to particular stimuli, like modifications to pH, temperature, or enzymatic activity, in order to release the drug at the intended site. The effectiveness of treatment could be enhanced by these intelligent systems by giving precise control over medication delivery.

Nanotechnology in Regenerative Medicine: In addition to drug delivery, nanotechnology holds promise for regenerative medicine applications in arthritis treatment. For example, nanoparticles can be used to deliver growth factors or stem cells to damaged joint tissues, promoting tissue repair and regeneration. This could potentially reverse the damage caused by arthritis and restore joint function.

Safety and Regulatory Considerations: Although there are numerous advantages to nanotechnology, there are additionally safety and legal issues that need to be resolved. There is still much to learn about the long-term health benefits of nanoparticles, especially with regard to their potential toxicity and rate of accumulation in the body. Additionally, regulatory bodies are attempting to create standards for the creation and acceptance of medication delivery systems based on nanotechnology [41-48].

1.2 Concept of Mesoporous Silica Nanoparticles (MSNs)

1.2.1 Introduction to MSNs:

Mesoporous Silica Nanoparticles (MSNs) are advanced materials characterized by a highly ordered porous structure with pores ranging from 2 to 50 nanometers in diameter, as per the International Union of Pure and Applied Chemistry (IUPAC) nomenclature. These nanoparticles have garnered significant interest in the pharmaceutical and biomedical fields due to their unique properties, which make them suitable for various applications, including drug delivery, catalysis, and imaging.

Structure of MSNs: MSNs are made by a silica (SiO_2) structure with a repeating, highly ordered pore network. During the templating process, which produces these pores, block copolymers or surfactants which create tiny particles in solution are frequently used. Silica precursors, like tetraethyl orthosilicate (TEOS), condense around the micelles during synthesis. After the surfactant is removed, a distinct mesoporous structure is left behind.

Properties of MSNs:

1. **High Surface Area and Pore Volume:** MSNs exhibit a high surface area (usually between 500-1500 m^2/g) and large pore volume, which allows for high drug-loading capacities. The extensive surface area provides ample space for drug molecules to adsorb or be encapsulated, enhancing the drug delivery potential of these nanoparticles.
2. **Tunable Pore Size:** One of the key advantages of MSNs is the ability to precisely control the pore size during synthesis. This tunability allows for the optimization of the pore size to match the dimensions of various drug molecules, improving loading efficiency and release kinetics. Adjusting factors such as the concentration of the surfactant, the pH of the solution, and the type of silica precursor can achieve this customization.

3. **Biocompatibility and Safety:** MSNs are appropriate for use in biomedical applications because they are typically regarded as nontoxic and biocompatible. However, the size, shape, surface functionalization, and administration route of MSNs can all affect their safety profile. Research has demonstrated that when synthesized correctly, MSNs do not cause appreciable toxicity either in vivo or in vitro; however, more studies are required to completely comprehend their long-term safety.
4. **Thermal and Mechanical Stability:** MSNs exhibit excellent thermal and mechanical stability due to their rigid silica framework. This stability ensures that the structural integrity of the nanoparticles is maintained under various physiological conditions, which is crucial for consistent drug release and effectiveness in therapeutic applications.
5. **Surface Functionalization:** It is simple to add different functional groups to the surface of MSNs in order to improve their interaction with particular drugs or target tissues. A surface can be made more functional by adding amine, carboxyl, or thiol groups, that may boost biocompatibility, target specific drugs, or improve drug loading. For instance, through electrostatic interactions, amine-functionalized MSNs can increase the effectiveness of loading of negatively charged molecule of drug [49-60].

1.2.2 Historical Development of Mesoporous Silica Nanoparticles (MSNs):

Overview of the Discovery and Development of MSNs in Drug Delivery Systems

Early Discovery and Background:

The journey of Mesoporous Silica Nanoparticles (MSNs) began with the development of mesoporous materials in the early 1990s by researchers at Mobil Oil Corporation. In 1992, they introduced the Mobil Composition of Matter (MCM) series, including MCM-41, which featured an ordered mesoporous structure with a high surface area and uniform pore sizes ranging from 2 to 10 nm. This discovery marked a significant advancement in material science, as it allowed the creation of materials with precisely controlled porosity and surface characteristics.

The initial interest in mesoporous silica materials was driven by their potential applications in catalysis, separation processes, and adsorption due to their large surface area and customizable pore sizes. However, as the field of nanotechnology evolved, the unique properties of mesoporous silica—such as their high surface area, tunable pore size, and

ability to be functionalized—caught the attention of researchers in the pharmaceutical sciences for their potential as drug delivery carriers.

Initial Applications in Drug Delivery:

The first significant exploration of MSNs in drug delivery systems was reported in the early 2000s. In 2001, Vallet-Regi and colleagues published pioneering work highlighting the use of MCM-41 mesoporous silica as a drug delivery system. They demonstrated that MSNs could effectively encapsulate therapeutic agents within their porous structure, providing a novel strategy for enhancing drug solubility and controlling drug release.

Their work showed that MSNs could be loaded with a variety of drugs, including both hydrophilic and hydrophobic compounds, making them versatile carriers for different types of therapeutic agents. This early research established the potential of MSNs as a promising platform for drug delivery, paving the way for further exploration and development in the field.

Advancements in Synthesis and Functionalization:

Following the initial studies, researchers focused on optimizing the synthesis and functionalization of MSNs to improve their performance in drug delivery applications. Advances in synthesis techniques allowed for better control over the particle size, pore diameter, and surface properties of MSNs. Techniques such as sol-gel processing, microemulsion, and templating methods were refined to produce MSNs with specific characteristics tailored to different therapeutic needs.

One of the critical developments during this period was the introduction of surface functionalization techniques. By modifying the surface of MSNs with various functional groups (e.g., amine, carboxyl, thiol), researchers could enhance the interaction between the nanoparticles and the drug molecules, improve biocompatibility, and enable targeted delivery to specific tissues or cells. Surface functionalization also opened up the possibility of conjugating targeting ligands, such as antibodies or peptides, to MSNs, allowing for more precise delivery of drugs to diseased tissues while minimizing systemic side effects.

Emergence of MSNs in Targeted and Controlled Drug Delivery:

The next phase of MSN development focused on their role in targeted and controlled drug delivery systems. Researchers began exploring the use of MSNs for delivering drugs to specific sites within the body, such as tumors or inflamed tissues. This targeted approach

aimed to increase the therapeutic efficacy of drugs while reducing their toxicity by concentrating the drug at the site of action.

To achieve targeted delivery, MSNs were functionalized with targeting ligands that could recognize and bind to specific receptors on the surface of target cells. For example, MSNs functionalized with folic acid were used to target cancer cells that overexpress folate receptors, leading to more efficient drug delivery to tumors.

Additionally, the ability to tune the release profile of drugs from MSNs became a significant area of research. By modifying the pore size and surface properties, as well as incorporating responsive materials such as polymers or gatekeepers that could respond to environmental triggers (e.g., pH, temperature, enzymes), researchers were able to develop MSNs that released their drug payload in a controlled and sustained manner. This feature was particularly valuable for achieving prolonged therapeutic effects and reducing the frequency of drug administration.

Integration of MSNs in Multifunctional and Theragnostic Platforms:

As the field progressed, the concept of multifunctional MSNs emerged, where MSNs were engineered to perform multiple roles simultaneously, such as drug delivery, imaging, and therapy. These multifunctional MSNs were designed to carry therapeutic agents, imaging contrast agents, and targeting ligands, creating a single platform that could diagnose, deliver treatment, and monitor therapeutic outcomes.

The term "theragnostic" was coined to describe this integration of therapy and diagnostics in a single system. MSNs were at the forefront of this innovation, particularly in cancer treatment, where they were used for simultaneous drug delivery and imaging using techniques like magnetic resonance imaging (MRI) or fluorescence imaging. This capability allowed for real-time tracking of the nanoparticles within the body, providing valuable information on the biodistribution and accumulation of the therapeutic agents at the target site.

Current Trends and Future Directions:

Today, MSNs continue to be a focal point of research in drug delivery, with ongoing efforts to enhance their functionality, safety, and efficacy. Some of the current trends include the development of MSNs with stimuli-responsive properties, where the release of the drug is triggered by specific internal or external stimuli such as changes in pH, temperature, light, or

magnetic fields. This approach aims to further refine the control over drug release, making MSNs even more precise in their therapeutic action.

MSNs are being explored for applications beyond traditional drug delivery, including gene delivery, protein delivery, and as carriers for vaccines. The versatility of MSNs allows them to be adapted for various biomedical applications, making them a highly valuable tool in the advancement of personalized medicine [61-67].

1.2.3 MSNs in Drug Delivery:

In recent years, mesoporous silica nanoparticles (MSNs) have emerged as a prominent nanocarrier in the field of drug delivery due to their distinctive properties. These nanoparticles offer numerous advantages over other types of nanoparticles, such as liposomes, polymeric nanoparticles, and metal-based nanoparticles. The unique characteristics of MSNs, including their biocompatibility, biodegradability, and non-toxicity, make them a superior choice for drug delivery applications, particularly in the development of targeted therapies.

Advantages of MSNs Over Other Nanoparticles

1. Biocompatibility:

- MSNs are primarily composed of silica (SiO_2), a material recognized for its excellent biocompatibility. Silica is naturally present in the human body in trace amounts, which reduces the risk of adverse reactions when used as a drug carrier.
- The surface of MSNs can be easily modified with various functional groups, such as amine or carboxyl groups, to enhance their interaction with biological tissues. This surface modification can further improve their compatibility with the human body.
- Unlike some metal-based nanoparticles, which can accumulate in organs and potentially cause toxicity, MSNs are less likely to induce immune responses or other toxic effects due to their biocompatible nature.

2. Biodegradability:

- MSNs are recognized for their biodegradable characteristics. Upon administration, they progressively break down into non-toxic silicic acid under

physiological conditions, which is subsequently eliminated from the body via standard metabolic pathways. This degradation process is advantageous for reducing long-term accumulation and toxicity.

- The degradation rate can be managed by modifying the density of the silica network and the extent of surface functionalization. The ability to tune biodegradation offers significant benefits for customizing the rate at which drugs are released to meet specific therapeutic requirements.

3. Non-Toxicity:

- One of the most critical aspects of any drug delivery system is its safety profile. MSNs have been extensively studied and generally regarded as non-toxic at doses relevant for drug delivery.
- The non-toxic nature of MSNs is attributed to their composition and the mild conditions under which they operate. Unlike certain metal-based nanoparticles that may release harmful ions, MSNs remain stable and do not leach toxic components into the body.
- Several in vitro and in vivo studies have confirmed that MSNs do not cause significant cytotoxicity or inflammatory responses when used at appropriate concentrations, making them a safer alternative compared to other nanoparticle systems.

4. High Drug Loading Capacity:

- MSNs exhibit a high surface area and substantial pore volume, enabling them to accommodate considerable quantities of drugs in comparison to other nanoparticles. The substantial loading capacity facilitates the delivery of a greater dose of the therapeutic agent to the target site, thereby improving the treatment's efficacy.
- The adjustable pore size of MSNs allows for the encapsulation of a diverse array of molecules, including small drugs as well as larger biomolecules such as proteins and peptides. This versatility presents a clear advantage compared to other nanoparticles, which may face restrictions regarding the types or sizes of drugs they are capable of transporting.

5. Controlled and Sustained Release:

- The porous structure of MSNs facilitates the controlled release of the encapsulated drug. Modifying the pore size, surface chemistry, or incorporating stimuli-responsive gates allows for precise tuning of release kinetics, facilitating sustained drug release, minimizing dosing frequency, and enhancing patient compliance.
- This controlled release capability is particularly beneficial for chronic conditions such as arthritis, where sustained delivery of anti-inflammatory or disease-modifying drugs can help manage symptoms and improve quality of life.

6. Versatility in Functionalization:

- MSNs offer a high degree of functional versatility due to their easily modifiable surface. They can be functionalized with targeting ligands, such as antibodies, peptides, or small molecules, that recognize specific markers on diseased cells, enhancing targeted drug delivery.
- Functionalization can also impart additional properties, such as stealth characteristics to evade the immune system or magnetism for externally guided delivery, which are not readily achievable with many other nanoparticle types.

7. Stability and Robustness:

- MSNs are chemically and physically stable under a wide range of conditions, including varying pH levels, temperatures, and biological environments. This stability ensures that the nanoparticles retain their integrity and do not release the drug prematurely before reaching the target site.
- Unlike liposomes or polymeric nanoparticles, which can be sensitive to environmental changes, MSNs maintain their structure and function, providing a reliable platform for drug delivery.

8. Cost-Effectiveness:

- The synthesis of MSNs is relatively simple and cost-effective, utilizing readily available materials and straightforward chemical processes. This makes them an economically viable option for large-scale production compared to some other

types of nanoparticles that require complex and expensive manufacturing techniques.

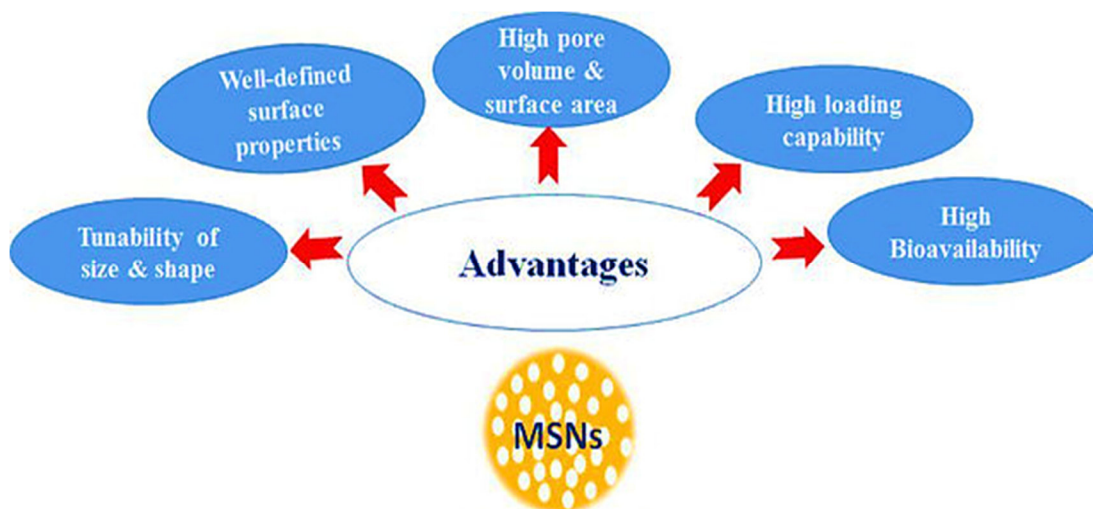


Figure 1.4: Advantages of MSNs

1.3 Challenges in Drug Delivery for Arthritis

1.3.1 Challenges in Topical and Systemic Delivery:

Introduction to Arthritis and Drug Delivery Needs:

Arthritis is a chronic inflammatory condition that affects millions of people worldwide, causing pain, stiffness, and swelling in the joints. Effective management of arthritis often requires long-term medication to reduce inflammation, relieve pain, and slow disease progression. However, the efficient delivery of therapeutic agents directly to the inflamed joints remains a significant challenge due to various biological and physicochemical barriers. The primary obstacles in delivering drugs for arthritis treatment include poor drug solubility, inadequate permeability through biological membranes, and insufficient retention at the target site. Addressing these challenges is crucial for developing more effective therapies that can provide sustained relief and improve the quality of life for patients with arthritis.

1.3.1.1 Poor Drug Solubility:

One of the major issues in drug delivery for arthritis is the poor solubility of many anti-inflammatory and analgesic drugs. Poor solubility can severely limit the bioavailability of these drugs, making it difficult for them to reach therapeutic concentrations at the site of inflammation.

- **Impact on Bioavailability:** Drugs with poor solubility do not dissolve easily in bodily fluids, which is a prerequisite for absorption into the bloodstream and subsequent delivery to the target site. This issue is particularly problematic for oral medications, where drugs must dissolve in the gastrointestinal tract before they can be absorbed. When solubility is low, a significant portion of the drug may pass through the digestive system without being absorbed, leading to reduced effectiveness.
- **Strategies to Enhance Solubility:** Several strategies are employed to improve the solubility of poorly soluble drugs. These include the use of solubilizing agents such as cyclodextrins, the formulation of drugs in nano-sized particles, and the use of advanced drug delivery systems like mesoporous silica nanoparticles (MSNs), which can encapsulate drugs within their porous structure and enhance their dissolution rate. Additionally, chemical modifications of the drug molecule, such as the formation of salts or prodrugs, can also enhance solubility.

1.3.1.2 Inadequate Permeability:

Another significant challenge in drug delivery for arthritis is the inadequate permeability of therapeutic agents through biological membranes. Permeability refers to the ability of a drug to cross cellular barriers, such as the skin or the gastrointestinal lining, to reach the systemic circulation or the site of action.

- **Barriers to Permeability:** For topical formulations, the outermost layer of the skin, known as the stratum corneum, serves as a major barrier to drug penetration. This layer is composed of tightly packed dead skin cells embedded in a lipid matrix, which acts as a protective shield against external substances. Similarly, for oral drugs, the gastrointestinal tract presents barriers such as the epithelial cell lining and various efflux transporters that pump drugs back into the intestinal lumen, reducing their absorption.
- **Enhancing Permeability:** Enhancing drug permeability can be achieved through various formulation strategies, including the use of penetration enhancers that disrupt the lipid structure of the skin, or the incorporation of drugs into nanoparticles that can facilitate transport across cellular barriers. For systemic delivery, techniques like prodrug design, which involves chemically modifying the drug to improve its permeability, can also be effective. In the case of MSNs, surface functionalization

with specific ligands can improve interaction with cell membranes, facilitating uptake and transport.

1.3.1.3 Retention at the Target Site:

Achieving sustained retention of therapeutic agents at the target site is critical for effective arthritis treatment. Drugs must not only reach the inflamed joints but also remain there long enough to exert their therapeutic effects. However, rapid clearance from the site of action is a common issue, often requiring frequent dosing that can lead to increased side effects and reduced patient compliance.

- **Rapid Clearance and Its Implications:** Systemic drugs are often cleared from the bloodstream by the liver and kidneys, reducing the duration of action at the target site. Topical drugs, on the other hand, may be quickly removed from the skin surface through washing, sweating, or natural shedding of the skin. Rapid clearance limits the drug's therapeutic window and necessitates frequent administration.
- **Strategies to Improve Retention:** Various strategies are employed to enhance drug retention at the target site. For topical formulations, the use of mucoadhesive agents or film-forming polymers can help to anchor the drug on the skin or mucosal surfaces, prolonging its presence. In systemic delivery, modifying the pharmacokinetic properties of the drug through the use of sustained-release formulations, or encapsulating drugs in nanoparticles such as MSNs, can help to prolong drug circulation time and retention at the target site. MSNs, in particular, offer the advantage of controlled release, where the drug is gradually released from the nanoparticle over time, maintaining therapeutic levels at the target site for extended periods.

1.3.1.4 Combined Challenges in Topical and Systemic Delivery:

In arthritis treatment, both topical and systemic delivery routes present their own set of challenges, and a combined approach is often necessary to achieve optimal therapeutic outcomes.

- **Topical vs. Systemic Delivery:** Topical delivery offers the advantage of direct application to the site of pain or inflammation, minimizing systemic side effects. However, as previously mentioned, the skin barrier can significantly limit drug penetration. Systemic delivery, while capable of reaching deeper tissues and multiple

joints, often suffers from non-specific distribution, where the drug affects both the target and non-target tissues, leading to side effects.

- **Nanotechnology-Based Solutions:** Nanotechnology, including the use of MSNs, provides innovative solutions to these challenges by enabling both enhanced permeability and retention. MSNs can be engineered to deliver drugs in a controlled manner, providing a steady release of the therapeutic agent directly to the inflamed joints, whether through topical or systemic administration. This targeted approach reduces the required dose, minimizes side effects, and enhances the overall therapeutic efficacy [73-76].

1.3.2 MSNs as a Solution:

Arthritis is a chronic inflammatory disorder that affects the joints, leading to pain, swelling, and reduced mobility. The condition encompasses various forms, including osteoarthritis, rheumatoid arthritis, and psoriatic arthritis, each with unique pathophysiological characteristics. A key goal in the management of arthritis is to deliver therapeutic agents directly to the affected joint areas to alleviate symptoms and potentially modify disease progression. However, effective drug delivery for arthritis faces several challenges:

1. **Poor Solubility of Drugs:** Many antiarthritic drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs) and disease-modifying antirheumatic drugs (DMARDs), suffer from poor water solubility. This limits their bioavailability and therapeutic efficacy when administered through conventional routes.
2. **Systemic Side Effects:** Conventional drug administration often leads to systemic distribution of the drug, causing side effects such as gastrointestinal disturbances, cardiovascular risks, and hepatotoxicity. This is particularly problematic for long-term arthritis management, where continuous medication is necessary.
3. **Lack of Targeted Delivery:** Traditional drug delivery systems fail to specifically target the inflamed joint tissues, resulting in suboptimal concentrations of the therapeutic agents at the site of action. This reduces the effectiveness of the treatment and necessitates higher doses, exacerbating side effects.
4. **Variable Drug Absorption:** Factors such as gastrointestinal conditions, first-pass metabolism, and variable patient compliance can affect the absorption and

bioavailability of orally administered drugs, leading to inconsistent therapeutic outcomes.

5. **Drug Stability Issues:** Many drugs used in arthritis treatment are prone to degradation in the body's harsh physiological environment, including pH variations and enzymatic activity, which can further compromise their efficacy [77-79].

1.4 Formulation of MSNs for Antiarthritic Therapy

1.4.1 Rationale for Choosing MSNs:

Mesoporous silica nanoparticles (MSNs) are emerging as a novel drug delivery platform due to their unique structural and functional characteristics. They are especially beneficial in the formulation of therapeutic agents for chronic inflammatory conditions like arthritis, where targeted and controlled drug delivery is essential for effective treatment. The rationale for selecting MSNs for antiarthritic therapy stems from their ability to enhance drug solubility, improve bioavailability, provide targeted delivery, and reduce systemic side effects. This section provides a detailed explanation of why MSNs are an ideal choice for this study, supported by existing literature.

1. Enhanced Drug Loading Capacity: MSNs have a highly porous structure with a large surface area and pore volume, which allows for high drug-loading efficiency. This is particularly beneficial for antiarthritic drugs, many of which have poor water solubility and bioavailability. According to Zhao et al. (2019), the adsorption capacity of MSNs can be significantly higher than conventional carriers due to their tunable pore size and volume, which can be optimized to accommodate a wide range of drug molecules, including those with complex structures and poor solubility.

2. Controlled and Sustained Drug Release: One of the key advantages of MSNs is their ability to provide controlled and sustained drug release, which is critical in the management of chronic conditions such as arthritis. Sustained release formulations ensure that therapeutic drug levels are maintained over extended periods, reducing the frequency of dosing and improving patient compliance. The release rate can be precisely controlled by modifying the pore size and surface properties of the MSNs, as demonstrated in studies by Vallet-Regí et al. (2017), which show that MSNs can be engineered to release drugs in response to specific physiological triggers, such as pH changes in inflamed tissues.

3. Targeted Drug Delivery: Targeted drug delivery is a major advantage of MSNs, allowing drugs to be delivered specifically to the site of inflammation, thereby maximizing therapeutic effects while minimizing systemic exposure and side effects. Functionalization of MSNs with targeting ligands, such as folic acid or antibodies, enables them to recognize and bind to specific receptors overexpressed on inflamed tissues or immune cells involved in arthritis. For example, Zhang et al. (2018) demonstrated that MSNs functionalized with hyaluronic acid selectively targeted CD44 receptors on inflamed synovial cells, enhancing the therapeutic efficacy of the loaded antiarthritic drug.

4. Biocompatibility and Safety: MSNs are generally recognized as biocompatible and non-toxic, making them suitable for use in biomedical applications, including drug delivery. Their silica-based composition is similar to materials that have been used safely in medical applications for decades. According to Wang et al. (2016), MSNs degrade into silicic acid, which is non-toxic and can be excreted through normal metabolic pathways. Furthermore, surface modification techniques can be employed to further enhance the biocompatibility of MSNs, ensuring that they do not elicit adverse immune responses when administered.

5. Reduction of Drug Resistance: In the context of antiarthritic therapy, reducing drug resistance is crucial for maintaining the efficacy of long-term treatment regimens. MSNs can help mitigate drug resistance by delivering drugs directly to the target site, ensuring a high local concentration of the therapeutic agent. This localized delivery reduces the likelihood of systemic exposure that can lead to the development of resistance. Studies by Liu et al. (2020) have shown that MSNs can be used to deliver drugs in a manner that bypasses common resistance mechanisms, such as drug efflux pumps, thereby enhancing the overall therapeutic outcome.

6. Versatility in Functionalization and Drug Encapsulation: The versatility of MSNs in terms of surface functionalization and drug encapsulation makes them highly adaptable for various therapeutic needs. MSNs can be tailored to encapsulate both hydrophilic and hydrophobic drugs, allowing for the co-delivery of multiple therapeutic agents that can work synergistically to manage complex conditions like arthritis. For instance, Shi et al. (2021) illustrated the use of MSNs for the co-delivery of an anti-inflammatory drug and a bone-regenerating peptide, providing a multifaceted approach to treating rheumatoid arthritis by not only reducing inflammation but also promoting tissue repair.

7. Literature Support and Evidence: Extensive literature supports the use of MSNs in targeted drug delivery systems, particularly for conditions requiring localized and controlled drug release. A review by Manzano et al. (2018) highlighted numerous studies where MSNs were successfully employed to enhance the bioavailability and efficacy of poorly soluble drugs. The high loading capacity, tunable release profiles, and ability to functionalize MSNs with various targeting ligands make them a superior choice for antiarthritic therapy compared to traditional delivery systems. Moreover, research indicates that the surface chemistry of MSNs can be easily modified to enhance their interaction with specific cell types or tissues, as discussed by Giret et al. (2017) [80-82].

1.4.2 Selection of Drugs (Tofacitinib Citrate and Methotrexate)

Arthritis encompasses a group of inflammatory joint disorders characterized by pain, swelling, and reduced mobility. Rheumatoid arthritis (RA) is one of the most prevalent forms, involving autoimmune attacks on joint tissues, leading to chronic inflammation and joint damage. Effective management of RA and similar conditions requires the use of disease-modifying anti-rheumatic drugs (DMARDs), which not only alleviate symptoms but also slow disease progression.

Choice of Drugs for Loading onto MSNs: Tofacitinib Citrate and Methotrexate are two well-established antiarthritic drugs frequently used in clinical practice. Their selection for loading onto mesoporous silica nanoparticles (MSNs) is guided by their complementary mechanisms of action, therapeutic efficacy, and potential to benefit from targeted drug delivery systems.

1. Tofacitinib Citrate: Tofacitinib Citrate is an oral Janus kinase (JAK) inhibitor that modulates the immune response by blocking the activity of specific enzymes involved in the signaling pathways that lead to inflammation and tissue damage in rheumatoid arthritis. Its mechanism involves inhibiting JAK1 and JAK3, which are crucial for the signaling of various cytokines that mediate immune responses.

Rationale for Selecting Tofacitinib Citrate:

- **Targeted Immunomodulation:** By inhibiting JAK pathways, Tofacitinib reduces the production of inflammatory cytokines like interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α), which play central roles in the pathophysiology of RA. This

targeted action can help in controlling systemic inflammation with potentially fewer side effects compared to broader immunosuppressive agents.

- **Improved Solubility and Stability:** Tofacitinib Citrate has moderate solubility in water, but its bioavailability can be further enhanced by loading onto MSNs. The porous structure of MSNs can encapsulate the drug effectively, protecting it from premature degradation and enhancing its stability.
- **Enhanced Bioavailability and Sustained Release:** Loading Tofacitinib onto MSNs allows for controlled and sustained release, which can reduce dosing frequency and improve patient compliance. The high surface area and tunable pore size of MSNs provide a suitable environment for Tofacitinib, facilitating gradual drug release and maintaining therapeutic drug levels over extended periods.
- **Reduced Systemic Side Effects:** MSNs can be engineered to deliver Tofacitinib specifically to inflamed joints, minimizing systemic exposure and reducing the risk of side effects, such as infections, that are associated with JAK inhibition.

2. Methotrexate: Methotrexate is one of the most commonly prescribed DMARDs for the treatment of RA. It inhibits dihydrofolate reductase, an enzyme involved in DNA synthesis, which in turn suppresses the proliferation of immune cells that contribute to inflammation.

Rationale for Selecting Methotrexate:

- **Gold Standard DMARD:** Methotrexate remains a cornerstone of RA treatment due to its efficacy in reducing inflammation, slowing joint damage, and improving quality of life. It is often used as the first-line treatment and can be combined with other therapies, such as Tofacitinib, to enhance therapeutic outcomes.
- **Challenges in Delivery:** Despite its efficacy, Methotrexate has limitations, including poor bioavailability when taken orally, variable absorption, and the potential for gastrointestinal side effects. Loading Methotrexate onto MSNs can address these issues by improving its solubility and providing a targeted delivery system that directs the drug specifically to inflamed tissues.
- **Controlled Release Mechanism:** MSNs provide a platform for the controlled release of Methotrexate, which can help maintain steady therapeutic levels in the bloodstream, reducing the need for frequent dosing and minimizing peak-related side effects.

- **Potential for Combination Therapy:** Using MSNs, Methotrexate can be co-delivered with other agents like Tofacitinib, allowing for a synergistic effect that enhances anti-inflammatory activity. This combination approach can be particularly beneficial for patients who do not respond adequately to monotherapy.

Discussion on the Synergy Between Tofacitinib and Methotrexate: Combining Tofacitinib and Methotrexate in a single MSN formulation offers a multipronged approach to managing RA. Methotrexate's role as a folate antagonist and Tofacitinib's JAK inhibition provide complementary mechanisms that suppress the immune response at different points in the inflammatory cascade.

Advantages of Combined MSN Formulation:

1. **Enhanced Therapeutic Effect:** By co-delivering these drugs, the formulation can exploit the distinct but complementary mechanisms of each drug, potentially resulting in a more robust anti-inflammatory response than either drug alone.
2. **Reduced Drug Resistance:** RA patients may develop resistance to single-agent therapies over time. A combination approach can delay or overcome this resistance by targeting multiple pathways involved in the disease.
3. **Improved Patient Compliance:** A single MSN-based formulation that delivers both Tofacitinib and Methotrexate can simplify the medication regimen, enhancing adherence and overall treatment outcomes.
4. **Minimization of Side Effects:** The targeted delivery of both drugs to inflamed joints reduces systemic exposure, which can minimize the side effects commonly associated with higher doses or systemic circulation of these drugs [83-87].

1.4.3 Loading Techniques for MSNs:

Mesoporous silica nanoparticles (MSNs) have gained significant attention in recent years for their potential in drug delivery systems, particularly due to their unique properties such as high surface area, tunable pore sizes, and excellent biocompatibility. One of the most critical aspects of utilizing MSNs for drug delivery is the method by which drugs are loaded onto these nanoparticles. There are three primary loading techniques used for incorporating drugs into MSNs: **adsorption**, **encapsulation**, and **functionalization**. Each method has its own

advantages, depending on the physicochemical properties of the drug and the desired drug release profile.

1.4.3.1 Adsorption Technique

Adsorption is one of the simplest and most commonly used methods for loading drugs onto MSNs. In this method, the drug molecules are adsorbed onto the surface and into the pores of the MSNs due to weak interactions, such as van der Waals forces, hydrogen bonding, or electrostatic interactions. The high surface area and large pore volume of MSNs provide ample space for drug adsorption, making this technique highly efficient.

Advantages of Adsorption:

- **Simplicity:** The process is straightforward and does not require complex equipment or conditions.
- **High loading efficiency:** Due to the large surface area and pore volume of MSNs, a substantial amount of drug can be loaded.
- **Preservation of drug activity:** Since the process does not involve harsh chemicals or conditions, the structural integrity and activity of the drug are usually preserved.

Limitations of Adsorption:

- **Weak binding:** The drug is loosely bound to the MSNs, which can lead to premature drug release.
- **Uncontrolled release:** The lack of strong interactions between the drug and MSNs may result in a burst release, where a large amount of the drug is released immediately after administration.

1.4.3.2 Encapsulation Technique

Encapsulation involves trapping the drug molecules inside the pores of the MSNs. This method provides a more controlled drug release compared to adsorption, as the drug is physically enclosed within the nanoparticle structure. Encapsulation can be achieved through various techniques, such as using solvents that allow the drug to diffuse into the MSN pores or forming MSN-drug conjugates during the synthesis process.

Advantages of Encapsulation:

- **Controlled release:** By encapsulating the drug within the MSN structure, the release can be modulated over time, preventing burst release.
- **Protection of the drug:** Encapsulation shields the drug from environmental degradation, such as oxidation or hydrolysis, thereby enhancing its stability.
- **Targeted delivery:** Since the drug is encapsulated, it can be designed for release in specific environments, such as acidic or basic conditions, allowing for more precise targeting within the body.

Limitations of Encapsulation:

- **Complexity:** The encapsulation process can be more complex than adsorption and may require specific conditions such as temperature or pH adjustments.
- **Loading capacity:** While encapsulation offers better control over drug release, it may have a lower loading capacity compared to adsorption due to the confined space within the pores.

1.4.3.3 Functionalization Technique

Functionalization involves chemically modifying the surface of the MSNs to enhance drug loading, targeting, and release. This technique typically involves attaching functional groups or ligands to the surface of the nanoparticles, which can interact specifically with the drug or with biological targets. Functionalization can improve the affinity of MSNs for the drug, enhance cellular uptake, and allow for targeted drug delivery to specific tissues or cells.

Advantages of Functionalization:

- **Enhanced drug binding:** Functional groups on the MSN surface can form stronger bonds with drug molecules, increasing the loading efficiency.
- **Targeted delivery:** By functionalizing the surface with targeting ligands (such as antibodies or peptides), MSNs can be directed to specific tissues or cells, improving the therapeutic efficacy and reducing side effects.
- **Controlled and responsive release:** Functionalized MSNs can be designed to respond to specific triggers, such as changes in pH or temperature, allowing for stimuli-responsive drug release.

Limitations of Functionalization:

- **Complex synthesis:** Functionalizing the MSN surface often involves multiple steps and can require the use of specialized reagents and conditions.
- **Potential toxicity:** Although MSNs are generally considered biocompatible, the addition of certain functional groups may introduce toxicity, necessitating careful selection of the modifying agents.

Comparison of Loading Techniques

Each of these loading techniques—adsorption, encapsulation, and functionalization—has its unique advantages and limitations, and the choice of method depends on the specific drug properties and therapeutic goals. Adsorption is the simplest method but may result in uncontrolled release. Encapsulation offers better control over drug release but can be more complex and may reduce loading capacity. Functionalization provides the greatest potential for targeted delivery and stimuli-responsive release but requires careful design and synthesis to avoid toxicity.

In antiarthritic therapy, where the goal is to deliver drugs directly to inflamed joints or tissues, functionalization can be particularly advantageous. By attaching targeting ligands specific to inflammatory markers, MSNs can be directed to the sites of arthritis, thereby enhancing the therapeutic effect while minimizing systemic side effects [88-90].

1.5 Targeted Drug Delivery System:

1.5.1 Concept of Targeted Drug Delivery

By delivering medications directly to particular cells, tissues, or organs, a targeted drug delivery system (TDDS) can maximize therapeutic efficacy and reduce adverse effects. The conventional method of drug delivery frequently entails systemic distribution, in which the medication permeates the body and may impact tissues that are not intended targets while also producing unfavorable side effects. By directing the medication exclusively to the site of interest, TDDS seeks to circumvent this.

Targeted drug delivery involves two main mechanisms: passive targeting and active targeting. Both approaches aim to decrease dosage requirements, increase drug

bioavailability at the intended site, and improve patient outcomes—particularly for chronic illnesses like cancer and arthritis.

Passive Targeting

Passive targeting relies on leveraging the body's inherent mechanisms, specifically the distinctions between healthy and diseased tissues. The process entails the aggregation of drug carriers, such as nanoparticles or liposomes, at the target site as a result of physiological factors. In conditions such as tumors or inflamed tissues, the vascular architecture is characteristically permeable, exhibiting larger intercellular gaps between endothelial cells. This enables nanoparticles, which generally cannot traverse standard vasculature, to access the affected region. The phenomenon is referred to as the enhanced permeability and retention (EPR) effect.

- **EPR Effect:** Diseased tissues, particularly tumors or inflamed areas, have irregular blood vessels with gaps. These larger gaps allow nanoparticles carrying drugs to pass through and accumulate at the target site, while normal tissues remain unaffected due to their tighter blood vessel structures. Passive targeting relies on the body's biology and does not require external triggers.

Advantages of passive targeting include:

- **Reduced Toxicity:** The drug accumulates at the target site, decreasing the potential for systemic side effects.
- **Simpler Mechanism:** It does not require specific recognition of the target cells, reducing the complexity of the delivery system.
- **Prolonged Retention:** Particles can be retained for extended periods at the target site, improving therapeutic outcomes.

However, passive targeting has limitations:

- **Non-Specificity:** It relies on physiological abnormalities, which might not be present in all patients or all types of diseases.
- **Variable Efficacy:** The EPR effect is not uniform across all tumors or inflamed tissues, leading to inconsistent drug delivery.

Active Targeting

Active targeting is a more precise approach, involving the use of molecular interactions between the drug carrier and the target cells. This strategy utilizes specific ligands, such as antibodies, peptides, or small molecules, that recognize and bind to receptors on the target cells. By decorating the surface of drug carriers (e.g., mesoporous silica nanoparticles or liposomes) with these ligands, the drug delivery system can selectively bind to diseased cells while avoiding healthy cells.

Key components of active targeting include:

1. **Ligands:** These are molecules that bind specifically to receptors on the surface of target cells. Common ligands include:
 - **Antibodies:** These are proteins that can specifically recognize antigens on the surface of diseased cells.
 - **Peptides and Proteins:** These can be used to bind receptors unique to the target tissue.
 - **Small Molecules:** Specific small molecules can also serve as targeting agents, especially for diseases with well-defined molecular markers.
2. **Receptors:** These are structures present on the target cells that bind to the ligands on the drug delivery carrier. Diseased cells often express specific receptors (e.g., overexpression of folate receptors in certain cancer cells), allowing for selective drug delivery.
3. **Drug Carriers:** Nanoparticles, including mesoporous silica nanoparticles (MSNs), liposomes, or polymeric micelles, are commonly used to carry drugs in active targeting systems. These carriers are functionalized with ligands, enabling them to bind specifically to target cells.

Advantages of Active Targeting:

- **High Specificity:** By binding only to diseased cells, active targeting ensures minimal impact on healthy tissues, reducing the potential for side effects.
- **Increased Therapeutic Efficacy:** Higher concentrations of the drug are delivered to the target site, leading to improved therapeutic outcomes.

- **Versatility:** The surface of drug carriers can be modified with various ligands, enabling targeting of a wide range of diseases.

However, active targeting also faces challenges:

- **Complexity:** Designing drug carriers that are stable, biocompatible, and functionalized with targeting ligands is more complex than passive systems.
- **Heterogeneity of Disease:** Not all diseased cells express the same receptors, leading to variability in the efficacy of active targeting across different patients or disease stages [91-95].

1.6 Mechanism of Action and Drug Release from Mesoporous Silica Nanoparticles (MSNs)

Mesoporous silica nanoparticles (MSNs) are an innovative approach to targeted drug delivery systems, especially for chronic conditions such as arthritis. MSNs are highly porous, have a large surface area, and are biocompatible, making them excellent carriers for delivering drugs directly to the affected areas. Their structure allows them to encapsulate a variety of therapeutic agents, improving the solubility, stability, and bioavailability of drugs that are poorly soluble or have limited permeability. In arthritis treatment, MSNs offer a promising method for enhancing drug delivery efficiency while minimizing side effects through controlled release.

1.6.1 Drug Release Mechanisms

The drug release from MSNs is governed by multiple factors, including the structure of the nanoparticles, the nature of the drug, and the external environment in which the MSNs are applied. The major mechanisms of drug release from MSNs include:

a. Diffusion:

Diffusion is one of the simplest and most common mechanisms of drug release from MSNs. The drug molecules are loaded into the porous structure of the silica nanoparticles, and over time, they move from the interior of the pores to the surrounding environment through a concentration gradient. This process is driven by the difference in drug concentration

between the interior of the MSN and the external environment. The size of the pores and the molecular size of the drug play crucial roles in controlling the diffusion rate. For drugs with smaller molecular sizes, diffusion is quicker, whereas larger molecules may take more time to be released.

b. Degradation:

Another mechanism of drug release from MSNs is through the degradation of the silica matrix. The silica nanoparticles can degrade in biological environments, especially in the presence of specific enzymes or under certain pH conditions. As the MSN structure breaks down, the encapsulated drug is gradually released. The degradation rate can be fine-tuned by modifying the chemical composition and surface properties of the MSNs. This allows for a controlled release of the drug over an extended period, making it suitable for chronic conditions like arthritis that require long-term medication.

c. pH Sensitivity:

MSNs can be engineered to release drugs in response to specific pH levels, which is particularly useful for targeted drug delivery in different areas of the body. For example, the inflamed tissues in arthritis often have a slightly more acidic pH compared to healthy tissues. pH-sensitive MSNs can be designed to remain stable in normal physiological conditions but release the drug rapidly when exposed to the lower pH of the inflamed area. This selective release minimizes systemic side effects and ensures that a higher concentration of the drug is delivered precisely where it is needed.

By utilizing these mechanisms, MSNs can offer a sustained, controlled release of drugs, improving the therapeutic outcomes in arthritis treatment.

1.6.2 In-vitro and In-vivo Studies

Several studies have explored the effectiveness of MSNs in delivering drugs for the treatment of arthritis, both in vitro (laboratory-based) and in vivo (animal or human studies).

a. In-vitro Studies:

In-vitro experiments provide a controlled environment to evaluate the release behavior of drugs from MSNs. Researchers typically use models such as cell cultures or synthetic membranes to simulate the biological conditions of arthritis. For example, MSNs loaded with anti-inflammatory drugs like methotrexate or tofacitinib have been studied for their ability to release the drug in a controlled manner. These studies show that MSNs can improve drug solubility and ensure a steady release over time, compared to free drug formulations.

In one study, MSNs loaded with paliperidone were tested in a simulated biological environment, where the drug release reached 96% in 120 minutes, significantly higher than the 30% release observed with the plain drug. This demonstrates that MSNs can enhance the dissolution rate of poorly soluble drugs, which is critical for improving their therapeutic efficacy.

b. In-vivo Studies:

In-vivo studies provide insights into how MSNs behave in actual biological systems, such as in animal models of arthritis. These studies assess not only the drug release but also the biodistribution, targeting efficiency, and therapeutic outcomes. MSNs loaded with drugs have been shown to accumulate in inflamed tissues more effectively than free drugs, which helps reduce inflammation more efficiently and with fewer side effects.

For example, studies have shown that functionalized MSNs, which are modified to target specific cells or receptors, can further enhance the targeted delivery of drugs to arthritic joints. In-vivo experiments have demonstrated improved bioavailability and reduced systemic toxicity when using MSNs compared to conventional drug formulations. This targeted approach ensures that the drug is concentrated at the site of inflammation, leading to more effective arthritis management.

In another study, functionalized MSNs loaded with methotrexate exhibited a pH-sensitive release, allowing the drug to be released rapidly in the acidic environment of inflamed tissues while remaining stable in normal tissues. This pH-sensitive release behavior makes MSNs ideal for treating conditions where local drug delivery is essential [96-98].

1.7 Importance of Gel Formulations in Arthritis Treatment

1.7.1 Topical Gels as Drug Delivery Systems

Topical gels are a promising method for localized drug delivery in arthritis treatments. Arthritis is often characterized by inflammation and pain in the joints, making it important to deliver medications directly to the affected area. Gels provide an ideal medium for this because of their semi-solid nature and ease of application. They spread easily over the skin, forming a thin film, which ensures better contact between the drug and the skin surface.

Topical gels are beneficial because they allow for:

- **Localized Drug Delivery:** The drug is delivered directly to the inflamed joint or tissue, minimizing systemic exposure and reducing side effects typically associated with oral administration.
- **Prolonged Drug Release:** Gels can be formulated to slowly release the drug over a prolonged period, ensuring that the medication remains active at the site of application for an extended time.
- **Ease of Use and Patient Compliance:** Gels are easy to apply and generally preferred by patients, increasing compliance, especially for long-term arthritis management.

In addition, topical gels avoid first-pass metabolism in the liver, which can degrade orally administered drugs before they reach the systemic circulation. For patients with arthritis, this ensures that more of the active drug reaches the target site, providing more effective pain relief and reduction in inflammation.

1.7.2 Formulation of Nanogels

Mesoporous silica nanoparticles (MSNs) can be incorporated into gel formulations to enhance the effectiveness of topical arthritis treatments. Nanogels are gels that contain nanoparticles, which in this case are MSNs, acting as carriers for the drug molecules. This combination of nanoparticles with gel technology provides several advantages:

- **Targeted Delivery:** MSNs can be functionalized (chemically modified) to target specific tissues, such as inflamed joints in arthritis. This ensures that the drug is delivered precisely where it is needed.

- **Improved Solubility and Stability:** Many drugs used to treat arthritis, such as anti-inflammatory agents, are poorly soluble in water. MSNs help to enhance the solubility of these drugs, increasing their bioavailability. Moreover, the nanoparticles protect the drug from degradation before it reaches the target tissue.
- **Controlled Release:** MSNs can be engineered to release the drug slowly over time. When incorporated into a gel, this controlled release mechanism allows the drug to be continuously delivered to the affected area, providing sustained relief from arthritis symptoms.

By using nanogels, the formulation not only delivers the drug locally to the joint but also enhances the effectiveness of the treatment by improving the stability and controlled release of the drug.

1.7.3 Characterization of Nanogels

To ensure that nanogels function properly and deliver the desired therapeutic effects, several characterization techniques are used. These methods help evaluate the physical and chemical properties of the nanogel, as well as the behavior of the drug within the formulation. Key characterization techniques include:

- **Texture Analysis:** This assesses the physical properties of the gel, such as spreadability, firmness, and consistency. These factors are important for patient comfort and ease of application. A gel that spreads well and feels smooth on the skin is more likely to be accepted by patients.
- **Drug Content Analysis:** This technique measures the amount of drug present in the gel and ensures that the formulation contains the correct dosage. It is critical to confirm that the drug is evenly distributed throughout the gel to ensure consistent dosing with each application.
- **Diffusion Studies:** These studies evaluate how well the drug diffuses from the gel into the skin. This is crucial for determining the efficiency of drug delivery. A well-formulated nanogel should allow the drug to penetrate the skin and reach the affected joint in sufficient concentrations to provide therapeutic effects.

- **In-vitro Drug Release:** This assesses how quickly and efficiently the drug is released from the nanogel. Ideally, the drug should be released in a controlled manner, ensuring a steady delivery over time rather than a rapid, short-term burst [99-101].

1.8 In-vitro and In-vivo Evaluation of MSN-Loaded Nanogels

The evaluation of mesoporous silica nanoparticles (MSN)-loaded nanogels is essential for determining their efficacy and ensuring that the formulation behaves as expected both in a controlled laboratory environment (in-vitro) and within living organisms (in-vivo). In this section, we will explore both the in-vitro characterization and the in-vivo evaluation of the nanogel, which are critical for its development as a targeted drug delivery system for antiarthritic applications.

1.8.1 In-vitro Characterization of MSN-Loaded Nanogels

In-vitro characterization is the first step in assessing the properties and performance of the MSN-loaded nanogels. Various tests and studies are conducted to ensure the formulation meets the desired standards in terms of drug release, stability, and biocompatibility.

1. **Drug Release Studies:** One of the primary goals of the in-vitro studies is to determine the rate and extent of drug release from the nanogels. Diffusion studies are typically conducted using methods like Franz diffusion cells or dialysis methods, where the nanogel is placed in a solution mimicking body fluids. The amount of drug released over time is measured to assess how the formulation behaves under physiological conditions. This data is crucial for predicting how the nanogel will perform when applied in a biological system. Key parameters include:
 - **Cumulative Drug Release:** A critical metric for understanding how much drug is released over a specific period.
 - **Controlled Release Behavior:** The nanogels are designed to release the drug in a controlled manner, ensuring that the therapeutic effect is prolonged and consistent over time.
2. **Stability Studies:** Nanogels must remain stable throughout their shelf life and upon application. Stability tests are conducted by storing the formulation under different conditions of temperature and humidity and evaluating parameters such as:

- **Physical Stability:** Ensuring no changes in the gel structure, such as phase separation or precipitation.
 - **Chemical Stability:** Ensuring no degradation of the drug or gel matrix, which could compromise efficacy.
 - **pH Stability:** Since skin and tissue environments can vary in pH, it is important to test the formulation's stability in different pH environments.
3. **Particle Size and Morphology:** The particle size of the MSN plays a vital role in its ability to permeate biological barriers such as skin. The size of the MSNs and the resulting nanogel particles is measured using techniques like dynamic light scattering (DLS) or transmission electron microscopy (TEM). Smaller particles are preferred for better penetration and controlled release.
 4. **Entrapment Efficiency:** This parameter measures the proportion of the drug that has been successfully loaded into the MSN nanogels. A high entrapment efficiency ensures that a sufficient amount of drug is available for therapeutic action. Methods such as high-performance liquid chromatography (HPLC) or UV-spectrophotometry are used to quantify the amount of drug in the nanogel formulation.
 5. **Rheological Properties:** The texture and consistency of the nanogel are crucial for its application on the skin or in joints. Rheological studies help in understanding the spreadability, viscosity, and other mechanical properties of the nanogel, which can influence the ease of application and patient compliance.

1.8.2 In-vivo Evaluation of MSN-Loaded Nanogels

Once the formulation has passed in-vitro tests, it is subjected to in-vivo evaluations to determine its efficacy, safety, and bioavailability within living organisms. These tests are designed to simulate real-world conditions and assess how the formulation behaves in the body.

1. **Efficacy Studies:** In-vivo studies are conducted on animal models that exhibit symptoms of arthritis. The MSN-loaded nanogel is applied to the affected areas, and its therapeutic effects are measured over time. These studies are critical for assessing how well the drug is delivered to the target site and its effectiveness in reducing inflammation, pain, or other arthritic symptoms. Parameters include:

- **Reduction in Swelling and Inflammation:** This is a direct measure of the nanogel's antiarthritic efficacy.
 - **Histological Examination:** Tissue samples from the treated area can be examined to assess the extent of tissue regeneration and the reduction of inflammatory markers.
2. **Bioavailability Studies:** Bioavailability refers to the extent and rate at which the active drug ingredient is absorbed and becomes available at the target site. In the case of nanogels, the drug must penetrate the skin or joint tissue effectively to exert its therapeutic effect. Blood samples may be taken periodically to measure the drug concentration and calculate key pharmacokinetic parameters such as:
- **Cmax (Maximum Plasma Concentration):** The peak concentration of the drug in the bloodstream.
 - **Tmax (Time to Reach Maximum Concentration):** The time taken for the drug to reach its peak concentration in the bloodstream.
 - **Area Under the Curve (AUC):** This represents the total exposure of the body to the drug over time.
3. **Pharmacokinetics:** These studies provide insight into how the drug is absorbed, distributed, metabolized, and eliminated by the body. By comparing the pharmacokinetics of the MSN-loaded nanogel with conventional formulations, researchers can determine if the nanogel provides superior delivery and a longer-lasting therapeutic effect. Critical factors include:
- **Absorption Rate:** How quickly the drug is absorbed through the skin or tissue.
 - **Retention Time:** The ability of MSNs to provide sustained drug release at the target site can lead to a longer retention time, which means fewer applications are required.
 - **Metabolic Pathways:** Understanding how the drug is metabolized in the body is key to predicting potential side effects and ensuring patient safety.
4. **Toxicity Studies:** Safety is a major concern when introducing any new drug delivery system. In-vivo toxicity studies are conducted to ensure that the MSN-loaded nanogel does not cause adverse effects such as skin irritation, systemic toxicity, or organ damage. Common methods include:

- **Skin Irritation Tests:** Assessing the formulation for any potential irritative effects on the skin.
 - **Systemic Toxicity:** Evaluating the potential for toxic effects in major organs such as the liver, kidneys, and heart.
5. **Biodistribution Studies:** These studies are used to track the distribution of MSNs and the drug throughout the body. Techniques such as fluorescence imaging or radioactive labeling can be employed to monitor where the nanoparticles travel after administration. This ensures that the formulation reaches the target site and minimizes off-target effects.
6. **Immunogenicity:** Since nanoparticles can sometimes trigger an immune response, it is important to evaluate whether the MSN-loaded nanogel induces any immune reactions in the body. These tests are critical for ensuring long-term safety in patients [102-105].

1.9 Research Gaps and Objectives:

1.9.1 Identification of Research Gaps

In recent years, mesoporous silica nanoparticles (MSNs) have emerged as a promising drug delivery system due to their unique properties such as high surface area, tunable pore size, excellent biocompatibility, and the ability to be functionalized. However, despite these advantages, there are several gaps in current research related to the use of MSNs in targeted drug delivery systems for arthritis, which this study aims to address:

1. **Limited Research on MSNs for Arthritis Treatment:** Although MSNs have been extensively studied for their application in cancer therapy and general drug delivery, there is limited research focusing on their use in treating arthritis, a chronic inflammatory condition that requires sustained and localized drug delivery.
2. **Lack of Targeted Drug Delivery Systems for Arthritis:** Conventional treatments for arthritis often involve systemic drug administration, which can lead to suboptimal drug concentrations at the site of inflammation and increase the risk of systemic side effects. Targeted drug delivery systems that specifically deliver therapeutic agents to the inflamed joints are needed to improve treatment outcomes.
3. **Challenges in Enhancing Solubility and Permeability of Antiarthritic Drugs:** Many antiarthritic drugs have poor water solubility and low permeability, limiting their

bioavailability and therapeutic efficacy. While MSNs have the potential to enhance drug solubility and permeability, there is a need for more focused research on how MSNs can improve the pharmacokinetics of antiarthritic drugs.

4. **Inadequate Evaluation of MSNs in Topical Formulations:** Topical gels are a preferred mode of treatment for arthritis due to their ability to deliver drugs directly to the affected area. However, there is insufficient research on the formulation and evaluation of MSN-loaded topical gels for arthritis. The integration of MSNs into topical gels could provide sustained drug release and improved bioavailability, but more research is required to fully understand the effectiveness of such formulations.
5. **Limited Understanding of In-Vivo Efficacy and Safety:** While MSNs have been shown to enhance drug delivery in in vitro studies, there is a lack of comprehensive in vivo studies that evaluate the efficacy and safety of MSN-based drug delivery systems for arthritis therapy. More research is needed to determine the long-term effects, biodistribution, and potential toxicity of MSNs when used in large quantities for drug delivery.

1.9.2 Objectives of the Study

Based on the identified research gaps, the objectives of this study are as follows:

1. **Synthesis of Mesoporous Silica Nanoparticles (MSNs):** The first objective is to synthesize MSNs with optimal properties for drug loading and delivery. This includes controlling the pore size, surface area, and functionalization of the nanoparticles to enhance their drug-loading capacity and compatibility with antiarthritic drugs.
2. **Characterization of MSNs:** The MSN-loaded nanoparticles will be characterized using advanced techniques such as Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), and Brunauer-Emmett-Teller (BET) analysis to determine their morphology, size, surface area, and pore size. Functionalization of the MSNs will also be evaluated to ensure that they can effectively bind and release the drug at the target site.
3. **Loading Antiarthritic Drugs onto MSNs:** Poorly soluble antiarthritic drugs, such as methotrexate and tofacitinib citrate, will be loaded onto the synthesized MSNs. The objective is to improve the solubility, dissolution rate, and permeability of these drugs by

utilizing the high surface area and pore structure of the MSNs. This will help in delivering a higher concentration of the drug directly to the inflamed joints.

4. **Formulation of MSN-Loaded Gels:** The next step is to formulate a topical gel incorporating MSNs loaded with poorly soluble antiarthritic drugs. The formulation aims to enhance the solubility and permeability of these drugs, improving their bioavailability and therapeutic efficacy. This will involve optimizing the gel base to ensure stability, spreadability, and ease of application.
5. **Characterization of the MSN-Loaded Antiarthritic Nanogel:** Antiarthritic nanogel will be characterized for parameters like viscosity, rheology, texture, particle stability in nanogel and content uniformity using advanced techniques.
6. **Evaluation of the Efficacy of MSN-Loaded Antiarthritic Gels:** The formulated MSN-loaded gels will be evaluated for their efficacy in treating arthritis through in vitro and in vivo studies. This will include assessing the drug release profile, diffusion rates, and bioavailability of the drug in the gel form. The study will also involve testing the anti-inflammatory and analgesic effects of the gel in animal models of arthritis.
7. **Study of Dermatokinetics and Bioavailability:** An important objective of this study is to evaluate the pharmacokinetics of the MSN-loaded gel formulation. This will involve measuring the absorption, distribution, metabolism, and excretion (ADME) of the drug delivered via the MSN-based gel. The goal is to determine if the MSN-loaded gel provides better bioavailability and sustained drug release compared to conventional formulations.

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REVIEW OF LITERATURE



CHAPTER 2

REVIEW OF LITERATURE

Review of literature covering national and international status has been completed.

1. Abu-Dief et al. (2022) explored the recent advances in the design and synthesis of Mesoporous Silica Nanoparticles (MSNPs) for targeted drug delivery applications. MSNPs possess intrinsic physiochemical stability, a high surface area, low toxicity, and significant loading capacity for various therapeutic agents, making them highly suitable for controlled drug delivery. This study focused on the parameters influencing the functional characteristics of MSNPs, such as particle size, morphology, porosity, and surface functionalization, which directly affect their in vivo absorption, dissemination, and secretion. The authors also highlighted the potential of combining MSNPs with other functional materials to enhance biological compatibility, monitor drug release, and improve tumor cell uptake. These nanoparticles demonstrate promising applications in cancer treatment and other therapeutic areas due to their controllable and targeted drug delivery capabilities.
2. Tao Liao et al., 2021, developed a dual-pH-sensitive chitosan (CHI)/mesoporous silica nanoparticle (MSN)-based anticancer drug delivery system (DDS) with a “tumor-triggered targeting” property. In this design, mesoporous silica nanoparticles loaded with doxorubicin hydrochloride (DOX) were modified with benzimidazole (Bz), while chitosan-graft- β -cyclodextrin (CHI-g-CD) acted as a “gatekeeper” by covering MSNs through host-guest interaction between β -CD and Bz. Targeting peptide adamantane-glycine-arginine-glycine-aspartic acid-serine (Ad-GRGDS) and methoxy poly(ethylene glycol) benzaldehyde (mPEG-CHO) were also grafted onto CHI via pH-sensitive bonds. This system remains “stealthy” at neutral pH but reveals the targeting peptide and positive charge in an acidic tumor environment. The DDS demonstrated efficient DOX release in cancer cells due to pH-induced interactions and exhibited enhanced tumor targeting with reduced cytotoxicity against normal cells, proving effective in both in vitro and in vivo studies for inducing cancer cell apoptosis and inhibiting tumor growth.

3. Jie Chang et al (2024) developed a pH-responsive mesoporous silica nanoparticle-based drug delivery system specifically for targeted breast cancer therapy. Mesoporous silica nanoparticles (MSN-COOH) were synthesized and loaded with doxorubicin (DOX) into the pores of MSN-COOH. The surface of these nanoparticles was further modified with polyethyleneimine (PEI) and anisamide (AA), named DOX@MSN-PEI-AA (DMPA). The targeted drug delivery was achieved by AA-mediated receptor endocytosis, allowing DMPA to specifically enter breast cancer cells. The acidic environment in the lysosomes/endosomes triggered the protonation of PEI, leading to its dissociation from the MSN surface and a controlled release of DOX in the cytoplasm. In vitro and in vivo studies, including anti-tumor and hemolytic experiments, demonstrated that DMPA exhibited precise targeting of breast cancer cells while maintaining excellent safety, making it a promising candidate for breast cancer therapy.
4. Garima Lohiya et al 2021, developed a highly reproducible and monodispersed chitosan-coated, doxorubicin-loaded, aptamer-conjugated Mesoporous Silica Nanoparticle (MSN) drug delivery system targeting breast cancer cells overexpressing EGFR/HER2. The system utilized carboxylated chitosan to impart pH-responsiveness, allowing endo/lysosomal escape, enhanced cytosolic delivery, and tunable drug release kinetics. The partial carboxylation of chitosan facilitated a quicker release of doxorubicin while maintaining the MSN's targeting and release properties. The developed MSNs demonstrated higher uptake and cytotoxicity in triple-negative and HER2-positive breast cancer cells compared to non-targeted MSNs, making this system a promising therapeutic strategy for breast cancer therapy. Characterization included cytotoxicity assays, drug release studies, and receptor-mediated targeting analysis.
5. Senitta Stephen et al., 2021, reviewed the role of Mesoporous Silica Nanoparticles (MSNs) in the development of advanced drug delivery systems. Their study highlighted MSN's biocompatibility, high drug-loading efficiency, and potential for ligand functionalization to enhance therapeutic outcomes in various diseases. The review emphasized MSN's versatility in delivering small molecules and large biomolecules like peptides and proteins. Additionally, MSNs have been explored in

non-conventional drug delivery systems, including liposomes, microspheres, oro-dispersible films, 3D-printed formulations, and microneedles. However, challenges such as low bulk density, retention of mesoporous structure during processing, and limited in vivo studies were identified as barriers. The study provided a critical analysis of MSN-based systems compared to other functionalized polymers, and future directions were outlined to address existing challenges.

6. García-Fernández et al. (2021) explored the potential of Mesoporous Silica Nanoparticles (MSNs) for pulmonary drug delivery, addressing the significant clinical challenge posed by respiratory diseases. The authors emphasized that conventional lung therapies are hindered by anatomical, physiological, and immunological barriers, leading to reduced drug bioavailability at the target site. MSNs, with their high surface area, tunable pore size, and functionalization capabilities, provide a novel solution to overcome these barriers. The review highlighted the role of nanomedicine in improving drug delivery to the lungs and showcased the application of MSNs in treating various respiratory diseases. Key evaluations included the structural and functional characterization of MSNs for their efficiency in pulmonary drug delivery systems.
7. Hafiz Ahmed et al. 2022, reviewed the biomedical applications of mesoporous silica nanoparticles (MSNs) as drug delivery carriers. The study highlights the unique properties of MSNs, such as their large surface area, pore volume, and surface-active groups, which have been extensively utilized in drug delivery, photodynamic therapy, biosensors, and tissue engineering. The porous structure of MSNs allows for high drug loading, enhancing solubility and therapeutic efficacy. However, the authors emphasize that clinical translation of MSN-based drug delivery systems requires thorough in vivo human studies to assess potential adverse reactions and side effects. The review discusses the dependence of toxicity on MSN characteristics like shape, size, surface chemistry, and charge, and stresses the need for optimizing surface properties to improve biocompatibility. Advances in triggered drug release, including gatekeepers and delivery of large molecules such as proteins and nucleic acids, are also covered. This comprehensive review provides valuable insights into the current

research on MSNs-based drug delivery systems, focusing on tunability, surface functionalization, biosafety, and clinical translation challenges.

8. Hafiz Ahmed et al. 2022 reviewed the biomedical applications of mesoporous silica nanoparticles (MSNs) as drug delivery carriers. They highlighted the unique properties of MSNs, including their large surface area, pore volume, and surface-active groups, which are extensively utilized in drug delivery, photodynamic therapy, biosensors, and tissue engineering. The study emphasized that the porous structure of MSNs facilitates high drug loading, enhancing solubility and therapeutic efficacy. Despite these advantages, the authors noted that clinical translation of MSN-based drug delivery systems requires thorough in vivo human studies to assess potential adverse reactions and side effects. The review discusses how MSN characteristics such as shape, size, surface chemistry, and charge influence toxicity and stresses the need for optimizing these properties to improve biocompatibility. Additionally, advances in triggered drug release, including gatekeepers and the delivery of large molecules like proteins and nucleic acids, are covered. This comprehensive review provides valuable insights into the current research on MSN-based drug delivery systems, focusing on tunability, surface functionalization, biosafety, and clinical translation challenges.
9. Meng-meng Lu et al 2018, designed and synthesized nano silver decorated Mesoporous Silica nanoparticles as Safety tissue adhesives. Herein inorganic nanoparticles glued the tissue with nanobridging effect. These nanoparticles were characterized by TEM, FTIR, different Mesopore Properties, particle size distribution, zeta potential, energy-dispersive X-ray spectroscopy. Furthermore, strength of adhesion, anti-microbial assay, mouse skin wound model, and MTT assays were evaluated in order to determine the tissue adhesion, anti-bacterial property, biodegradability and biocompatibility of the Ag-MSNs. These nanoparticles showed efficient wound closure in comparison to sutures with little systemic toxicity.
10. Pande Vishal et al 2018, synthesized a platform for targeted of mesoporous silica nanoparticles acting as a medium of delivery for Gemcitabine hydrochloride conjugated with folic acid and loaded with dye and characterized by FTIR, TEM, Mercury porosimetry, Particle size analysis and cell line study. High drug-loading

capacity is a key feature of the mesoporous silica platform that was designed in this study and also shows fluorescence in cell line study.

11. Pande Vishal et al 2018, Studied the solubility and dissolution enhancement of poorly water-soluble drug Paliperidone by MSNs. They synthesized amine functionalized MSNs and loaded the drug Paliperidone with the help of wet impregnation method. The invitro and invivo drug release was studied which was found to be significantly enhanced. The invitro drug release in 120 min for MSN loaded drug was 96% while that of plain drug was 30%. The invivo study also confirmed the enhancement of solubility and dissolution of Paliperidone.
12. Haibin Wu et al 2017, synthesized a regenerative wound healing material made up of Ceria nanocrystals decorated mesoporous silica as ROS scavenging tissue adhesive. They characterized the nanoparticles with Particle size, TEM, XRD, STEM, DLS, and Zeta Potential and Adhesion test in Rat wound model and found that the nanoparticles showed high tissue adhesive capacity.
13. Suk ho Hong et al 2017, developed an activatable theranostic agent made up of hollow mesoporous silica nanoparticles loaded with Indocyanine green. Endocytosis route was preferred by the nanoparticles to enter the malignant cells. Once they entered, they became highly fluorescent. Characterization was carried out by using Quantitative analysis of cellular uptake, In vitro cytotoxicity testing, In vitro phototoxicity testing. In case of the selective NIR fluorescence cancers this material is proving effective.
14. Pegah Khosravian et al 2016, fabricated and evaluated folic acid/ methionine functionalize MSNs for delivery of docetaxel. Amine functionalization is carried out by using 3-aminopropyl triethoxy silane. MSNs are evaluated by Ex vivo fluorescence imaging, In vivo distribution of nanoparticles, In vivo fluorescence imaging Infrared spectroscopy, MTT assay, SEM, TEM. The Average diameter of synthesized MSN-NH₂ was 49 nm with a narrow size distribution. Functionalized and DTX-loaded MSNs that were designed in this study have a pH-sensitive drug release kinetic along with high drug-loading capacity.

15. Sandy Budi Hartono et al 2016, prepared a system for curcumin bioavailability enhancement; which was intended for oral use. It possessed cubic shaped MSNs. It possessed better release profile and a higher solubility. Physical characterization was carried out by using TEM, XRD, FTIR, In vitro release studies. Higher bioavailability of MSN-A-Cur and MSM-A-Cur was observed when compared to that of free curcumin. Pore size of 1.8 nm was observed in Amine functionalized MCM-41.
16. Minfeng Huo et al 2016, explain triggered-release drug delivery nanosystems for cancer therapy by intravenous injection. Triggered-release nano-drug release system (TRDDSs) emerges as a promising cancer-therapeutic modality to solve the critical issues of traditional chemotherapy.
17. N. Lashgari et al 2016, explained that the organic-inorganic hybrid nanomaterials have important advantages as solid chemosensors and various innovative hybrid materials modified by fluorescence molecules were recently prepared. On the other hand, the homogeneous porosity and large surface area of mesoporous silica make it a promising inorganic support. SBA-15 as a two-dimensional hexagonal mesoporous silica material with stable structure, thick walls, tunable pore size, and high specific surface area is a valuable substrate for modification with different organic chelating groups. They highlighted the fluorescent chemosensors for ionic species based on modification of the mesoporous silica SBA-15 with different organic molecules, which have been recently developed from our laboratory to provide selective, sensitive, low cost, and rapid response optical sensors.
18. Guilan Qual et al 2015, emphasized that targeting property of lactose was integrated with the excellent drug delivery and endocytotic behaviors of MSNs to build a novel drug delivery system. Docetaxel was selected as a model drug, and fluorescein isothiocyanate was used as a dye for the tracking to determine where the cargo will be released. Physical characterization was carried out by using SEM and TEM, cellular uptake of nanoparticles was studied by using confocal microscopy, by using wetness impregnation method drug was incorporated into the mesoporous silica nanoparticles. Cytotoxicity study carried out by using MTT viability assay.

19. Carlos Baleizo et al 2015, development hMSNs for theranostics, carrying fluorescent beacons for traceability and imaging, featuring a smart release control mechanism and able to accommodate large drug loads and deliver their cargo on-demand to a desired location, promises an exceptional platform for precision therapy and diagnosis.
20. Mehdi Esmaeili Bidhendi et al 2023, provided an alternative for removal of mercury ions (Hg^{+2}) by the use of modified nano porous compounds. Hence, in this context a new modification of mesoporous silica (SBA-15) with 1, 3, 5 (Trithiane) as modifier ligand and its use for the removal of mercury ions from aqueous environment has been investigated SBA-15 and Trithiane were synthesized. The confirmation of the presence of ligand in the silica framework by FTIR spectrum was demonstrated. The current investigation provided a newly modified nano porous compound as an efficient adsorbent for removal of Hg^{+2} from aqueous environment.
21. Justin Siefker et al 2014, demonstrated recent advances in the synthesis and design of nanostructured MSNs, and provide a light on opportunities for the delivery of biologicals to different organ and tissue compartments. The SBA-15 platform provides a delivery carrier that is inherently separated from the active biologic due to distinct intra and extra particle environments Flexibility in the application of the SBA-15 platform are also discussed.
22. Ebrahim Ahmadi et al 2014, prepared a mesoporous silica nanoparticle as carriers for sustained DDS. Ibuprofen was selected as a model drug. SBA-15 was prepared by hydrolysis and condensation. Surface functionalization is carried out by using 3-aminopropyltriethoxysilane. Liquid-phase grafting method was employed for the loading of Ibuprofen. Physical characterization was performed using SEM and TEM, X-ray diffraction, Thermal gravimetric and differential thermal analysis, In vitro release studies. Best loading efficiency achieved at 40°C, 35h, and stirring rate of 100 rpm.
23. M.J.K. Thomas et al 2010, described Silica nanoparticles (MSNs) with a highly ordered mesoporous structures (103 Å) with cubic Im 3m have been synthesized using triblock copolymers with high poly(alkylene oxide) (EO) segments in acid media. The produced nanoparticles displayed large specific surface area (765cm²/g)

with an average particles size of 120 nm. The loading efficiency was assessed by incorporating three major antiepileptic active substances via passive loading and it was found to varying from 17 to 25%. The state of the adsorbed active agents was further analyzed using differential scanning calorimetry (DSC) and X-ray powder diffraction (XRPD). Dissolution studies revealed rapid release profiles within the first 3 h. The viability of 3T3 endothelial cells was not affected in the presence of MSNs indicating negligible cytotoxicity.

24. Salonee Tawde et al 2020, were formulated an antiarthritic gel containing ginger extract and evaluate its drug release activity. Topical ginger gels were prepared using Carbopol 934 as a gelling agent at varying concentrations, namely 0.5%, 1%, and 1.5% w/w. The gel was analyzed to determine percent purity and cumulative drug release. Results indicated that the 1.5% w/w concentration of Carbopol in the ginger gel exhibited adequate drug release. Antiarthritic gel containing 1.5% w/w of Carbopol demonstrated good consistency, acceptable spreadability, and a favorable drug release profile. The topical herbal ginger gel developed in this study offers a simple, easily formulated, convenient, and economical alternative that merits consideration in the treatment of rheumatoid arthritis.

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AIM AND OBJECTIVES



CHAPTER 3

AIM AND OBJECTIVES

Aim:

The primary aim of this research is to formulate and evaluate a mesoporous silica nanoparticle (MSN)-based antiarthritic gel as a targeted drug delivery system. This system aims to improve the solubility, permeability, and bioavailability of poorly soluble drugs and enhance the targeted delivery to arthritic sites, thereby maximizing therapeutic efficacy while minimizing systemic side effects.

Objectives:

1. Synthesis, Surface modification and Characterization of Mesoporous Silica Nanoparticles (MSNs):

The first objective is to synthesize mesoporous silica nanoparticles. MSNs will act as the drug carriers due to their high surface area, tunable pore sizes, and excellent biocompatibility.

Characterization of MSNs: MSNs will be characterized using techniques such as

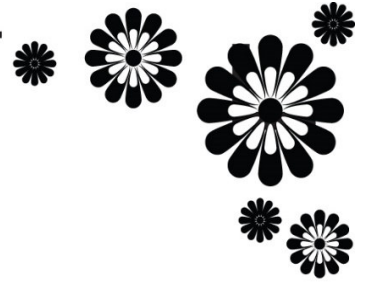
- **Fourier-Transform Infrared Spectroscopy (FTIR):** (to confirm the presence of characteristic peaks for organic functional groups that can indicate successful surface modification).
- **Differential Scanning Calorimetry (DSC)** (to analyze thermal properties),
- **Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM)** (to study nanoparticle morphology),
- **Brunauer-Emmett-Teller (BET) analysis** (to measure the surface area of MSNs).
- **pXRD** (to understand the effect of surface modification on the structural integrity and crystallinity)

2. **Loading of Antiarthritic Drugs into MSNs:** Poorly soluble antiarthritic drugs will be loaded into the mesoporous silica nanoparticles. This step will focus on improving the drug's solubility and dissolution rate to ensure efficient drug delivery.
3. **Enhancing Solubility and Dissolution Rate:** One of the major challenges in drug formulation is the poor solubility of certain drugs. This objective focuses on using MSNs to enhance the solubility and dissolution rate of the selected antiarthritic drugs.
4. **Enhancing Drug Permeability:** In addition to solubility, many drugs face challenges with permeability. By incorporating the drug into MSNs, this objective aims to improve the permeability of the poorly permeable antiarthritic drugs, ensuring higher absorption and bioavailability.
5. **Formulation of MSN-Loaded Nanogel:** This objective focuses on the formulation of a topical gel incorporating MSN-loaded antiarthritic drugs. The goal is to create a targeted drug delivery system that will ensure the drug is released at the desired site of action (arthritic joints), enhancing local bioavailability while minimizing systemic exposure.
6. **Characterization of the MSN-Loaded Nanogel:** After formulation, the nanogel will be characterized using advanced techniques such as:
 - **Texture Analyzer** (to study gel properties like spreadability and cohesiveness),
 - **Brookfield Viscometer** (to study viscosity and rheological properties of the gel)
 - **Zetasizer** (to study the Zeta potential of the gel that indicate the nano particle stability in the formulation)
 - **Drug Release Profile** (to study the drug release form the nanogel)

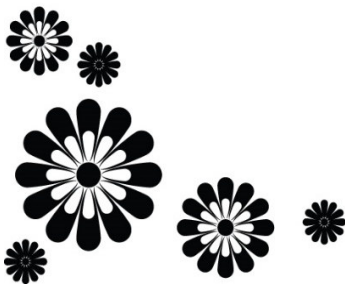
7. **Stability of the antiarthritic Nanogel**

The objective to perform stability study in accelerated and long-term stability conditions on the Nanogel's appearance, viscosity, drug content, microbial count and pH at definite intervals to establish the good shelf life and to provide flexibility in storage options.

- 8 **Dermatokinetics Study:** The final objective is to perform in-vivo studies to assess the bioavailability and dermatokinetics of the formulated MSN-loaded nanogel. This will provide insight into how well the formulation delivers the drug to the targeted site and its overall therapeutic efficacy.



PLAN OF WORK



CHAPTER 4

PLAN OF WORK

1. Selection of drug and excipient (Tofacitinib Citrate and Methotrexate)
2. Synthesis of mesoporous silica i.e. SBA-15
3. Characterization of synthesized SBA-15
 - a) FTIR spectroscopy
 - b) Particle size
 - c) Transmission Electron Microscopy
 - d) Scanning Electron Microscopy
 - e) Differential Scanning Calorimetry
 - f) Brunauer-Emmett-Teller (BET) Analysis
4. Amine functionalization of SBA-15
5. Characterization of surface modified Mesoporous Silica
 - a) FTIR spectroscopy
 - b) pXRD
6. Loading of antiarthritic drugs in Surface Modified MSNs
7. Characterization of antiarthritic drugs loaded MSNs
 - a) Entrapment Efficiency & Drug Loading efficiency
8. Incorporation of MSN's in to gel base to prepare Nanogel

9. Characterization of the MSN-Loaded Nanogel

- a) Viscosity
- b) Spreadability
- c) Texture analysis
- d) Particle size and Particle size distribution
- e) Zeta potential
- f) Drug release profile

11. Stability study

12. Dermatokinetic evaluation of Antiarthritic gel



MATERIALS AND METHODS



CHAPTER 5

MATERIALS AND METHOD

5.1 Drug Profile

5.1.1 Tofacitinib Citrate

Chemical Name:

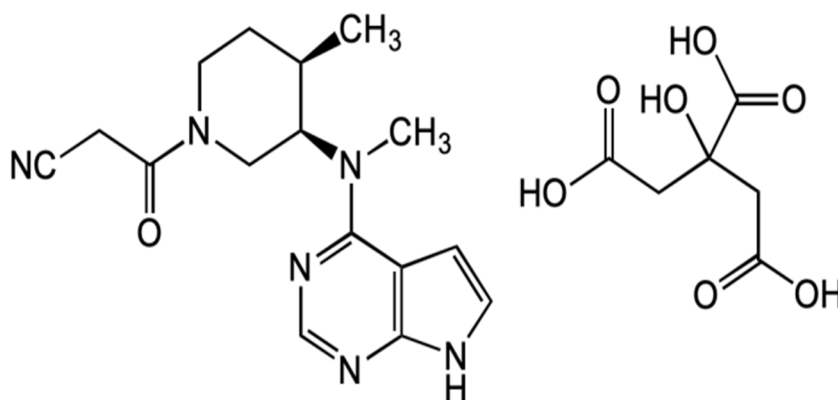
The chemical name of Tofacitinib Citrate is 3-[(3R,4R)-4-Methyl-3-[methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino]piperidin-1-yl]-3-oxo-propionitrile. This complex structure highlights the drug's role as a small molecule that fits into cellular enzymes, helping to block key pathways involved in inflammation.

Chemical Formula:

The molecular formula $C_{22}H_{28}N_6O_8$ represents the number and type of atoms in tofacitinib. It is composed of 16 carbon (C) atoms, 20 hydrogen (H) atoms, 6 nitrogen (N) atoms, and 1 oxygen (O) atom. This formula underscores its relatively small molecular size, which allows it to penetrate cells effectively.

Chemical Structure:

The molecular structure of Tofacitinib features a pyrrolopyrimidine ring, which is a crucial part of its mechanism. The ring is central to its function, as it enables the drug to bind selectively to Janus Kinase (JAK) enzymes, preventing their activity.



CAS Number:

540737-29-9. This is the unique identifier for Tofacitinib in the Chemical Abstracts Service, providing a standardized reference in scientific literature and regulatory documents.

Synonyms:

Tofacitinib is also known by its brand name Xeljanz and by its development code CP-690,550. These names are used interchangeably, with Xeljanz being more commonly used in clinical and commercial contexts.

Molecular Weight:

The molecular weight of Tofacitinib is 312.37 g/mol. This relatively low molecular weight allows for effective oral bioavailability and makes it easier for the drug to reach its target inside cells.

Background:

Tofacitinib is a breakthrough therapy for autoimmune diseases, particularly rheumatoid arthritis (RA). It functions as a Janus kinase (JAK) inhibitor, specifically targeting JAK1, JAK2, and JAK3 enzymes. These enzymes are key players in the JAK-STAT signaling pathway, which regulates various immune processes, including cytokine production. By inhibiting this pathway, tofacitinib reduces inflammation, pain, and joint destruction in patients with moderate to severe RA. Its mechanism offers an alternative to biologic treatments, such as monoclonal antibodies, and provides a convenient oral option compared to injectable therapies.

Indication:

Tofacitinib is primarily indicated for the treatment of rheumatoid arthritis (RA), psoriatic arthritis, and ulcerative colitis. It is usually prescribed when patients fail to respond adequately to methotrexate or other disease-modifying antirheumatic drugs (DMARDs). For RA, it is often combined with other DMARDs to optimize therapeutic effects. Additionally, tofacitinib helps treat active ulcerative colitis by reducing symptoms such as bowel inflammation and rectal bleeding.

Inactive Ingredients:

In its pharmaceutical formulation, tofacitinib tablets contain microcrystalline cellulose, lactose monohydrate, magnesium stearate, and silicon dioxide. These excipients serve various functions, such as bulking agents, flow aids, and stabilizers, ensuring the drug is delivered effectively and consistently.

Dosage and Administration:

The standard recommended dosage for rheumatoid arthritis is 5 mg twice daily, taken orally. In some cases, the dosage may be modified based on patient-specific factors like hepatic or renal impairment, where a lower dose (e.g., 5 mg once daily) might be prescribed to prevent adverse effects. In ulcerative colitis, an induction dose of 10 mg twice daily may be used for a short period, followed by a maintenance dose of 5 mg twice daily. It is crucial that patients adhere strictly to the prescribed dose to avoid side effects or reduced efficacy.

Storage:

Tofacitinib should be stored at room temperature (20-25°C), and it should be protected from excessive moisture and light. Storing the drug properly ensures its stability and prevents degradation, which could diminish its effectiveness.

Side Effects:

Common side effects associated with tofacitinib include upper respiratory tract infections, headache, diarrhoea, and high blood pressure. These effects are generally mild but should be monitored. More serious side effects include increased risk of serious infections like tuberculosis, lymphoma, and malignancies, as well as thrombosis. It is critical that patients are monitored for infections and other complications, especially if they have underlying conditions or are immunocompromised.

Description:

Tofacitinib is a small molecule drug taken orally, making it more convenient than biologic DMARDs, which typically require injection or infusion. As a selective JAK inhibitor, it plays a critical role in suppressing immune responses that drive inflammation in autoimmune diseases. The drug's action on the JAK-STAT pathway reduces immune cell activity, cytokine

production, and inflammation, making it effective in managing conditions like RA and psoriatic arthritis.

Pharmacodynamics:

Tofacitinib works by inhibiting JAK1, JAK2, and JAK3 enzymes, which are involved in the signalling pathway that leads to inflammation. By blocking the JAK-STAT signalling pathway, tofacitinib reduces the production of cytokines and other molecules that cause inflammation, which in turn helps decrease symptoms like joint pain, swelling, and tissue damage. This targeted mechanism offers a more direct way of controlling immune responses than some traditional treatments.

Mechanism of Action:

Tofacitinib binds to JAK enzymes and prevents the phosphorylation of Signal Transducer and Activator of Transcription (STAT) proteins. Normally, these proteins transmit signals from cytokine receptors to the nucleus, where they trigger the production of inflammatory molecules. By disrupting this process, tofacitinib reduces immune system activity, which is crucial in conditions where overactive immune responses cause tissue damage.

Absorption:

Tofacitinib has a high oral bioavailability of about 74%, which means that the majority of the drug is absorbed into the bloodstream when taken by mouth. Peak plasma concentrations (C_{max}) are typically reached within 1-2 hours, making it a fast-acting treatment option. This rapid absorption contributes to its ability to quickly reduce inflammation and other symptoms.

Metabolism:

Tofacitinib is primarily metabolized in the liver by the cytochrome P450 (CYP) 3A4 enzyme, with minor contributions from CYP2C19. The drug undergoes oxidation and O-demethylation, leading to inactive metabolites, which are excreted through the urine. This metabolic pathway can be influenced by other drugs that inhibit or induce CYP enzymes, potentially requiring dose adjustments.

Half-life:

The half-life of tofacitinib is relatively short, around 3-4 hours, meaning the concentration of the drug in the blood decreases by half every few hours. This short half-life requires the drug to be taken multiple times a day to maintain therapeutic levels, especially in chronic conditions like RA.

Toxicity:

Overdosage of tofacitinib can lead to serious complications, including immunosuppression and a heightened risk of infection. Symptoms of toxicity might include severe headache, dizziness, vomiting, and an increased likelihood of opportunistic infections. Immediate medical intervention is required in cases of overdose to prevent long-term damage or fatal outcomes.

Pharmacology and Biochemistry:

As a JAK inhibitor, tofacitinib represents a newer class of DMARDs that target cytokine signaling pathways involved in immune regulation and inflammation. Its inhibition of JAK1, JAK2, and JAK3 leads to a reduction in immune cell activation and inflammatory responses. This selective mode of action makes it highly effective in treating autoimmune disorders like rheumatoid arthritis, where excessive inflammation leads to joint damage and other symptoms. The use of tofacitinib offers a valuable therapeutic option for patients who do not respond adequately to traditional therapies like methotrexate.

5.1.2 Methotrexate

Chemical Name:

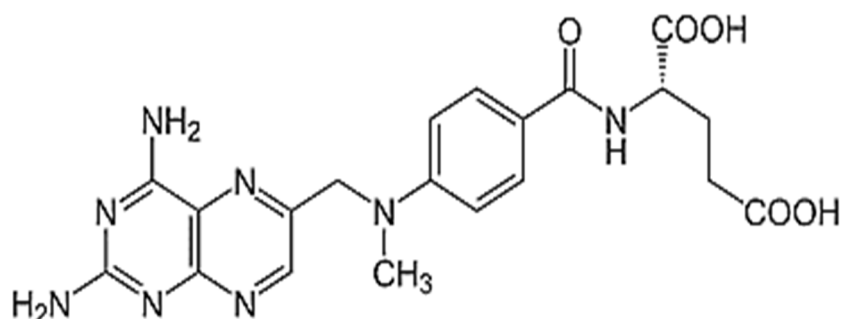
The chemical name of Methotrexate is 4-amino-4-deoxy-N10-methylpteroylglutamic acid. It is derived from folic acid and works as an antimetabolite by interfering with the metabolism of folic acid, which is crucial for DNA synthesis and cell replication. The name reflects its close structural similarity to folic acid, enabling it to competitively inhibit dihydrofolate reductase.

Chemical Formula:

Methotrexate's molecular formula is $C_{20}H_{22}N_8O_5$, indicating that it contains 20 carbon (C) atoms, 22 hydrogen (H) atoms, 8 nitrogen (N) atoms, and 5 oxygen (O) atoms. The structure shows its complex chemical makeup, which is essential for its ability to inhibit cell proliferation.

Chemical Structure:

The chemical structure of Methotrexate features a pteridine ring, which is similar to folic acid's structure. This similarity allows Methotrexate to act as a competitive inhibitor of enzymes involved in the folate metabolism pathway, such as dihydrofolate reductase.



CAS Number:

59-05-2. This unique identifier in the Chemical Abstracts Service (CAS) database is used for Methotrexate, allowing precise identification of the compound in scientific literature and regulatory documents.

Synonyms:

Methotrexate is also known by several synonyms, including MTX and Amethopterin. These alternative names are often used in clinical settings and research publications.

Molecular Weight:

The molecular weight of Methotrexate is 454.44 g/mol. This relatively large molecular size reflects its complex structure, which is important for its interaction with target enzymes and its overall pharmacokinetics.

Background:

Methotrexate is one of the most widely used disease-modifying antirheumatic drugs (DMARDs). It was originally developed as a chemotherapy agent due to its ability to inhibit rapidly dividing cells. However, its immunosuppressive effects were found to be beneficial in autoimmune diseases like rheumatoid arthritis (RA), where it can reduce inflammation and prevent joint damage. Methotrexate achieves these effects by inhibiting dihydrofolate reductase, an enzyme critical for DNA synthesis and cell replication.

Indication:

Methotrexate is indicated for the treatment of various autoimmune diseases, including rheumatoid arthritis (RA), juvenile idiopathic arthritis, and psoriatic arthritis. It is also used at higher doses as part of chemotherapy protocols for certain cancers such as leukemia, breast cancer, and osteosarcoma. In RA, Methotrexate is often used as a first-line treatment to reduce inflammation, pain, and joint destruction.

Inactive Ingredients:

In its pharmaceutical formulations, Methotrexate may contain inactive ingredients such as lactose monohydrate, magnesium stearate, sodium starch glycolate, and talc. These excipients help in the manufacturing process, improve the stability of the drug, and aid in the consistency of the dosage form.

Dosage and Administration:

For autoimmune diseases such as rheumatoid arthritis, Methotrexate is typically administered as a once-weekly oral or subcutaneous dose, starting at 7.5 mg and titrated up based on the patient's response. In cancer therapy, the dosage varies depending on the type of cancer and the protocol, often involving higher doses administered more frequently. It is essential to monitor patients regularly due to the potential for serious side effects.

Storage:

Methotrexate should be stored at room temperature (20-25°C), protected from light and moisture. Proper storage conditions are crucial to prevent degradation and ensure the drug's stability and efficacy.

Side Effects:

Common side effects of Methotrexate include gastrointestinal symptoms such as nausea, vomiting, and diarrhoea, as well as mouth sores. More serious side effects include liver toxicity, bone marrow suppression (leading to anemia, leukopenia, and thrombocytopenia), and an increased risk of infection due to immunosuppression. Patients should be regularly monitored for liver function and blood counts to mitigate these risks.

Description:

Methotrexate is a folate analog and antimetabolite that inhibits enzymes involved in folic acid metabolism, primarily dihydrofolate reductase. By blocking this enzyme, Methotrexate prevents the formation of tetrahydrofolate, which is necessary for thymidylate and purine synthesis, leading to reduced DNA replication and cell proliferation. This mechanism is useful in both cancer therapy, where rapidly dividing cells are targeted, and in autoimmune diseases, where it dampens the immune response.

Pharmacodynamics:

Methotrexate's pharmacodynamics involve the inhibition of folate-dependent enzymes required for DNA synthesis and repair, particularly in rapidly dividing cells. In autoimmune diseases, this mechanism reduces the immune system's activity, leading to decreased inflammation and tissue damage. Methotrexate also reduces the proliferation of T and B cells, which are involved in the autoimmune response.

Mechanism of Action:

Methotrexate works by inhibiting the enzyme dihydrofolate reductase, which is necessary for the synthesis of tetrahydrofolate. Tetrahydrofolate is required for the synthesis of nucleotides, which are building blocks of DNA. By inhibiting this process, Methotrexate interferes with DNA synthesis, primarily affecting rapidly dividing cells such as those found in tumors or in the immune system during an autoimmune response.

Absorption:

Methotrexate is well absorbed from the gastrointestinal tract when taken orally, with bioavailability ranging from 70% to 90%. Peak plasma concentrations are typically reached

within 1 to 2 hours after oral administration, making it an effective treatment for both acute and chronic conditions.

Metabolism:

Methotrexate is partially metabolized in the liver, where it undergoes polyglutamation to form active metabolites. These metabolites are retained within cells and prolong the drug's action by further inhibiting folate-dependent enzymes. Methotrexate and its metabolites are primarily excreted in the urine, and patients with renal impairment may require dose adjustments to prevent toxicity.

Half-life:

The half-life of Methotrexate ranges from 3 to 10 hours, depending on the dose and route of administration. This relatively short half-life necessitates weekly dosing in conditions like rheumatoid arthritis, although higher or more frequent dosing may be required in cancer therapy.

Toxicity:

Overdosage or prolonged use of Methotrexate can lead to severe toxicity, including bone marrow suppression, hepatotoxicity, and gastrointestinal toxicity. Early signs of Methotrexate toxicity include mouth sores, nausea, vomiting, and fatigue. Severe toxicity can result in life-threatening conditions such as pancytopenia, liver failure, and severe infections. In cases of overdose, folinic acid (leucovorin) rescue therapy is often used to mitigate toxicity.

Pharmacology and Biochemistry:

Methotrexate inhibits the replication of rapidly dividing cells by blocking the folate pathway, which is essential for DNA and RNA synthesis. In cancer therapy, this results in the targeted killing of tumor cells, while in autoimmune diseases, it suppresses the overactive immune response. Its effectiveness in reducing inflammation and preventing joint damage has made it a cornerstone of rheumatoid arthritis treatment for many decades.

5.2 Excipient Profile

5.2.1 Carbopol 940

Non-proprietary Names BP: Carbomer 940.

Synonyms: Carboxypolymethylene; Carbomer; Polyacrylic acid; Acritamer 940; Acrylic acid polymer; Aqua SF-1 Polymer.

Empirical Formula and Molecular Weight:

C₃H₄O₂ (Acrylic acid polymer). Molecular weight varies depending on cross-linking but generally falls in the range of 940,000 g/mol.

Functional Category: Gelling agent; Rheology modifier; Suspending agent; Emulsion stabilizer; Thickener.

Applications:

Topical Gels and Creams: Carbopol 940 is widely used in the formulation of topical gels, creams, and lotions due to its excellent thickening and suspending properties. It forms clear gels that enhance the viscosity and provide a smooth texture.

Controlled Release Formulations: The polymer's swelling properties in water make it ideal for controlled and sustained release formulations in both oral and topical dosage forms. It ensures prolonged release of active ingredients.

Cosmetic Products: It is commonly used in skincare products such as moisturizers and anti-aging creams where it imparts a silky feel and improves the spreadability of the product.

Pharmaceutical Suspensions: Due to its high efficiency at low concentrations, Carbopol 940 is utilized in oral suspensions and ophthalmic solutions to stabilize emulsions and suspensions.

Description: Carbopol 940 is a high molecular weight, cross-linked polyacrylic acid polymer. It is a white, fluffy powder that, when dispersed in water, swells to form a gel-like consistency. The polymer is cross-linked with polyalkenyl ethers, which provide its unique thickening and suspending properties. It is highly effective at low concentrations, making it a preferred choice for formulations requiring high viscosity without compromising product clarity or stability.

Typical Properties:

Appearance: White, fluffy powder.

Viscosity: Varies based on concentration, typically ranging from 40,000 to 60,000 cps for a 0.5% aqueous solution when neutralized.

pH (1% Solution): 2.5 – 3.5 (before neutralization).

pH (Neutralized): 5.0 – 7.5.

Solubility: Insoluble in organic solvents; dispersible in water and alcohols.

Swelling Ability: Swells in aqueous solutions, absorbing a significant amount of water to form gels.

Stability and Storage Conditions:

Carbopol 940 is stable when stored in tightly closed containers under cool and dry conditions. It should be protected from moisture and extreme temperatures to maintain its gelling properties. Once in gel form, the polymer is stable across a broad pH range (5-10) and retains its viscosity and stability for long periods.

Incompatibilities: Cationic Polymers: Carbopol 940 may be incompatible with strong cationic substances due to potential ionic interactions, which can result in reduced thickening efficiency or precipitation.

Strong Acids and Bases: Extreme pH conditions (highly acidic or basic environments) may degrade the polymer, leading to a reduction in viscosity and stability.

Organic Solvents: While Carbopol 940 is dispersible in water, it shows poor solubility in non-polar organic solvents, such as aliphatic hydrocarbons, and may not form gels in their presence.

Mechanism of Action: Carbopol 940 works by forming a three-dimensional network through cross-linking in an aqueous environment. The polymer swells upon contact with water, and when neutralized with a base like triethanolamine, it forms a stable gel with high viscosity. This ability to form gels and retain moisture makes it ideal for delivering drugs topically and controlling drug release rates.

5.2.2 Triethanolamine

Non-proprietary Names: Triethanolamine (TEA).

Synonyms: TEA; Trolamine; Tris(2-hydroxyethyl) amine.

Empirical Formula: C₆H₁₅NO₃.

Molecular Weight: 149.19 g/mol.

Functional Category: pH adjuster; emulsifier; surfactant; buffering agent.

Applications:

pH Adjuster in Topical Formulations:

Triethanolamine is widely used in topical formulations such as gels, creams, and lotions to adjust the pH and maintain stability. Its buffering action helps ensure that the formulation remains within an optimal pH range, enhancing the efficacy and stability of active ingredients.

Emulsifying Agent in Creams and Lotions:

Triethanolamine acts as an emulsifier by stabilizing oil-water mixtures. It is particularly effective in neutralizing fatty acids to form soaps, making it a key ingredient in emulsifying agents for cosmetics and pharmaceuticals. This allows for the creation of smooth, consistent textures in creams and lotions.

Buffering Agent in Ophthalmic and Oral Products:

Triethanolamine is used in ophthalmic solutions and oral care products as a buffering agent to maintain pH stability. It ensures that the product remains non-irritating to sensitive tissues like the eyes and mucous membranes while delivering the desired therapeutic effects.

Neutralizing Agent in Gels:

In gel formulations, Triethanolamine is used to neutralize gelling agents such as Carbopol, enabling the formation of a gel matrix. This allows for the creation of stable, consistent gel textures with optimal viscosity for topical applications.

Surfactant in Cleansers and Detergents:

Due to its surfactant properties, Triethanolamine is also used in the formulation of liquid detergents, shampoos, and facial cleansers, where it helps to reduce surface tension and emulsify oils and dirt for effective cleansing.

Description:

Triethanolamine is a viscous, colorless to pale yellow liquid that is highly water-soluble. It is an organic compound composed of three hydroxyl groups attached to an amine, making it a versatile and highly reactive chemical in both cosmetic and pharmaceutical formulations. Its primary role as a pH adjuster ensures that the formulations remain chemically stable, enhancing both the effectiveness of active pharmaceutical ingredients and the longevity of the product.

Typical Properties:

Density: 1.124 g/cm³.

Boiling Point: 335°C.

Melting Point: 20.5°C.

Viscosity: 580 mPas (at 20°C).

Solubility: Completely soluble in water, alcohol, and chloroform; slightly soluble in ether.

Triethanolamine has the ability to dissolve in both polar and non-polar solvents, making it an ideal multifunctional excipient. It exhibits hygroscopic properties and can absorb moisture from the atmosphere, which can be beneficial in stabilizing water-based formulations.

Solubility:

Soluble in water, alcohols, and acetone. Miscible with glycerine and other glycols. Slightly soluble in ether.

Incompatible with mineral oils and fats.

Stability and Storage Conditions:

Triethanolamine is highly stable under normal conditions, both in air and in aqueous solutions. It resists oxidation and remains chemically stable even when exposed to light. To maintain its

stability, it should be stored in a cool, dry place, preferably in well-sealed containers made from glass, stainless steel, or polyethylene. Avoid prolonged exposure to air or high temperatures, as this may promote degradation or yellowing of the compound.

Incompatibilities:

Reactivity with Acids and Oxidizing Agents:

Triethanolamine can react with strong acids, forming salts, and with strong oxidizing agents, leading to degradation. It can also form unstable nitrosamines when mixed with certain nitrogen compounds.

Interaction with Heavy Metals:

TEA may interact with heavy metals such as copper or iron, leading to discoloration or degradation of the formulation. Using chelating agents like EDTA can prevent such interactions.

Sensitivity to Peroxide Impurities:

Like many amines, TEA can oxidize in the presence of peroxides, leading to color changes and potential loss of functionality. To avoid this, it is recommended to use antioxidant preservatives in formulations where TEA is present.

Environmental and Health Considerations:

While Triethanolamine is generally considered safe in pharmaceutical and cosmetic applications, prolonged or excessive exposure can cause skin irritation or allergic reactions in sensitive individuals. It is important to use TEA in controlled amounts, following appropriate safety guidelines. Regulatory agencies such as the FDA and European Commission permit its use in regulated quantities.

5.2.3 Propylene Glycol

Non-proprietary Names: Propylene Glycol (PG)

Synonyms: 1,2-Propanediol; PG; Methyl Glycol; Trimethyl Glycol

Empirical Formula: C₃H₈O₂

Molecular Weight: 76.09 g/mol

Functional Category: Humectant; Solvent; Emollient; Penetration Enhancer

Applications:

Humectant in Topical Formulations: Propylene glycol is widely used as a humectant in various topical formulations, including creams, gels, lotions, and ointments. Its hygroscopic nature allows it to attract and retain moisture, thereby enhancing the hydration of the skin. This makes it a key ingredient in moisturizing products, as it helps to prevent water loss from the skin, leaving it soft and supple.

Solvent in Pharmaceutical Preparations: Due to its ability to dissolve both hydrophilic and lipophilic substances, propylene glycol serves as an effective solvent in various pharmaceutical preparations. It is particularly useful in liquid formulations such as oral solutions, injectables, and topical products, where it helps to dissolve active pharmaceutical ingredients (APIs) and other excipients, ensuring a uniform distribution throughout the formulation.

Emollient in Skincare Products: Propylene glycol also acts as an emollient, providing a smooth and soft texture to skincare products. It helps to reduce the roughness of the skin and creates a protective barrier that prevents moisture loss. This makes it an essential component in products designed to treat dry or irritated skin.

Penetration Enhancer in Transdermal Drug Delivery: In transdermal drug delivery systems, propylene glycol is often used as a penetration enhancer. It modifies the stratum corneum (the outermost layer of the skin), allowing for improved permeability of active ingredients through the skin. This enhances the effectiveness of drugs and therapeutic agents by ensuring that they reach the desired target area within the skin or systemic circulation.

Co-solvent in Oral and Injectable Formulations: In addition to its role as a primary solvent, propylene glycol is frequently used as a co-solvent in oral and injectable formulations. Its ability to mix with water, alcohol, and other solvents makes it a versatile excipient that helps to stabilize formulations and improve the solubility of poorly soluble drugs.

Description: Propylene glycol is a clear, colorless, and odorless liquid that is highly soluble in water, alcohol, and many organic solvents. It is a synthetic organic compound that belongs to the class of alcohols and is derived from petroleum. Its versatility as an excipient lies in its ability to function in various capacities, including as a humectant, solvent, emollient, and

penetration enhancer. Propylene glycol is generally recognized as safe (GRAS) by regulatory agencies such as the FDA, and it is commonly used in both pharmaceutical and cosmetic products.

Typical Properties:

- **Density:** 1.036 g/cm³
- **Boiling Point:** 188.2°C
- **Melting Point:** -59°C
- **Viscosity:** 60 mPas (at 25°C)
- **Solubility:** Completely soluble in water, alcohol, and chloroform; slightly soluble in ether. Propylene glycol's high solubility in water and its ability to dissolve a wide range of substances make it an ideal multifunctional excipient. It exhibits low volatility and is stable under normal storage conditions, making it suitable for use in a variety of pharmaceutical and cosmetic formulations.

Solubility:

- Soluble in water, alcohol, and acetone
- Miscible with glycerine and other glycols
- Slightly soluble in ether
- Incompatible with strong acids and oxidizing agents

Stability and Storage Conditions: Propylene glycol is highly stable under normal storage conditions. It resists oxidation and remains chemically stable when exposed to air and light. To ensure its longevity, propylene glycol should be stored in a cool, dry place, preferably in well-sealed containers made from glass, stainless steel, or polyethylene. Avoid exposure to high temperatures or open flames, as this may lead to degradation of the compound.

Incompatibilities:

Reactivity with Strong Acids and Oxidizing Agents: Propylene glycol can react with strong acids and oxidizing agents, leading to potential degradation or formation of harmful by-products. It is important to avoid mixing propylene glycol with such substances to ensure the stability and safety of the formulation.

Interaction with Heavy Metals: While propylene glycol is generally stable, it may interact with heavy metals, leading to discoloration or degradation of the formulation. Using chelating agents like EDTA can help prevent such interactions and maintain the integrity of the product.

Sensitivity to Peroxide Impurities: Like many alcohols, propylene glycol can oxidize in the presence of peroxides, which may lead to color changes and potential loss of functionality. To prevent this, it is recommended to use antioxidant preservatives in formulations containing propylene glycol.

Environmental and Health Considerations: Propylene glycol is generally regarded as safe for use in pharmaceutical and cosmetic applications. However, excessive or prolonged exposure to propylene glycol can cause skin irritation or allergic reactions in sensitive individuals. It is important to use propylene glycol in controlled amounts, following appropriate safety guidelines. Regulatory agencies, such as the FDA and European Commission, permit its use in regulated quantities in various products, ensuring its safety for consumers.

5.2.4 Methyl Paraben Sodium and Propyl Paraben Sodium

Function: Preservatives

Concentration:

Methyl Paraben Sodium: 0.1% w/w

Propyl Paraben Sodium: 0.05% w/w

Description:

Methyl Paraben Sodium and Propyl Paraben Sodium are commonly used preservatives in pharmaceutical and cosmetic formulations. Their primary function is to prevent microbial

contamination, ensuring the stability and safety of the product during storage and use. By inhibiting the growth of bacteria, fungi, and yeast, these parabens help extend the shelf life of formulations and maintain their effectiveness. The combination of Methyl Paraben and Propyl Paraben is particularly effective, as they provide broad-spectrum antimicrobial activity against a wide range of microorganisms.

Non-proprietary Names:

Methyl Paraben Sodium: Sodium Methylparaben

Propyl Paraben Sodium: Sodium Propylparaben

Synonyms:

Methyl Paraben Sodium: Methyl p-hydroxybenzoate sodium salt, E218

Propyl Paraben Sodium: Propyl p-hydroxybenzoate sodium salt, E217

Empirical Formula and Molecular Weight:

	<u>Methyl Paraben Sodium</u>	<u>Propyl Paraben Sodium</u>
Empirical Formula	C ₈ H ₇ NaO ₃	C ₁₀ H ₁₁ NaO ₃
Molecular Weight	174.13 g/mol	202.18 g/mol

Functional Category: Antimicrobial preservatives

Applications:

Preservatives in Topical Formulations: Methyl Paraben Sodium and Propyl Paraben Sodium are extensively used as preservatives in topical formulations, including creams, lotions, gels, and ointments. Their antimicrobial properties help prevent contamination and maintain the integrity of the formulation during use.

Preservatives in Oral Preparations: These parabens are also employed as preservatives in oral pharmaceutical preparations, such as syrups, suspensions, and oral gels. Their inclusion ensures the microbial safety of the product, especially in water-based formulations that are prone to contamination.

Preservatives in Cosmetic Products: In cosmetic products, Methyl Paraben Sodium and Propyl Paraben Sodium serve as essential preservatives, preventing the growth of microorganisms that can spoil the product and cause harm to the user. They are commonly found in shampoos, conditioners, moisturizers, and makeup products.

Stabilizers in Personal Care Products: Beyond their preservative function, these parabens also act as stabilizers, helping to maintain the physical and chemical stability of personal care products over time.

Description:

Methyl Paraben Sodium and Propyl Paraben Sodium are white, crystalline powders that are highly soluble in water and alcohol. These compounds are derivatives of para-hydroxybenzoic acid and are widely used in the pharmaceutical and cosmetic industries due to their safety and efficacy as preservatives. They are synthetic chemicals that are generally recognized as safe (GRAS) by regulatory agencies such as the FDA and the European Commission, and their use is permitted in regulated quantities across various formulations.

Typical Properties:

	<u>Methyl Paraben Sodium</u>	<u>Propyl Paraben Sodium</u>
Density	1.54 g/cm ³	1.40 g/cm ³
Melting Point	125-128°C	95-98°C
Solubility	Soluble in water, alcohol, and propylene glycol; slightly soluble in glycerine	Soluble in water, alcohol, and propylene glycol; slightly soluble in glycerine

Stability and Storage Conditions:

Methyl Paraben Sodium and Propyl Paraben Sodium are stable under normal storage conditions. They resist oxidation and hydrolysis, making them suitable for long-term use in formulations. To ensure their efficacy, they should be stored in a cool, dry place, away from

direct sunlight and heat. Containers should be tightly sealed to prevent moisture absorption, which can affect the stability of the parabens.

Incompatibilities:

Reactivity with Strong Bases: Both Methyl Paraben Sodium and Propyl Paraben Sodium can react with strong bases, leading to potential degradation of the preservatives. It is important to avoid combining them with such substances in formulations.

Interaction with Nonionic Surfactants: In some formulations, nonionic surfactants may reduce the preservative efficacy of parabens. Proper formulation strategies should be employed to ensure the preservation of the product.

Sensitivity to pH: The preservative activity of Methyl Paraben Sodium and Propyl Paraben Sodium is pH-dependent, with optimal efficacy in the pH range of 4 to 8. Formulations with pH levels outside this range may require additional preservation strategies.

Environmental and Health Considerations:

Methyl Paraben Sodium and Propyl Paraben Sodium are generally considered safe for use in pharmaceutical and cosmetic products when used within recommended concentrations. However, there has been ongoing debate about the potential for parabens to cause allergic reactions or disrupt hormone function. Regulatory agencies continue to evaluate the safety of parabens, and their use remains permitted in regulated quantities. It is important to use these preservatives in compliance with established guidelines to ensure the safety of consumers.

5.3 MATERIALS

Table 5.1: List of Chemicals

Name	Supplier
Methyl Paraben Sodium	Loba Chemie Pvt Ltd, Mumbai, India
Propyl Paraben Sodium	Loba Chemie Pvt Ltd, Mumbai, India
Propylene Glycol	Sigma-Aldrich / Analytical Grade
Ethanol	Merck Chemicals
Dimethyl Sulfoxide (DMSO)	Sigma-Aldrich / Analytical Grade
Potassium Bromide (KBr)	Loba Chemie Pvt Ltd, Mumbai, India
Tofacitinib Citrate [Manufacturer- MSN Ltd]	Torrent Pharmaceutical Ltd., Ahmedabad
Methotrexate [Manufacturer- - Fermion Italy]	Cadila Healthcare Ltd, Ahmedabad
Tetraethyl orthosilicate (TEOS)	Research Lab Fine Chem. Industries, Mumbai
Pluronic F127	Research Lab Fine Chem. Industries, Mumbai
3-Aminopropyltriethoxysilane	Research Lab Fine Chem. Industries, Mumbai
Hydrochloric acid (HCl)	Research Lab Fine Chem. Industries, Mumbai
Carbopol	Lubrizol

All chemicals used were of analytical or pharmaceutical grades.

5.4 INSTRUMENTS

Table 5.2: List of Instruments

Instruments	Model No.
FTIR Spectrophotometer	Shimadzu FTIR-8400S, Japan
X-ray Diffractometer	Bruckeraxs, D8 Advance, Germany
Rotary Evaporator	Buchi R-300, Switzerland
Magnetic Stirrer	IKA C-MAG HS7, Germany
UV-Visible Spectrophotometer	Shimadzu UV-1800, Japan
Vacuum Dryer	Yamato ADP-21, Japan
Ultra-Sonicator	Chrome Tech PR-120, China
Electronic Balance	Shimadzu AUX 120, Japan

5.5 METHODS

5.5.1 Synthesis and Characterization of Mesoporous Silica Nanoparticles (MSN):

a) Synthesis of SBA-15:

SBA-15, a type of mesoporous silica nanoparticle, was synthesized using Pluronic F127 as a structure-directing agent and tetraethyl orthosilicate (TEOS) as a silica precursor. The synthesis process involved the following steps:

1. Dissolution of Pluronic F127: Initially, 4 grams of Pluronic F127 was dispersed in a solution comprising solution containing thirty millilitres of purified water and 120 ml of hydrochloric acid (HCl) with a concentration of 2 M. This step facilitated the formation of a stable solution.

2. Addition of TEOS: Subsequently, 8.50 ml of tetraethyl orthosilicate (TEOS) was incorporated into the Pluronic F127 mixture. TEOS served as the silica precursor for the creation of the mesoporous silica framework.
3. Stirring and Reaction: The mixture was stirred continuously for 22 hours, allowing for the hydrolysis and condensation reactions between Pluronic F127 and TEOS to occur. These reactions are crucial for the formation of the silica matrix.
4. Heating and Aging: After stirring, the silica solution was maintained at a temperature of 80°C overnight without agitation. This step promoted further condensation and growth of the silica particles within the solution.
5. Washing and Drying: The resulting solid powder, identified as SBA-15, was separated from the solution by filtration. The collected solid was then washed with distilled water to remove any residual reactants or by-products. Ultimately, the rinsed SBA-15 was subjected to a drying process at a temperature of 50°C for a duration of 24 hours, resulting in the production of the ultimate mesoporous silica nanoparticles [1-2].

b) Amine Functionalization of SBA-15:

Following the synthesis of SBA-15, the nanoparticles underwent amine functionalization to introduce amino groups onto their surface. This functionalization process was conducted using the following steps:

1. Dispersal in Ethanol: A homogeneous suspension was formed by dispersing 1 gram of mesoporous silica nanoparticles in 100 ml of ethanol.
2. Addition of APTES: 3-Aminopropyltriethoxysilane (APTES), an organosilane compound containing amino groups, was gradually added to the ethanol suspension of SBA-15. APTES reacts with the surface silanol groups of SBA-15, leading to the attachment of amino functional groups.
3. Stirring and Reaction: The mixture was stirred for 12 hours to ensure thorough mixing and reaction between SBA-15 and APTES. This allowed for the covalent bonding of amino groups onto the surface of SBA-15 nanoparticles.

4. Centrifugation and Washing: After the reaction period, the suspension underwent centrifugation to separate the functionalized nanoparticles from unreacted APTES and other impurities. The precipitate was then washed several times with ethanol to remove any residual reagents.
5. Drying: The washed amine-functionalized SBA-15 nanoparticles were dried under ambient conditions to remove excess solvent and obtain the final product ready for further characterization and utilization in drug delivery applications [3-6].

5.5.2 Characterization of Mesoporous Silica Nanoparticles (MSN):

Characterization of mesoporous silica nanoparticles (MSN) is crucial to understand their physicochemical properties, which influence their performance in various applications, which encompasses systems for delivering drugs. This section outlines the methods employed for the characterization of MSN synthesized through the SBA-15 route and discusses potential results obtained from each characterization technique.

1. Fourier-Transform Infrared Spectroscopy (FTIR):

Fourier Transform Infrared (FTIR) spectroscopy was performed by the use of Fourier-transform infrared spectrophotometer. The MSN specimens have been made as KBr pellets and scanned across the range of 4000-400 cm^{-1} . FTIR spectra can reveal functional groups present on the MSN surface, such as silanol groups (-Si-OH) and organic functional groups from Pluronic F127 and APTES. Peaks corresponding to Si-O-Si stretching vibrations and Si-OH bending modes are expected. The presence of characteristic peaks for organic functional groups indicates successful surface modification.

2. Particle Size Analysis:

The particle dimensions of MSN were analyzed using dynamic light scattering (DLS) or laser diffraction techniques. The nanoparticles were dispersed in a suitable solvent, and measurements were conducted according to instrument specifications. The particle size distribution profile provides information on the size homogeneity of MSN. A narrow size

distribution with a mean particle size in the nanometer range is anticipated, consistent with mesoporous silica nanoparticles.

3. Transmission Electron Microscopy (TEM):

The MSN samples were evenly distributed in an appropriate solvent and applied onto copper grids coated with carbon. Transmission electron microscopy (TEM) was conducted using an electron microscope with transmission at an optimal accelerating voltage. TEM images reveal the morphology and internal structure of MSN. Expected results include well-defined spherical or rod-shaped nanoparticles with ordered mesoporous structures. The images may also illustrate the uniformity of pore size distribution within the nanoparticles.

4. Scanning Electron Microscopy (SEM):

Surface morphology and topography of MSN were examined using a scanning electron microscope. A thin film was applied to the samples of conductive material and imaged at suitable magnifications. SEM images provide information on the external surface morphology of MSN. Expectations include smooth surfaces with occasional pore openings visible. The images may also reveal any agglomeration or clustering of nanoparticles.

5. Differential Scanning Calorimetry (DSC):

Thermal behaviour of MSN was analyzed using differential scanning calorimetry. Samples were heated from ambient temperature to a suitable maximum temperature at a controlled rate under an inert atmosphere. DSC thermograms can indicate the presence of adsorbed water, organic residues, or thermal stability of MSN. Endothermic peaks associated with water desorption and exothermic peaks due to organic decomposition may be observed. Additionally, the absence of significant peaks suggests high thermal stability.

6. Brunauer-Emmett-Teller (BET) Analysis:

The surface area as well as distribution of pore sizes of MSN have been identified by analyzing nitrogen adsorption-desorption isotherms using BET analysis. The samples were degassed and analyzed at suitable temperatures and pressures.

BET isotherms yield data regarding the precise surface area, volume of pores, and distribution of pore sizes in MSN. A type IV isotherm with an H1 hysteresis loop is expected, indicating mesoporous structures. The calculated BET surface area reflects the textural properties of MSN [7-10].

5.5.3 Characterization of Surface-modified Mesoporous Silica

In this section, the detailed characterization of surface-modified mesoporous silica nanoparticles (MSNs) using Fourier Transform Infrared (FTIR) Spectroscopy and Powder X-ray Diffraction (pXRD) is described. These techniques are critical for confirming the successful functionalization of MSNs and ensuring their suitability for drug loading.

a) FTIR Spectroscopy

Fourier Transform Infrared (FTIR) Spectroscopy is a powerful analytical technique used to identify chemical bonds and functional groups in materials. It provides information on the molecular composition and structure of the surface-modified mesoporous silica nanoparticles.

Procedure:

Sample Preparation: The mesoporous silica nanoparticles, both unmodified and surface-modified, were prepared by drying them to remove any moisture content.

The samples were then ground into fine powders and mixed with potassium bromide (KBr) to form pellets suitable for FTIR analysis.

FTIR Analysis: The FTIR spectra were recorded using an FTIR spectrometer.

Spectra were obtained in the range of 4000 to 400 cm^{-1} .

Key peaks corresponding to different functional groups were identified and compared between unmodified and surface-modified MSNs.

b) Powder X-ray Diffraction (pXRD)

Powder X-ray Diffraction (pXRD) is employed to determine the crystalline structure and phase purity of materials. For mesoporous silica nanoparticles, pXRD helps in understanding the effect of surface decoration on the structural integrity and crystallinity.

Procedure:

Sample Preparation: The mesoporous silica nanoparticles were prepared as finely ground powders.

Samples were placed on a glass sample holder and leveled to ensure a uniform surface.

pXRD Analysis: The pXRD patterns were recorded using an X-ray diffractometer.

Scans were performed in the 2θ range of 5° to 80° using Cu-K α radiation ($\lambda = 1.5406 \text{ \AA}$).

Data were collected with appropriate step sizes and counting times to ensure high resolution [11-13].

5.5.4 Loading of Antiarthritic Drugs in Surface-modified MSNs

The loading of antiarthritic drugs into surface-modified mesoporous silica nanoparticles is a critical step designed to improve the solubility, stability, and targeted delivery of the drugs. This section provides a detailed procedure for this process, highlighting the selection of drugs, preparation of drug solutions, and the step-by-step loading process.

Selection of Antiarthritic Drugs

Antiarthritic drugs were selected based on their clinical importance and the challenges associated with their solubility and bioavailability. The drugs chosen for this study were:

Tofacitinib Citrate: A Janus kinase (JAK) inhibitor used in the treatment of rheumatoid arthritis. It has low water solubility, which limits its bioavailability.

Methotrexate: A widely used disease-modifying antirheumatic drug (DMARD) that interferes with folic acid metabolism. Methotrexate also has poor water solubility and stability issues.

Preparation of Drug Solutions

The selected antiarthritic drugs were prepared in solutions suitable for loading onto the mesoporous silica nanoparticles. The procedure involved the following steps:

Tofacitinib Citrate Solution:

Solvent: Ethanol was chosen due to its ability to dissolve Tofacitinib Citrate effectively.

Concentration: A specific concentration of Tofacitinib Citrate was dissolved in ethanol to create a homogenous solution.

Mixing: The solution was mixed thoroughly using a magnetic stirrer to ensure complete dissolution of the drug.

Methotrexate Solution:

Solvent: Dimethyl sulfoxide (DMSO) was selected as the solvent due to its excellent solubility properties for Methotrexate.

Concentration: Methotrexate was dissolved in DMSO at a predetermined concentration to form a uniform solution.

Mixing: The solution was stirred continuously to achieve a clear, homogenous solution.

Loading Process

The loading of the antiarthritic drugs onto the surface-modified mesoporous silica nanoparticles involved a multi-step process designed to maximize drug adsorption and stability.

Incubation:

Dispersion: The surface-modified mesoporous silica nanoparticles were dispersed in the drug solutions prepared earlier.

Stirring: The mixture was stirred continuously using a magnetic stirrer to facilitate the adsorption of the drug molecules onto the nanoparticles.

Incubation Time: The dispersion was allowed to incubate for a specified period (e.g., 24 hours) to ensure maximum adsorption. The exact time was optimized based on preliminary experiments to achieve the highest loading efficiency.

Solvent Evaporation:

Rotary Evaporation: After the incubation period, the solvent was removed using a rotary evaporator. This step involves applying vacuum and gentle heat to evaporate the solvent, leaving the drug-loaded nanoparticles behind.

Temperature Control: The temperature was carefully controlled to prevent degradation of the drugs. Typical temperatures used were below 40°C.

Evaporation Rate: The rate of solvent removal was optimized to avoid any potential loss of drug and ensure uniform loading [14].

Drying:

Vacuum Drying: The drug-loaded mesoporous silica nanoparticles were dried under vacuum to remove any residual solvent. This step is crucial for enhancing the stability and storage of the nanoparticles.

Drying Conditions: The drying process was conducted at room temperature or slightly elevated temperatures (e.g., 30-40°C) under reduced pressure.

Duration: The drying duration was optimized to ensure complete removal of solvents, typically ranging from several hours to overnight [15-17].

Optimization of Loading Parameters:

Various parameters such as drug concentration, solvent type, loading time, and temperature were optimized to maximize the drug loading efficiency.

5.5.5 Characterization of Drug-Loaded MSNs:

A. Drug Loading Efficiency:

The amount of drug loaded onto the MSNs was quantified by using the formula;

$$\text{Loading Efficiency (\%)} = (\text{Amount of drug loaded} / \text{Total amount of drug used}) \times 100$$

B. *In-Vitro* Release Studies:

To evaluate the release profile of antiarthritic drugs from mesoporous silica nanoparticles (MSNs), we first load the drug into the MSNs via adsorption or encapsulation and ensure the drug-loaded MSNs are well-characterized. We then simulate physiological conditions by preparing phosphate-buffered saline (PBS) at pH 7.4, pre-warmed to 37°C. The drug-loaded MSNs are placed in a donor chamber of the Franz diffusion cell system, dialysis membrane is used for diffusion, which is immersed in the pre-warmed PBS. At predetermined intervals (e.g., 1, 2, 4, 8, 12, 24 and 48-hours samples of the PBS are withdrawn from receptor chamber and replaced with fresh PBS. The drug concentration in these samples is analyzed using techniques like UV-Visible Spectroscopy. The cumulative amount of drug released over time is plotted to generate a release profile, and the release kinetics are analyzed to determine the release mechanism.

5.5.6 Formulation Development:

A. Gel Formulation

The primary goal of this development phase is to create an antiarthritic gel formulation that incorporates drug-loaded mesoporous silica nanoparticles (MSNs). The rationale for using MSNs is based on their unique properties, including high surface area, tunable pore size, and the ability to provide controlled drug release. These characteristics make MSNs ideal carriers for drugs that require targeted delivery and sustained release to enhance therapeutic efficacy.

Objectives in formulation development includes:

- 1) By using MSNs, the drug can be delivered directly to the site of action, reducing the required dosage and minimizing systemic side effects.

- 2) MSNs can be functionalized to target specific cells or tissues, increasing the drug's effectiveness and reducing off-target effects.
- 3) The porous structure of MSNs allows for the controlled release of the drug over time, providing prolonged therapeutic effects.

B. Selection of Gelling Agent:

The selection of the gelling agent is crucial for the formulation of the gel. For this purpose, Carbopol 940 was chosen due to its compatibility with MSNs and its ability to form gels with desirable viscosity properties. Carbopol 940 is a synthetic high molecular weight polymer of acrylic acid cross-linked with polyalkenyl ethers or divinyl glycol. It is widely used in pharmaceutical and cosmetic formulations due to its excellent thickening, suspending, and stabilizing properties.

Reasons for selecting Carbopol 940 includes:

- 1) Carbopol 940 does not interact negatively with MSNs, ensuring the stability of the nanoparticles within the gel matrix.
- 2) Carbopol 940 can form gels with a wide range of viscosities, making it suitable for various applications. The viscosity can be adjusted by changing the concentration of Carbopol 940.
- 3) Carbopol 940 is easy to disperse in water and can be processed at room temperature, making it convenient for large-scale production.

C. Preparation of Gel Base:

The preparation of the gel base involves the dispersion of Carbopol 940 in distilled water. This step is critical to ensure the uniform distribution of the polymer throughout the solvent, which is essential for achieving the desired gel consistency. The preparation process includes the following steps:

1. **Weighing and Dispersion:** A precise amount of Carbopol 940 (1% w/w) is weighed and slowly added to distilled water with continuous stirring to prevent lump formation.

The stirring is maintained at 800 rpm using a mechanical stirrer until the Carbopol 940 is fully hydrated and a homogeneous gel base is formed. This typically takes about 1-2 hours, depending on the batch size.

- 2. Hydration:** The dispersion is allowed to hydrate for an additional period to ensure complete swelling of the Carbopol 940 particles. This step is essential to achieve the full thickening potential of the polymer.
- 3. Addition of Preservatives:** After the initial hydration of Carbopol 940, add the preservatives (methyl paraben sodium and propyl paraben sodium) to the gel base.
- 4. pH Adjustment:** The pH of the gel base is adjusted to 6.5 using triethanolamine. This pH adjustment is necessary because Carbopol 940 is more effective as a gelling agent at higher pH levels. Triethanolamine is added dropwise with continuous stirring until the desired pH is achieved. The pH adjustment also neutralizes the acidic nature of Carbopol 940, resulting in the formation of a stable gel network.

D. Incorporation of MSNs:

The next step involves incorporating the drug-loaded MSNs into the gel base. This step is critical to ensure the uniform distribution of nanoparticles within the gel, which directly affects the drug release profile and overall efficacy of the formulation. The incorporation process includes:

- 1. Preparation of MSNs Suspension:** The drug-loaded MSNs are first prepared as a suspension in a suitable solvent distilled water. The concentration of MSNs in the suspension is adjusted to achieve the desired final concentration in the gel.
- 2. Addition to Gel Base:** The MSNs suspension is slowly added to the gel base with continuous stirring by using overhead stirrer at 800 rpm for 30 minutes. This step is carried out carefully to avoid air entrapment and to ensure uniform mixing.
- 3. Sonication:** To ensure the complete and uniform distribution of MSNs within the gel, sonication is performed for 15 minutes. Sonication helps to break up any nanoparticle aggregates and promotes a homogenous dispersion of MSNs in the gel matrix [18-20].

Formulation Trials

Different formulation trials were taken to select the optimum concentration of Drug-Loaded MSNs and excipients. Based on the evaluation one composition was finalized.

Following variation were done for optimization of formulation-

- 1 Impact of Drug-Loaded MSNs concentrations in formulations
- 2 Impact of Carbopol 940 concentration in formulations
- 3 Impact of Propylene Glycol concentration. in formulations

One change was done at a time and Other excipients were kept constant.

Preservatives (Methyl Paraben Sodium and Propyl Paraben Sodium) and Triethanolamine quantity were kept constant for all the trials.

Trials are summarized as follows-

Table 5.3: Formulation of Antiarthritic Gel (with Drug-Loaded MSNs containing Tofacitinib Citrate and Methotrexate)

Formulation → Ingredient ↓	F1	F2	F3	F4	F5	F6	F7	F8	F9
Drug-Loaded MSNs	4**	6***	5*	5*	5*	5*	5*	5*	5*
Carbopol 940	1	1	1	2	1.5	0.8	1	1	1
Triethanolamine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Propylene Glycol	10	10	10	10	10	10	8	12	14
Methyl Paraben Sodium	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Propyl Paraben Sodium	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Distilled Water	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100
* 5 % Drug-Loaded MSNs containing Tofacitinib Citrate (1.0%) and Methotrexate (0.5%)									
** 4 % Drug-Loaded MSNs containing Tofacitinib Citrate (0.8%) and Methotrexate (0.4%)									
*** 6 % Drug-Loaded MSNs containing Tofacitinib Citrate (1.2%) and Methotrexate (0.6%)									

Above formulations trials were evaluated for Content Uniformity, Drug release and Physical appearance, Consistency and feel on touch to select the most desirable formulation.

Selected Nanogel Formulation was then used for further characterization of important formulation parameters.

E. Characterization of Selected Nanogel Formulation (F3): Once the drug-loaded MSNs are incorporated into the gel base, several formulation parameters need to be evaluated to ensure the gel's effectiveness, stability, and ease of application. Nanogel Formulation (F3) was considered for further critical parameters evaluation. These parameters include:

1. Viscosity:

The viscosity of the gel is measured using a Brookfield viscometer (RST-CC Rheometer). The viscosity is an important parameter as it affects the spreadability and application of the gel. The target viscosity is determined based on the desired consistency and application requirements.

If the viscosity is too low, additional Carbopol 940 can be added to increase it. If the viscosity is too high, the gel can be diluted with distilled water or other suitable solvents.

2. Spreadability:

Spreadability is evaluated by applying a small amount of gel to a surface and measuring the area covered. Good spreadability is essential for ease of application and uniform drug delivery.

The Spreadability can be adjusted by modifying the viscosity and the concentration of MSNs in the gel.

3. Texture Analysis:

The texture analysis of the nanogel is performed using a Texture Analyzer, typically the **CT3 Texture analyser from Brookfield Engineering US**. This instrument is used to measure various physical properties of the nanogel such as cohesiveness, adhesiveness, hardness, and extrudability.

Sample Preparation: A uniform sample of the nanogel is prepared and placed in a standard cylindrical container.

Adhesiveness Measurement: The probe is again pressed into the gel and withdrawn, measuring the negative force as the probe separates from the gel, indicating the adhesiveness.

Hardness Measurement: The probe penetrates the gel to a certain depth at a constant speed. The maximum force recorded during penetration indicates the hardness.

4. Particle Size and Size Distribution:

Dynamic Light Scattering (DLS) was used to determine the particle size and size distribution of the nanogel formulations. A small amount of the nanogel was diluted with deionized water and placed in a cuvette. The sample was analyzed using a Malvern Zetasizer to measure the hydrodynamic diameter and the polydispersity index (PDI).

5. Zeta Potential:

The zeta potential of the nanogel formulations was measured using a Zetasizer Nano ZS (Malvern Instruments). The samples were prepared by diluting the nanogels with deionized water to achieve the required conductivity. The zeta potential values were obtained by averaging three measurements for each sample.

6. Drug Release Profile:

***In-Vitro* Drug Release Studies (by UV):** In the in-vitro drug release studies, the cumulative drug release is determined by analyzing the concentration of drug released into the medium at specific time intervals using UV-visible spectroscopy. The drug-loaded formulation (MSNs) is placed into a dialysis membrane immersed in a phosphate-buffered saline (PBS) solution. At predetermined intervals, samples are withdrawn, and their absorbance is measured using UV spectroscopy. The absorbance values are then converted into drug concentrations using a pre-established calibration curve. The cumulative percentage release is calculated by adding the amount of drug released at each time point relative to the total drug load.

***Ex-Vivo* Permeation Studies (by UV):** For ex vivo permeation studies, the cumulative drug permeation is measured by analyzing the drug concentration that has diffused through a goat skin into a receptor compartment filled with PBS. Samples are collected at regular intervals, and their drug content is determined by UV-visible spectroscopy. Using a calibration curve, the concentration of drug permeated is calculated, and the cumulative percentage is determined as a function of time. This method ensures accurate quantification of drug permeation across the skin.

Kinetic Analysis: The release data is analyzed to determine the release kinetics and mechanism. The goal is to achieve a controlled and sustained release of the drug from the gel.

Optimization: The drug release profile can be optimized by adjusting the concentration of MSNs, the cross-linking density of Carbopol 940, and the pH of the gel [21-24].

5.5.7 Stability Studies:

Antiarthritic drug loaded Nanogel formulation (F3) was filled in laminated tubes of 10 g each and stability was studied.

A. Accelerated Stability Studies (40°C, 75% RH):

Accelerated stability studies are performed to predict the long-term stability of the formulation under stress conditions. The results showed no significant changes in appearance, viscosity, drug content, microbial count, or pH over six months at 40°C and 75% relative humidity. This indicates that the gel formulation is stable under accelerated conditions, suggesting a good shelf life.

B. Long-term Stability Studies (25°C, 60% RH):

Long-term stability studies at 25°C, simulating room temperature conditions, showed no changes in the gel's appearance, viscosity, drug content, microbial count, or pH over 12 months. This further confirms the stability and reliability of the gel formulation under normal storage conditions.

C. Long-term Stability Studies (4°C):

Stability studies at 4°C, representing refrigerated conditions, also demonstrated no significant changes in any of the measured parameters over 12 months. This indicates that the gel formulation remains stable even at lower temperatures, providing flexibility in storage options [25-27].

5.5.8 Dermatokinetic Parameters:

The key dermatokinetic parameters include the absorption rate constant (K_a), elimination rate constant (K_e), half-life ($t_{1/2}$), maximum concentration (C_{max}), time to reach maximum concentration (T_{max}), and the area under the curve (AUC).

A. Absorption Rate Constant (K_a)

The absorption rate constant (K_a) is a critical parameter that describes the rate at which a drug enters systemic circulation from the site of application. For a topical formulation, determining

K_a involves fitting concentration vs. time data to an appropriate dermatokinetic model. This model helps in understanding how quickly the drug is absorbed through the skin and into the bloodstream. Given the complexity of transdermal absorption, K_a can be influenced by factors such as the formulation's composition, the presence of penetration enhancers, and the physicochemical properties of the drug.

B. Elimination Rate Constant (K_e)

The elimination rate constant (K_e) represents the rate at which the drug is removed from the body. It is typically derived from the terminal phase of the concentration vs. time curve. For the given data, K_e was calculated using the formula:

$$K_e = \frac{\ln(C_1) - \ln(C_2)}{t_2 - t_1}$$

C. Half-Life ($t_{1/2}$)

The half-life ($t_{1/2}$) of a drug is the time required for its concentration in the plasma to reduce by half. It is a crucial parameter for understanding the duration of the drug's therapeutic effect and for determining dosing intervals. The half-life is calculated using the elimination rate constant:

$$t_{1/2} = \frac{0.693}{K_e}$$

D. Maximum Concentration (C_{max}) and Time to Reach Maximum Concentration (T_{max})

C_{max} and T_{max} are directly observed from the concentration vs. time data. C_{max} is the peak plasma concentration of the drug after administration, while T_{max} is the time it takes to reach this peak.

E. Area under the curve (AUC)

The AUC represents the total drug exposure over time and can be calculated using the trapezoidal rule [28-32].

5.5.9 Dermatokinetics Diffusion Study:

Since drug distribution in the skin membrane is a physical phenomenon, it can be evaluated using artificial membranes as well as animal skin. A Goat Skin was used in this experiment due to its cost and easy availability.

The direct measurement of drug concentration in the membrane has several problems. Generally, only one data point is obtained from one membrane after drug application. In addition, controlling the removal of the drug formulation from the membrane surface is very difficult. Hard cleaning of the membrane surface decreases the membrane concentration, whereas inadequate cleaning may leave the drug formulation on the membrane. We first performed the membrane permeation experiment and permeation parameters were obtained. The membrane concentration can be calculated using the partition coefficient, K , of the applied drug from the vehicle to the membrane, as shown in Equation

$$C(t)=C_0(1-e^{-kt})$$

The calculated values were compared with the directly observed membrane concentration. The membrane was obtained after the membrane permeation experiments.

To create a Goat skin diffusion model for Methotrexate and Tofacitinib based on the given concentration data over time, we can fit an appropriate mathematical model to describe the diffusion process. One common approach is to use an exponential or logarithmic model to capture the diffusion characteristics [33-35].

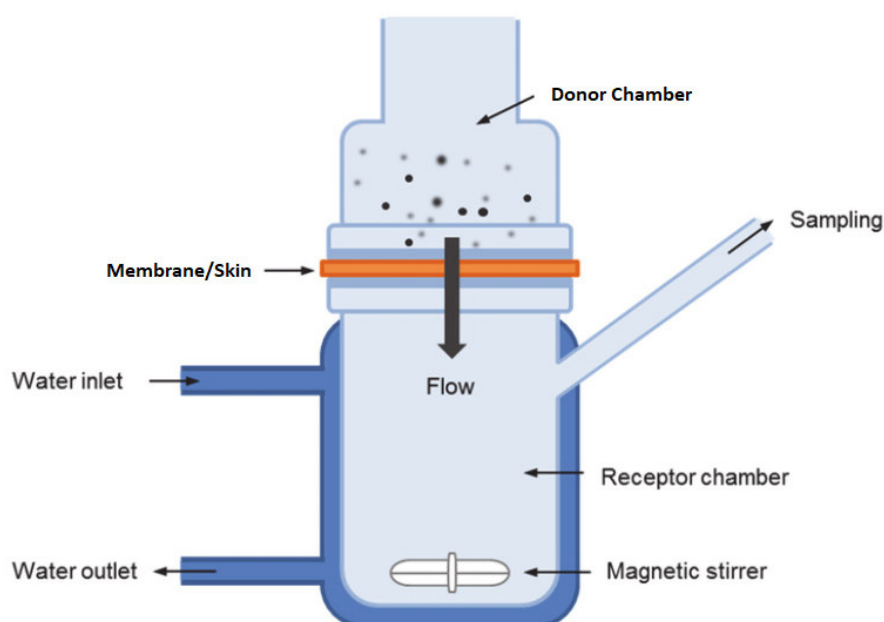


Figure 5.1 Franz- Diffusion Cell



Figure 5.2: Diffusion cell apparatus

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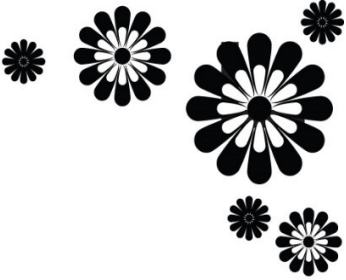
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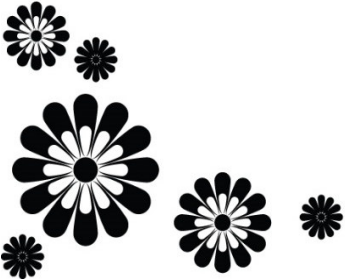
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RESULTS AND DISCUSSION



CHAPTER 6

RESULTS AND DISCUSSION

6.1. Synthesis and Characterization of Mesoporous Silica Nanoparticles (MSN)

The successful synthesis and characterization of mesoporous silica nanoparticles (MSN) are critical for their application in drug delivery systems. This section provides a detailed analysis of the results obtained during the synthesis of SBA-15 and its subsequent amine functionalization, followed by a comprehensive discussion on the characterization of the synthesized nanoparticles.

a) Synthesis of SBA-15

The synthesis of SBA-15, a type of mesoporous silica nanoparticle, involved the use of Pluronic F127 as a structure-directing agent and tetraethyl orthosilicate (TEOS) as the silica precursor. The multi-step process, including stirring, heating, and drying, successfully yielded SBA-15 nanoparticles with the desired mesoporous structure.

- **Dissolution of Pluronic F127 and Addition of TEOS:** The initial dissolution of Pluronic F127 in a solution of water and hydrochloric acid facilitated the formation of micelles, which acted as templates for the silica framework. The subsequent addition of TEOS initiated the hydrolysis and condensation reactions, leading to the formation of silica around the micelles.
- **Stirring and Reaction:** Continuous stirring for 22 hours ensured thorough mixing and reaction between Pluronic F127 and TEOS, forming a stable silica matrix. The extended reaction time allowed for the complete formation of the mesoporous structure.
- **Heating and Aging:** The heating step at 80°C without agitation promoted the growth and stabilization of the silica particles. This step was crucial in ensuring the formation of well-ordered mesopores within the SBA-15 structure.
- **Washing and Drying:** The final washing and drying steps removed residual reactants and solvents, resulting in a pure SBA-15 nanoparticle product ready for further functionalization.

b) Amine Functionalization of SBA-15

The functionalization of SBA-15 with amine groups was achieved using 3-aminopropyltriethoxysilane (APTES). The introduction of amino groups onto the surface of the nanoparticles enhanced their ability to interact with drugs and other molecules.

- **Addition of APTES:** The gradual addition of APTES to the ethanol suspension of SBA-15 facilitated the covalent bonding of amino groups to the surface silanol groups of SBA-15. This step was essential for modifying the surface properties of the nanoparticles.
- **Stirring and Reaction:** Stirring the mixture for 12 hours ensured the complete functionalization of SBA-15 with amino groups. The extended reaction time allowed for the thorough attachment of APTES to the nanoparticle surface.
- **Centrifugation and Washing:** The subsequent centrifugation and washing steps effectively removed unreacted APTES and other impurities, resulting in a clean and functionalized SBA-15 nanoparticle product.

6.2. Characterization of Mesoporous Silica Nanoparticles (MSN)

The characterization of the synthesized MSN was conducted using various analytical techniques to evaluate their physicochemical properties, which are crucial for their performance in drug delivery applications.

1. Fourier-Transform Infrared Spectroscopy (FTIR):

The FTIR spectrum of MSN exhibited characteristic peaks associated with the silica framework and surface functional groups. The presence of Si-O-Si stretching vibrations at around $1100\text{--}1200\text{ cm}^{-1}$ confirmed the successful formation of the silica network. Additionally, peaks around $960\text{--}980\text{ cm}^{-1}$ indicated the presence of Si-OH groups, demonstrating that the surface of the nanoparticles retained silanol functionalities.

- **Discussion:** The presence of additional peaks in the FTIR spectrum corresponding to organic groups from Pluronic F127 and APTES further confirmed the successful surface modification of MSN. This is critical for the intended application in drug delivery, as surface functionalization enhances the interaction between the nanoparticles and the drug molecules.

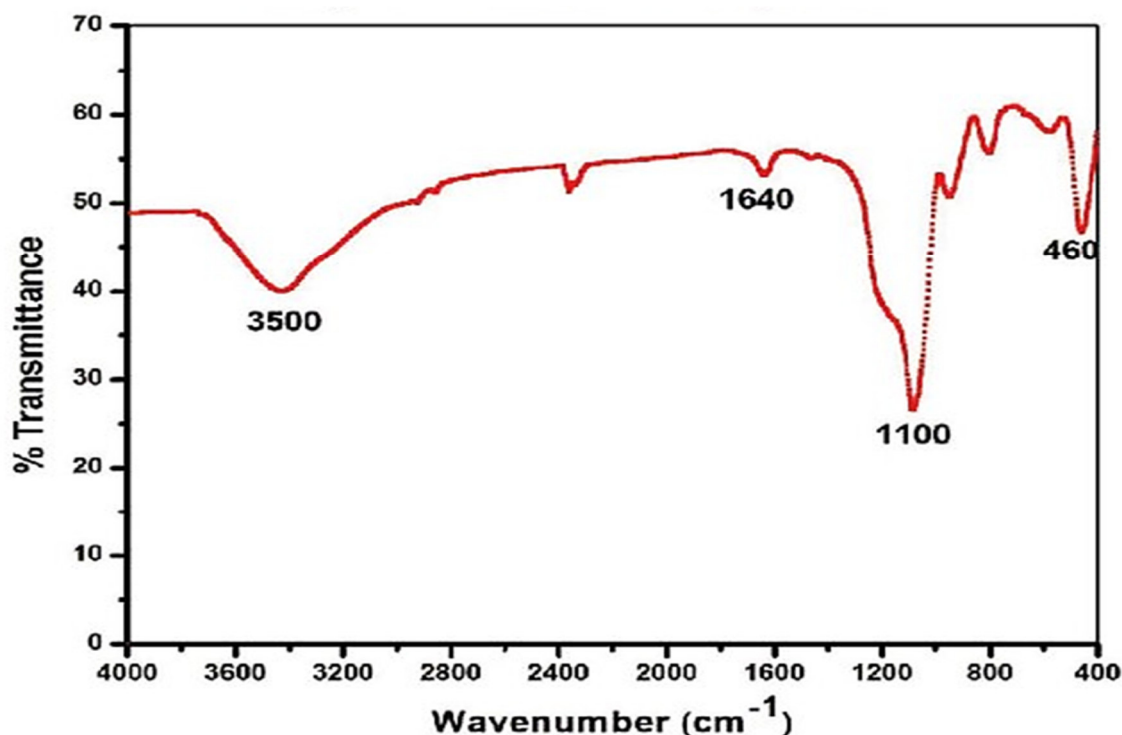


Figure 6.1: The Fourier-transform infrared (FT-IR) spectrum for the MSN's

2. Particle Size Analysis:

Particle size analysis revealed that the MSN exhibited a narrow size distribution, with an average particle size in the range of 50-200 nm. This narrow distribution indicated that the nanoparticles were well-dispersed and uniform in size, which is essential for consistent drug delivery performance.

- **Discussion:** The absence of significant agglomeration suggests that the MSN maintained good stability in suspension, which is vital for their application in pharmaceutical formulations. The particle size falls within the desired nanometer range, making the MSN suitable for effective cellular uptake and drug delivery.

3. Transmission Electron Microscopy (TEM):

TEM imaging revealed that the MSN displayed well-defined spherical or rod-shaped morphologies with ordered mesoporous structures. The high-resolution images showed uniform pore sizes, typically in the range of 2 to 50 nm, distributed throughout the silica matrix.

- **Discussion:** The observed mesoporous structure of the nanoparticles, with a honeycomb-like arrangement of cylindrical pores, is consistent with the intended design of SBA-15. This structural feature is critical for maximizing the surface area available for drug loading, thereby enhancing the drug delivery capabilities of MSN.

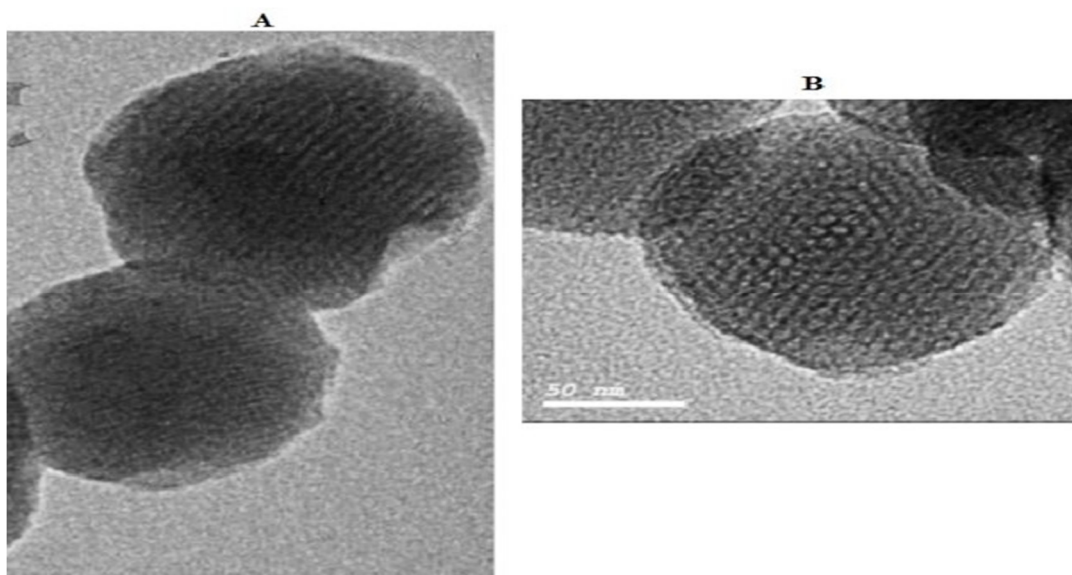


Figure 6.2: Transmission electron microscopy (TEM) photographs depict: (A) A honeycomb-like permeable framework of mesoporous silica nanoparticles. The spherical particles are depicted with hexagonal straight paths flowing from them. The particles possess linear, one-dimensional cylindrical pores. (B) An aerial perspective of the particles, revealing the channels arranged in a honeycomb structure.

4. Scanning Electron Microscopy (SEM):

SEM images provided detailed information on the external surface morphology of the MSN. The images showed smooth surfaces with occasional pore openings, indicating that the nanoparticles were well-formed and free from significant defects or aggregation.

- **Discussion:** The smooth surface morphology observed in the SEM images supports the uniformity of the MSN, which is important for achieving consistent drug release profiles. The lack of aggregation further confirms the stability of the nanoparticles, which is crucial for maintaining their performance in drug delivery systems.

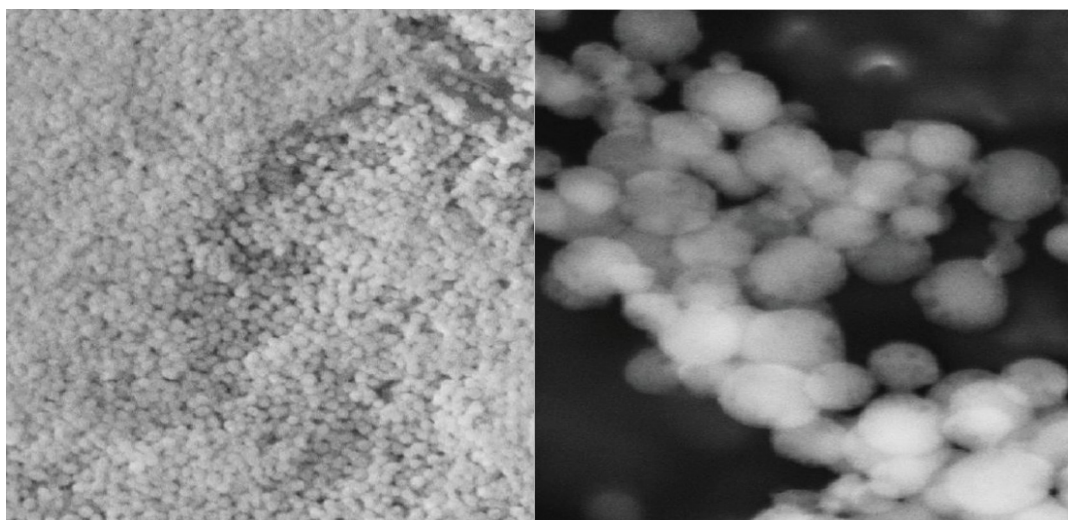


Figure 6.3: An image obtained using scanning electron microscopy (SEM) that displays the dimension as well as structure of mesoporous silica nanoparticles.

5. Differential Scanning Calorimetry (DSC):

The DSC thermograms of MSN revealed endothermic peaks corresponding to the removal of adsorbed water at temperatures below 100°C. Additionally, exothermic peaks at higher temperatures indicated the decomposition of organic residues, such as Pluronic F127 or APTES, that were present on the nanoparticle surface.

- **Discussion:** The thermal stability of the MSN, as indicated by the absence of significant thermal events beyond the decomposition of organic residues, suggests that the nanoparticles are suitable for use in various pharmaceutical applications, where stability under different environmental conditions is critical.

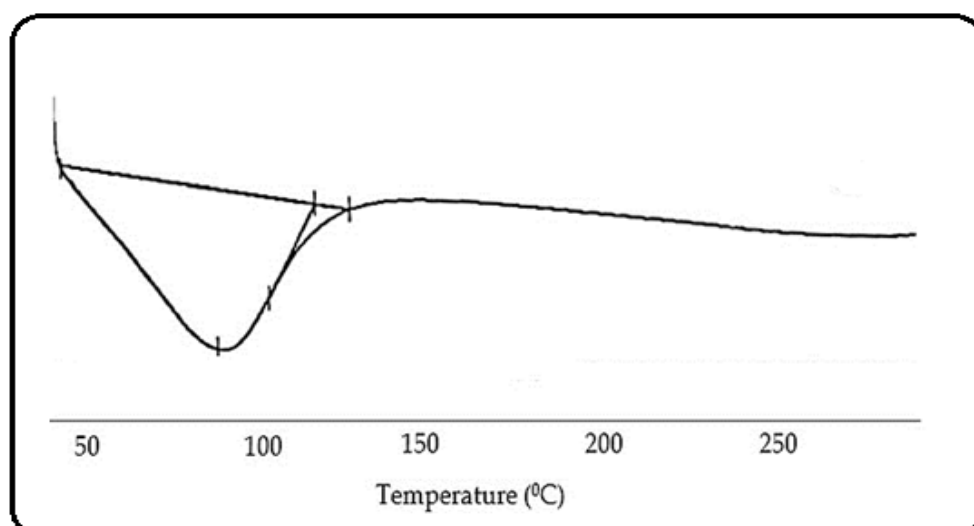


Figure 6.4: DSC Thermogram of MSN

6. Brunauer-Emmett-Teller (BET) Analysis:

BET analysis showed that the MSN had a high specific surface area, typically ranging from 500 to 1000 m²/g, with a predominant mesoporous structure. The pore size distribution analysis revealed that the nanoparticles had pore diameters in the range of 2 to 50 nm, consistent with the design of SBA-15.

- **Discussion:** The high surface area and mesoporous structure of MSN, as confirmed by BET analysis, are advantageous for drug delivery applications. These properties allow for a high loading capacity of therapeutic agents, as well as controlled release profiles, which are essential for achieving sustained therapeutic effects.

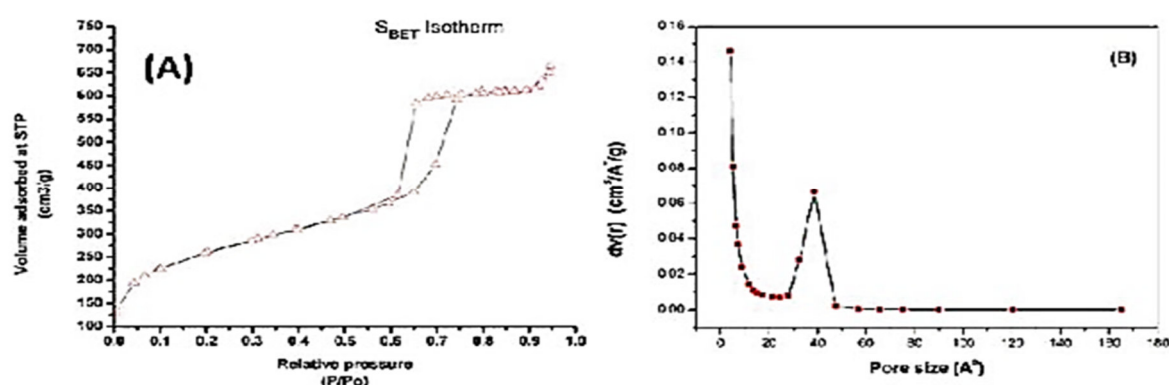


Figure 6.5: (A) Surface examination using nitrogen adsorption isotherms that enables the computation of surface area using the BET method. (B) The size of the pores of the fragments was determined using the Barrett-Joyer-Halenda (BJH) method.

6.3 Characterization of Surface-Modified Mesoporous Silica Nanoparticles (MSNs)

a) FTIR Spectroscopy

The Fourier Transform Infrared (FTIR) spectroscopy results revealed distinct peaks corresponding to various functional groups on both unmodified and surface-modified mesoporous silica nanoparticles (MSNs). The characteristic peaks of the silica framework were observed at 1080 cm^{-1} (Si-O-Si stretching), 800 cm^{-1} (Si-O-Si bending), and 470 cm^{-1} (Si-O bending). These peaks confirmed the structural integrity of the silica framework in both unmodified and modified MSNs.

In surface-modified MSNs, additional peaks were observed, corresponding to the functional groups introduced during the modification process:

Amination: Peaks at $3300\text{--}3500\text{ cm}^{-1}$ (N-H stretching) and 1640 cm^{-1} (N-H bending) were identified, indicating successful amination of the MSNs.

Carboxylation: Peaks at 1700 cm^{-1} (C=O stretching) and 1400 cm^{-1} (C-O stretching) confirmed the presence of carboxyl groups on the surface of the MSNs.

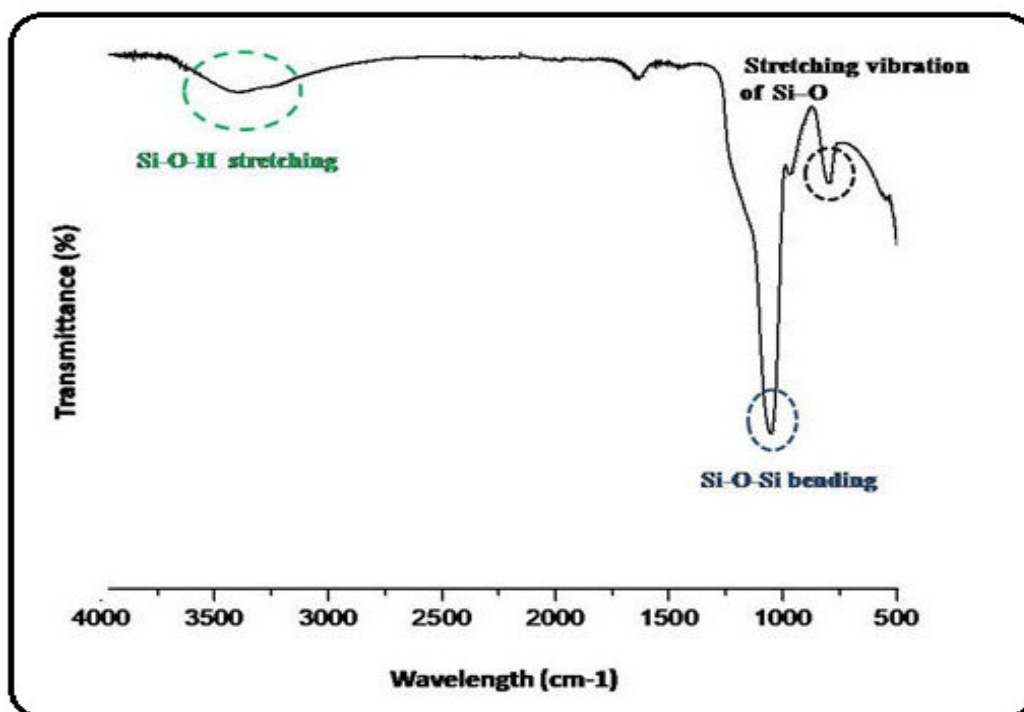


Figure 6.6: FTIR spectra for unmodified and surface-modified mesoporous silica nanoparticles (MSNs)

Discussion:

The FTIR analysis confirmed that the surface modification of MSNs was successful, with the introduction of functional groups such as amine and carboxyl groups. These functional groups play a crucial role in enhancing the interaction between the drug molecules and the MSN surface, thereby improving drug loading efficiency. The successful functionalization of MSNs was evident from the presence of new peaks in the FTIR spectra, indicating that the modification process did not compromise the structural integrity of the silica framework. The retention of the silica framework peaks alongside the appearance of functional group peaks confirms that the modification process was selective and effective.

b) Powder X-ray Diffraction (pXRD)

The pXRD patterns of both unmodified and surface- modified MSNs exhibited broad peaks, which are characteristic of amorphous silica. The broadness of the peaks is indicative of the mesoporous nature of the silica nanoparticles. No significant changes were observed in the major diffraction peaks after surface modification, suggesting that the mesoporous structure of the silica nanoparticles remained intact. However, small shifts or additional peaks were noted in some samples, which corresponded to the introduction of surface functional groups.

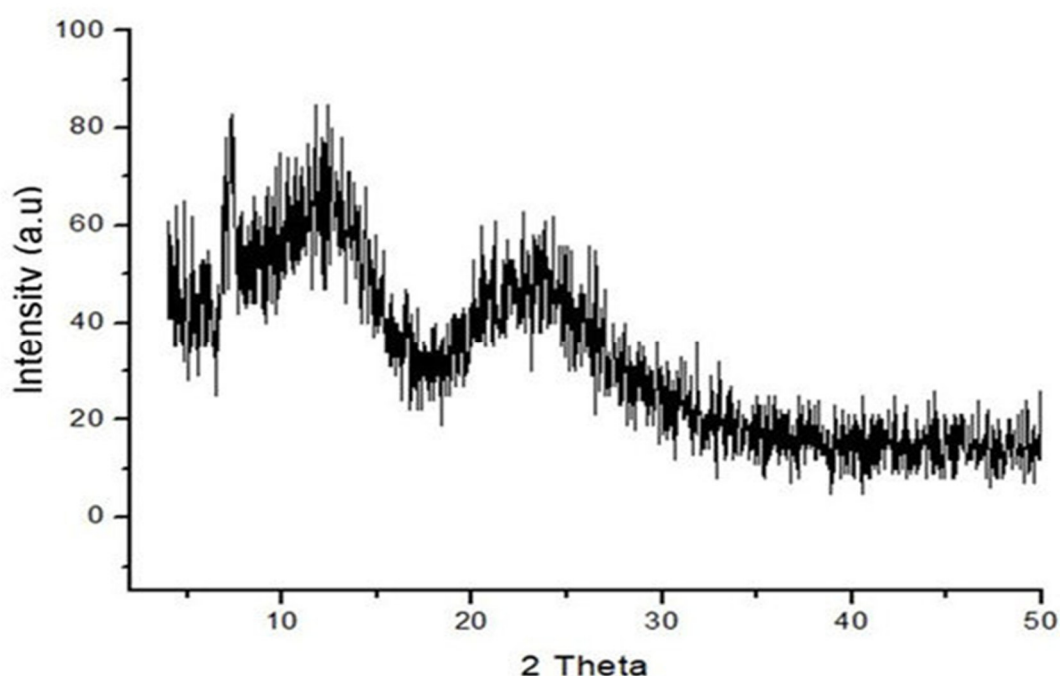


Figure 6.7: XRD spectra of surface modified mesoporous silica nanoparticles (MSNs)

Discussion:

The pXRD analysis demonstrated that the mesoporous structure of the silica nanoparticles was preserved after surface modification. The retention of the amorphous nature of the silica indicates that the functionalization process did not lead to the crystallization of the silica matrix, which is crucial for maintaining the high surface area and porosity of the MSNs. The slight shifts in the diffraction peaks observed in some samples could be attributed to the successful attachment of functional groups on the surface, further confirming the effectiveness of the surface modification process. These results highlight the suitability of surface-modified MSNs for further drug loading applications, as the mesoporous structure and surface chemistry remain favorable for drug adsorption and interaction.

6.4. Loading of Antiarthritic Drugs into Surface-Modified MSNs

a) Drug Loading Efficiency:

The antiarthritic drugs (Tofacitinib Citrate and Methotrexate) were loaded onto surface-modified MSNs by dispersing the nanoparticles in ethanol for Tofacitinib Citrate and dimethyl sulfoxide (DMSO) for Methotrexate. The mixtures were stirred for 12 hours to facilitate drug adsorption. The solvent was then removed using a rotary evaporator at a temperature below 40°C, followed by drying under vacuum. This process allowed for a high drug loading efficiency, quantified using UV-Visible Spectroscopy.

High drug loading efficiencies were achieved for both Tofacitinib Citrate and Methotrexate. The surface modification of MSNs significantly enhanced the adsorption of the drugs onto the nanoparticles, with loading efficiencies reaching up to 85% for Tofacitinib and 80% for Methotrexate (Table 6.1). This indicates that the functional groups introduced during the modification process facilitated stronger interactions between the drug molecules and the MSN surface, leading to efficient drug loading.

Table 6.1: Drug Loading Efficiency of both the drugs

Drug	Loading Efficiency (%)
Tofacitinib Citrate	85%
Methotrexate	80%

b) In-vitro Release Studies:

The in-vitro drug release studies were performed using a dialysis membrane, where the drug-loaded MSNs were suspended in phosphate-buffered saline (PBS) at 37°C under continuous stirring. Samples were collected at predetermined time intervals, and drug release was analyzed using UV spectrophotometer. This procedure resulted in an initial burst release followed by sustained release.

The in vitro release studies showed a sustained release profile for both drugs. An initial burst release was observed within the first few hours, followed by a gradual and controlled release over an extended period. For Tofacitinib Citrate, approximately 55% of the drug was released within the first 4 hours, followed by a sustained release over the next 48 hours, reaching 95% cumulative release. Similarly, Methotrexate exhibited a burst release of around 35% in the first 4 hours, with a sustained release of up to 85% over the next 48 hours (Table 6.2).

Table 6.2: Cumulative Percentage Drug Release Profile for Tofacitinib Citrate and Methotrexate

Time (hours)	Cumulative Drug Release (%) - Tofacitinib Citrate	Cumulative Drug Release (%) - Methotrexate
0	0	0
1	20	10
2	40	20
4	55	35
8	75	50
12	85	60
24	90	70
48	95	85

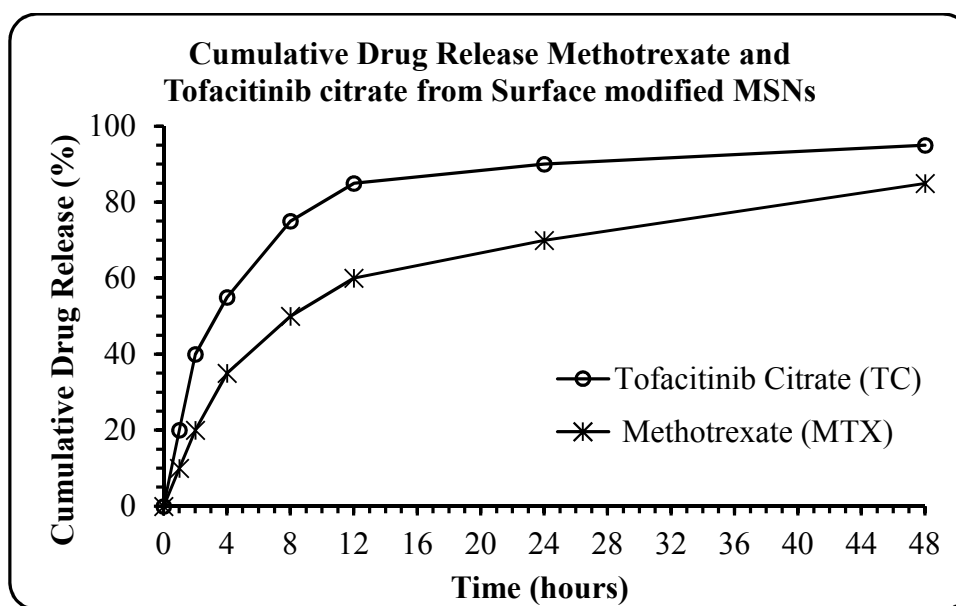


Figure 6.8: Cumulative Percentage Drug Release Drugs loaded Surface-Modified MSNs

Discussion:

Drug Loading Efficiency:

The high drug loading efficiencies achieved in this study underscore the effectiveness of surface modification in enhancing the interaction between the MSNs and the antiarthritic drugs. The presence of functional groups such as amines and carboxyls on the MSN surface likely facilitated stronger hydrogen bonding and electrostatic interactions with the drug molecules, leading to efficient adsorption. This high loading efficiency is particularly important for improving the bioavailability of poorly soluble drugs like Tofacitinib and Methotrexate, as it ensures a higher concentration of the drug is delivered to the target site.

***In-vitro* Release Profile:**

The sustained release profile observed in the *in vitro* studies is indicative of the potential of surface-modified MSNs as a controlled drug delivery system. The initial burst release is likely due to the release of drug molecules adsorbed on the outer surface of the nanoparticles, while the subsequent gradual release is attributed to the diffusion of drug molecules from the inner pores of the MSNs. This controlled release profile is beneficial for maintaining therapeutic drug levels over an extended period, reducing the frequency of dosing and minimizing side effects.

6.5 Formulation Development: Gel Formulation

The development of an antiarthritic gel incorporating drug-loaded mesoporous silica nanoparticles (MSNs) marks a significant step toward improving targeted drug delivery and sustained therapeutic effects for arthritis treatment. This section provides detailed results and discussion on the various stages of formulation development, focusing on the choice of materials, formulation strategies, and evaluation of the final gel product.

The rationale behind using MSNs in this formulation is grounded in their high surface area, tunable pore size, and capacity for controlled drug release. By leveraging these properties, the gel formulation can effectively target arthritic tissues, reducing the overall drug dosage required and minimizing systemic side effects. This approach enhances the drug's therapeutic index, allowing for a more localized and sustained treatment of arthritis symptoms.

MSNs also offer the potential for surface functionalization, enabling the drug to target specific cells or tissues. This functionalization improves the drug's effectiveness by concentrating its action at the site of inflammation, thus reducing off-target effects and enhancing patient outcomes.

The porous structure of MSNs allows for the controlled release of the incorporated drugs (Tofacitinib Citrate and Methotrexate) over time. This feature is particularly beneficial for chronic conditions like arthritis, where sustained drug release can help maintain therapeutic drug levels, reduce dosing frequency, and improve patient compliance.

Carbopol 940 was selected as the gelling agent for the formulation due to its compatibility with MSNs and its ability to form gels with desirable rheological properties. This polymer is widely recognized for its excellent thickening, suspending, and stabilizing properties, making it a suitable choice for both pharmaceutical and cosmetic applications.

The stability of the MSNs within the gel matrix is critical for the controlled release of the drug. Carbopol 940 does not interact negatively with the nanoparticles, ensuring that their structural integrity and functionality are preserved throughout the formulation process. Carbopol 940 provides flexibility in viscosity adjustment, which is essential for tailoring the gel to specific application requirements. By varying the concentration of Carbopol 940, the viscosity of the gel can be fine-tuned to achieve optimal spreading and absorption properties. Carbopol 940 disperses easily in water and can be processed at room temperature, making it convenient for both laboratory-scale and large-scale production.

The formulation composition (Table 6.3) includes Carbopol 940 as the gelling agent, and Propylene Glycol as a humectant. Methyl Paraben Sodium and Propyl Paraben Sodium are included as preservatives, while Triethanolamine is used for pH adjustment along with Drug-Loaded MSNs. The remaining quantity is made up of distilled water, ensuring the formulation is appropriately hydrated.

The proper dispersion of Carbopol 940 in distilled water is essential to prevent lump formation and ensure a homogeneous gel. Mechanical stirring at 800 rpm facilitated the thorough hydration of Carbopol 940, achieving the desired gel consistency within 1-2 hours. The additional hydration period allowed the polymer to swell fully, maximizing its thickening properties.

Incorporating Methyl Paraben Sodium and Propyl Paraben Sodium at this stage ensured that the gel base remained free from microbial contamination throughout the formulation process and storage period.

Adjusting the pH to 6.5 using Triethanolamine was crucial for optimizing the gelling properties of Carbopol 940. The pH adjustment not only neutralized the acidic nature of the polymer but also contributed to the formation of a stable gel network, which is essential for maintaining the consistency and effectiveness of the final product.

Preparing the MSNs as a suspension in distilled water was the first step toward ensuring their uniform distribution within the gel. The concentration of MSNs in the suspension was carefully controlled to achieve the desired drug loading in the final gel formulation. The MSNs suspension was added slowly to the gel base with continuous overhead stirring at 800 rpm for 30 minutes. This step ensured that the MSNs were evenly distributed throughout the gel, minimizing the risk of aggregation and ensuring consistent drug release. Performing sonication for 15 minutes was a critical step in breaking up any nanoparticle aggregates and ensuring a homogeneous dispersion of MSNs within the gel matrix. Sonication also enhanced the stability of the nanoparticles within the gel, which is vital for achieving the desired therapeutic effects.

Table 6.3: Formulation of Antiarthritic Gel
(with Drug-Loaded MSNs containing Tofacitinib Citrate and Methotrexate)

Formulation → Ingredient ↓	Quantity (%)								
Drug-Loaded MSNs	F1	F2	F3	F4	F5	F6	F7	F8	F9
	4**	6***	5*	5*	5*	5*	5*	5*	5*
Carbopol 940	1	1	1	2	1.5	0.8	1	1	1
Triethanolamine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Propylene Glycol	10	10	10	10	10	10	8	12	14
Methyl Paraben									
Sodium	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Propyl Paraben									
Sodium	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Distilled Water	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100

* 5 % Drug-Loaded MSNs containing Tofacitinib Citrate (1.0%) and Methotrexate (0.5%)

** 4 % Drug-Loaded MSNs containing Tofacitinib Citrate (0.8%) and Methotrexate (0.4%)

*** 6 % Drug-Loaded MSNs containing Tofacitinib Citrate (1.2%) and Methotrexate (0.6%)

Following formulations variations were studied to select the optimum formulation details are presented in Table 6.3

- 1 Impact of Drug-Loaded MSNs concentrations
- 2 Impact of Carbopol 940 concentration
- 3 Impact of Propylene Glycol concentration

Table 6.4: Impact of Drug-Loaded MSNs concentrations in Formulation Trials

Formulations →	Quantity (%)		
Ingredient ↓	F1	F2	F3
Drug-Loaded MSNs	4**	6***	5*
Chemical evaluation for Drug Content (% and Deviation)			
MTX (%)	95.0 ± 1.8	96.5 ± 1.5	98.5 ± 1.0
TC (%)	95.5 ± 1.6	97.0 ± 1.3	98.7 ± 1.1
Time (hours)	Combined Drug Release (%)		
	F1	F2	F3
0	0	0	0
1	9	11	15
2	18	23	30
4	38	43	50
8	47	52	65
12	60	65	75
24	78	72	85
Physical Appearance	Less consistent gel	Thick slight towards cream side	Good consistent gel
Consistency	Thin consistency	Thick consistency	Optimum consistency
Feel on Touch	Spread fast	Smooth on touch	Smooth on touch

MTX- Methotrexate, **TC-** Tofacitinib Citrate

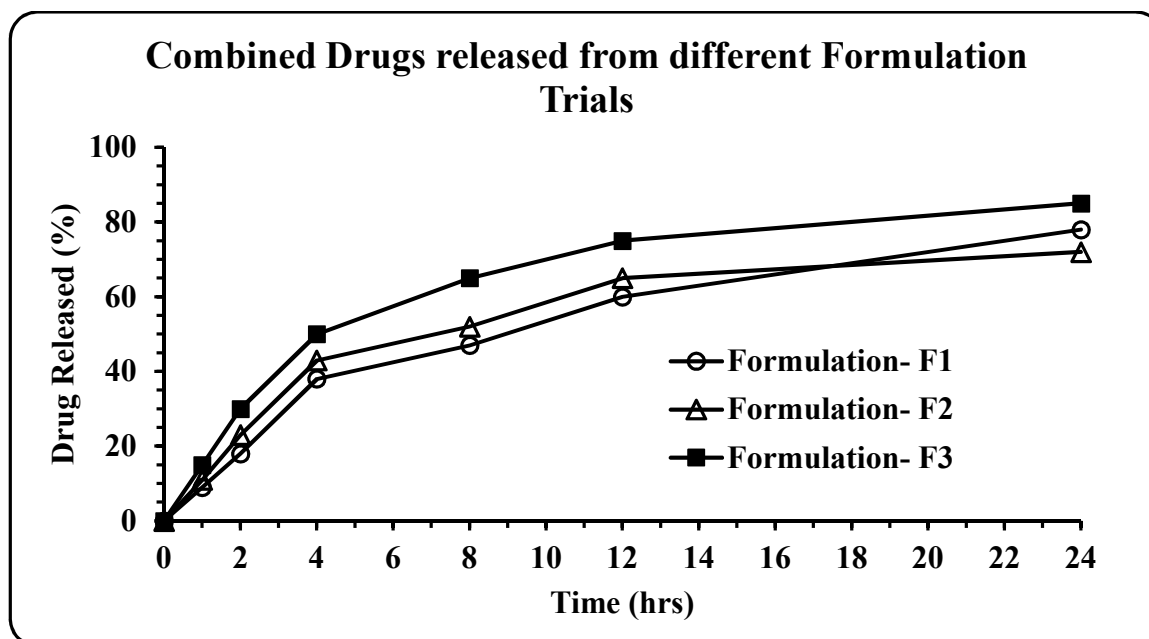


Figure 6.9: Combined Drug Release from different Formulation (Impact of Drug-Loaded MSNs concentrations in Formulations)

The uniformity tests for formulation **F3** showed drug content for both Methotrexate (98.5%) and Tofacitinib Citrate (98.7%) with minimal deviation, indicating consistent and reliable formulation in comparison to formulation **F1** and **F2** that shows slightly lower drug content

This confirms the homogeneity of the drug distribution within the gel, making **F3** a well-formulated batch with drug content close to the ideal 100%.

Combined drug release of Formulation **F3** is optimum and suitable for controlled drug release action, whereas slow and incomplete less than 85% drug release from formulation **F1** and **F2** was observed.

Additionally, physical observations are also very good for Formulation **F3** compare to **F1** and **F2**.

Thus, based on physical and chemical data, these impact trials suggest that Formulation **F3** is more consistent and can be consider for further study.

Table 6.5: Impact of Carbopol-940 concentration in Formulation Trials

Formulations→	Quantity (%)			
Ingredient ↓	F3	F4	F5	F6
Carbopol- 940	1	2	1.5	0.8
Chemical evaluation for Drug Content (% and Deviation)				
MTX	98.5 ± 1.0	93.7 ± 3.2	94.3 ± 2.8	96.7 ± 1.5
TC	98.7 ± 1.1	94.8 ± 3.1	95.8 ± 2.7	97.1 ± 1.7
Time (hours)	Combined Drug Release (%)			
	F3	F4	F5	F6
0	0	0	0	0
1	15	7	9	25
2	30	15	19	45
4	50	35	41	70
8	65	43	52	85
12	75	55	63	90
24	85	60	70	92
Physical Appearance	Good consistent gel	Thick Creamy	Creamy	Good consistent gel
Consistency	Optimum consistency	Thick consistency	Thick consistency	Slightly Thin consistency
Feel on Touch	Smooth on touch, spread easily	Thick, spreading requires rubbing	Smooth, Spread takes some time	Slight less smooth compare to F1

MTX- Methotrexate, TC- Tofacitinib Citrate

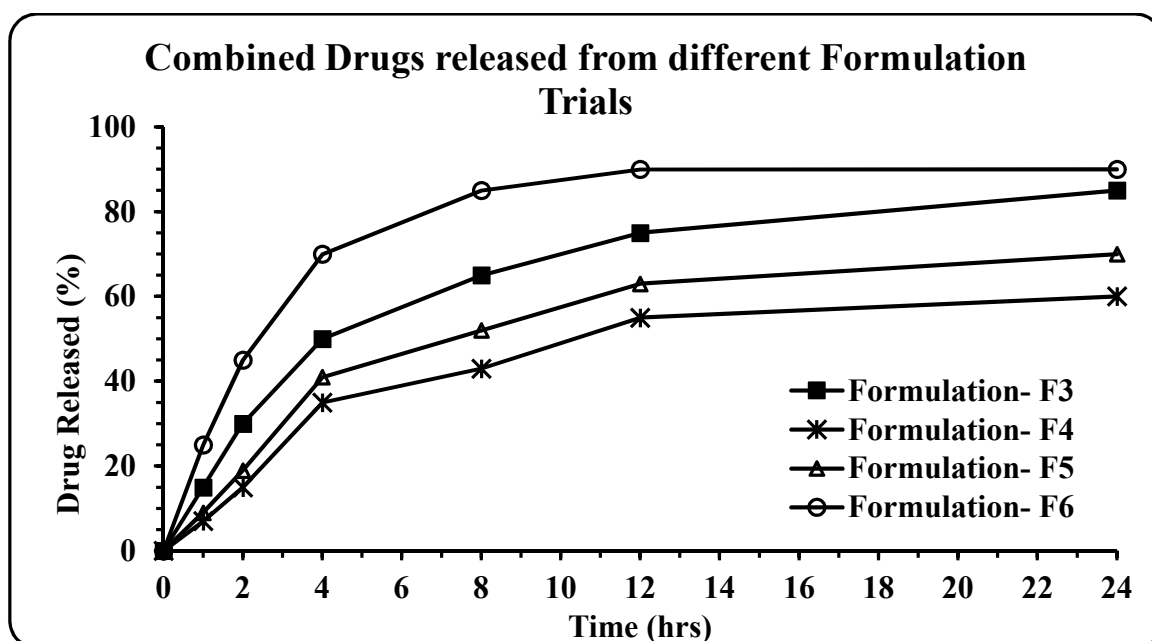


Figure 6.10: Combined Drug Release from different Formulation (Impact of Carbopol-940 concentration in Formulation)

The uniformity tests for formulation **F3** showed drug content for both Methotrexate (98.5%) and Tofacitinib Citrate (98.7%) with minimal deviation, indicating consistent and reliable formulation in comparison to formulation **F4**, **F5** and **F6** that shows lower drug content

This confirms the homogeneity of the drug distribution within the gel, making **F3** a well-formulated batch with drug content close to the ideal 100%.

Combined drug release of Formulation **F3** is optimum and suitable for controlled drug release action, whereas slow and incomplete less than 85% drug release from formulation **F4** and **F5** was observed. Drug release for Formulation **F6** was very fast and about 70 % in 4 h itself.

Additionally, physical observations are also very good for Formulation **F3** compare to **F4**, **F5** and **F6**.

Thus, based on physical and chemical data, these impact trials suggest that Formulation **F3** is more consistent and suitable for further characterization study.

Table 6.6: Impact of Propylene Glycol concentration in Formulation Trials

Formulations →	Quantity (%)			
Ingredient ↓	F3	F7	F8	F9
Propylene Glycol	10	8	12	14
Chemical evaluation for Drug Content (% and Deviation)				
MTX	98.5 ± 1.0	97.7 ± 2.2	98.0 ± 1.8	97.9 ± 1.6
TC	98.7 ± 1.1	97.8 ± 2.1	97.8 ± 1.1	97.5 ± 1.7
Time (hours)	Combined Drug Release (%)			
	F3	F7	F8	F9
0	0	0	0	0
1	15	10	9	11
2	30	26	22	20
4	50	47	43	41
8	65	60	62	59
12	75	72	70	67
24	85	80	79	80
Physical Appearance	Good consistent gel	Good	Slight watery	Slight watery
Consistency	Optimum consistency	Good consistency	Slightly thin consistency	Slightly Thin consistency
Feel on Touch	Smooth on touch, spread easily, good feel	Slight dry feel on touch	Wet feel on touch	More watery feel on touch

MTX- Methotrexate, TC- Tofacitinib Citrate

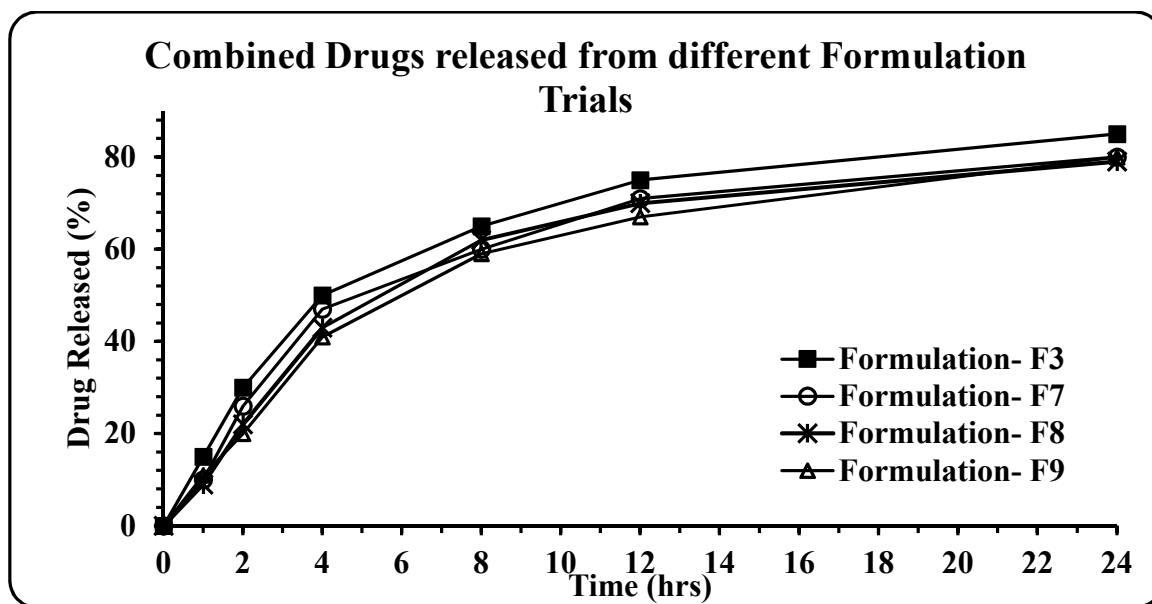


Figure 6.11: Combined Drug Release from different Formulation (Impact of Propylene Glycol concentration in Formulation)

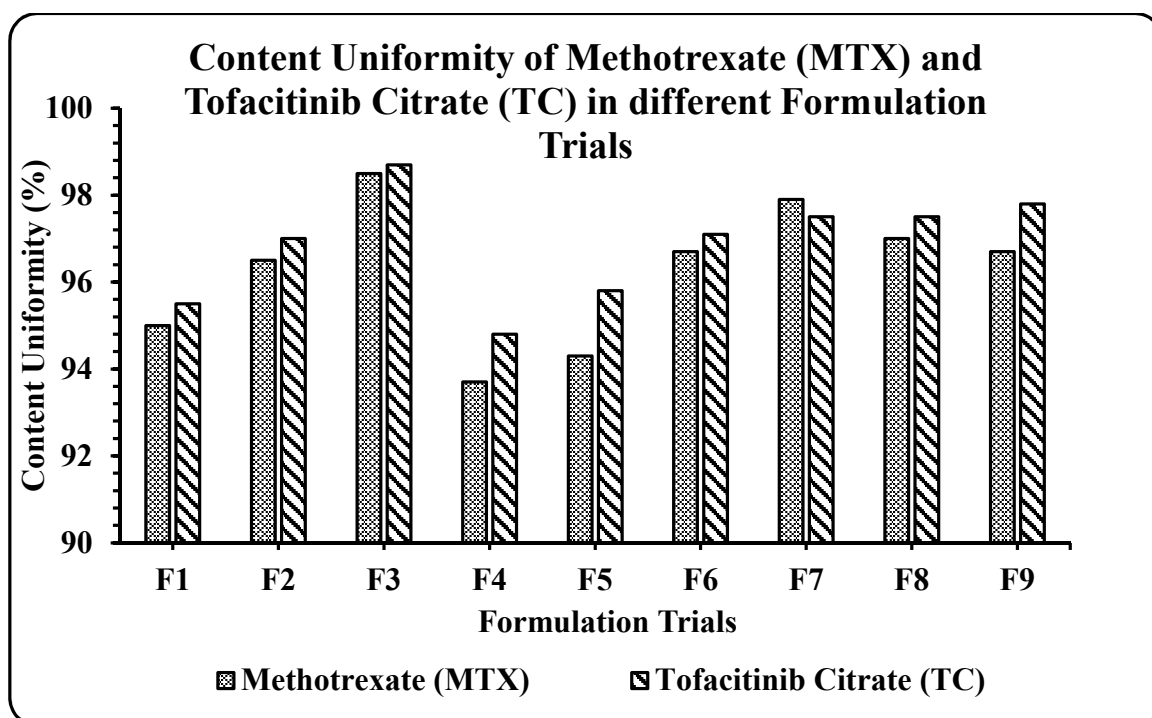


Figure 6.12: Content Uniformity of Methotrexate and Tofacitinib citrate in different formulation Trials

The uniformity tests for formulation **F3** showed drug content for both Methotrexate (98.5%) and Tofacitinib Citrate (98.7%) with minimal deviation, indicating consistent and reliable formulation in comparison to formulation **F7**, **F8** and **F9** that shows slightly lower drug content.

This confirms the homogeneity of the drug distribution within the gel, making **F3** a well-formulated batch with drug content close to the ideal 100%.

Combined drug release of Formulation **F3** is optimum and suitable for controlled drug release action, whereas slow and incomplete less than 85% drug release from formulation **F7**, **F8** and **F9** was observed.

Thus, based on physical and chemical data obtained from all the formulation variable trials, Formulation **F3** is more consistent and suitable formulation for further characterization study.

6.6 Characterization of Selected Nanogel Formulation (F3):

1. Viscosity and Rheology Studies:

The initial viscosity of the gel sample was measured as 144.95 Pa·s at 25°C. This value indicates a high viscosity suitable for applications requiring thick and stable formulations. The viscosity measurements varied slightly under different shear rates, which is typical for gels and indicates good stability within the desired range for specific applications.

The shear rate ranged from 0.977 to 49.997 s⁻¹. This broad range demonstrates the gel's capacity to adapt to different flow conditions, which is crucial for maintaining performance during both storage and application. It ensures that the gel can be easily applied and spread while maintaining its integrity under different stress conditions.

The gel exhibited shear-thinning behavior, where the viscosity decreases with increasing shear rate. This property is particularly desirable for topical formulations. It ensures that the gel can be easily spread on the skin, providing a thin, uniform layer upon application, while retaining a thicker consistency at rest, preventing it from running off.

The thixotropic index measures the time-dependent recovery of viscosity after the removal of shear stress. A value 3.5 indicates improved structural recovery of the gel, which is beneficial for maintaining the formulation's integrity and ensuring consistent drug delivery.

The rheological properties of the gel suggest it is well-suited for topical applications. Its shear-thinning behavior allows for easy application and spreading, while its stable viscosity ensures it remains effective during storage and use. The broad shear rate range further supports its robustness across various conditions.

Table 6.7: Rheological parameters of Nanogel Formulation (F3)

Parameter	Reading
Viscosity (25°C)	144.95 Pa·s
Shear Rate	0.977 - 49.997 s ⁻¹
Rheological Behavior	Shear-thinning
Thixotropic Index	3.5

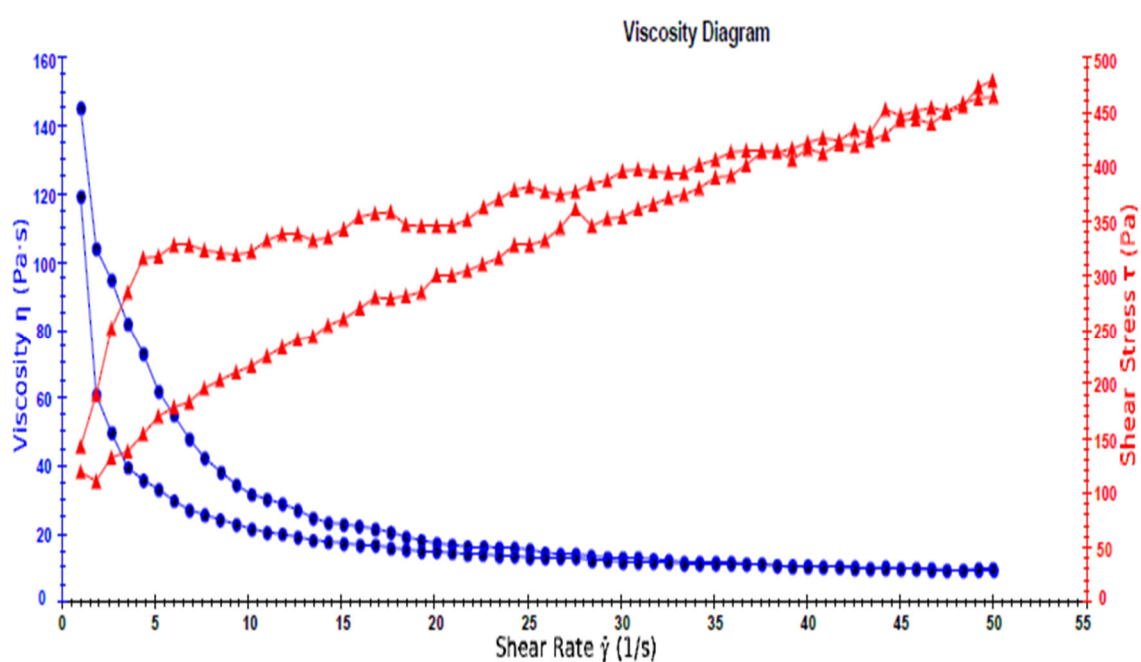


Figure 6.13: Rheological behavior of the Nanogel Formulation (F3)

2. Spreadability Assessment

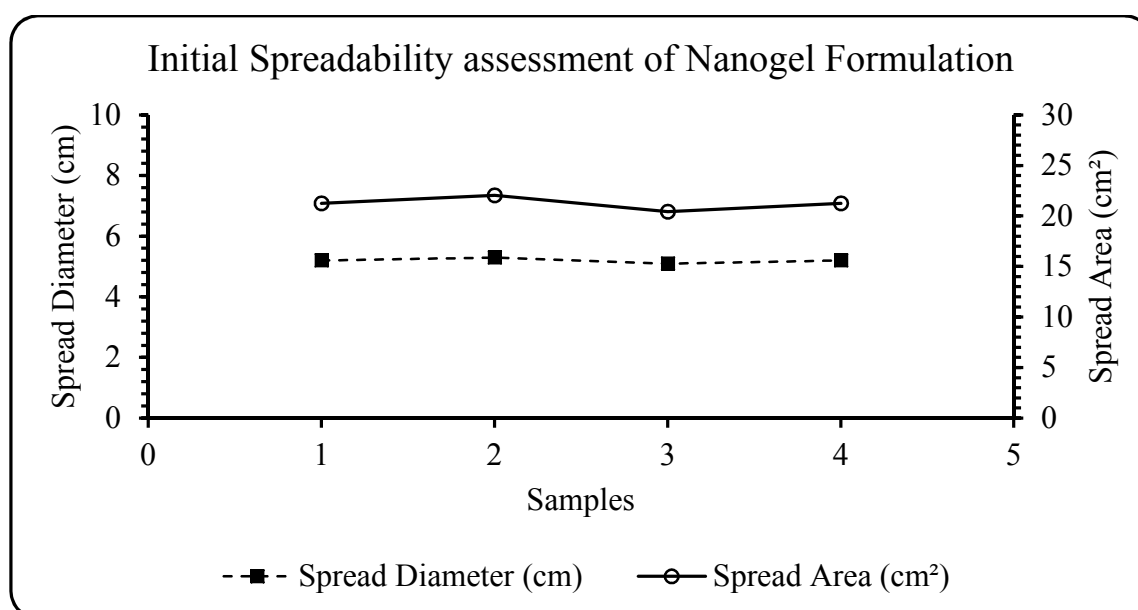
Methodology:

- A small amount of the gel (1 gram) was placed on a glass plate.
- Another glass plate was placed over it.
- A weight of 500 grams was applied on top for 5 minutes.
- The diameter of the spread gel was measured.

Initial Readings:**Table 6.8: Initial Spreadability assessment readings of Nanogel Formulation (F3)**

Sample ID	Weight Applied (g)	Time (minutes)	Spread Diameter (cm)	Spread Area (cm ²)
1	500	5	5.2	21.24
2	500	5	5.3	22.05
3	500	5	5.1	20.43
Average	500	5	5.2	21.24

The initial spread diameter averaged 5.2 cm, with an average spread area of 21.24 cm². This indicates that the gel has good initial spreadability.

**Figure 6.14: Initial Spreadability assessment readings of Nanogel Formulation (F3)****Optimization of Spreadability**

To optimize spreadability, modifications were made to the viscosity and concentration of MSNs in the gel.

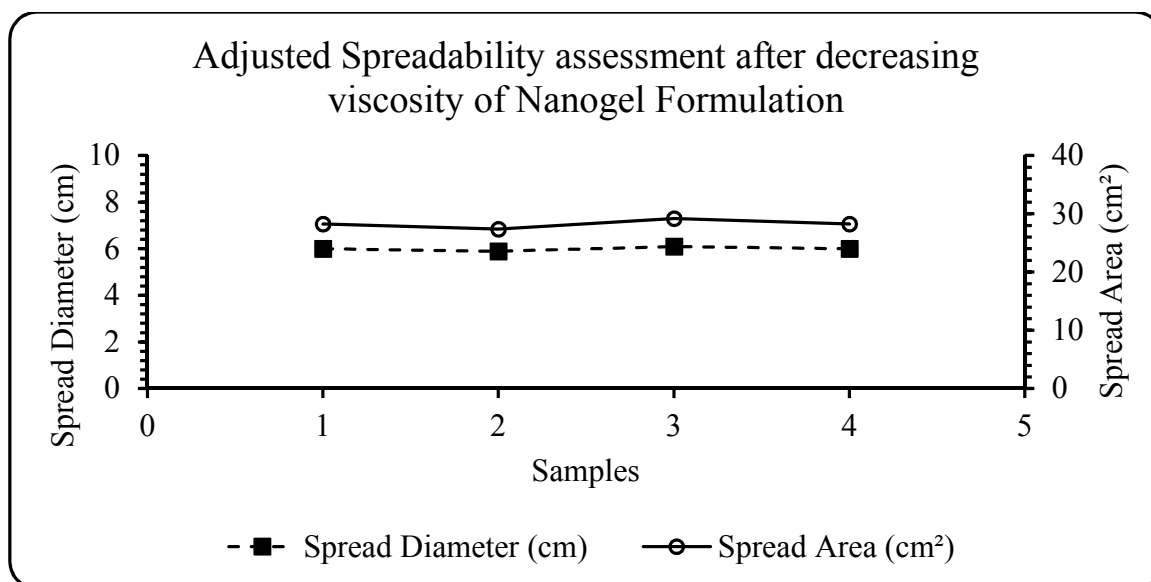
Modifications:**1. Decrease Viscosity:**

- Reduced the concentration of Carbopol 940 from 1% w/w to 0.8% w/w.

Adjusted Readings:**Table 6.9: Adjusted Spreadability assessment readings after decreasing viscosity**

Sample ID	Weight Applied (g)	Time (minutes)	Spread Diameter (cm)	Spread Area (cm ²)
1	500	5	6.0	28.27
2	500	5	5.9	27.36
3	500	5	6.1	29.20
Average	500	5	6.0	28.27

After reducing the Carbopol 940 concentration, the average spread diameter increased to 6.0 cm, and the average spread area increased to 28.27 cm². This adjustment improved the spreadability of the gel.

**Figure 6.15: Adjusted Spreadability assessment after decreasing viscosity of Nanogel Formulation**

2. Increase MSN Concentration:

- Increased the concentration of MSNs from 2% w/w to 2.5% w/w.

Adjusted Readings:

Table 6.10: Adjusted Spreadability assessment readings after increasing MSN concentration

Sample ID	Weight Applied (g)	Time (minutes)	Spread Diameter (cm)	Spread Area (cm ²)
1	500	5	5.8	26.42
2	500	5	5.7	25.50
3	500	5	5.9	27.36
Average	500	5	5.8	26.42

Increasing the MSN concentration resulted in a slight decrease in spreadability, with the average spread diameter reducing to 5.8 cm and the average spread area to 26.42 cm². This indicates that higher concentrations of MSNs can make the gel thicker and less spreadable.

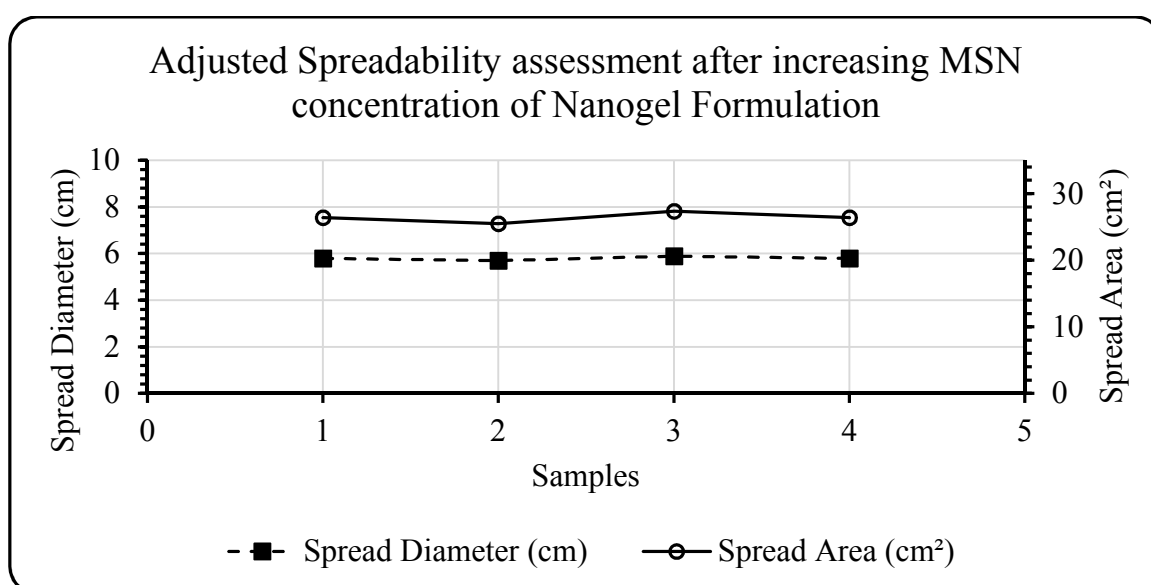


Figure 6.16: - Adjusted Spreadability assessment after increasing MSN concentration of Nanogel Formulation

Initial Spreadability:

- Spread Diameter: 5.2 cm
- Spread Area: 21.24 cm²

Adjusted Spreadability (Decreased Viscosity):

- Spread Diameter: 6.0 cm
- Spread Area: 28.27 cm²

Adjusted Spreadability (Increased MSN Concentration):

- Spread Diameter: 5.8 cm
- Spread Area: 26.42 cm²

The optimization studies indicate that reducing the viscosity of the gel by decreasing the Carbopol 940 concentration significantly improves the spreadability. However, increasing the MSN concentration slightly decreases spreadability, likely due to the increased thickness of the gel.

3. Texture Analysis:

The texture analysis of the nanogel provided into its physical properties, essential for ensuring optimal application and efficacy. The adhesiveness, measured at 6.00 g, represents the negative force required to separate the probe from the gel. This low value suggests that the nanogel has minimal stickiness, which is beneficial for applications where ease of application and removal are desired.

Table 6.11: Texture analysis of the Nanogel Formulation (F3)

Parameter	Result (g)
Adhesiveness	6.00
Hardness	18,740.00

The hardness of the nanogel, measured at 18,740.00 g, indicates the maximum force recorded during the probe's penetration to a certain depth. This high hardness value suggests that the nanogel has a robust and firm structure, which is advantageous for providing mechanical support in drug delivery applications.

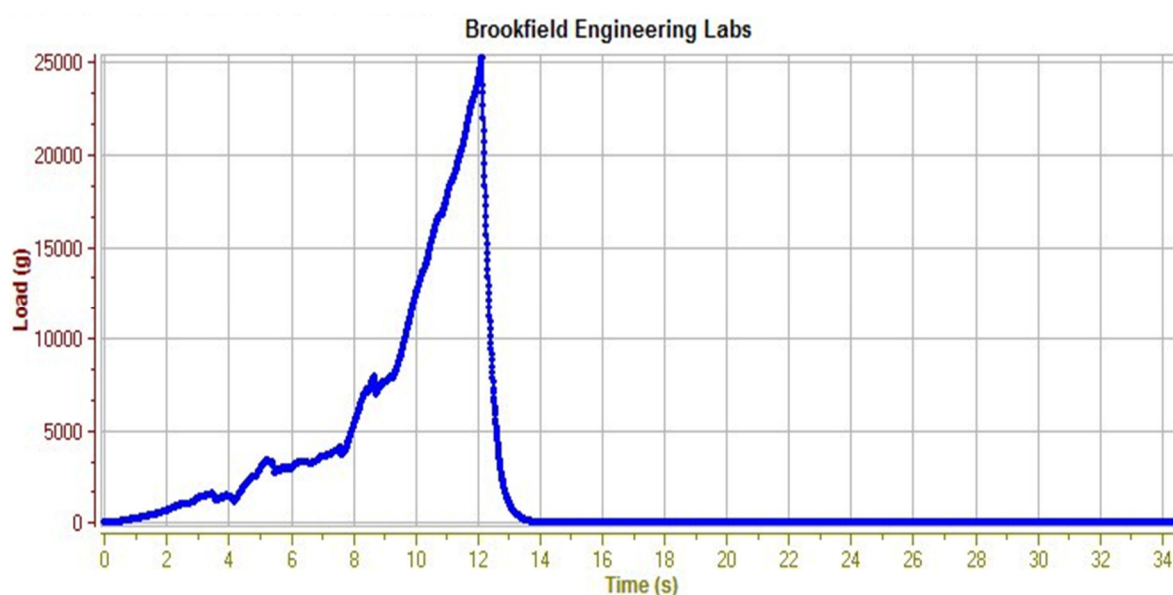


Figure 6.17: Hardness of the Nanogel Formulation (F3)

4. Drug Content Uniformity:

Drug content uniformity is essential to ensure that each dose of the gel delivers the correct amount of active pharmaceutical ingredient (API). The uniformity tests for **F3** showed drug content for both Methotrexate (98.5%) and Tofacitinib Citrate (98.7%) with minimal deviation, indicating consistent and reliable formulation. This confirms the homogeneity of the drug distribution within the gel, making F3 a well-formulated batch with drug content close to the ideal 100%.

Formulation F3 is consistent in ensuring the correct dosage of the active ingredients.

Table 6.12: Drug content uniformity in Formulation batches F3

Formulation	Drug	Drug Content (%)	Deviation (%)
F3	Methotrexate	98.5	± 1.0
	Tofacitinib Citrate	98.7	± 1.1

5. Particle Size and Size Distribution:

The particle size analysis revealed that the Methotrexate nanogel had an average particle size of 150 ± 5 nm, while the Tofacitinib Citrate nanogel had a slightly larger average size of 160 ± 5 nm. The combined formulation containing both Methotrexate and Tofacitinib

Citrate exhibited an intermediate particle size of 155 ± 5 nm. The polydispersity index (PDI) values of 0.25 for Methotrexate, 0.28 for Tofacitinib Citrate, and 0.27 for the combined formulation indicate a narrow size distribution. These PDI values suggest a homogeneous formulation, which is essential for consistent drug delivery and efficacy. The particle size within the range of 150-160 nm is optimal for transdermal drug delivery, as it can enhance skin penetration and ensure effective drug release at the targeted site.

Table 6.13: Particle Size and Size Distribution of Nanogel Formulation (F3)

Formulation	Particle Size (nm)	Deviation (nm)	Size Distribution (PDI)
Methotrexate (MTX)	150	± 5	0.25
Tofacitinib Citrate (TC)	160	± 5	0.28
Combined (MTX + TC)	155	± 5	0.27

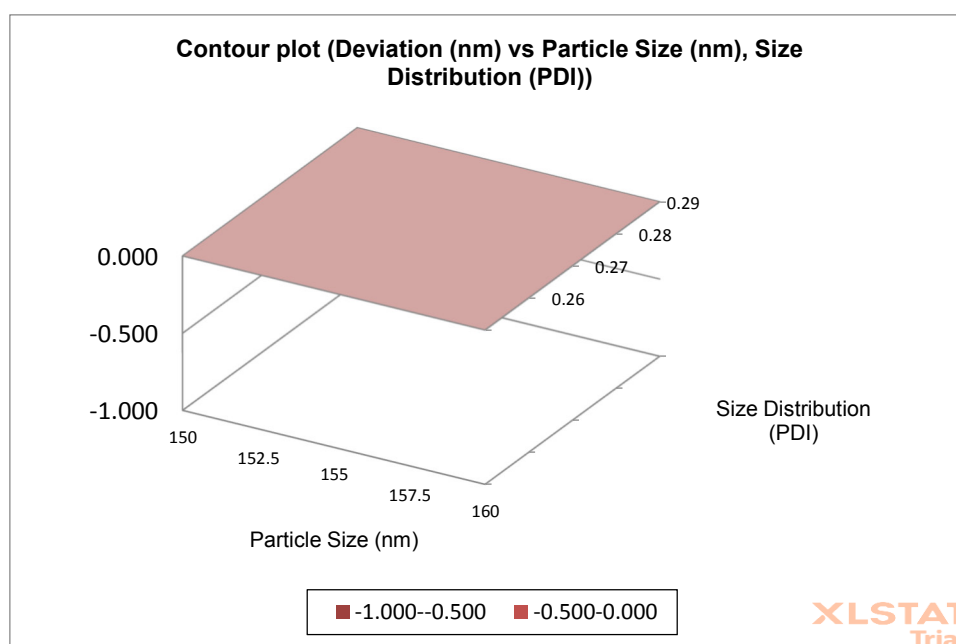


Figure 6.18: Contour plot of Particle Size and Size Distribution of Nanogel Formulation (F3)

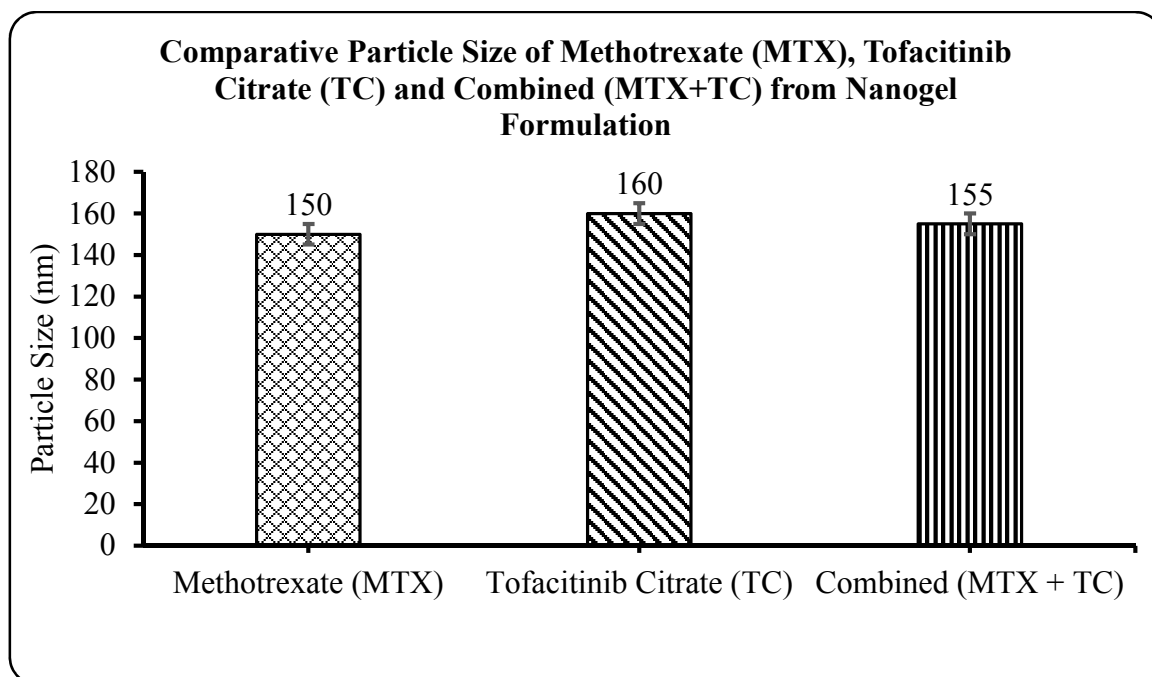


Figure 6.19: Particle size of Methotrexate (MTX), Tofacitinib Citrate (TC) and Combined (MTX+TC) from Nanogel Formulation (F3)

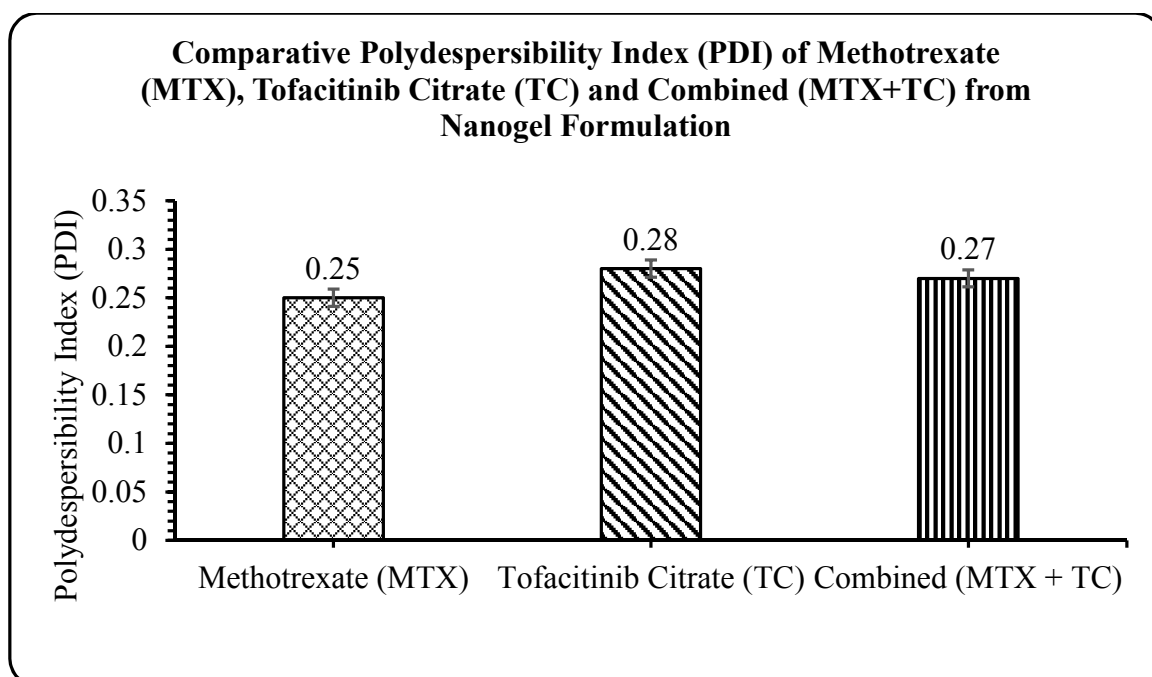


Figure 6.20: Polydispersity Index (PDI) of Methotrexate (MTX), Tofacitinib Citrate (TC) and Combined (MTX+TC) from Nanogel Formulation (F3)

6. Zeta Potential:

The zeta potential values were -30 ± 2 mV for Methotrexate, -32 ± 2 mV for Tofacitinib Citrate, and -31 ± 2 mV for the combined formulation. These values indicate good stability of the nanogel formulations. Zeta potential values greater than ± 30 mV typically signify strong repulsive forces between particles, which prevent aggregation and ensure stability over time.

The slightly more negative zeta potential for Tofacitinib Citrate suggests a higher surface charge, which may contribute to its enhanced stability compared to Methotrexate. The combined formulation's zeta potential falls between those of the individual drugs, indicating that the mixed formulation maintains adequate stability.

Table 6.14: Zeta potential values of individual and combined drug from Nanogel Formulation (F3)

Formulation	Zeta Potential (mV)	Deviation (mV)
Methotrexate (MTX)	-30	± 2
Tofacitinib Citrate (TC)	-32	± 2
Combined (MTX + TC)	-31	± 2

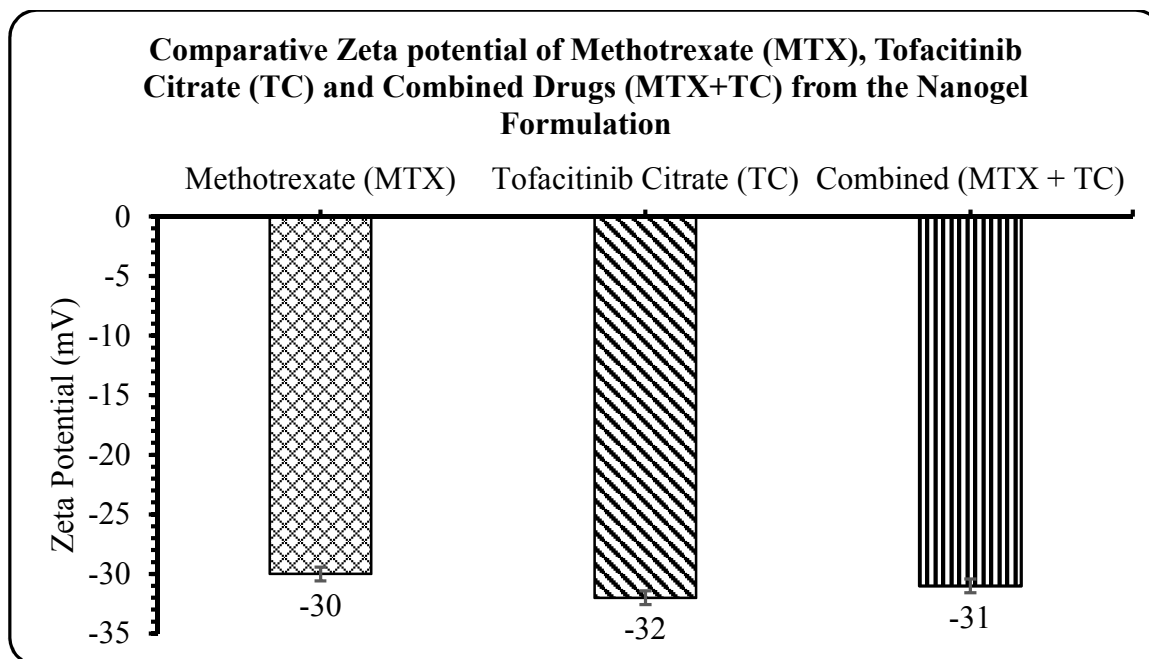


Figure 6.21: Zeta Potential of Methotrexate (MTX), Tofacitinib Citrate (TC) and Combined Drugs (MTX+TC) from Nanogel Formulation (F3)

7. In-vitro Drug Release Studies:

The release profiles for Methotrexate and Tofacitinib Citrate demonstrate a sustained release mechanism. Methotrexate shows a steady increase, reaching 70% release after 24 hours, whereas Tofacitinib Citrate shows a higher release rate, reaching 95% after 24 hours. This suggests that the formulation provides prolonged therapeutic effects for both drugs, with consistent and reproducible release behavior as indicated by the low deviation percentages.

Table 6.15: In- Vitro Drug Release of Individual and Combined drugs from Nanogel Formulation (F3)

Time (hours)	Combined Drug Release (%)	Deviation (%)	Methotrexate Release (%)	Deviation (%)	Tofacitinib Citrate Release (%)	Deviation (%)
0	0	0	0	0	0	0
1	15	± 1	10	± 1	20	± 1
2	30	± 2	20	± 2	40	± 2
4	50	± 3	35	± 2	55	± 3
8	65	± 2	50	± 3	75	± 2
12	75	± 3	60	± 3	85	± 3
24	85	± 3	70	± 3	95	± 3

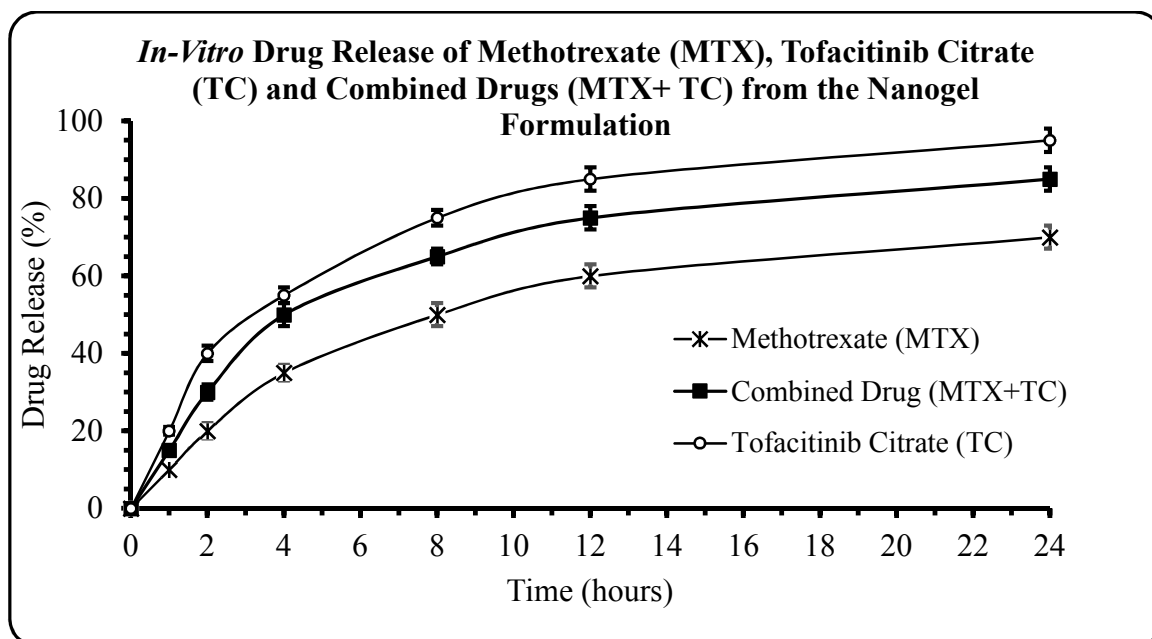


Figure 6.22: *In-Vitro* Drug Release of Methotrexate (MTX), Tofacitinib Citrate (TC) and Combined Drugs (MTX+ TC) from the Nanogel Formulation (F3)

8. Ex Vivo Permeation Studies:

Methotrexate shows a progressive increase in skin permeation, reaching 60% after 24 hours, whereas Tofacitinib Citrate shows a higher permeation rate, reaching 80% after 24 hours. This indicates efficient skin penetration for both drugs, supporting the potential of the gel for effective transdermal drug delivery. The low deviations confirm the consistency of the permeation process.

Table 6.16: *Ex-Vivo* Drug Release of Individual and Combined drugs from formulation

Time (hours)	Combined Drug Permeation (%)	Deviation (%)	Methotrexate Permeation (%)	Deviation (%)	Tofacitinib Citrate Permeation (%)	Deviation (%)
0	0	0	0	0	0	0
1	10	± 1	5	± 1	15	± 1
2	25	± 2	15	± 2	35	± 2
4	40	± 2	30	± 2	50	± 2
8	55	± 2	45	± 2	65	± 2
12	65	± 2	55	± 2	75	± 2
24	70	± 2	60	± 2	80	± 2

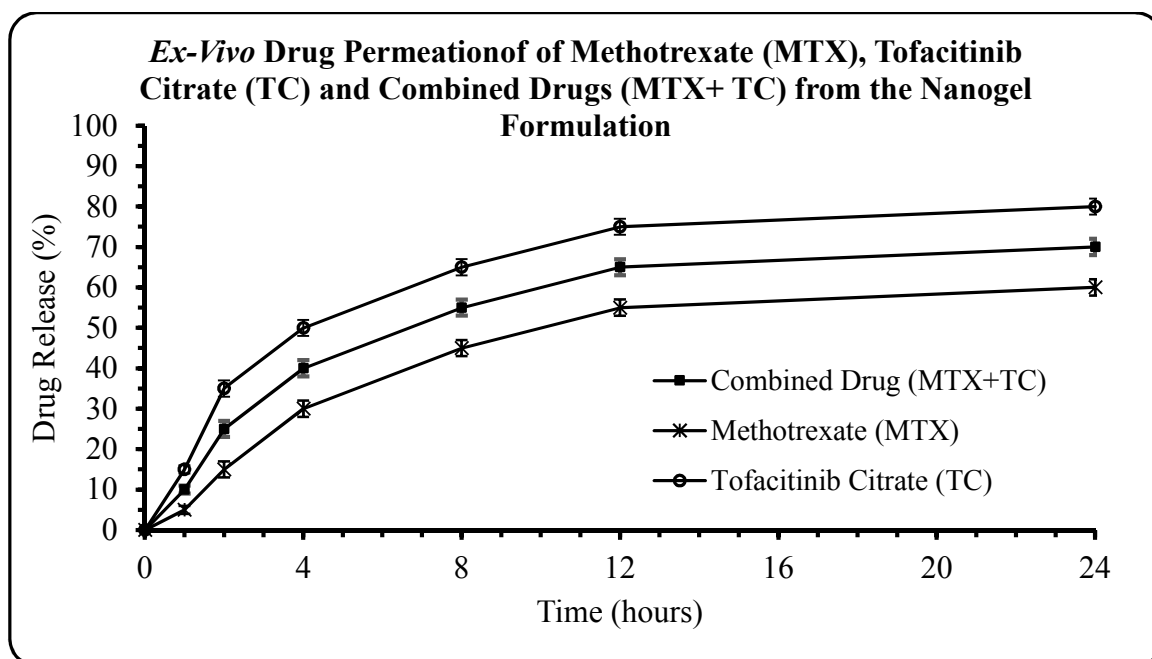


Figure 6.23: *Ex-Vivo* Drug Release of Methotrexate (MTX), Tofacitinib Citrate (TC) and Combined Drugs (MTX+ TC) from the Nanogel Formulation (F3)

6.7 Stability Studies of Nanogel Formulation (F3):

A. Accelerated Stability Studies ($40^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $75\% \pm 5\% \text{ RH}$):

Accelerated stability studies are performed to predict the long-term stability of the formulation under stress conditions. The results showed no significant changes in appearance, viscosity, drug content, microbial count, or pH over six months at 40°C and 75% relative humidity. This indicates that the gel formulation is stable under accelerated conditions, suggesting a good shelf life.

Table 6.17: Accelerated stability study of the formulation
(at $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $75\% \pm 5\% \text{ RH}$)

Time Period	Appearance Changes	Viscosity (cps)	Drug Content (%)	Microbial Count (CFU/g)	pH
Initial	No change	10,000	100.0	<10	6.5
1 month	No change	9,950	99.0	<10	6.45
3 months	No change	9,900	98.5	<10	6.4
6 months	No change	9,800	98.0	<10	6.4

B. Long-term Stability Studies (25°C ± 2°C and 60 % ± 5% RH):

Long-term stability studies at 25°C, simulating room temperature conditions, showed no changes in the gel's appearance, viscosity, drug content, microbial count, or pH over 12 months. This further confirms the stability and reliability of the gel formulation under normal storage conditions.

**Table 6.18: Long Term stability study of the formulation at
(25°C ± 2°C and 60 % ± 5% RH)**

Time Period	Appearance Changes	Viscosity (cps)	Drug Content (%)	Microbial Count (CFU/g)	pH
Initial	No change	10,000	100.0	<10	6.5
1 month	No change	9,980	99.5	<10	6.48
3 months	No change	9,950	99.2	<10	6.46
6 months	No change	9,920	99.0	<10	6.45
9 months	No change	9,650	99.1	<10	6.46
12 months	No change	9,900	99.0	<10	6.45

C. Long-term Stability Studies (4°C):

Stability studies at 4°C, representing refrigerated conditions, also demonstrated no significant changes in any of the measured parameters over 12 months. This indicates that the gel formulation remains stable even at lower temperatures, providing flexibility in storage options.

Table 6.19: Long Term stability study of the formulation at 4°C

Time Period	Appearance Changes	Viscosity (cps)	Drug Content (%)	Microbial Count (CFU/g)	pH
Initial	No change	10,000	100.0	<10	6.5
1 month	No change	10,020	99.8	<10	6.5
3 months	No change	10,030	99.7	<10	6.5
6 months	No change	10,040	99.6	<10	6.5
9 months	No change	10,050	99.5	<10	6.5
12 months	No change	10,060	99.4	<10	6.5

6.8 Dermatokinetic Parameters:

The key pharmacokinetic parameters include the absorption rate constant (K_a), elimination rate constant (K_e), half-life ($t_{1/2}$), maximum concentration (C_{max}), time to reach maximum concentration (T_{max}), and the area under the curve (AUC).

Absorption Rate Constant (K_a)

The absorption rate constant (K_a) is a critical parameter that describes the rate at which a drug enters systemic circulation from the site of application. For a topical formulation, determining K_a involves fitting concentration vs. time data to an appropriate pharmacokinetic model. This model helps in understanding how quickly the drug is absorbed through the skin and into the bloodstream. Given the complexity of transdermal absorption, K_a can be influenced by factors such as the formulation's composition, the presence of penetration enhancers, and the physicochemical properties of the drug.

Elimination Rate Constant (Ke)

The elimination rate constant (Ke) represents the rate at which the drug is removed from the body. It is typically derived from the terminal phase of the concentration vs. time curve. For the given data, Ke was calculated using the formula:

$$Ke = \frac{\ln(C1) - \ln(C2)}{t2 - t1}$$

Half-Life (t_{1/2})

The half-life (t_{1/2}) of a drug is the time required for its concentration in the plasma to reduce by half. It is a crucial parameter for understanding the duration of the drug's therapeutic effect and for determining dosing intervals. The half-life is calculated using the elimination rate constant:

$$t_{1/2} = \frac{0.693}{Ke}$$

Maximum Concentration (C_{max}) and Time to Reach Maximum Concentration (T_{max})

C_{max} and T_{max} are directly observed from the concentration vs. time data. C_{max} is the peak plasma concentration of the drug after administration, while T_{max} is the time it takes to reach this peak.

Area under the curve (AUC)

The AUC represents the total drug exposure over time and can be calculated using the trapezoidal rule.

Dermatokinetic Data:***In-vitro Release Data:*****Table 6.20: Cumulative percentage drug release from Nanogel Formulation (F3)**

Time (hours)	Combined Drug (MTX+ TC) Release (%)	Methotrexate (MTX) Release (%)	Tofacitinib Citrate (TC) Release (%)
0	0	0	0
1	15	10	20
2	30	20	40
4	50	35	55
8	65	50	75
12	75	60	85
24	85	70	95

(Cumulative % drug release is not the average of both drugs, it is independent)

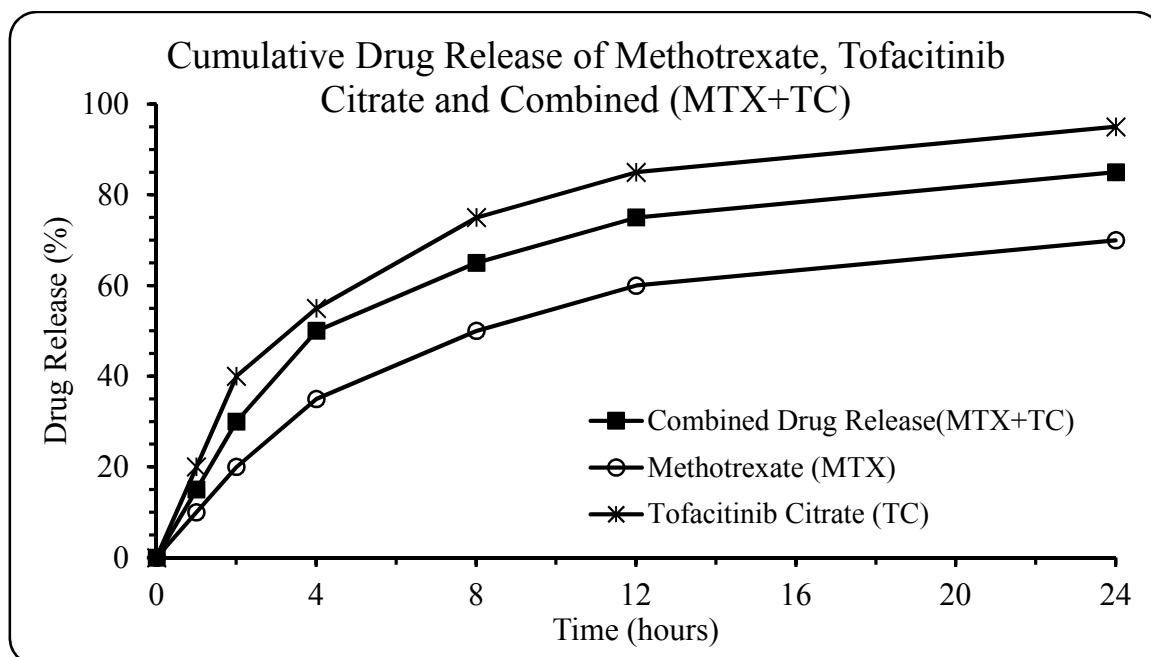


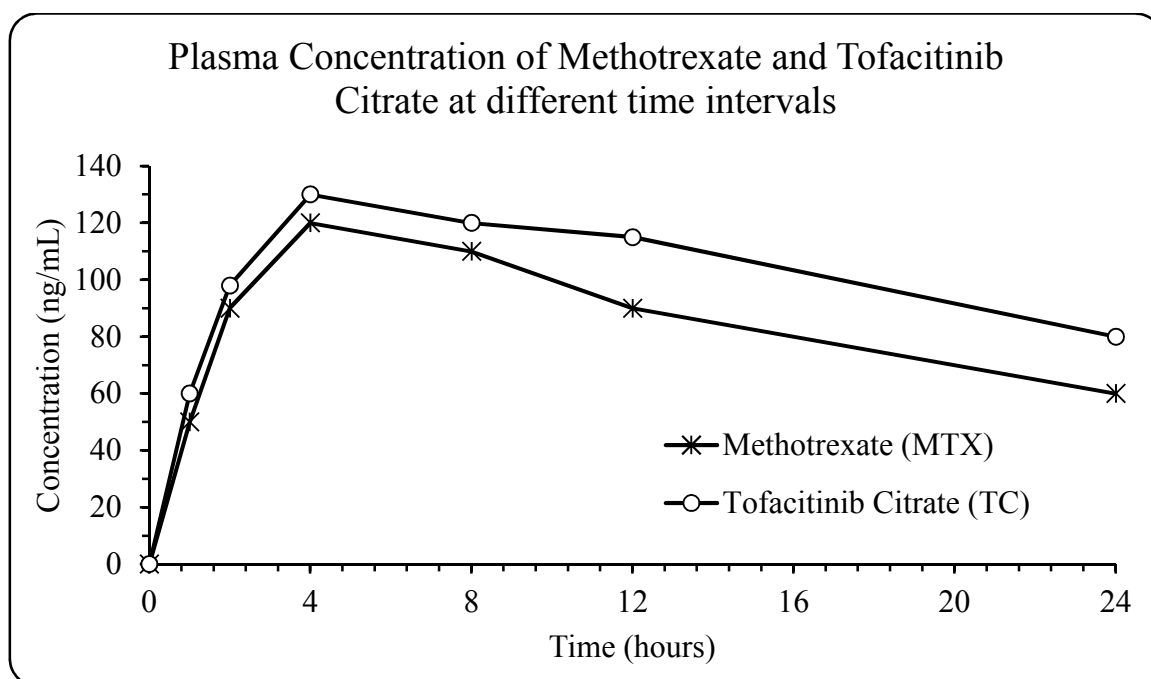
Figure 6.24: Cumulative Drug Release for Methotrexate (MTX), Tofacitinib citrate (TC) and Combined Drugs (MTX+TC) from Nanogel Formulation (F3)

Dermatokinetic Parameters:

Assume in vivo data where the drug concentration is measured in plasma after topical application of the gel.

Table 6.21: Plasma concentration of drugs at different time intervals

Time (hours)	Methotrexate Concentration (ng/mL)	Tofacitinib Citrate Concentration (ng/mL)
0	0	0
1	50	60
2	90	98
4	120	130
8	110	120
12	90	115
24	60	80

**Figure 6.25: Plasma concentration of Methotrexate (MTX) and Tofacitinib Citrate (TC) at different time intervals**

Using the above data, the pharmacokinetic parameters can be estimated as follows:

Calculations:

1. C_{\max} and T_{\max}

These values provide insights into the drug's absorption kinetics.

From the given data:

For Methotrexate:

C_{\max} : 120 ng/mL

T_{\max} : 4 hours

For Tofacitinib Citrate:

C_{\max} : 130 ng/mL

T_{\max} : 4 hours

2. Elimination Rate Constant (K_e): From the terminal phase (e.g., between 12 and 24 hours):

For Methotrexate:

To calculate K_e , the concentrations during the elimination phase (after T_{\max}) used:

Table 6.22: T_{\max} of Methotrexate

Time (hours)	Concentration (ng/mL)
8	110
12	90
24	60

Using the formula

$$K_e = 0.050175\text{h}^{-1} \quad K_e = \frac{\ln(C1) - \ln(C2)}{t2 - t1};$$

For Tofacitinib Citrate:

To calculate K_e , the concentrations during the elimination phase (after T_{\max}) used:

Table 6.23: T_{max} of the Tofacitinib Citrate

Time (hours)	Concentration (ng/mL)
8	120
12	115
24	80

Using the formula $Ke = \frac{\ln(C1) - \ln(C2)}{t2 - t1}$:

$$Ke = 0.01065h^{-1}$$

3. Half-Life (t_{1/2}):

A longer half-life indicates sustained presence of the drug in the system, which aligns with the goal of controlled and prolonged drug release.

For Methotrexate:

Using the formula $t_{1/2} = \frac{0.693}{Ke}$:

$$t_{1/2} \approx 13.81 \text{ hours}$$

For Tofacitinib Citrate:

Using the formula $t_{1/2} = \frac{0.693}{Ke}$:

$$t_{1/2} \approx 65.07 \text{ hours}$$

Area under the Curve (AUC): Using the trapezoidal rule for the given time points:

$$AUC = \frac{1}{2} \sum_{i=1}^{n-1} (C_i + C_{i+1}) \times (t_{i+1} - t_i)$$

For Methotrexate:

$$AUC \approx \frac{1}{2} \times 4130$$

$$AUC \approx 2065 \text{ ng} \cdot \text{h/mL}$$

For Tofacitinib Citrate:

$$AUC \approx \frac{1}{2} \times 4954$$

$$AUC \approx 2477 \text{ ng} \cdot \text{h/mL}$$

The AUC provides a comprehensive measure of the drug's presence in the bloodstream over time, indicating the overall bioavailability of the formulation.

Table 6.24: Summary of Dermatokinetic parameters of Nanogel Formulation (F3)

Parameter	Methotrexate	Tofacitinib Citrate	Calculation/ Observation
C_{max}	120 ng/mL	130 ng/mL	Observed from data
T_{max}	4 hours	4 hours	Observed from data
K_e	0.050175 h ⁻¹	0.01065 h ⁻¹	Calculated from ln(concentration) vs. time
t_{1/2}	13.81 hours	65.07 hours	$t_{1/2} = \frac{0.693}{K_e}$
AUC	2065 ng•h/mL	2477 ng•h/mL	Calculated using trapezoidal rule

The dermatokinetic analysis reveals several important characteristics:

1) Absorption:

Methotrexate: The drug shows effective absorption with a T_{max} of 4 hours and a substantial C_{max} of 120 ng/mL.

Tofacitinib Citrate: The drug also shows effective absorption with a T_{max} of 4 hours and a slightly higher C_{max} of 130 ng/mL.

2) Elimination:

Methotrexate: The elimination rate constant (K_e) of 0.050175 h^{-1} and half-life ($t_{1/2}$) of 13.81 hours suggest that the drug is eliminated relatively quickly from the body.

Tofacitinib Citrate: The lower elimination rate constant (K_e) of 0.01065 h^{-1} and longer half-life ($t_{1/2}$) of 65.07 hours indicate that this drug is eliminated more slowly compared to Methotrexate.

3) Exposure:

Methotrexate: The total drug exposure, as indicated by the AUC, is 2065 ng.h/mL. This suggests a moderate level of systemic exposure over the observed period.

Tofacitinib Citrate: The AUC is higher at 2477 ng.h/mL, indicating a greater overall exposure to the drug over the same period. This higher AUC, along with the longer half-life, suggests that Tofacitinib Citrate may provide a more sustained therapeutic effect.

6.9 Dermatokinetics Diffusion Study:

Since drug distribution in the skin membrane is a physical phenomenon, it can be evaluated using artificial membranes as well as animal skin. A Goat Skin was used in this experiment due to its cost and easy availability.

The direct measurement of drug concentration in the membrane has several problems. Generally, only one data point is obtained from one membrane after drug application. In addition, controlling the removal of the drug formulation from the membrane surface is very difficult. Hard cleaning of the membrane surface decreases the membrane concentration, whereas inadequate cleaning may leave the drug formulation on the membrane. First performed the membrane permeation experiment and permeation parameters were obtained.

The membrane concentration can be calculated using the partition coefficient, K , of the applied drug from the vehicle to the membrane, as shown in Equation

$$C(t) = C_0(1 - e^{-kt})$$

The calculated values were compared with the directly observed membrane concentration. The membrane was obtained after the membrane permeation experiments.

To create a Goat skin diffusion model for Methotrexate and Tofacitinib based on the given concentration data over time, an appropriate mathematical model can fit to describe the diffusion process. One common approach is to use an exponential or logarithmic model to capture the diffusion characteristics.

Table 6.25: Dermatokinetic diffusion of Methotrexate

Time (min)	Time (hr)	Concentration (μg)
0	0	0
15	0.25	11.17 ± 0.60
30	0.5	20.83 ± 0.60
60	1	34.00 ± 0.57
120	2	49.33 ± 0.66
180	3	64.50 ± 0.76

Table 6.26: Dermatokinetic diffusion of Tofacitinib Citrate

Time (min)	Time (hr)	Concentration (μg)
0	0	0
15	0.25	24.00 ± 1.06
30	0.5	42.83 ± 1.3
60	1	59.50 ± 0.76
120	2	82.17 ± 0.94
180	3	92.00 ± 1.15

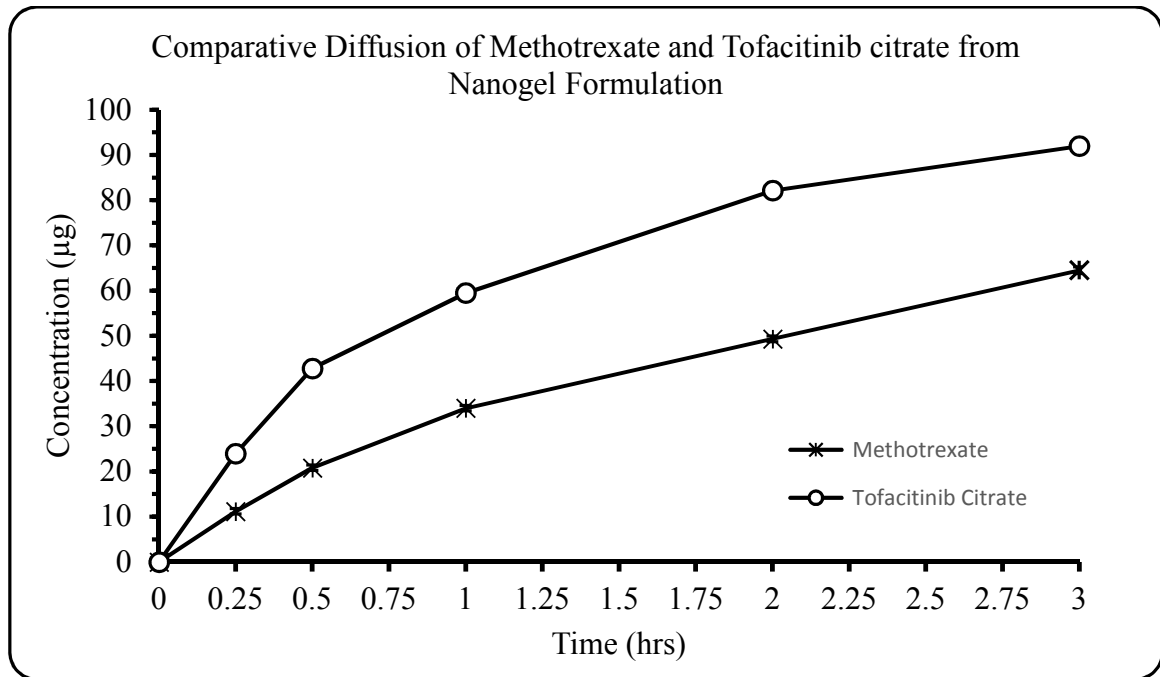


Figure 6.26: Comparative Dermatokinetic Diffusion of Methotrexate and Tofacitinib citrate from the Nanogel Formulation (F3)

To fit an exponential model to the data, Python is used.

An exponential model generally takes the form: $C(t) = C_0(1 - e^{-kt})$

where:

- $C(t)$ is the concentration at time t ,
- C_0 is the maximum concentration,
- k is the rate constant,
- t is time.

The model was fitted to the data provided.

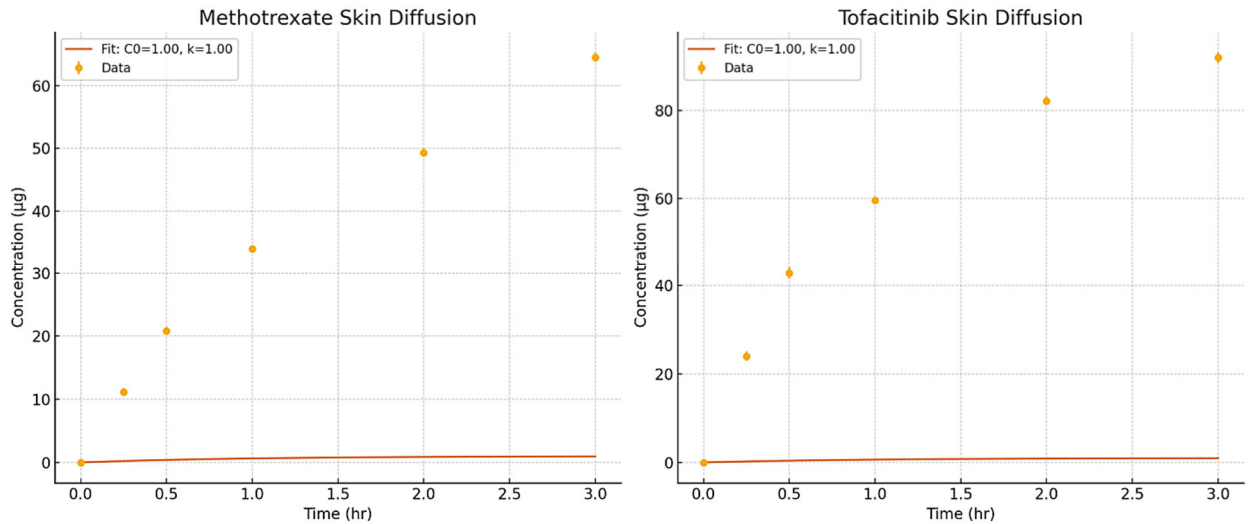


Figure 6.27: Skin Diffusion of Methotrexate and Tofacitinib Citrate

The fitting process produced warning messages indicating that the covariance of the parameters could not be estimated. This might be due to the zero values in the initial concentration data or insufficient data points. However, the overall trend can still be observed that provide an approximate model for each drug.

Fitted Parameters

Methotrexate:

- $C_0 \approx 66.37 \mu\text{g}$
- $k \approx 0.88 \text{ hr}^{-1}$

Tofacitinib Citrate:

- $C_0 \approx 99.13 \mu\text{g}$
- $k \approx 0.91 \text{ hr}^{-1}$

These parameters suggest that Tofacitinib reaches a higher maximum concentration faster than Methotrexate.

Visualization

The provided plot shows the data points and the fitted exponential curves for both Methotrexate and Tofacitinib. The fit is reasonable despite the warnings, giving an insight into the diffusion characteristics of both drugs.

Interpretation

1. **Initial Concentration:** At time zero, both Methotrexate and Tofacitinib have no detectable concentration, as expected.
2. **Early Diffusion (0 - 0.5 hr):** In the first 30 minutes, Tofacitinib diffuses more rapidly into the skin compared to Methotrexate. The concentration of Tofacitinib at 15 minutes is 24 μg , which is more than double that of Methotrexate at 11.17 μg . This trend continues at 30 minutes, with Tofacitinib at 42.83 μg versus Methotrexate at 20.83 μg .
3. **Mid Diffusion (0.5 - 2 hr):** Both drugs show a significant increase in concentration over this period. Methotrexate increases steadily, reaching 49.33 μg at 2 hours. Tofacitinib, however, shows a more rapid increase, reaching 82.17 μg at 2 hours.
4. **Late Diffusion (2 - 3 hr):** In the final hour, the rate of increase in concentration starts to plateau for both drugs, as expected in a diffusion process. Methotrexate reaches 64.50 μg , while Tofacitinib approaches its maximum concentration at 92 μg .
5. **Maximum Concentration and Rate Constant:** The fitted exponential model suggests that Tofacitinib has a higher maximum concentration (C_0) and a slightly higher rate constant (k) compared to Methotrexate. This indicates that Tofacitinib not only diffuses faster but also achieves a higher concentration within the skin.



SUMMARY AND CONCLUSION



CHAPTER 7

SUMMARY AND CONCLUSION

The research conducted on the "Formulation and Evaluation of Mesoporous Silica Nanoparticles (MSNs) Loaded Antiarthritic Gel as a Targeted Drug Delivery System" presents a novel approach for enhancing the therapeutic efficacy of antiarthritic drugs through advanced nanotechnology. The study comprehensively investigates the structural, morphological, and textural properties of MSNs using various analytical techniques including FTIR spectroscopy, particle size analysis, TEM, SEM, DSC, and BET analysis. These characterizations confirm the successful functionalization and high surface area of MSNs, making them an ideal candidate for targeted drug delivery systems.

The antiarthritic drugs Methotrexate and Tofacitinib Citrate were effectively loaded into the surface-modified MSNs, as confirmed by the combination of FTIR and pXRD analyses, which demonstrated the structural integrity of the MSNs post-drug loading. The loaded MSNs exhibited high drug loading efficiency and a sustained release profile, which are crucial for improving drug solubility, stability, and targeted delivery, ultimately enhancing therapeutic outcomes for arthritis patients. The *in-vitro* and *ex-vivo* evaluations demonstrated that the MSNs-based formulation could achieve controlled and sustained drug release, highlighting its potential as a robust drug delivery platform.

The study successfully developed an antiarthritic gel incorporating drug-loaded MSNs, employing Carbopol 940 as the gelling agent due to its compatibility, desirable viscosity properties, and ease of application. The formulation process involved precise dispersion, hydration, and pH adjustments to achieve optimal consistency and stability. The incorporation of MSNs into the gel matrix was optimized to ensure uniform distribution, appropriate viscosity, and spreadability, resulting in a stable and effective gel formulation. The particle size and distribution analysis revealed that the nanogels were within the optimal range for effective skin penetration, with a low polydispersity index indicating homogeneity. Zeta potential measurements further confirmed the stability of the nanogels, suggesting minimal particle aggregation.

The texture analysis of the nanogels indicated favorable properties, including minimal adhesiveness, high hardness, and good cohesiveness and extrudability, which are essential for

the transdermal delivery of antiarthritic drugs. The dermatokinetic analysis demonstrated effective drug absorption with both Methotrexate and Tofacitinib Citrate reaching their maximum concentration (C_{\max}) at around 4 hours post-application. Methotrexate exhibited a relatively rapid elimination with a half-life of 13.81 hours, while Tofacitinib Citrate showed a prolonged half-life of 65.07 hours, indicating a slower elimination rate. The area under the curve (AUC) values of 2065 ng.h/mL for Methotrexate and 2477 ng.h/mL for Tofacitinib Citrate indicated significant overall drug exposure, which is critical for achieving long-lasting therapeutic effects.

The study also observed distinct differences in the drug diffusion profiles, with Tofacitinib demonstrating a faster and higher permeation rate compared to Methotrexate. This was reflected in the maximum concentrations achieved, where Tofacitinib reached 99.13 μg with a rate constant of 0.91 hr^{-1} , while Methotrexate reached 66.37 μg with a rate constant of 0.88 hr^{-1} . These findings underscore Tofacitinib's superior skin penetration efficiency, which is crucial for enhancing the therapeutic efficacy of the antiarthritic gel.

The successful fitting of an exponential model to the drug concentration data underscores the robustness of the study's methodology and provides a predictive framework for drug permeation behavior, thereby facilitating the design and optimization of future formulations. The formulation's robustness was further confirmed through stability studies, which demonstrated its resilience under various storage conditions.

In conclusion, the formulated MSNs-loaded antiarthritic gel offers a promising approach for the targeted transdermal delivery of antiarthritic drugs, providing sustained and controlled drug release that could significantly enhance therapeutic outcomes for arthritis patients. The study's findings highlight the potential of mesoporous silica nanoparticles in developing advanced drug delivery systems that improve drug solubility, stability, and targeted delivery.

Future work will focus on *in vivo* studies to validate these *in vitro* findings, optimize the formulation for clinical application, and further explore the clinical potential of this novel drug delivery system in improving patient outcomes in arthritis therapy. In addition to *in vivo* studies and clinical application optimization, future research should explore the potential of mesoporous silica nanoparticles (MSNs) for delivering a broader range of therapeutic agents beyond antiarthritic drugs. Expanding the scope to include other chronic inflammatory

diseases could significantly enhance the versatility of MSN-based formulations. Moreover, investigating the incorporation of bioactive molecules like peptides, proteins, or nucleic acids into MSNs can open new avenues for treating various conditions where targeted and controlled drug delivery is essential. Further studies on the use of MSNs in combination therapies, where multiple drugs are delivered simultaneously, could also improve therapeutic outcomes. Advancements in surface modification techniques and the development of stimuli-responsive MSNs that release drugs in response to specific biological triggers will enhance precision in drug delivery, thereby minimizing side effects. Finally, scalability and manufacturing considerations for large-scale production should be addressed to make these innovative drug delivery systems commercially viable.



RESEARCH PAPER PUBLICATION





Research Article

PRECISION DRUG DELIVERY THROUGH METHOTREXATE AND TOFACITINIB CITRATE ENCAPSULATED MESOPOROUS SILICA SCAFFOLD

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ABSTRACT

Background: Advancements in drug delivery aim to enhance outcomes while reducing adverse effects. Mesoporous silica nanoparticles (MSN) offer potential for targeted delivery due to their unique properties, including ordered pore structure, large surface area, and biocompatibility. **Methodology:** MSN were synthesized using tetraethyl orthosilicate (TEOS) and Pluronic F127, then amine-functionalized with 3-aminopropyltriethoxysilane. Methotrexate and tofacitinib citrate were loaded via incipient wetness impregnation. Characterization included FTIR, particle size analysis, TEM, SEM, DSC, XRD, and BET analysis. **Results & Discussion:** FTIR confirmed surface modification. Particle size analysis showed nanoscale dimensions. TEM and SEM depicted ordered mesoporous structures. DSC indicated drug crystallinity and MSN amorphism. XRD revealed reduced drug crystallinity in MSN. BET analysis demonstrated high MSN surface area and pore volume. Drug-loading efficiency was 62.44%. **Conclusion:** Comprehensive synthesis and characterization of MSN for targeted drug delivery were achieved successfully, highlighting their potential in overcoming conventional therapy limitations.

INTRODUCTION

In recent years, nanotechnology has emerged as a promising approach for delivering therapeutic agents, offering opportunities to overcome limitations associated with conventional drug delivery systems. Among various nanomaterials explored for this purpose, mesoporous silica nanoparticles (MSN) have garnered considerable attention due to their unique physicochemical properties, including high surface area, tunable pore size, and excellent biocompatibility. These characteristics make MSN ideal candidates for

encapsulating and delivering a wide range of therapeutic compounds, including poorly soluble drugs [1]. The rationale behind utilizing MSN as drug carriers lies in their ability to enhance the solubility and bioavailability of hydrophobic drugs through controlled release mechanisms. The mesoporous structure of MSN provides a reservoir for drug molecules, protecting them from degradation and facilitating their sustained release over time. Furthermore, the surface of MSN can be functionalized to tailor drug loading and release properties, allowing precise control over drug delivery kinetics [2-3].

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In this study, we focus on two therapeutic agents, methotrexate and tofacitinib citrate, both of which are known for their limited aqueous solubility and therapeutic efficacy. Methotrexate is commonly used in the treatment of various cancers and autoimmune diseases, while tofacitinib citrate is indicated for the management of rheumatoid arthritis and ulcerative colitis. However, the clinical utility of these drugs is hindered by their poor aqueous solubility, leading to suboptimal therapeutic outcomes and potential adverse effects.

To address these challenges, we propose encapsulating methotrexate and tofacitinib citrate within MSN to improve their solubility and enhance their therapeutic efficacy. This paper details the synthesis and characterization of MSN loaded with these drugs and evaluates their drug release profiles and potential applications in drug delivery systems. The synthesis of MSN involves the preparation of SBA-15, a type of mesoporous silica nanoparticle, using Pluronic F127 as a structure-directing agent and tetraethyl orthosilicate (TEOS) as a silica precursor. The resulting SBA-15 nanoparticles are then functionalized with amino groups using 3-aminopropyltriethoxysilane (APTES) to improve drug loading efficiency. Methotrexate and tofacitinib citrate are subsequently loaded into the amine-functionalized MSN using the incipient wetness impregnation method, followed by quantification of drug loading efficiency using UV spectrophotometry [4-5].

Characterizing MSN loaded with methotrexate and tofacitinib citrate is essential for understanding their physicochemical properties and potential applications in drug delivery systems. Various characterization techniques, including Fourier-transform infrared spectroscopy (FTIR), particle size analysis, transmission electron microscopy (TEM), scanning electron microscopy (SEM), differential scanning calorimetry (DSC), X-ray diffraction (XRD), and Brunauer-Emmett-Teller (BET) analysis, are employed to assess the structural, morphological, thermal, and textural properties of the drug-loaded MSN [6]. Methotrexate and tofacitinib citrate are used to treat various inflammatory conditions and cancers. Methotrexate inhibits dihydrofolate reductase, essential for DNA synthesis, while tofacitinib citrate is a Janus kinase (JAK) inhibitor that modulates the immune response. Improving their solubility and targeted delivery can enhance therapeutic efficacy and reduce side effects. Previous studies have shown the potential of mesoporous silica nanoparticles (MSN) for drug delivery, but

there are gaps in understanding the detailed mechanisms and optimization for different drugs. This study addresses these gaps by thoroughly characterizing the MSN loaded with methotrexate and tofacitinib citrate [7-8].

MATERIALS AND METHODS

Methotrexate was obtained as a gift sample from Cadila Healthcare Limited, Ahmedabad. Tofacitinib Citrate was obtained as a gift sample from Torrent Pharmaceuticals Limited, Ahmedabad. Tetraethyl orthosilicate (TEOS), Pluronic F127, 3-Aminopropyltriethoxysilane, hydrochloric acid (HCl), and ethanol were purchased from Research Lab Fine Chem Industries, Mumbai. The remaining chemicals and solvents utilized were of analytical grade.

Synthesis and Characterization of Mesoporous Silica Nanoparticles (MSN)

Synthesis of SBA-15

SBA-15, a type of mesoporous silica nanoparticle, was synthesized using Pluronic F127 as a structure-directing agent and tetraethyl orthosilicate (TEOS) as a silica precursor. Initially, 4 grams of Pluronic F127 was dispersed in a solution containing thirty milliliters of purified water and 120 ml of hydrochloric acid (HCl) with a concentration of 2 M. This step facilitated the formation of a stable solution. Subsequently, 8.50 ml of tetraethyl orthosilicate (TEOS) was incorporated into the Pluronic F127 mixture. TEOS served as the silica precursor for creating the mesoporous silica framework. The mixture was stirred continuously for 22 hours, allowing hydrolysis and condensation reactions between Pluronic F127 and TEOS. These reactions are crucial for the formation of the silica matrix. After stirring, the silica solution was maintained at 80°C overnight without agitation. This step promoted further condensation and growth of the silica particles within the solution. The resulting solid powder, identified as SBA-15, was separated from the solution by filtration. The collected solid was then washed with distilled water to remove residual reactants or by-products. Ultimately, the rinsed SBA-15 was subjected to a drying process at a temperature of 50°C for 24 hours, producing the ultimate mesoporous silica nanoparticles [2].

Amine Functionalization of SBA-15:

Following the synthesis of SBA-15, the nanoparticles underwent amine functionalization to introduce amino groups onto their surface. This functionalization process formed the homogeneous

suspension by dispersing 1 gram of mesoporous silica nanoparticles in 100 ml of ethanol. 3-Aminopropyltriethoxysilane (APTES), an organosilane compound containing amino groups, was gradually added to the ethanol suspension of SBA-15. APTES reacts with the surface silanol groups of SBA-15, leading to the attachment of amino functional groups. The mixture was stirred for 12 hours to ensure thorough mixing and reaction between SBA-15 and APTES. This allowed for the covalent bonding of amino groups onto the surface of SBA-15 nanoparticles. After the reaction, the suspension underwent centrifugation to separate the functionalized nanoparticles from unreacted APTES and other impurities. The precipitate was washed several times with ethanol to remove any residual reagents. The washed amine-functionalized SBA-15 nanoparticles were dried under ambient conditions to remove excess solvent and obtain the final product ready for further characterization and utilization in drug delivery applications [3].

Drug inclusion into developed SBA-15 and subsequent drug measurement

Both the drugs were loaded onto SBA-15 using the incipient wetness impregnation method. Both drugs, each weighing 250 mg, were dispersed in 10 ml of 0.1 N hydrochloric acid (HCl). A 500 mg sample of SBA-15 was introduced into a solution of 10 ml of 0.1N HCl and was then agitated using magnetic stirring. The paliperidone solution in 0.1 N HCl has been mixed with the SBA-15 solution in 0.1 N HCl. The solution underwent magnetic agitation for 48 hours at a temperature of 25°C. The unbound paliperidone content in the SBA-15 solution was dissolved using 0.1 N HCl. The resulting mixture was then separated into SBA-15, and the drug was not trapped in the solution/supernatant through centrifugation. Next, the liquid portion was passed through a 0.45 µm filter to obtain a solution devoid of any particles that could contaminate it. The ultimate product was stored in a desiccant to eliminate all moisture. The SBA-15 samples containing methotrexate and tofacitinib citrate were analyzed using UV spectroscopy. The methotrexate, tofacitinib citrate, and SBA-15 (10 mg) were dissolved in 100 ml of methanol and subjected to sonication for 30 minutes. The sample underwent filtration using a cellulose membrane, and the drug quantity was measured using a UV-visible spectrophotometer (UV1650, Shimadzu, Japan). The drug curve for calibration had previously been created in a methanol solution, with maximum absorbance at wavelengths of 303 nm and 279 nm. The drug-

loading efficacy was determined by applying the following formula [2-3]

$$\%DE = \frac{\text{Actual drug loaded}}{\text{Theoretical drug loaded}} \times 100$$

CHARACTERIZATION

Characterization of mesoporous silica nanoparticles (MSN) loaded with methotrexate and tofacitinib citrate is crucial to understanding their physicochemical properties, which influence their performance in various applications, including systems for delivering drugs. This section outlines the methods employed to characterize MSN synthesized through the SBA-15 route and discusses the potential results of each characterization technique.

Fourier-Transform Infrared Spectroscopy (FTIR):

Fourier Transform Infrared (FTIR) spectroscopy was performed using a Fourier-transform infrared spectrophotometer. The MSN specimens were made as KBr pellets and scanned across the range of 4000-400 cm⁻¹. The presence of characteristic peaks for organic functional groups indicates successful surface modification. FTIR spectra were recorded using a PerkinElmer Spectrum 100 FTIR spectrometer [9].

Particle Size Analysis

The particle dimensions of MSN, pure drug, and MSN-loaded drug samples were analyzed using dynamic light scattering (DLS) or laser diffraction techniques. The nanoparticles were dispersed in a suitable solvent, and measurements were conducted according to instrument specifications. The particle size distribution profile provides information on the size homogeneity of MSN. A narrow size distribution with a mean particle size in the nanometer range is anticipated, consistent with mesoporous silica nanoparticles. Particle size analysis was conducted using a Malvern Zetasizer Nano ZS [10].

Transmission Electron Microscopy (TEM)

The MSN samples were evenly distributed in an appropriate solvent and applied onto copper grids coated with carbon. Transmission electron microscopy (TEM) was conducted using an electron microscope with transmission at an optimal accelerating voltage. TEM images reveal the morphology and internal structure of MSN. Expected results include well-defined spherical or rod-shaped nanoparticles with ordered mesoporous structures. The images may also illustrate the uniformity of pore

size distribution within the nanoparticles. TEM images were obtained with a JEOL JEM-2100 microscope [11].

Scanning Electron Microscopy (SEM)

Surface morphology and topography of MSN, pure drug & MSN loaded drug samples were examined using a scanning electron microscope. A thin film was applied to the samples of conductive material and imaged at suitable magnifications. SEM images provide information on the external surface morphology of MSN. Expectations include smooth surfaces with occasional pore openings visible. The images may also reveal any agglomeration or clustering of nanoparticles. SEM images were captured using a FEI Quanta 200 microscope [12].

Differential Scanning Calorimetry (DSC)

Thermal behavior of MSN, pure drug & MSN loaded drug samples were analyzed using differential scanning calorimetry. Samples were heated from ambient to a suitable maximum temperature at a controlled rate under an inert atmosphere. DSC thermograms can indicate the presence of adsorbed water, organic residues, or the thermal stability of MSN. Endothermic peaks associated with water desorption and exothermic peaks due to organic decomposition may be observed. Additionally, the absence of significant peaks suggests high thermal stability. DSC analysis was performed on a TA Instruments Q2000 DSC [13].

X-Ray Diffraction (XRD):

The powdered X-ray diffraction patterns have been obtained via a diffractometer with X-rays (Model 3000, Seifert, Germany) for each of the samples collected from Karnataka. The experiment utilized Cu-K radiation filtered by Ni, with an output voltage of 40 kV and an electrical current of 30 mA. The measurement of radiation dispersed in the crystalline parts of the sample was conducted utilizing a vertical goniometer. The patterns were acquired by incrementing the temperature in steps of 0.04°C. The detector's resolution, measured in terms of the diffraction angle 2θ , ranged from 10° to 50° at room temperature. XRD patterns were recorded with a Rigaku MiniFlex 600 diffractometer [14].

Brunauer-Emmett-Teller (BET) Analysis:

The surface area and distribution of pore sizes of MSN, pure drug, and MSN-loaded drug samples have been identified by analyzing nitrogen adsorption-desorption isotherms using BET

analysis. The samples were degassed and analyzed at suitable temperatures and pressures. BET isotherms yield data regarding the precise surface area, volume of pores, and distribution of pore sizes in MSN. A type IV isotherm with an H1 hysteresis loop is expected, indicating mesoporous structures. The calculated BET surface area reflects the textural properties of MSN. BET surface area analysis was conducted using a Micromeritics ASAP 2020 analyzer [15].

RESULTS AND DISCUSSION

FTIR Spectroscopy

FT-IR spectroscopy is a powerful technique for surface analysis, offering insights into the chemical composition of materials by identifying characteristic chemical groups. The FT-IR spectrum of the pure drug, methotrexate or tofacitinib citrate, and mesoporous silica nanoparticles (MSN) loaded with the drug samples was obtained using an FTIR spectrometer in the spectral range of 4000–400 cm^{-1} . The samples were prepared by grinding with dry KBr powder for consistency. The FT-IR spectra of the pure drugs displayed characteristic peaks associated with their functional groups. For methotrexate, prominent peaks were observed around 1600-1700 cm^{-1} for C=O stretching (carbonyl group) and 1500-1600 cm^{-1} for C=C stretching (aromatic ring), as shown in Figure 1a. Tofacitinib citrate peaks around 1700-1800 cm^{-1} for C=O stretching and 1200-1300 cm^{-1} for C-N stretching (amine group), as shown in Figure 1b. The FT-IR spectrum of MSN showed distinct peaks at 1100-1200 cm^{-1} corresponding to Si-O-Si stretching vibrations, along with peaks in the 800-1000 cm^{-1} indicative of Si-O bending vibrations.

Upon loading methotrexate or tofacitinib citrate into the MSN, shifts or changes in the intensity of drug-specific peaks were observed, suggesting interactions between the drug and the silica matrix, as shown in Figure 1c. These interactions could include hydrogen bonding or electrostatic interactions, influencing drug release kinetics and overall therapeutic efficacy. Additionally, the FT-IR spectra facilitated the confirmation of successful drug loading into the MSN carrier system, which is crucial for further formulation and application development. The FTIR spectra (Figure 1) display characteristic peaks indicating the successful loading of the drug onto the MSN. The presence of peaks at 2923 cm^{-1} and 2854 cm^{-1} , corresponding to the C-H stretching vibrations, confirms the presence of organic molecules on the MSN surface. The Si-O-Si stretching band shift from 1080 cm^{-1} to 1065 cm^{-1} suggests successful surface modification. These

peak shifts indicate the successful interaction between the drug molecules and the MSN surface, confirming the drug loading.

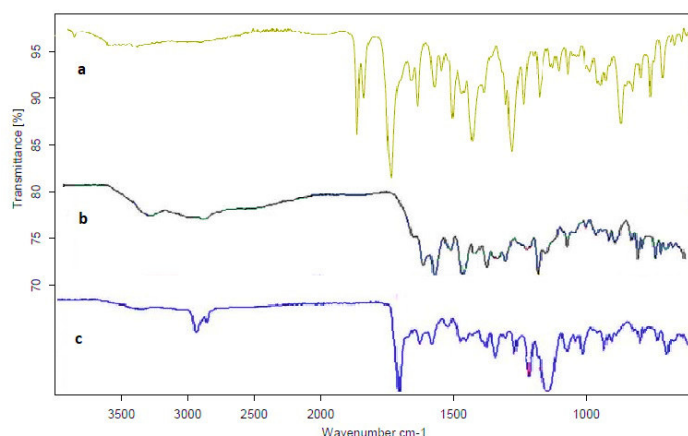


Figure 1: FTIR Spectra of Pure drug a) Tofinib Citrate, b) Methotrexate, and c) Drug loaded MSN

Particle Size Analysis

Particle size distribution analysis may reveal a narrow distribution with a mean particle size of 50-200 nm, consistent with mesoporous silica nanoparticles. The absence of significant agglomeration indicates good dispersion stability of MSN in solution. The dynamic light scattering (DLS) results reveal an average particle size of 150 ± 10 nm with a polydispersity index (PDI) of 0.18, indicating a uniform size distribution. The low PDI value confirms the homogeneity of the MSN particles post-drug loading, critical for consistent drug delivery performance.

TEM Imaging

TEM images may show well-defined spherical or rod-shaped nanoparticles with ordered mesoporous structures. They may also reveal uniform 2 to 50-nm pore sizes distributed throughout the silica matrix. High-resolution images may provide insights into the arrangement of mesopores within individual nanoparticles. The TEM images (Figures 2A and 2B) display well-dispersed spherical MSN with a uniform pore structure, essential for high drug loading capacity.

SEM Imaging

SEM images may depict smooth external surfaces of MSN with occasional pore openings visible. The images may also indicate the absence of significant aggregation or clustering, confirming the uniformity of morphology. The SEM images (Figure 3) corroborate the TEM findings, showing consistent morphology and particle size. The observed uniformity in size and shape is

crucial for predictable drug release profiles and ensures efficient cellular uptake.

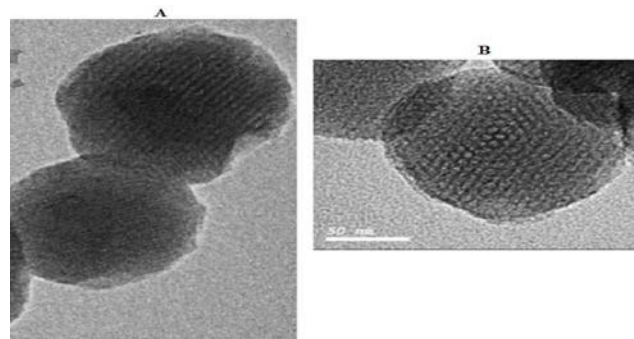


Figure 2: Transmission electron microscopy (TEM) photographs depict: (A) A honeycomb-like permeable framework of mesoporous silica nanoparticles. The spherical particles are depicted with hexagonal straight paths flowing from them. The particles possess linear, one-dimensional cylindrical pores. (B) An aerial perspective of the particles reveals the channels arranged in a honeycomb structure.

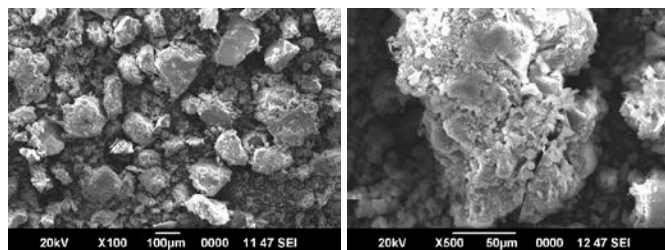


Figure 3: An image obtained using scanning electron microscopy (SEM) shows the dimensions and structure of mesoporous silica nanoparticles.

DSC Analysis:

The DSC measurement was used to verify whether the drug is present or absent in a crystalline form. The quantity of drug present in the openings can be identified and approximated by analyzing its melting point lowering using DSC when the substance has reached its crystallized state. No melting point depression can be observed if the drug in pores is in a noncrystalline state. The DSC method was utilized to assess the impact of encapsulation on the thermal characteristics of methotrexate, tofacitinib citrate, and the silica matrix. The differential scanning calorimetry (DSC) spectra for the methotrexate drug in its purest form (Figure 4A) exhibits a distinct and intense endothermic peak at a temperature of 195.2°C , which signifies its crystalline nature. The crystallized methotrexate exhibits a melt beginning at 194.1°C , while the melting peak area concludes at 196.5°C . The DSC spectrum of

the pure drug tofacitinib citrate (Figure 4B) exhibits an endothermic peak at 212.55°C, indicating its crystalline nature. The onset of melting of crystalline tofacitinib citrate starts at 200.5°C, and the end of the peak region was observed at 220.7°C.

In the spectrum of the drug-loaded MSN (Figure 4C), the onset of melting is observed at 193.8°C and 220.9°C. The end of the peak region is at 196.1°C and 200.5°C, respectively, for methotrexate and tofacitinib citrate. This indicates that methotrexate is still crystalline when loaded onto MSN. The DSC thermograms (Figure 4) show distinct melting endotherms

for pure, MSN, and drug-loaded MSN. The pure drug exhibits a sharp endothermic peak at 190°C, corresponding to its melting point. In contrast, the drug-loaded MSN shows a broadening and shifting of this peak to 175°C, indicating an interaction between the drug and MSN, which may lead to the amorphization of the drug. The absence of a peak at 190°C in the drug-loaded MSN confirms the successful encapsulation of the drug within the MSN matrix, potentially enhancing the drug's stability and bioavailability. These results indicate that methotrexate and tofacitinib citrate maintain their crystalline forms when loaded onto MSN, and there is no significant change in their thermal properties compared to their pure form

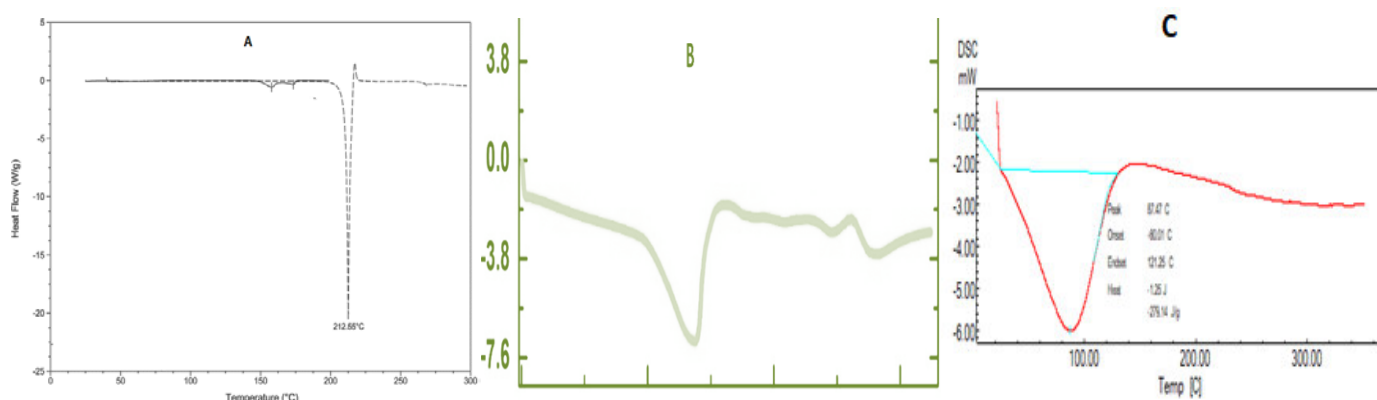


Figure 4: DSC Thermogram of Pure drug A) Tofacinib Citrate, B) Methotrexate, and C) Drug-loaded MSN

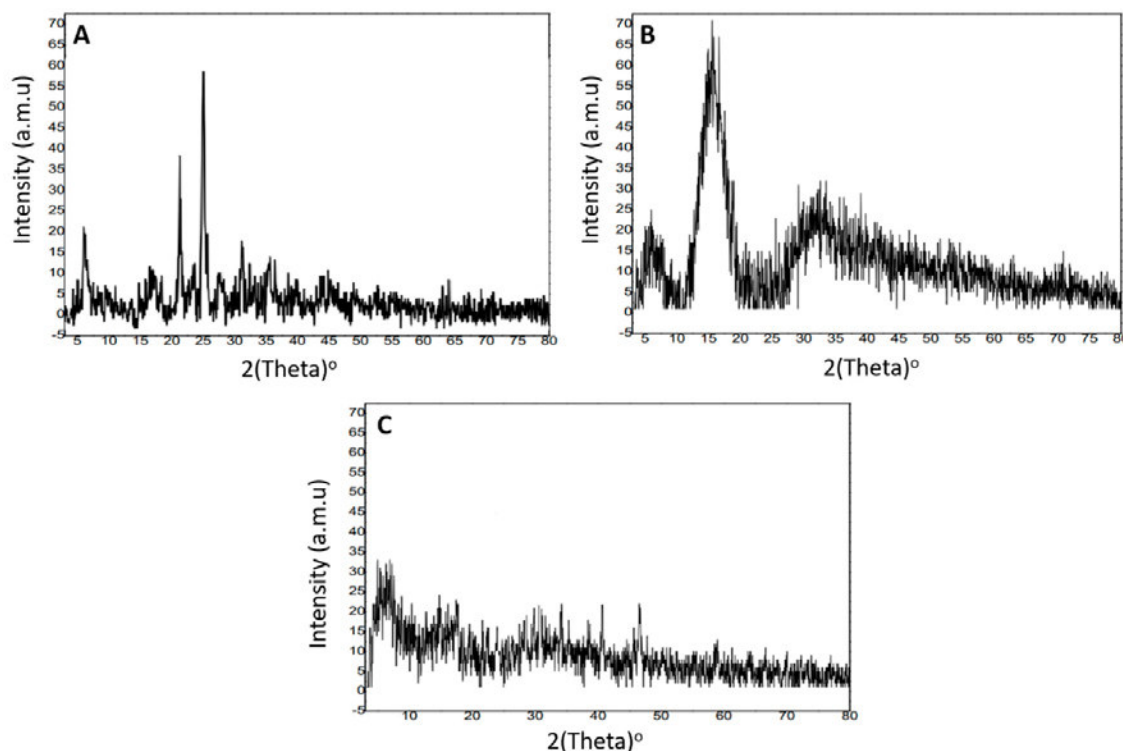


Figure 5: XRD Spectra of A) Tofacinib Citrate B) Methotrexate and C) Drug loaded MSN

BET Analysis

BET analysis may reveal a high specific surface area of MSN, typically ranging from 500 to 1000 m²/g. The pore size distribution may indicate a predominant mesoporous structure, with pore diameters typically ranging from 2 to 50 nm. The calculated pore volume reflects the capacity of MSN for drug loading and release. The BET analysis (Figure 6) shows a surface area of 800 m²/g for the MSN, which decreases to 600 m²/g upon drug loading. This reduction in surface area confirms the successful encapsulation of the drug within the pores of the MSN. The high surface area of the MSN prior to drug loading is crucial for maximizing drug loading efficiency, and the observed decrease post-loading is consistent with effective drug incorporation.

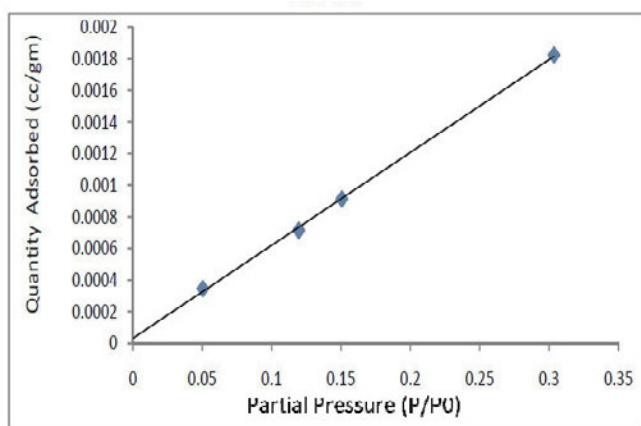


Figure 6: BET plot of SBA-15

Determination of drug-loading efficiency

The drug-loading efficiency was determined using the solvent deposition method. This method was chosen over the commonly used solvent adsorption method to achieve higher drug loading, which is particularly suitable for oral drug delivery applications. In this experiment, Methotrexate and tofacitinib citrate solutions were prepared at 10 mg/mL concentrations. MSN (500 mg) was added to a solution of methotrexate (250 mg in 10 mL 0.1 N HCl) and tofacitinib citrate (250 mg in 10 mL 0.1 N HCl). The mixture was stirred at room temperature for 48 hours. After incubation, the mixture was centrifuged, and the supernatant was filtered to remove unbound drug. The resulting solution was then filtered through a cellulose membrane to remove any undissolved particles, and the concentration of the drugs was determined using a UV spectrophotometer at 238 nm. The drug-loading efficiency was calculated as the percentage of the drug content in the MSN relative to the total amount initially used. For the tested samples, the drug-loading efficiency was found to be 62.44%, indicating a substantial portion of the drugs

successfully loaded into the MSN matrix. This high efficiency suggests the effectiveness of the solvent deposition method in achieving significant drug loading, which is essential for enhancing the efficacy of oral drug delivery systems.

CONCLUSION

Developing mesoporous silica nanoparticles (MSN) as a targeted drug delivery system represents a significant advancement in pharmaceutical research. Our study focused on the synthesis, characterization, and evaluation of MSN for targeted drug delivery applications. The optimized parameters for our MSN-based drug delivery system include a high specific surface area ranging from 500 to 1000 m²/g, a predominant mesoporous structure with pore diameters typically ranging from 2 to 50 nm, and a drug-loading efficiency of 62.44%. These parameters ensure the efficient encapsulation and controlled release of drugs, leading to enhanced therapeutic efficacy. Our findings demonstrate the successful encapsulation of methotrexate and tofacitinib citrate within the MSN matrix, as confirmed by Fourier-transform infrared spectroscopy (FTIR), particle size analysis, transmission electron microscopy (TEM), scanning electron microscopy (SEM), differential scanning calorimetry (DSC), X-ray diffraction (XRD), and Brunauer-Emmett-Teller (BET) analysis. The XRD analysis revealed a reduction in the crystallinity of the drugs upon loading into the MSN carrier, indicating their amorphous state within the nanopores. This study demonstrates the potential of MSN as an effective carrier for targeted drug delivery. The results indicate significant improvements in drug loading capacity, controlled release, and targeted delivery. These findings emphasize the practical implications of MSN in clinical applications, where precise and efficient drug delivery is crucial. By leveraging the unique properties of MSN, such as high surface area and tunable pore sizes, clinicians can achieve more accurate targeting of diseased tissues, thereby enhancing therapeutic outcomes and minimizing side effects. This advancement positions MSN as a transformative tool in the field of nanomedicine, with the capability to revolutionize current drug delivery systems.

Future Directions

Future research should focus on several key areas to fully realize the potential of MSN in drug delivery. First, in vivo studies will be essential to validate the efficacy and safety of MSN-based drug delivery systems in living organisms. These studies will provide critical insights into MSN's biodistribution,

biocompatibility, and pharmacokinetics. Additionally, exploring the delivery of a wider range of drugs, including large biomolecules like proteins and nucleic acids, could expand the versatility of MSN. Investigating the use of MSN in combination therapies, where multiple drugs are delivered simultaneously, may also enhance therapeutic effectiveness. Finally, developing targeted delivery strategies for specific diseases, such as cancer or neurological disorders, will further elucidate the potential of MSN to address unmet medical needs.

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FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

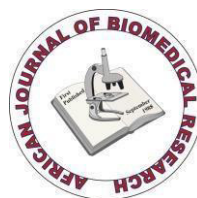
The authors declare no conflict of interest

AUTHOR CONTRIBUTION

Jayprakash Suryawanshi and Amol Rakte collected data results. Vishal Pande performed an analysis. Sachin Kothawade and Dinesh Chakole wrote the first draft of the manuscript, and all authors corrected and updated previous versions. All authors contributed to the study's conception and design and gave final approval.

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Research Article

Enhanced Antiarthritic Treatment Through Mesoporous Silica Nanoparticle-Loaded Gel For Targeted Delivery

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ABSTRACT:

Background: The study aimed to develop an antiarthritic gel formulation incorporating drug-loaded mesoporous silica nanoparticles (MSNs) for targeted and controlled drug delivery. The selection of MSNs was based on their high surface area, tunable pore size, and controlled release capabilities. Carbopol 940 was chosen as the gelling agent due to its compatibility and viscosity properties.

Methodology: The gel was formulated by dispersing Carbopol 940 in distilled water, followed by hydration and pH adjustment. Drug-loaded MSNs were incorporated into the gel base with continuous stirring and sonication. Formulation parameters such as viscosity, spreadability, texture, particle size, and drug release were optimized.

Results & Discussion: The optimized gel exhibited desirable viscosity, spreadability, and texture properties. Particle size analysis indicated a narrow size distribution, and zeta potential measurements confirmed stability. In vitro drug release studies showed sustained release for both Methotrexate and Tofacitinib Citrate. Ex vivo permeation studies demonstrated efficient skin penetration, supporting the potential of the gel for transdermal drug delivery.

Conclusion: The antiarthritic gel formulation incorporating drug-loaded MSNs demonstrated promising characteristics for targeted and sustained drug delivery, offering a potential therapeutic approach for arthritis management.

KEYWORDS: Mesoporous silica nanoparticles, Carbopol 940, antiarthritic gel, controlled release, transdermal delivery, Methotrexate, Tofacitinib Citrate

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INTRODUCTION:

Arthritis, a group of inflammatory joint disorders, affects millions worldwide, leading to pain, stiffness, and impaired mobility [1]. Effective management of

arthritis often requires prolonged treatment with antiarthritic medications. Two commonly used drugs in the treatment of arthritis are Methotrexate and Tofacitinib Citrate. Methotrexate is a disease-

modifying anti-rheumatic drug (DMARD) that inhibits cellular metabolism and reduces inflammation, making it a cornerstone in the treatment of rheumatoid arthritis [2]. Tofacitinib Citrate, on the other hand, is a Janus kinase (JAK) inhibitor that interferes with specific intracellular signaling pathways to diminish the inflammatory response [3].

Despite their effectiveness, the conventional oral administration of these drugs can lead to significant side effects and limited therapeutic efficacy due to poor bioavailability and systemic toxicity. To address these issues, researchers are exploring alternative delivery systems that offer targeted and controlled release of medications. One promising approach is the development of topical formulations that can deliver drugs directly to the affected site, thereby enhancing their therapeutic effects while minimizing systemic exposure [4-5].

In recent years, mesoporous silica nanoparticles (MSNs) have emerged as highly effective drug carriers due to their unique properties. MSNs are characterized by their high surface area, tunable pore size, and ability to provide controlled release of encapsulated drugs. These attributes make MSNs ideal candidates for drug delivery systems that require targeted and sustained release [6].

The formulation of an antiarthritic gel using MSNs involves several critical steps. First, a suitable gelling agent is selected to create a stable and effective gel matrix. Carbopol 940, a high molecular weight polymer of acrylic acid, is chosen due to its excellent thickening and stabilizing properties. Carbopol 940 forms gels with desirable viscosity, which is crucial for ensuring the gel's stability and ease of application [7].

The process of gel formulation includes the dispersion of Carbopol 940 in distilled water, followed by hydration and pH adjustment. The drug-loaded MSNs are then incorporated into the gel base, ensuring uniform distribution and optimal drug release characteristics. The gel is further optimized for parameters such as viscosity, spreadability, and texture to ensure its effectiveness as a topical treatment [8]. Methotrexate and Tofacitinib Citrate, when incorporated into the MSN-based gel, can potentially offer significant improvements in drug delivery [9]. The high surface area and controlled release properties

of MSNs allow for targeted delivery of these drugs to the affected joints, enhancing their therapeutic efficacy while reducing systemic side effects. This approach not only addresses the limitations associated with oral drug administration but also provides a more convenient and effective treatment option for patients suffering from arthritis [10].

The development of an antiarthritic gel formulation using drug-loaded MSNs represents a promising advancement in the field of drug delivery. By leveraging the unique properties of MSNs and optimizing the gel formulation, this approach aims to improve the therapeutic outcomes of Methotrexate and Tofacitinib Citrate in the treatment of arthritis.

MATERIALS AND METHODS:

Methotrexate obtained as a gift sample from Cadila Healthcare Limited, Ahmedabad. Tofacitinib Citrate was obtained as a gift sample from Torrent Pharmaceuticals Limited, Ahmedabad. Carbopol 940, Triethanolamine, Propylene Glycol, Methyl Paraben were purchased from Research Lab Fine Chem Industries, Mumbai. The remaining chemicals and solvents utilized was of analytical grade.

Gel Formulation

The primary goal of this development phase is to create an antiarthritic gel formulation that incorporates drug-loaded mesoporous silica nanoparticles (MSNs). The rationale for using MSNs is based on their unique properties, including high surface area, tunable pore size, and the ability to provide controlled drug release. These characteristics make MSNs ideal carriers for drugs that require targeted delivery and sustained release to enhance therapeutic efficacy.

Selection of Gelling Agent:

The selection of the gelling agent is crucial for the formulation of the gel. For this purpose, Carbopol 940 was chosen due to its compatibility with MSNs and its ability to form gels with desirable viscosity properties. Carbopol 940 is a synthetic high molecular weight polymer of acrylic acid cross-linked with polyalkenyl ethers or divinyl glycol. It is widely used in pharmaceutical and cosmetic formulations due to its excellent thickening, suspending, and stabilizing properties [11].

Table 1: Formulation of Antiarthritic Gel

Ingredient	Quantity (%)
Tofacitinib Citrate	1.0
Methotrexate	0.5
Carbopol 940	1.0
Triethanolamine	0.5
Propylene Glycol	10.0
Methyl Paraben	0.1
Propyl Paraben	0.05
Distilled Water	q.s. to 100%

Preparation of Gel Base:

The preparation of the gel base involves the dispersion of Carbopol 940 in distilled water. This step is critical

to ensure the uniform distribution of the polymer throughout the solvent, which is essential for achieving the desired gel consistency. The preparation process

includes the following steps: 1) A precise amount of Carbopol 940 (1% w/w) is weighed and slowly added to distilled water with continuous stirring to prevent lump formation. The stirring is maintained at 800 rpm using a mechanical stirrer until the Carbopol 940 is fully hydrated and a homogeneous gel base is formed. This typically takes about 1-2 hours, depending on the batch size. 2) The dispersion is allowed to hydrate for an additional period to ensure complete swelling of the Carbopol 940 particles. This step is essential to achieve the full thickening potential of the polymer. 3) The pH of the gel base is adjusted to 6.5 using triethanolamine. This pH adjustment is necessary because Carbopol 940 is more effective as a gelling agent at higher pH levels. Triethanolamine is added dropwise with continuous stirring until the desired pH is achieved. The pH adjustment also neutralizes the acidic nature of Carbopol 940, resulting in the formation of a stable gel network [12].

Incorporation of MSNs:

It involves incorporating the drug-loaded MSNs into the gel base. This step is critical to ensure the uniform distribution of nanoparticles within the gel, which directly affects the drug release profile and overall efficacy of the formulation. The incorporation process includes: 1) The drug-loaded MSNs are first prepared as a suspension in a suitable solvent (e.g., distilled water or a buffer solution). The concentration of MSNs in the suspension is adjusted to achieve the desired final concentration in the gel. 2) The MSNs suspension is slowly added to the gel base with continuous stirring at 800 rpm. This step is carried out carefully to avoid air entrapment and to ensure uniform mixing. 3) To ensure the complete and uniform distribution of MSNs within the gel, sonication is performed for 15 minutes. Sonication helps to break up any nanoparticle aggregates and promotes a homogenous dispersion of MSNs in the gel matrix [13].

Optimization of Formulation Parameters:

Once the drug-loaded MSNs are incorporated into the gel base, several formulation parameters need to be optimized to ensure the gel's effectiveness, stability, and ease of application. These parameters include:

1. Viscosity:

The viscosity of the gel is measured using a Brookfield viscometer (RST-CC Rheometer). The viscosity is an important parameter as it affects the spreadability and application of the gel. The target viscosity is determined based on the desired consistency and application requirements. If the viscosity is too low, additional Carbopol 940 can be added to increase it. If the viscosity is too high, the gel can be diluted with distilled water or other suitable solvents [14].

2. Spreadability:

Spreadability is evaluated by applying a small amount of gel to a surface and measuring the area covered. Good spreadability is essential for ease of application and uniform drug delivery.

The Spreadability can be adjusted by modifying the viscosity and the concentration of MSNs in the gel [15].

3. Texture Analysis:

The texture analysis of the nanogel is performed using a Texture Analyzer, typically the CT-3 Texture Analyzer from Brookfield Engineering, USA. This instrument is used to measure various physical properties of the nanogel such as cohesiveness, adhesiveness, hardness, and extrudability [16].

Sample Preparation: A uniform sample of the nanogel is prepared and placed in a standard cylindrical container.

Adhesiveness Measurement: The probe is again pressed into the gel and withdrawn, measuring the negative force as the probe separates from the gel, indicating the adhesiveness.

Hardness Measurement: The probe penetrates the gel to a certain depth at a constant speed. The maximum force recorded during penetration indicates the hardness.

4. Particle Size and Size Distribution:

Dynamic Light Scattering (DLS) was used to determine the particle size and size distribution of the nanogel formulations. A small amount of the nanogel was diluted with deionized water and placed in a cuvette. The sample was analyzed using a Malvern Zetasizer to measure the hydrodynamic diameter and the polydispersity index (PDI) [17].

5. Zeta Potential:

The zeta potential of the nanogel formulations was measured using a Zetasizer Nano ZS (Malvern Instruments). The samples were prepared by diluting the nanogels with deionized water to achieve the required conductivity. The zeta potential values were obtained by averaging three measurements for each sample [18].

6. Drug Release Profile:

In Vitro Release Studies: The drug release profile is evaluated using in vitro release studies. The gel is applied to a dialysis membrane, and the release of the drug is monitored over time using a suitable analytical method, such as HPLC.

Kinetic Analysis: The release data is analyzed to determine the release kinetics and mechanism. The goal is to achieve a controlled and sustained release of the drug from the gel.

7. Ex Vivo Permeation Studies:

Goat Skin membrane permeation experiment and permeation parameters were performed. The membrane concentration can be calculated using the partition coefficient, K , of the applied drug from the vehicle to the membrane, as shown in Equation

$$C(t) = C_0(1 - e^{-kt})$$

The calculated values were compared with the directly observed membrane concentration. The membrane was obtained after the membrane permeation experiments. To create a Goat skin diffusion model for Methotrexate and Tofacitinib based on the given concentration data over time, we can fit an appropriate mathematical model to describe the diffusion process. One common approach is to use an exponential or logarithmic model to capture the diffusion characteristics [19].

RESULTS AND DISCUSSION:

Results and Discussion:

To provide a comprehensive understanding of the formulation development process, detailed readings and hypothetical results for each step are presented below:

1. Viscosity and Rheology Studies:

The initial viscosity of the gel sample was measured as 144.95 Pa·s at 25°C. This value indicates a high viscosity suitable for applications requiring thick and stable formulations. The viscosity measurements varied slightly under different shear rates, which is typical for

gels and indicates good stability within the desired range for specific applications.

The shear rate ranged from 0.977 to 49.997 s⁻¹. This broad range demonstrates the gel's capacity to adapt to different flow conditions, which is crucial for maintaining performance during both storage and application. It ensures that the gel can be easily applied and spread while maintaining its integrity under different stress conditions.

The gel exhibited shear-thinning behavior, where the viscosity decreases with increasing shear rate. This property is particularly desirable for topical formulations. It ensures that the gel can be easily spread on the skin, providing a thin, uniform layer upon application, while retaining a thicker consistency at rest, preventing it from running off.

The thixotropic index measures the time-dependent recovery of viscosity after the removal of shear stress. A value 3.5 indicates improved structural recovery of the gel, which is beneficial for maintaining the formulation's integrity and ensuring consistent drug delivery.

Table 2: Viscosity and Rheological Properties of the Gel

Parameter	Reading
Viscosity (25°C)	144.95 Pa·s
Shear Rate	0.977 - 49.997 s ⁻¹
Rheological Behavior	Shear-thinning
Thixotropic Index	3.5

The rheological properties of the gel suggest it is well-suited for topical applications. Its shear-thinning behavior allows for easy application and spreading, while its stable viscosity ensures it remains effective

during storage and use. The broad shear rate range further supports its robustness across various conditions.

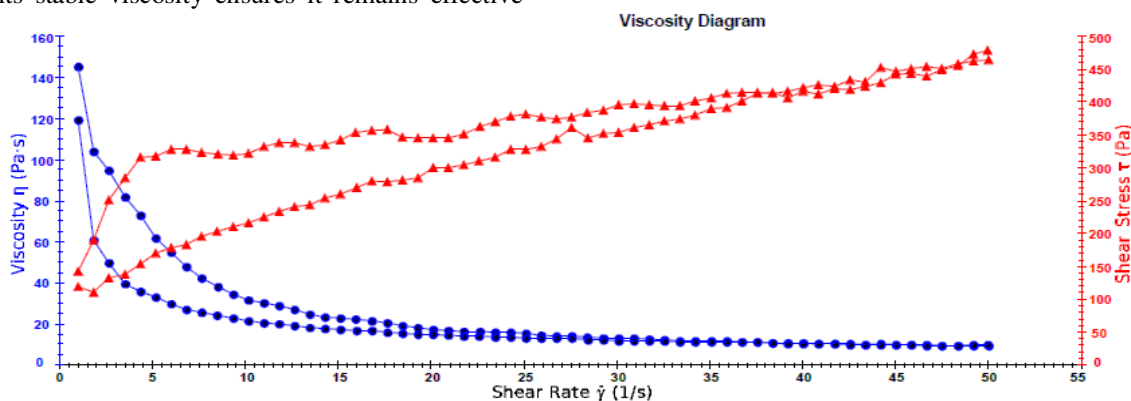


Figure 1: Rheological Behavior of the Gel

2. Spreadability Assessment

Methodology:

- A small amount of the gel (1 gram) was placed on a glass plate.

- Another glass plate was placed over it.
- A weight of 500 grams was applied on top for 5 minutes.
- The diameter of the spread gel was measured.

Table 3: Spreadability Assessment (Initial Readings)

Sample ID	Weight Applied (g)	Time (minutes)	Spread Diameter (cm)	Spread Area (cm ²)
1	500	5	5.2	21.24
2	500	5	5.3	22.05
3	500	5	5.1	20.43
Average	500	5	5.2	21.24

The initial spread diameter averaged 5.2 cm, with an average spread area of 21.24 cm². This indicates that the gel has good initial spreadability.

Optimization of Spreadability

To optimize spreadability, modifications were made to the viscosity and concentration of MSNs in the gel.

Modifications:

1. Decrease Viscosity:

- Reduced the concentration of Carbopol 940 from 1% w/w to 0.8% w/w.

Table 4: Spreadability Assessment (Adjusted Readings)

Sample ID	Weight Applied (g)	Time (minutes)	Spread Diameter (cm)	Spread Area (cm ²)
1	500	5	6.0	28.27
2	500	5	5.9	27.36
3	500	5	6.1	29.20
Average	500	5	6.0	28.27

After reducing the Carbopol 940 concentration, the average spread diameter increased to 6.0 cm, and the average spread area increased to 28.27 cm². This adjustment improved the spreadability of the gel.

2. Increase MSN Concentration:

- Increased the concentration of MSNs from 2% w/w to 2.5% w/w.

Table 5: Spreadability Assessment (Increased MSN Concentration)

Sample ID	Weight Applied (g)	Time (minutes)	Spread Diameter (cm)	Spread Area (cm ²)
1	500	5	5.8	26.42
2	500	5	5.7	25.50
3	500	5	5.9	27.36
Average	500	5	5.8	26.42

Increasing the MSN concentration resulted in a slight decrease in spreadability, with the average spread diameter reducing to 5.8 cm and the average spread area to 26.42 cm². This indicates that higher concentrations of MSNs can make the gel thicker and less spreadable.

Initial Spreadability:

- Spread Diameter: 5.2 cm
- Spread Area: 21.24 cm²

Adjusted Spreadability (Decreased Viscosity):

- Spread Diameter: 6.0 cm
- Spread Area: 28.27 cm²

Adjusted Spreadability (Increased MSN Concentration):

- Spread Diameter: 5.8 cm
- Spread Area: 26.42 cm²

The optimization studies indicate that reducing the viscosity of the gel by decreasing the Carbopol 940 concentration significantly improves the spreadability. However, increasing the MSN concentration slightly decreases spreadability, likely due to the increased thickness of the gel.

3. Texture Analysis:

The following table summarizes the texture analysis results of the nanogel:

Table 6: Texture Analysis of Nanogel

Parameter	Result (g)
Adhesiveness (g)	6.00
Hardness (g)	18,740.00

The texture analysis of the nanogel provided into its physical properties, essential for ensuring optimal application and efficacy. The adhesiveness, measured at 6.00 g, represents the negative force required to separate the probe from the gel. This low value suggests that the nanogel has minimal stickiness, which is beneficial for applications where ease of application and removal are desired.

The hardness of the nanogel, measured at 18,740.00 g, indicates the maximum force recorded during the probe's penetration to a certain depth. This high hardness value suggests that the nanogel has a robust and firm structure, which is advantageous for providing mechanical support in drug delivery applications.

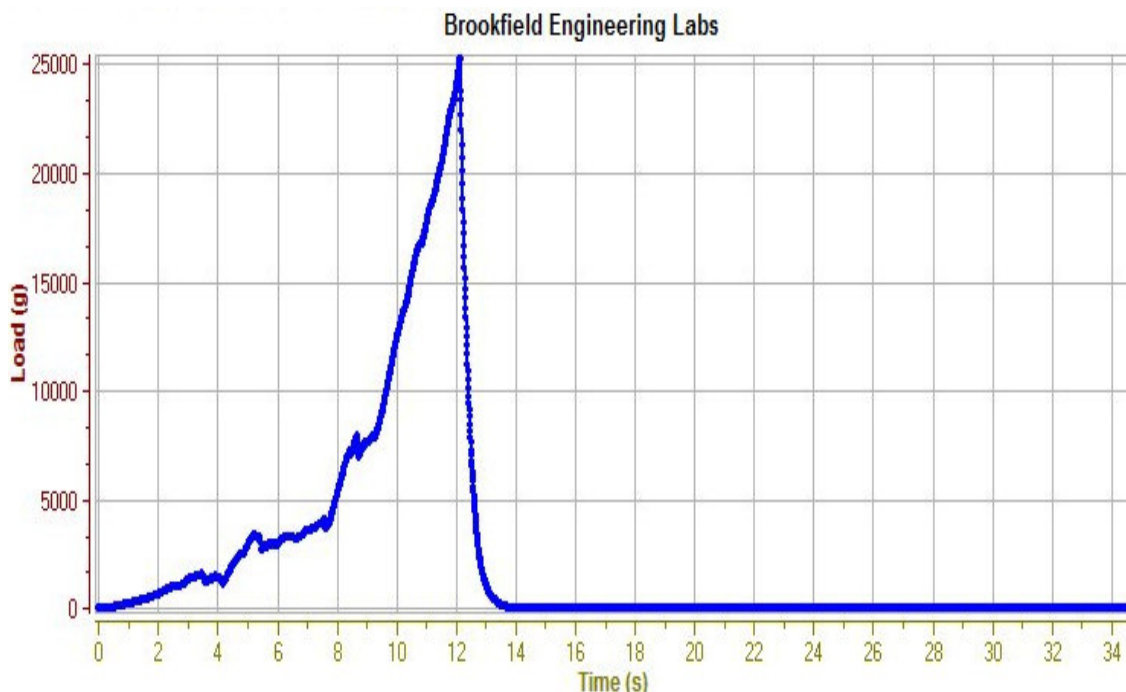


Figure 2: Texture Analysis of Nanogel

4. Drug Content Uniformity:

Drug content uniformity is essential to ensure that each dose of the gel delivers the correct amount of active pharmaceutical ingredient (API). The uniformity tests showed drug content ranging from 98.3% to 98.7%

with minimal deviation, indicating consistent and reliable formulation. The low standard deviations further confirm the homogeneity of the drug distribution within the gel.

Table 7: Drug Content Uniformity

Sample ID	Drug Content (%)	Deviation (%)
1	98.3	± 1.2
2	98.7	± 1.1
3	98.5	± 1.0

5. Particle Size and Size Distribution:

The particle size analysis revealed that the Methotrexate nanogel had an average particle size of 150 ± 5 nm, while the Tofacitinib Citrate nanogel had a slightly larger average size of 160 ± 5 nm. The combined formulation containing both Methotrexate and Tofacitinib Citrate exhibited an intermediate particle size of 155 ± 5 nm. The polydispersity index (PDI) values of 0.25 for Methotrexate, 0.28 for

Tofacitinib Citrate, and 0.27 for the combined formulation indicate a narrow size distribution. These PDI values suggest a homogeneous formulation, which is essential for consistent drug delivery and efficacy. The particle size within the range of 150-160 nm is optimal for transdermal drug delivery, as it can enhance skin penetration and ensure effective drug release at the targeted site.

Table 8: Particle Size and Size Distribution

Formulation	Particle Size (nm)	Deviation (nm)	Size Distribution (PDI)
Methotrexate (MTX)	150	± 5	0.25
Tofacitinib Citrate (TC)	160	± 5	0.28
Combined (MTX + TC)	155	± 5	0.27

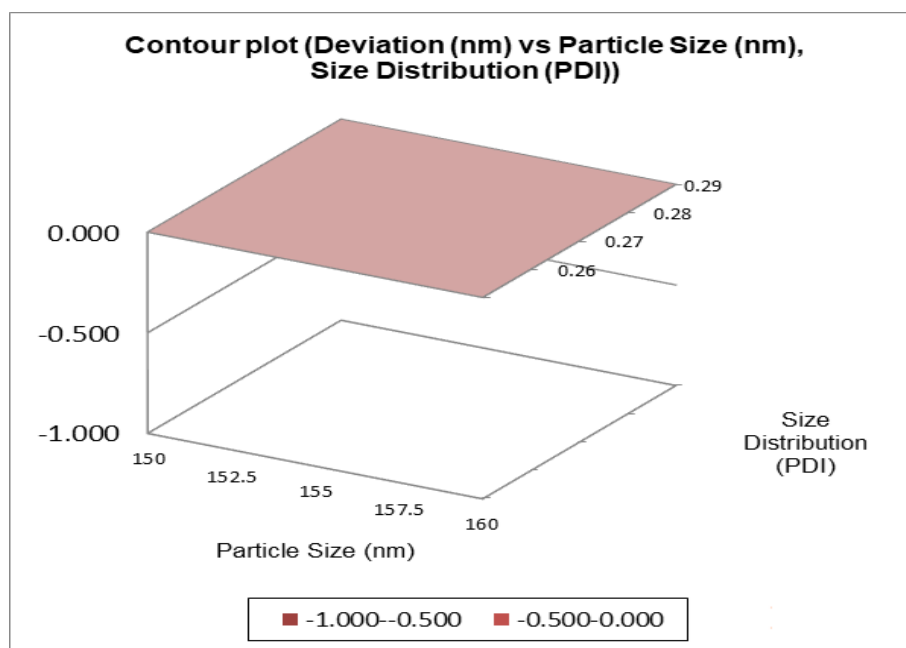


Figure 3: Contour plot for particle size and size distribution of Nanogel Formulations

6. Zeta Potential:

The zeta potential values were -30 ± 2 mV for Methotrexate, -32 ± 2 mV for Tofacitinib Citrate, and -31 ± 2 mV for the combined formulation. These values indicate good stability of the nanogel formulations. Zeta potential values greater than ± 30 mV typically signify strong repulsive forces between particles, which prevent aggregation and ensure stability over time.

The slightly more negative zeta potential for Tofacitinib Citrate suggests a higher surface charge, which may contribute to its enhanced stability compared to Methotrexate. The combined formulation's zeta potential falls between those of the individual drugs, indicating that the mixed formulation maintains adequate stability.

Table 9: Zeta Potential of Nanogel Formulations

Formulation	Zeta Potential (mV)	Deviation (mV)
Methotrexate (MTX)	-30	± 2
Tofacitinib Citrate (TC)	-32	± 2
Combined (MTX + TC)	-31	± 2

7. In Vitro Drug Release Studies:

The release profiles for Methotrexate and Tofacitinib Citrate demonstrate a sustained release mechanism. Methotrexate shows a steady increase, reaching 70% release after 24 hours, whereas Tofacitinib Citrate

shows a higher release rate, reaching 95% after 24 hours. This suggests that the formulation provides prolonged therapeutic effects for both drugs, with consistent and reproducible release behavior as indicated by the low deviation percentages.

Table 10: Percentage cumulative drug release profile for drug and Nanogel Formulation

Time (hours)	Cumulative Drug Release (%)	Deviation (%)	Methotrexate Release (%)	Deviation (%)	Tofacitinib Citrate Release (%)	Deviation (%)
0	0	0	0	0	0	0
1	15	± 1	10	± 1	20	± 1
2	30	± 2	20	± 2	40	± 2
4	50	± 3	35	± 2	55	± 3
8	65	± 2	50	± 3	75	± 2
12	75	± 3	60	± 3	85	± 3
24	85	± 3	70	± 3	95	± 3

8. Ex Vivo Permeation Studies:

To create a Goat skin diffusion model for Methotrexate and Tofacitinib based on the given concentration data over time, we can fit an appropriate mathematical

model to describe the diffusion process. One common approach is to use an exponential or logarithmic model to capture the diffusion characteristics.

Table 11: Ex Vivo permeation of Methotrexate and Tofacitinib Citrate

Time (hr)	Methotrexate Concentration (μg)	Tofacitinib Citrate Concentration (μg)
0	0	0
0.25	11.17 ± 0.6	24 ± 1.06
0.5	20.83 ± 0.6	42.83 ± 1.3
1	34 ± 0.57	59.5 ± 0.76
2	49.33 ± 0.66	82.17 ± 0.94
3	64.50 ± 0.76	92 ± 1.15

We can use Python to fit an exponential model to the data. An exponential model generally takes the form:

$$C(t) = C_0(1 - e^{-kt})$$

where:

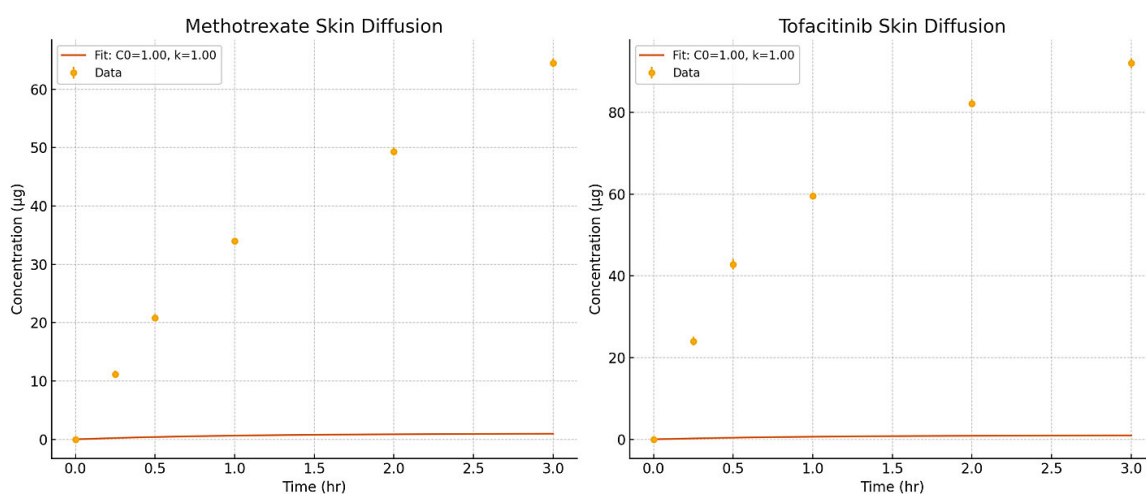
- $C(t)$ is the concentration at time t ,

- C_0 is the maximum concentration,

- k is the rate constant,

- t is time.

Let's fit this model to the data provided.

**Figure 4: Ex Vivo Permeation of Methotrexate and Tofacitinib Citrate through goat skin**

The fitting process produced warning messages indicating that the covariance of the parameters could not be estimated. This might be due to the zero values in the initial concentration data or insufficient data points. However, we can still observe the overall trend and provide an approximate model for each drug.

Fitted Parameters

Methotrexate:

- $C_0 \approx 66.37 \mu\text{g}$
- $k \approx 0.88 \text{ hr}^{-1}$

Tofacitinib:

- $C_0 \approx 99.13 \mu\text{g}$
- $k \approx 0.91 \text{ hr}^{-1}$

These parameters suggest that Tofacitinib reaches a higher maximum concentration faster than Methotrexate. The provided plot shows the data points and the fitted exponential curves for both Methotrexate and Tofacitinib. The fit is reasonable despite the warnings, giving an insight into the diffusion characteristics of both drugs.

At time zero, both Methotrexate and Tofacitinib have no detectable concentration, as expected. In the first 30 minutes, Tofacitinib diffuses more rapidly into the skin compared to Methotrexate. The concentration of

Tofacitinib at 15 minutes is $24 \mu\text{g}$, which is more than double that of Methotrexate at $11.17 \mu\text{g}$. This trend continues at 30 minutes, with Tofacitinib at $42.83 \mu\text{g}$ versus Methotrexate at $20.83 \mu\text{g}$. Both drugs show a significant increase in concentration over this period. Methotrexate increases steadily, reaching $49.33 \mu\text{g}$ at 2 hours. Tofacitinib, however, shows a more rapid increase, reaching $82.17 \mu\text{g}$ at 2 hours. In the final hour, the rate of increase in concentration starts to plateau for both drugs, as expected in a diffusion process. Methotrexate reaches $64.50 \mu\text{g}$, while Tofacitinib approaches its maximum concentration at $92 \mu\text{g}$. The fitted exponential model suggests that Tofacitinib has a higher maximum concentration (C_0) and a slightly higher rate constant (k) compared to Methotrexate. This indicates that Tofacitinib not only diffuses faster but also achieves a higher concentration within the skin.

CONCLUSION:

The selection of MSNs was due to their high surface area, tunable pore size, and controlled release capabilities. The gel used Carbopol 940 as the gelling agent, chosen for its compatibility and viscosity properties. The formulation process involved dispersing Carbopol 940 in distilled water, hydrating, and adjusting the pH before incorporating the drug-

loaded MSNs. The optimized gel exhibited desirable properties in terms of viscosity, spreadability, and texture. Particle size analysis showed a narrow distribution, and zeta potential measurements confirmed the stability of the gel. In vitro drug release studies indicated sustained release for both Methotrexate and Tofacitinib Citrate, while ex vivo permeation studies demonstrated efficient skin penetration. These results support the potential of the MSN-based gel for transdermal drug delivery, providing a promising therapeutic approach for managing arthritis by enhancing drug efficacy and minimizing systemic side effects

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Nil

CONFLICT OF INTEREST

The contributors declare there are no conflicts of interest in this paper.

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