CHAPTER 5

MATERIALS AND METHOD

5.1 Drug Profile

5.1.1 Tofacitinib Citrate

Chemical Name:

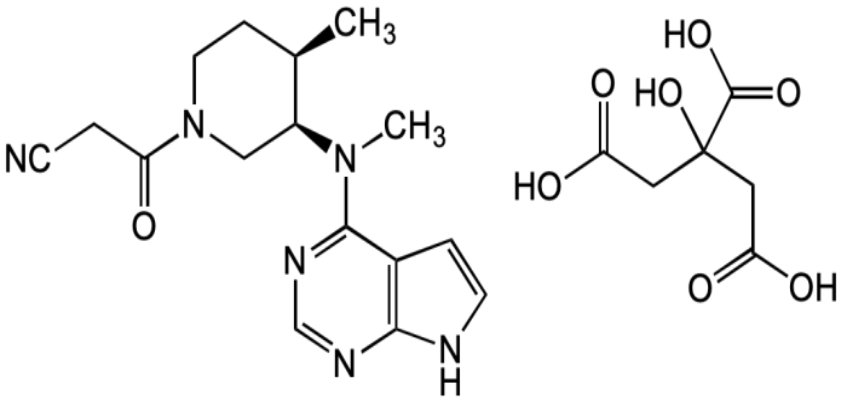
The chemical name of Tofacitinib Citrate is 3-[(3R,4R)-4-Methyl-3-[methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino]piperidin-1-yl]-3-oxo-propionitrile. This complex structure highlights the drug's role as a small molecule that fits into cellular enzymes, helping to block key pathways involved in inflammation.

Chemical Formula:

The molecular formula C22H28N6O8 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:

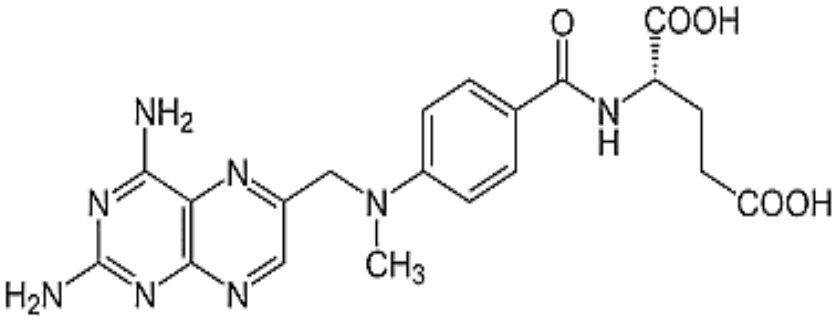
The chemical name of Methotrexate is 4-amino-4-deoxy-N10-methylpteroylglutamic acid. It is derived from folic acid and works as an antimetabolite by interfering with the metabolism of folic acid, which is crucial for DNA synthesis and cell replication. The name reflects its close structural similarity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

Chemical Formula:

Methotrexate's molecular formula is C20H22N8O5, 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: $C_3H_8O_2$

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:

	Methyl Paraben Sodium	**Propyl Paraben Sodium**
Empirical Formula	C8H7NaO3	C10H11NaO3
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:

	Methyl Paraben Sodium	**Propyl Paraben Sodium**
Density	1.54 g/cm³	1.40 g/cm³
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-Kα radiation (λ = 1.5406 Å).

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;

Loading Efficiency (%) = (Amount of drug loaded / Total amount of drug used) × 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 ↓	**Quantity (%)**								
	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 (Ka), elimination rate constant (Ke), half-life (t1/2), maximum concentration (Cmax), time to reach maximum concentration (Tmax), and the area under the curve (AUC).

A. Absorption Rate Constant (Ka)

The absorption rate constant (Ka) 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

Ka 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, Ka 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 (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:

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

C. Half-Life (t1/2)

The half-life (t1/2) 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:

$$t1/2 = \frac{0.693}{Ke}$$

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

Cmax and Tmax are directly observed from the concentration vs. time data. Cmax is the peak plasma concentration of the drug after administration, while Tmax 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

$$C(t)=C0(1-e-kt)$$

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].

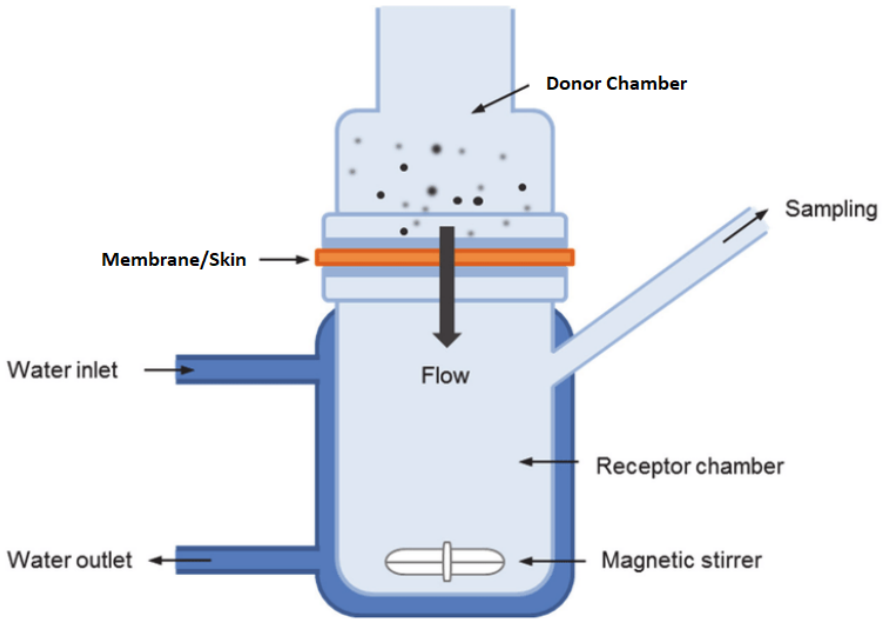


Figure 5.1 Franz- Diffusion Cell

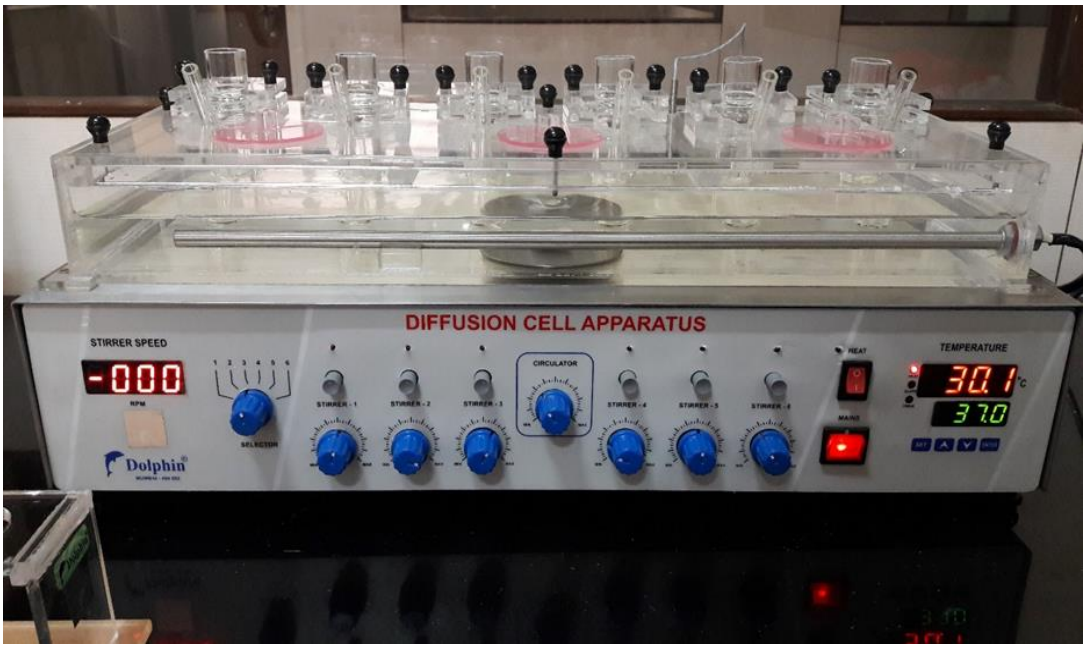


Figure 5.2: Diffusion cell apparatus

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