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 1100-1200 cm^{-1} confirmed the successful formation of the silica network. Additionally, peaks around 960-980 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.

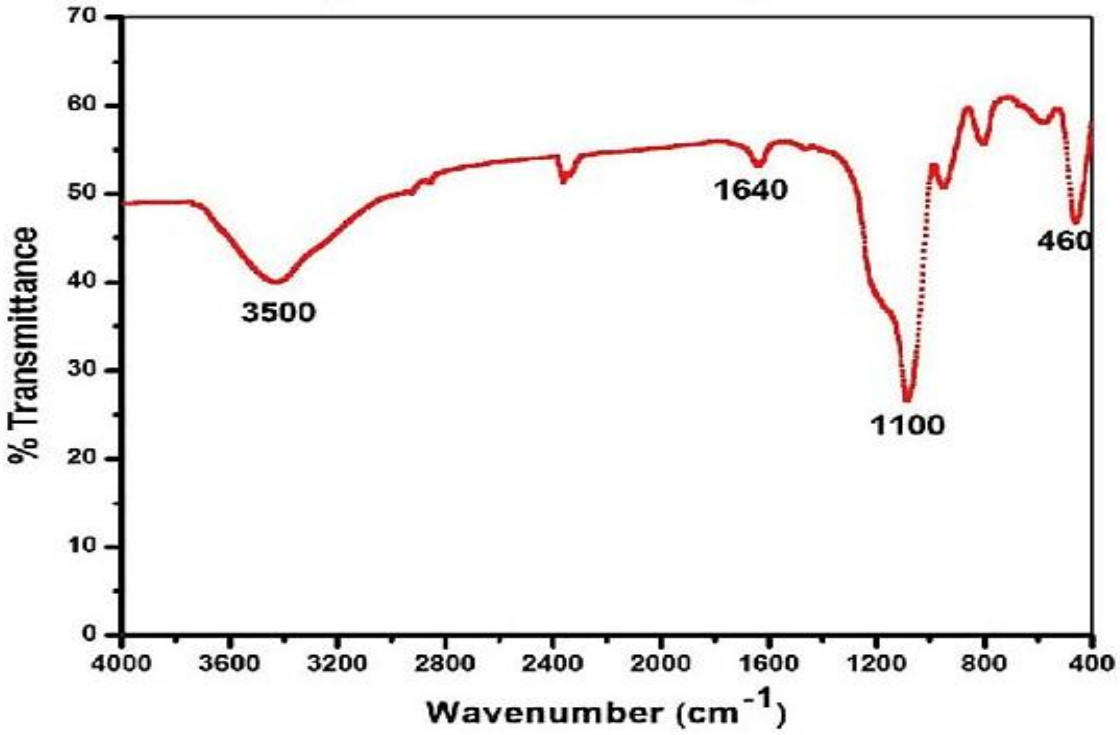

Figure 6.1: The Fourier-transform infrared (FT-IR) spectrum for the MSN's

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.

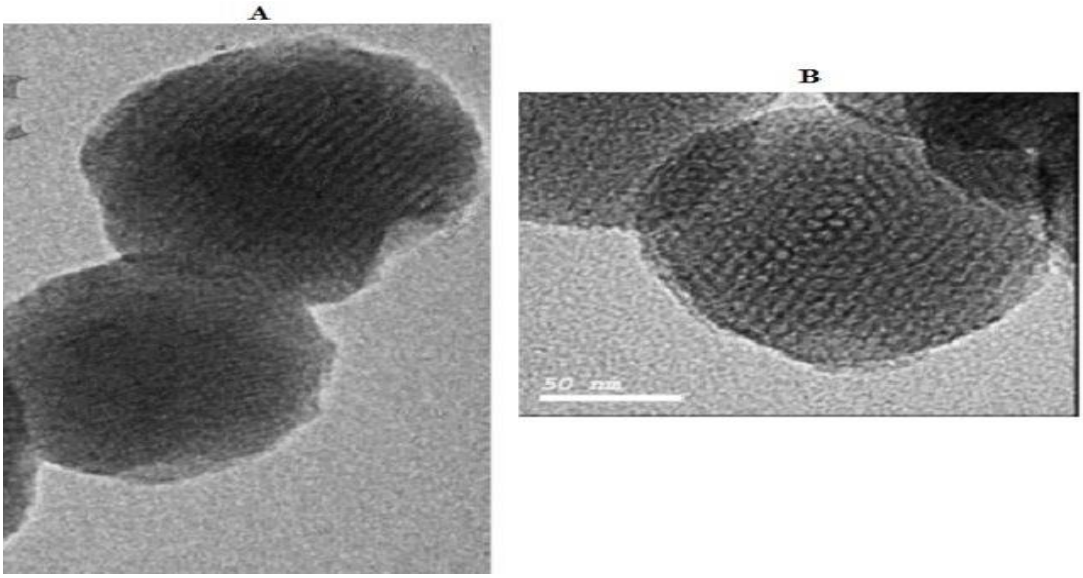

Figure 6.2: Transmission electron microscopy (TEM) photographs depict: (A) A honeycomb-like permeable framework of mesoporous silica nanoparticles. The spherical particles are depicted with hexagonal straight paths flowing from them. The particles possess linear, one-dimensional cylindrical pores. (B)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

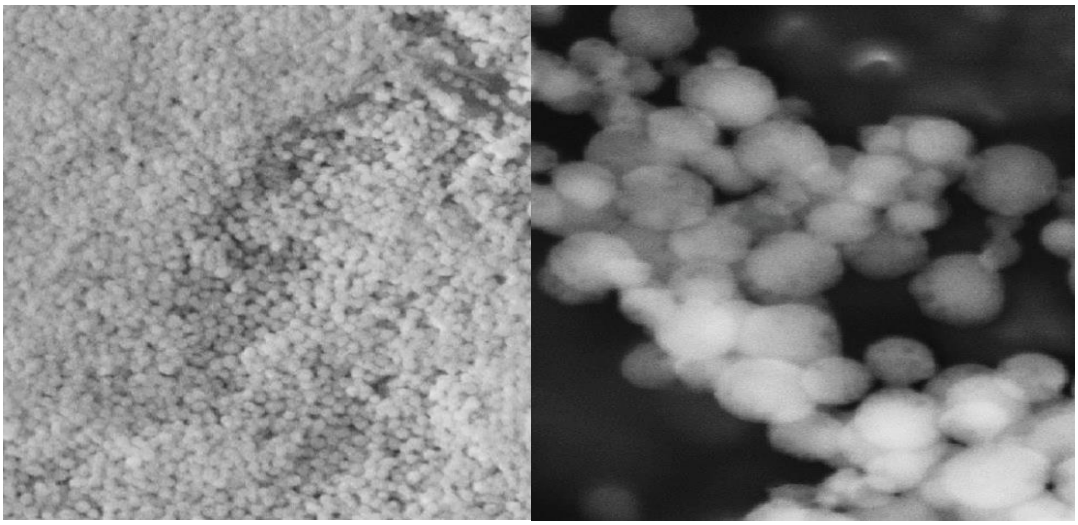

Figure 6.3: An image obtained using scanning electron microscopy (SEM) that displays the dimension as well as structure of mesoporous silica nanoparticles.

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.

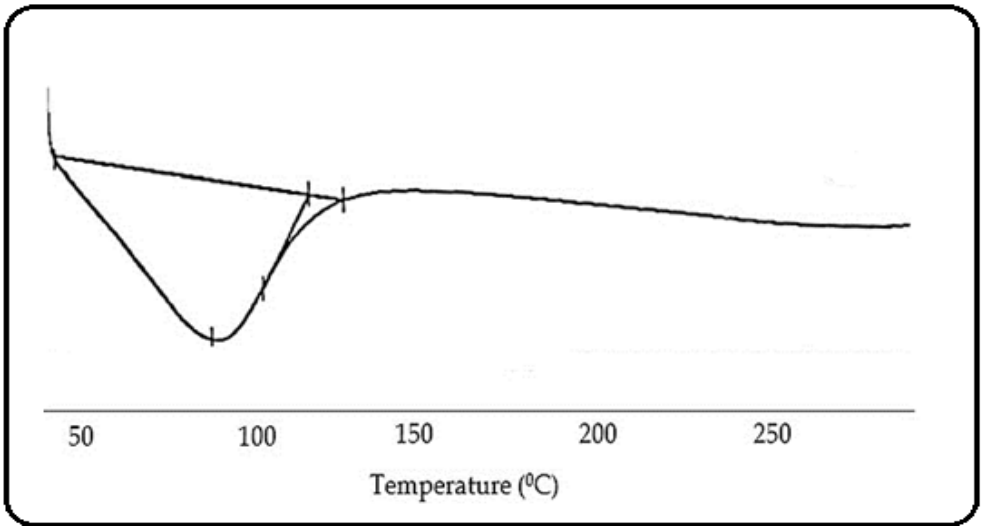

Figure 6.4: DSC Thermogram of MSN

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

BET analysis showed that the MSN had a high specific surface area, typically ranging from 500 to 1000 m^2/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.

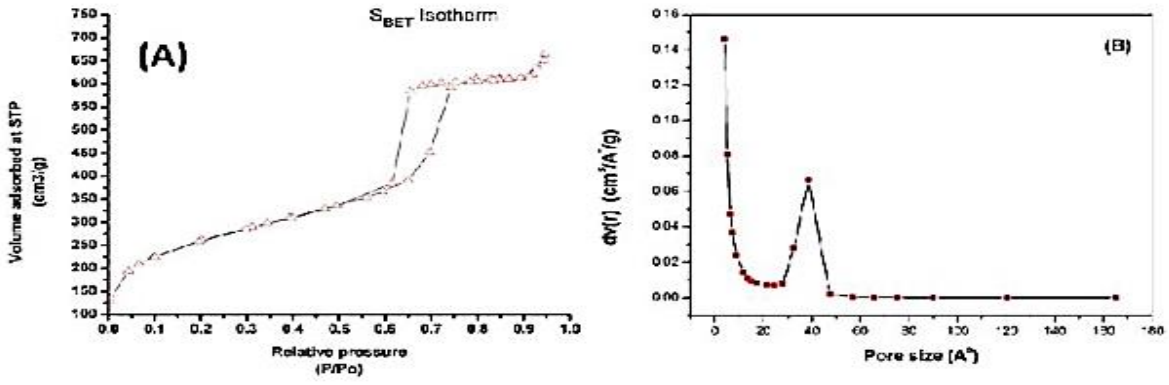

Figure 6.5: (A) Surface examination using nitrogen adsorption isotherms that enables the computation of surface area using the BET method. (B) The size of the pores of the fragments was determined using the Barrett-Joyer-Halenda (BJH) method.

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 3300-3500 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.

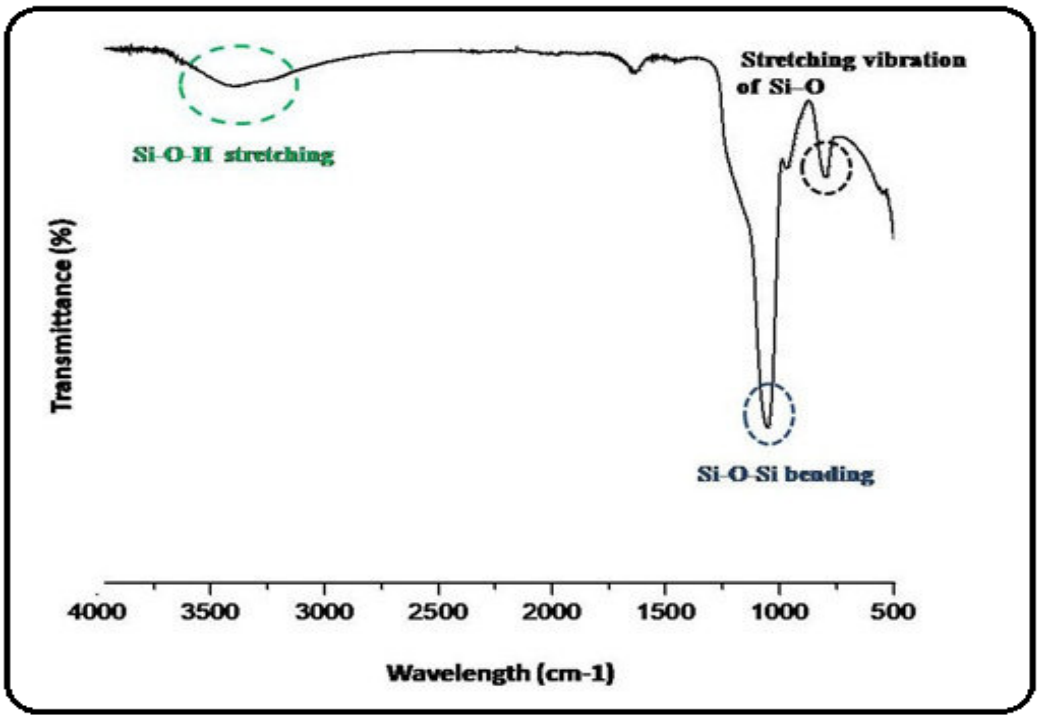

Figure 6.6: FTIR spectra for unmodified and surface-modified mesoporous silica nanoparticles (MSNs)

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.

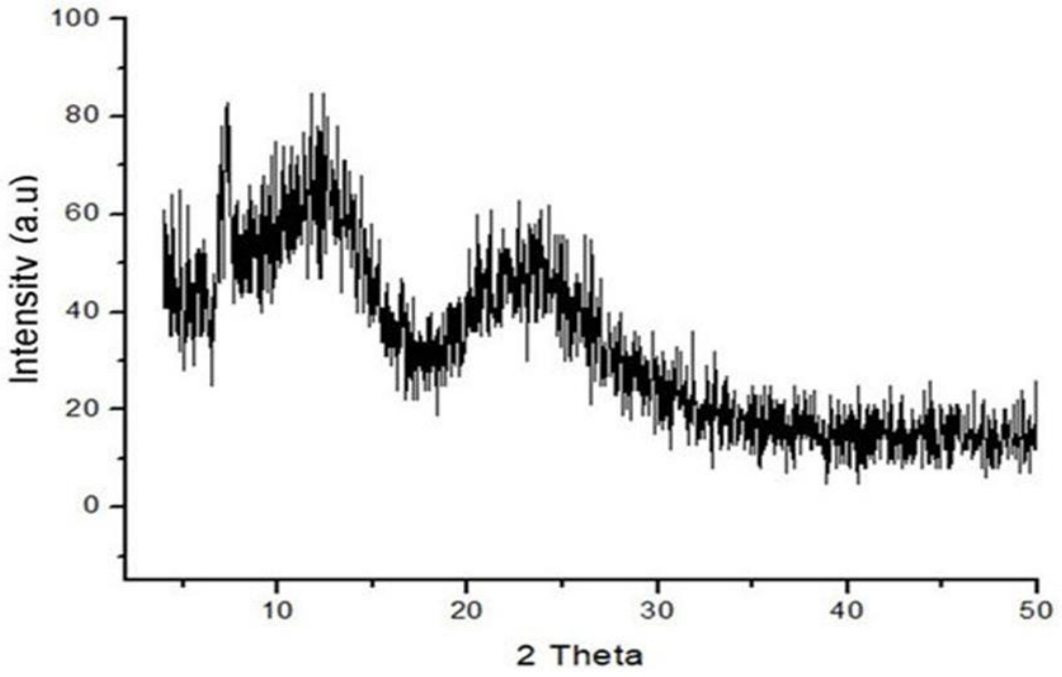

Figure 6.7: XRD spectra of surface modified mesoporous silica nanoparticles (MSNs)

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

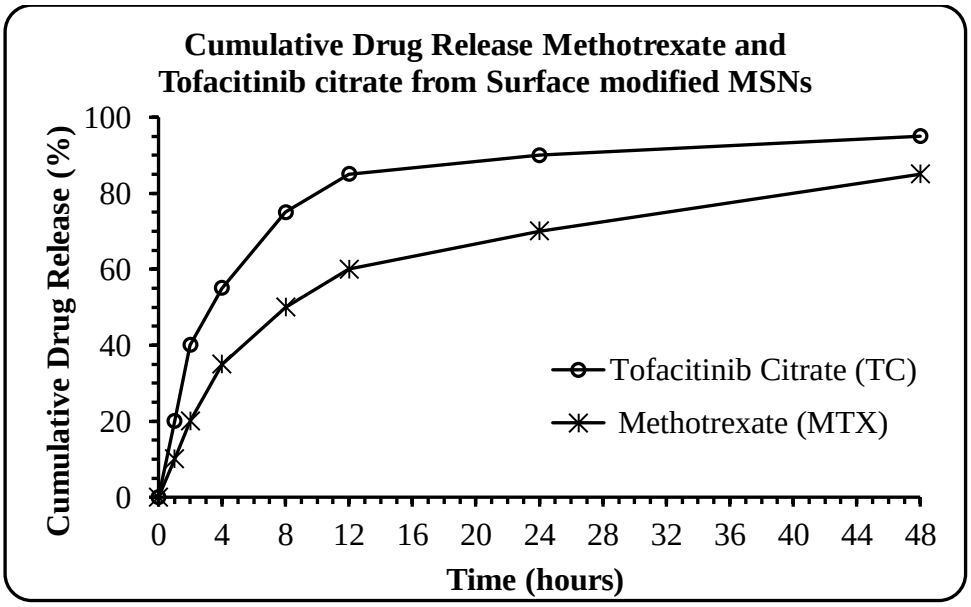

Figure 6.8: Cumulative Percentage Drug Release Drugs loaded Surface-Modified MSNs

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 (%)**								
	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%)

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

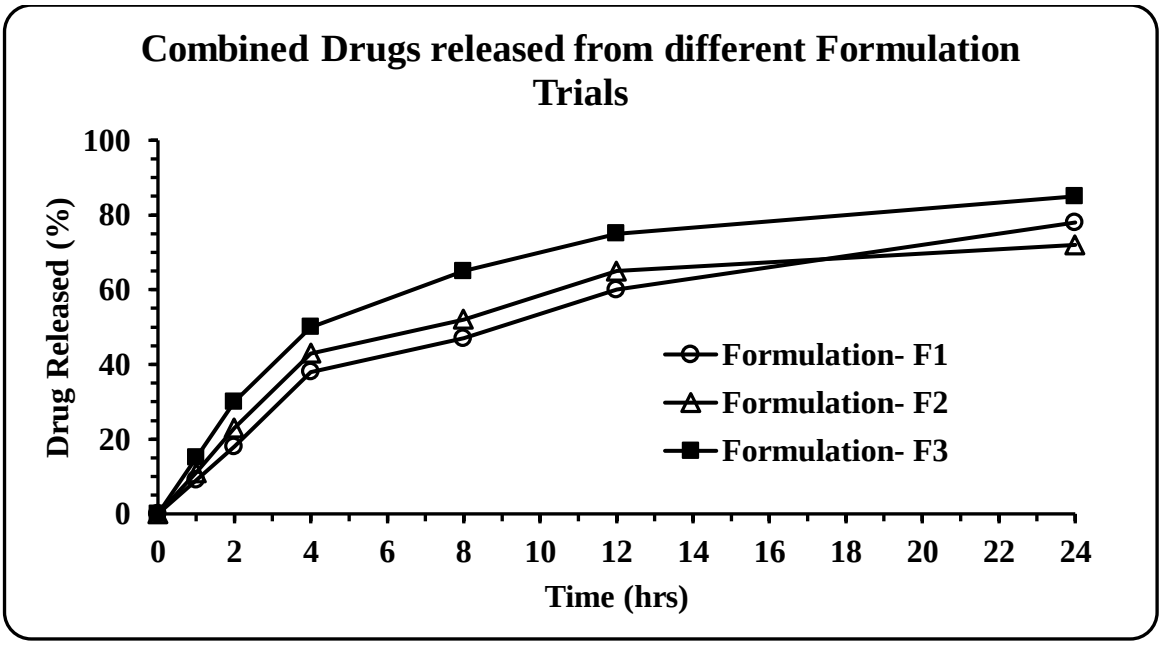

Figure 6.9: Combined Drug Release from different Formulation (Impact of Drug-Loaded MSNs concentrations in Formulations)

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

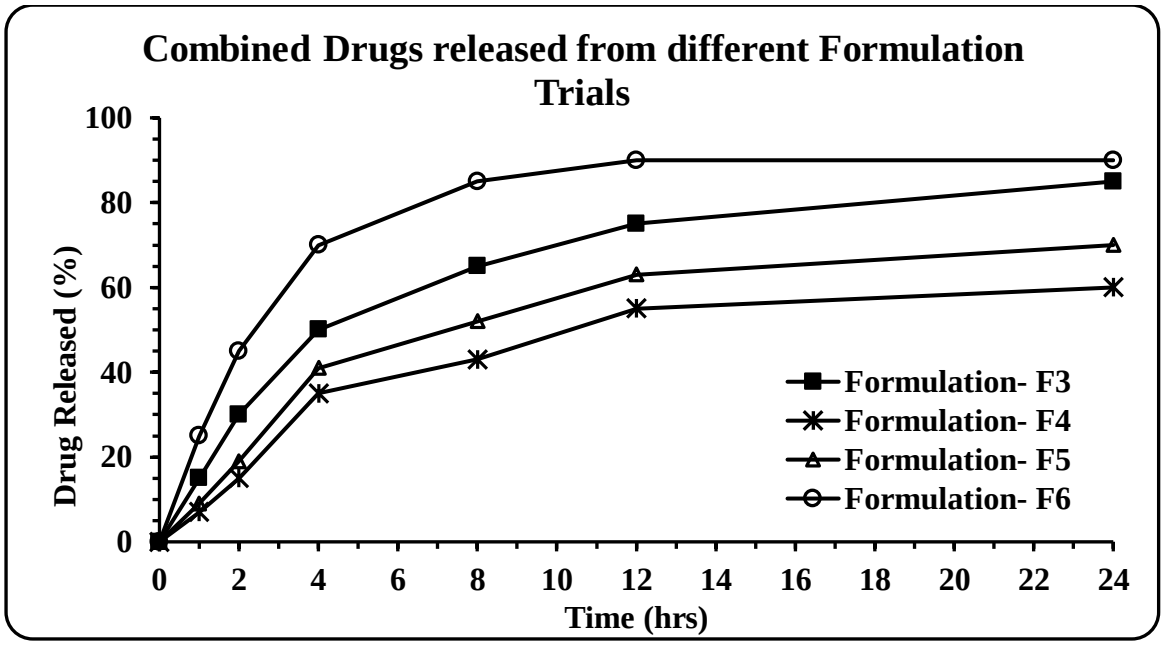

Figure 6.10: Combined Drug Release from different Formulation (Impact of Carbopol-940 concentration in Formulation)

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

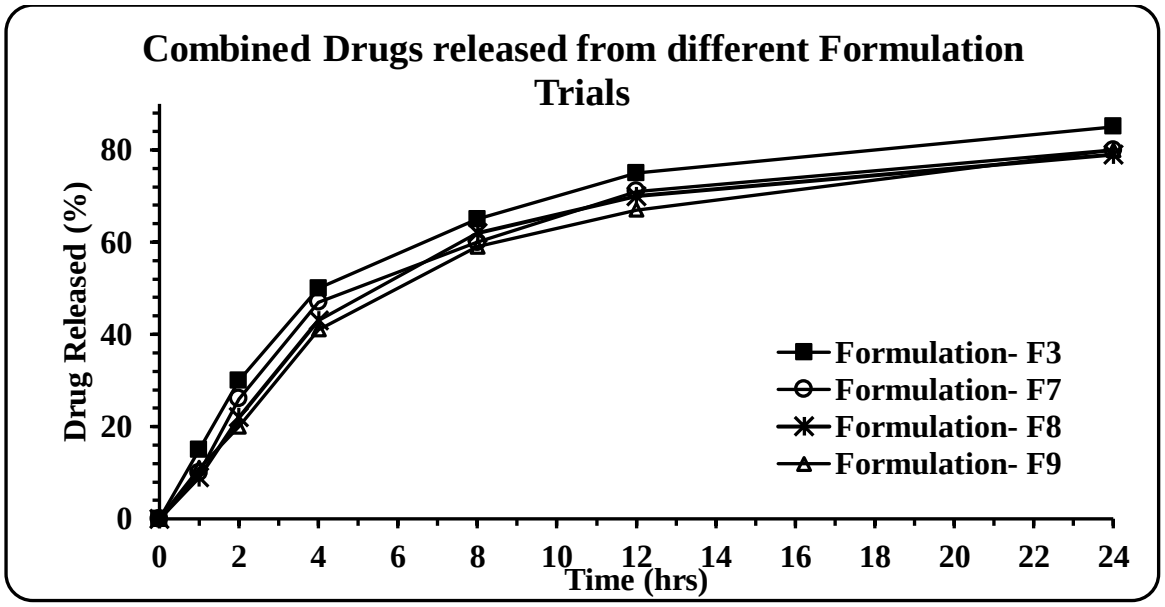

Figure 6.11: Combined Drug Release from different Formulation (Impact of Propylene Glycol concentration in Formulation)

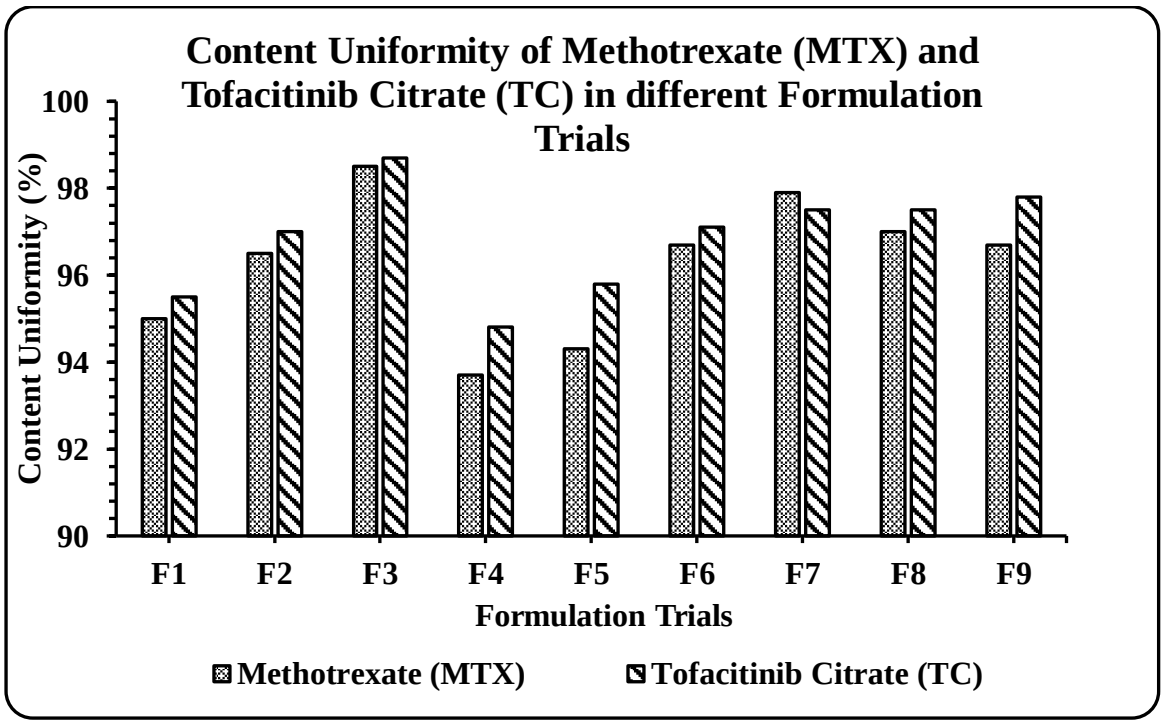

Figure 6.12: Content Uniformity of Methotrexate and Tofacitinib citrate in different formulation Trials

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

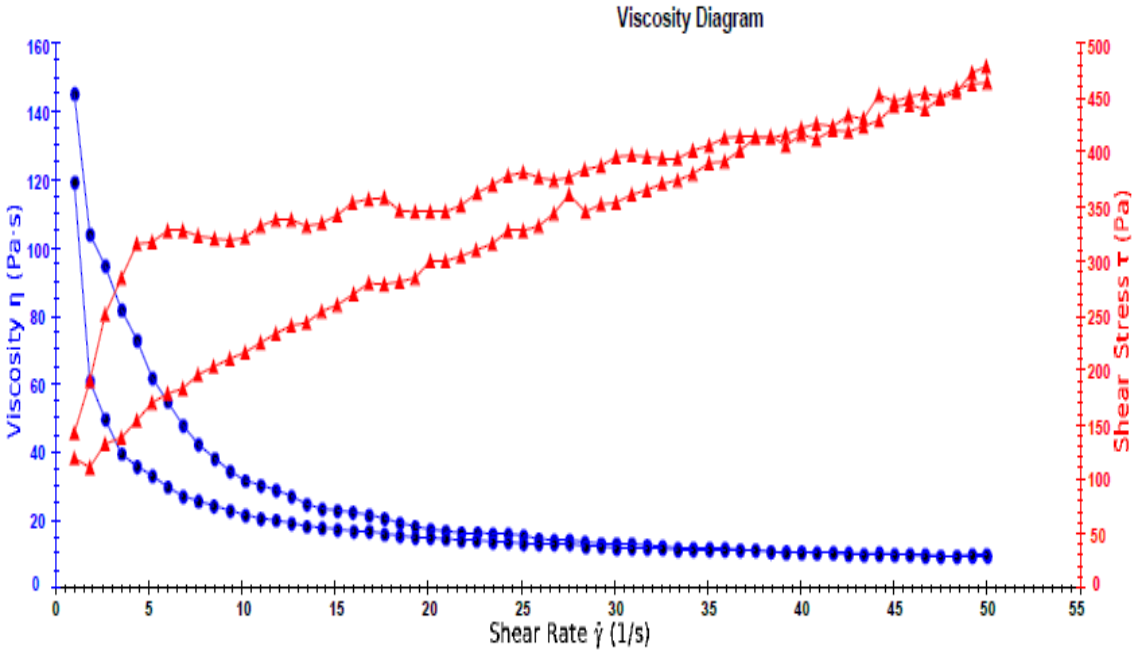

Figure 6.13: Rheological behavior of the Nanogel Formulation (F3)

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 cm^2. This indicates that the gel has good initial spreadability.

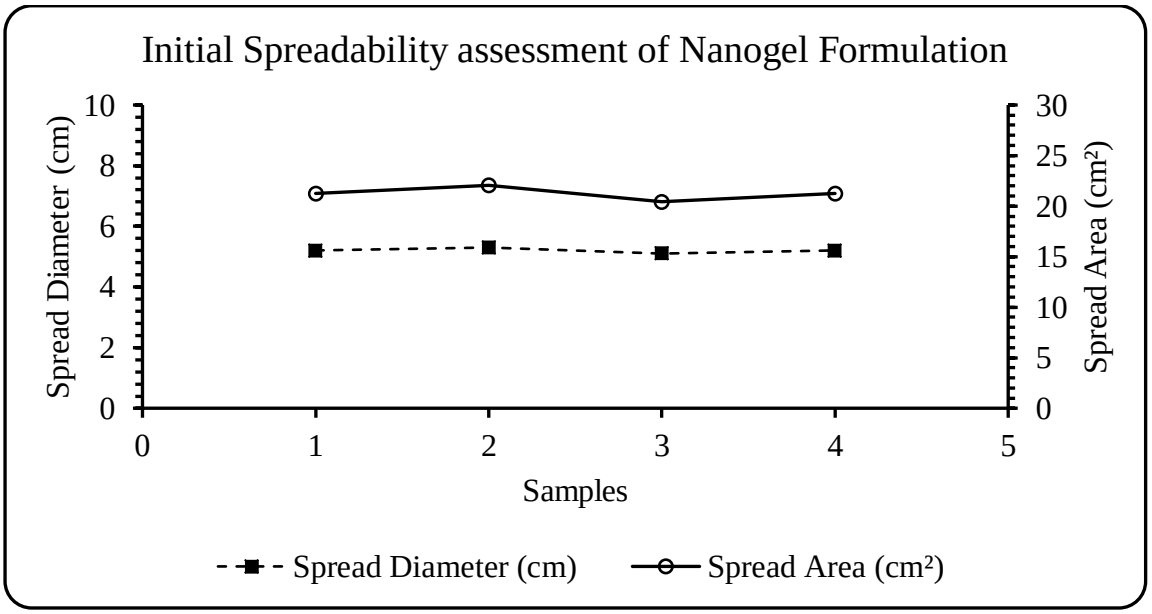

Figure 6.14: Initial Spreadability assessment readings of Nanogel Formulation (F3)

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 cm^2. This adjustment improved the spreadability of the gel.

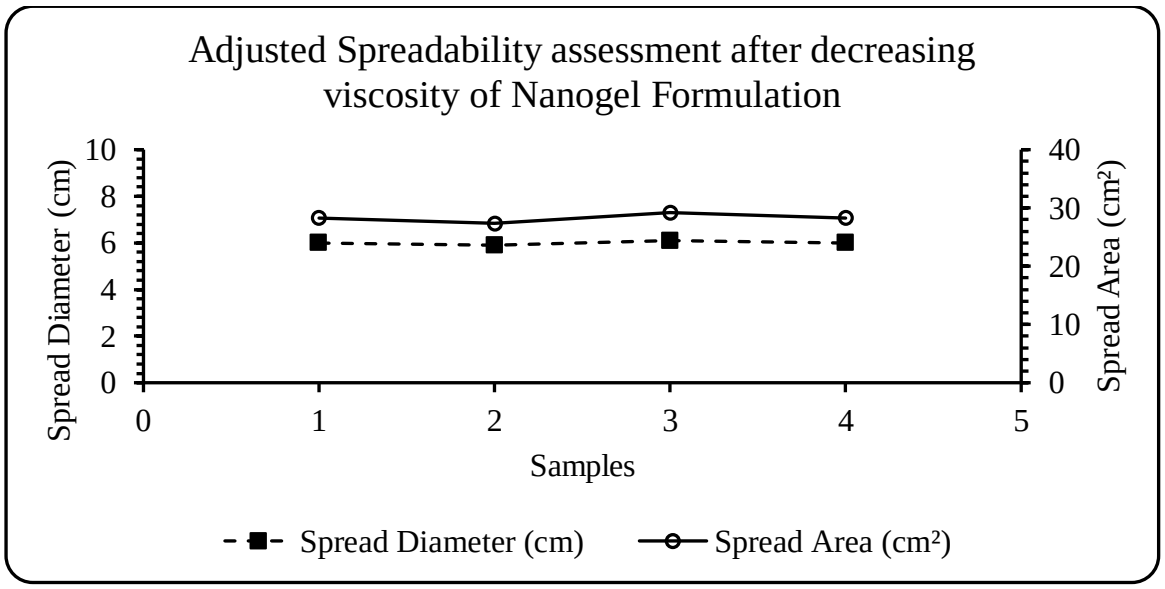

Figure 6.15: Adjusted Spreadability assessment after decreasing viscosity of Nanogel Formulation

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 cm^2. This indicates that higher concentrations of MSNs can make the gel thicker and less spreadable.

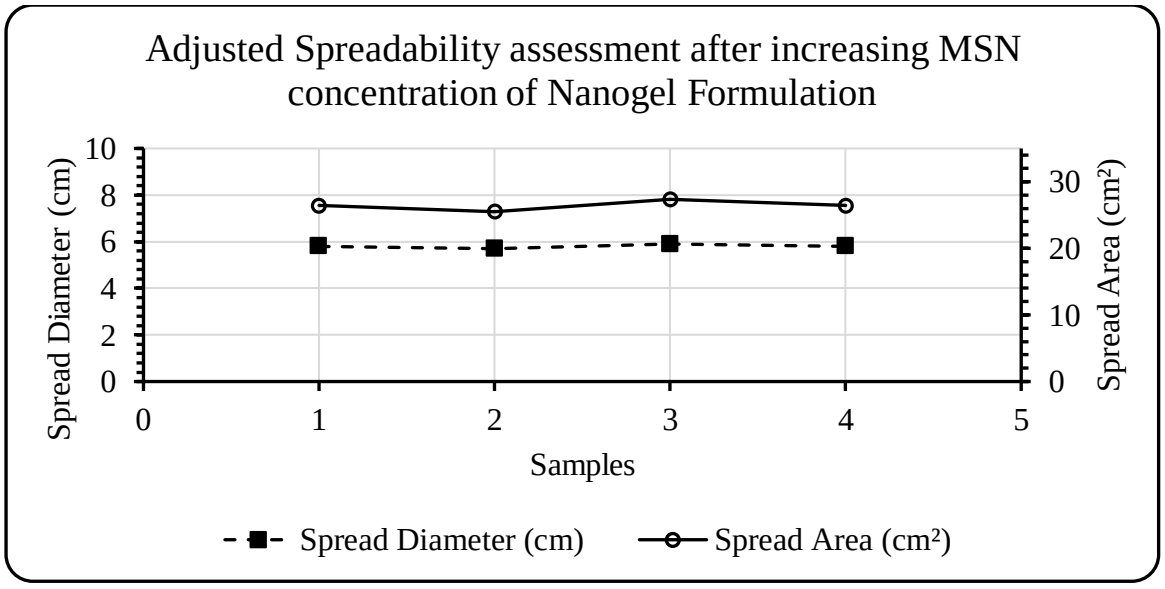

Figure 6.16: - Adjusted Spreadability assessment after increasing MSN concentration of Nanogel Formulation

Initial Spreadability:

- Spread Diameter: 5.2 cm
- Spread Area: 21.24 cm^2

Adjusted Spreadability (Decreased Viscosity):

- Spread Diameter: 6.0 cm
- Spread Area: 28.27 cm^2

Adjusted Spreadability (Increased MSN Concentration):

- Spread Diameter: 5.8 cm
- Spread Area: 26.42 cm^2

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.

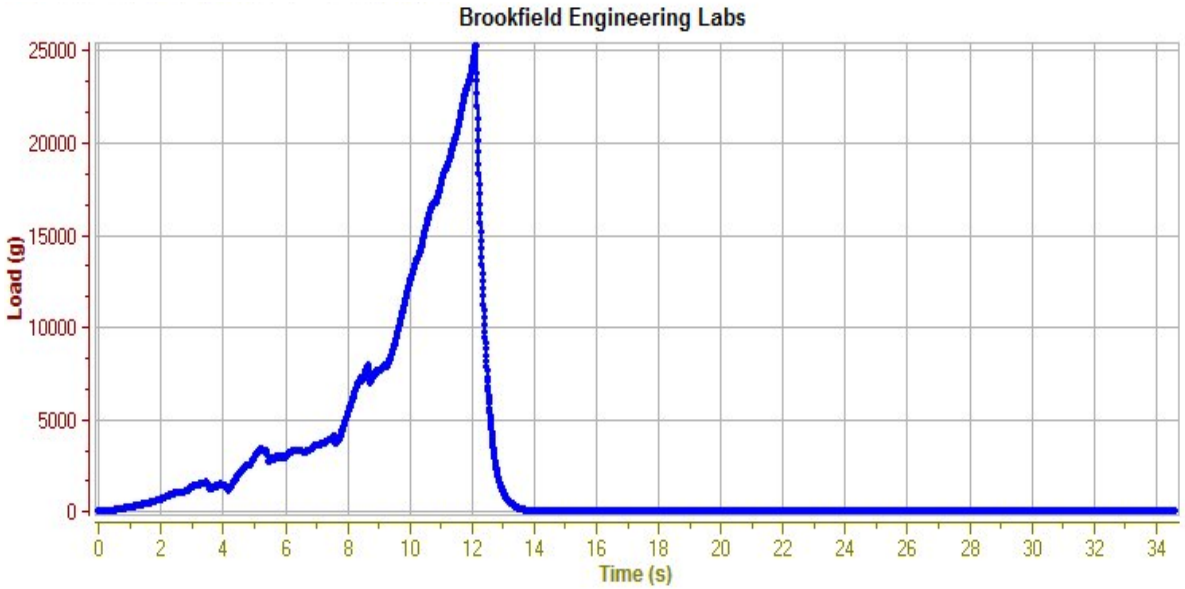

Figure 6.17: Hardness of the Nanogel Formulation (F3)

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

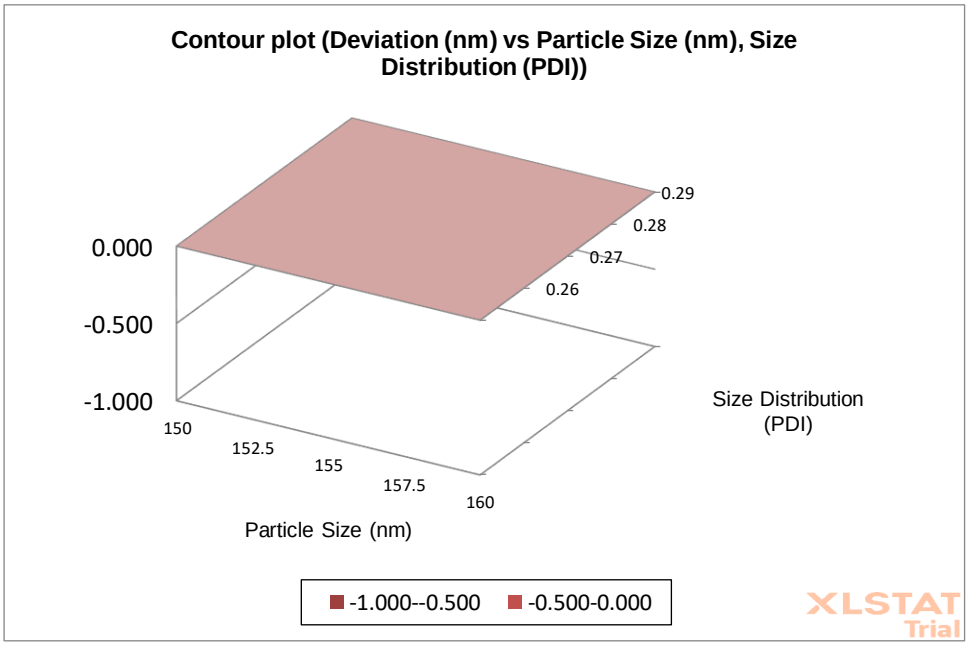

Figure 6.18: Contour plot of Particle Size and Size Distribution of Nanogel Formulation (F3)

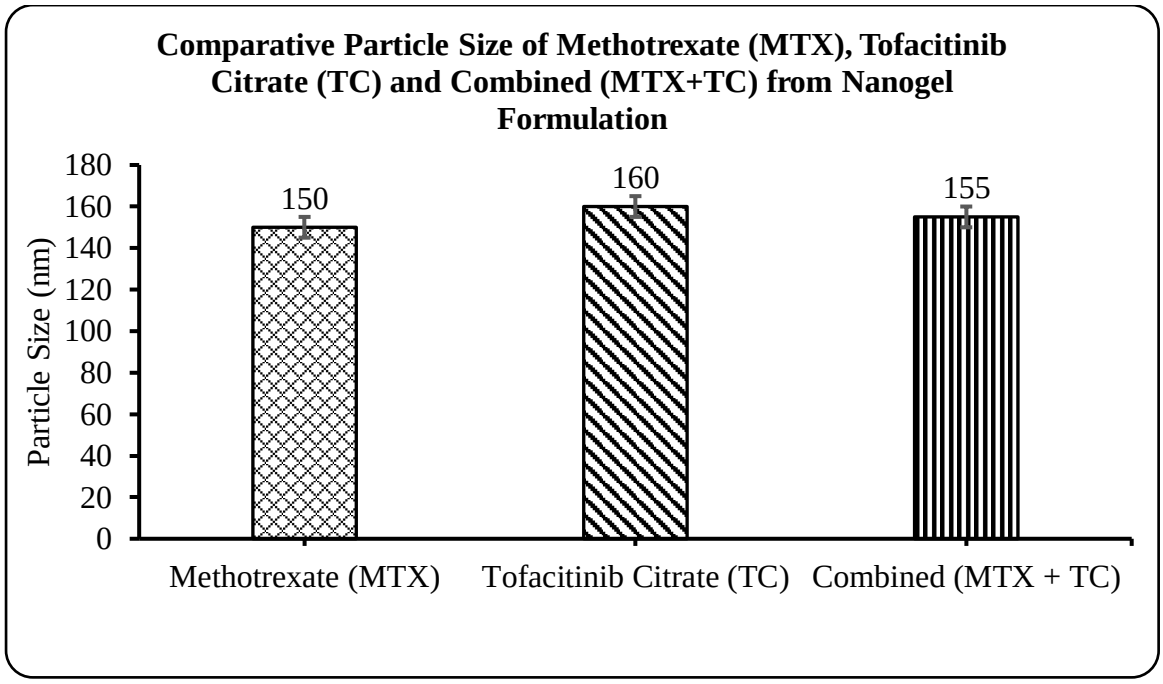

Figure 6.19: Particle size of Methotrexate (MTX), Tofacitinib Citrate (TC) and Combined (MTX+TC) from Nanogel Formulation (F3)

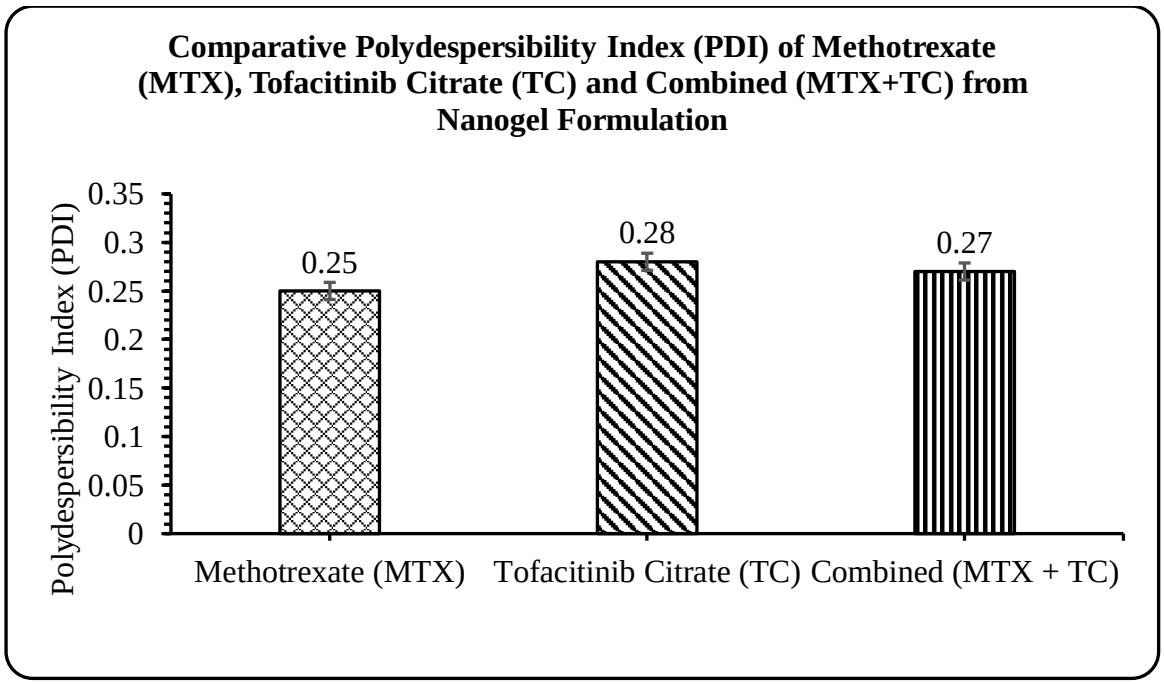

Figure 6.20: Polydespersibility Index (PDI) of Methotrexate (MTX), Tofacitinib Citrate (TC) and Combined (MTX+TC) from Nanogel Formulation (F3)

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

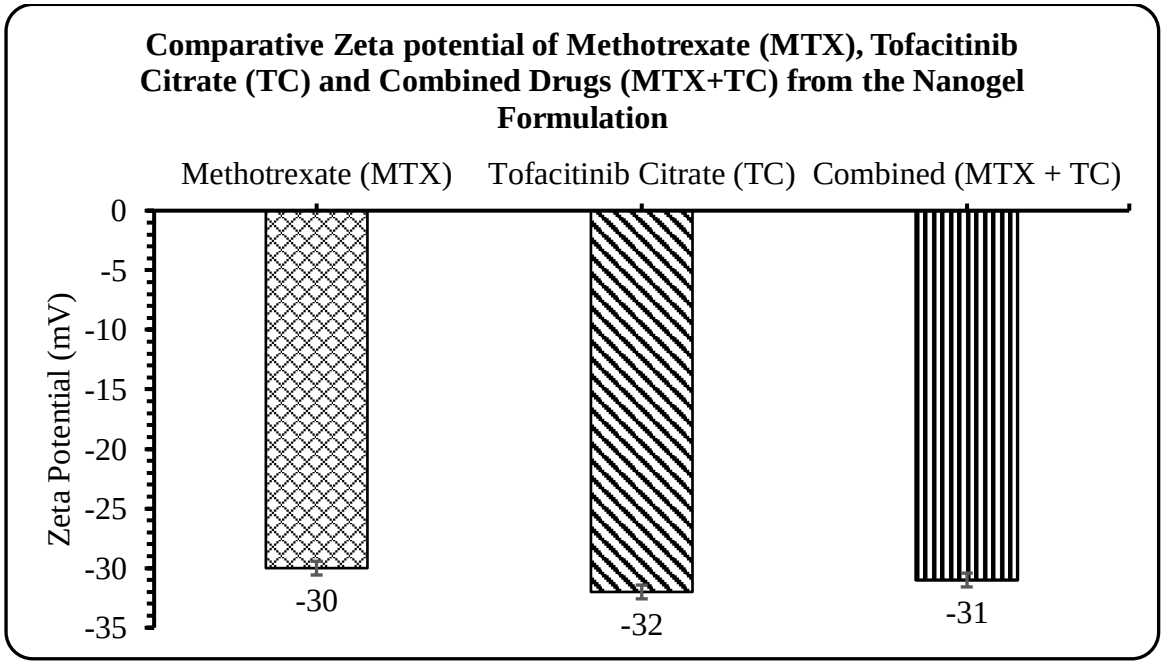

Figure 6.21: Zeta Potential of Methotrexate (MTX), Tofacitinib Citrate (TC) and Combined Drugs (MTX+TC) from Nanogel Formulation (F3)

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

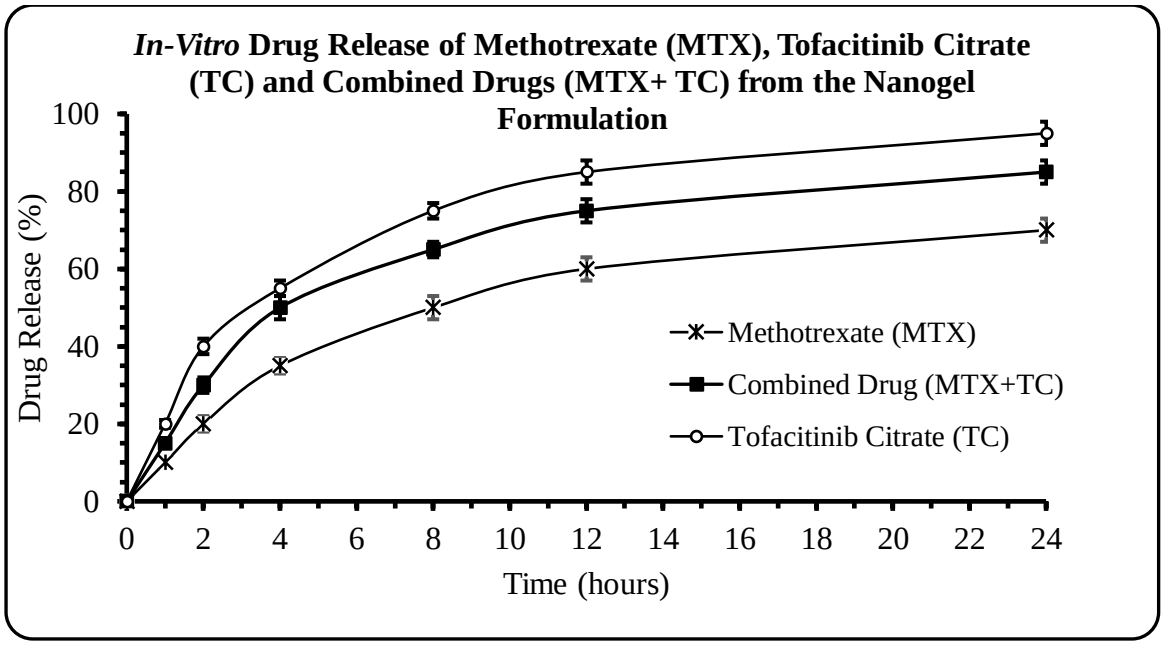

Figure 6.22: *In-Vitro* Drug Release of Methotrexate (MTX), Tofacitinib Citrate (TC) and Combined Drugs (MTX+ TC) from the Nanogel Formulation (F3)

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

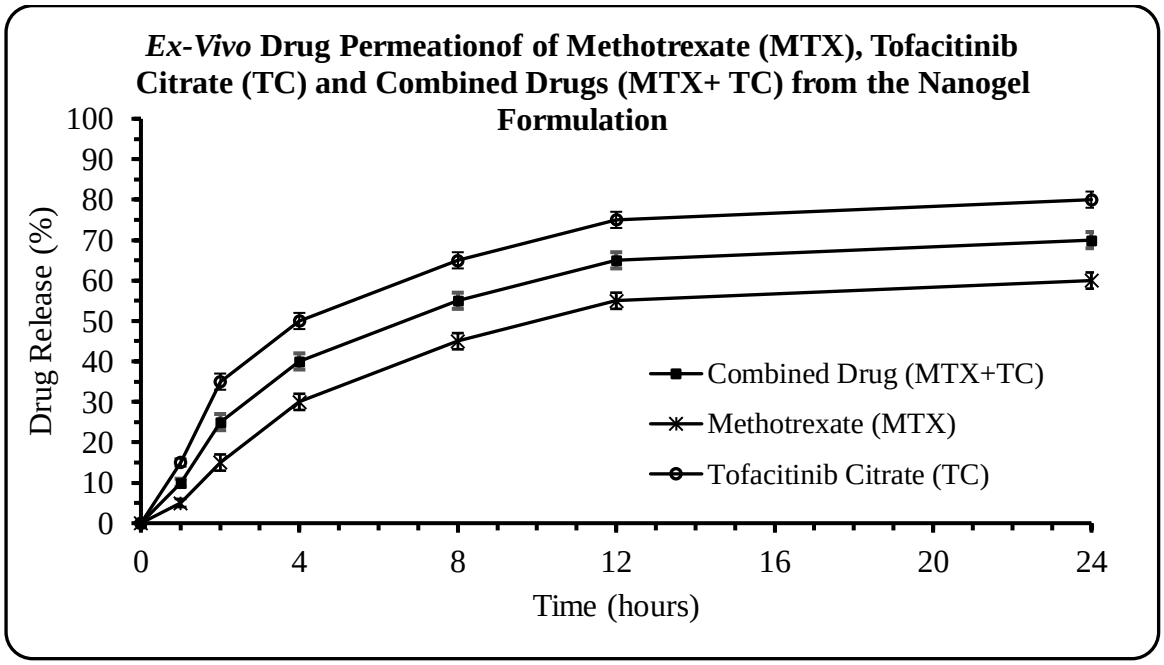

Figure 6.23: *Ex-Vivo* Drug Release of Methotrexate (MTX), Tofacitinib Citrate (TC) and Combined Drugs (MTX+ TC) from the Nanogel Formulation (F3)

6.7 Stability Studies of Nanogel Formulation (F3):

A. Accelerated Stability Studies (40°C ± 2°C and 75 % ± 5% 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°C ± 2°C and 75% ± 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	9,950	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 (Ka), elimination rate constant (Ke), half-life ($t_{1/2}$), maximum concentration (C_{max}), time to reach maximum concentration (Tmax), and the area under the curve (AUC).

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 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, Ka 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:

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

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

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.

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)

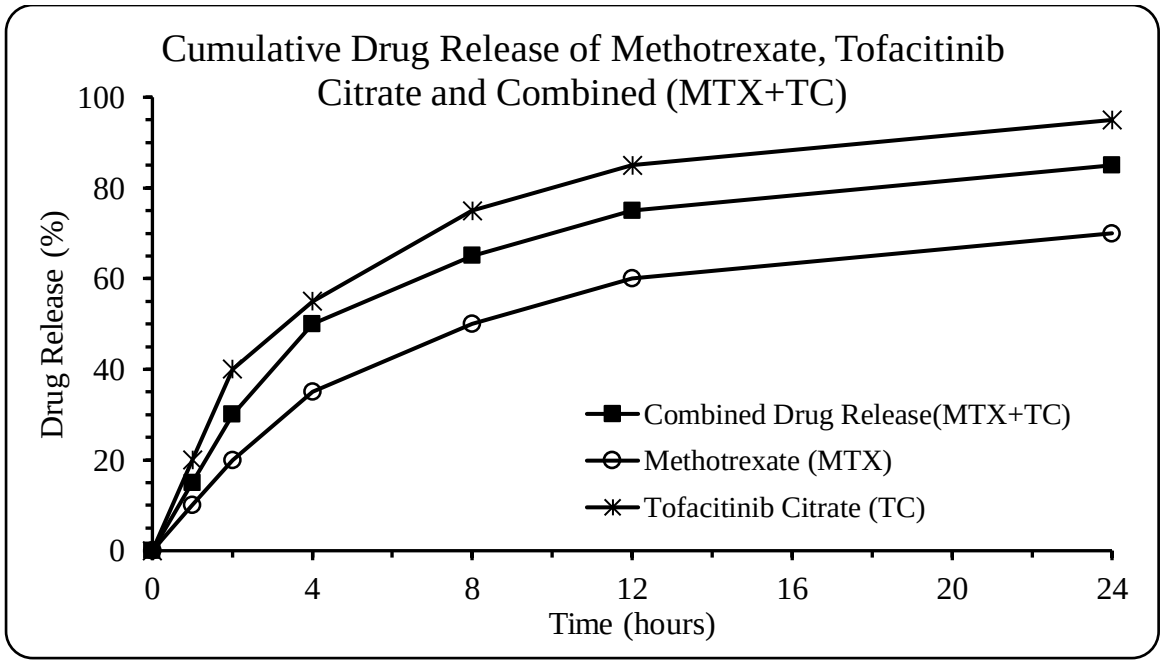

Figure 6.24: Cumulative Drug Release for Methotrexate (MTX), Tofacitinib citrate (TC) and Combined Drugs (MTX+TC) from Nanogel Formulation (F3)

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

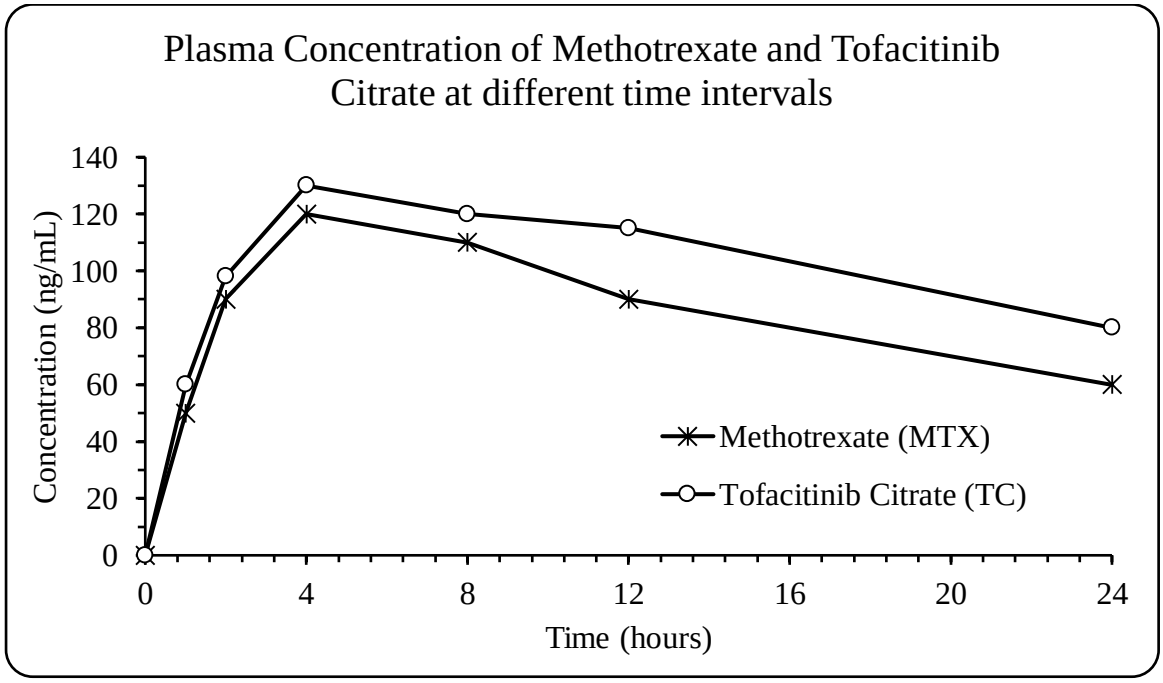

Figure 6.25: Plasma concentration of Methotrexate (MTX) and Tofacitinib Citrate (TC) at different time intervals

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 (Ke): From the terminal phase (e.g., between 12 and 24 hours):

For Methotrexate:

To calculate Ke, the concentrations during the elimination phase (after Tmax) used:

Table 6.22: T_{max} of Methotrexate

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

Using the formula

Ke = 0.050175h^{-1} $Ke = \frac{\ln(C1)-\ln(C2)}{t2-t1}$:

For Tofacitinib Citrate:

To calculate Ke, the concentrations during the elimination phase (after Tmax) 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 (t1/2):

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

$$t1/2 \approx 13.81 \text{ hours}$$

For Tofacitinib Citrate:

Using the formula $t1/2 = \frac{0.693}{Ke}$:

$$t1/2 \approx 65.07 \text{ hours}$$

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
Cmax	120 ng/mL	130 ng/mL	Observed from data
Tmax	4 hours	4 hours	Observed from data
Ke	0.050175 h^{-1}	0.01065 h^{-1}	Calculated from ln(concentration) vs. time
t1/2	13.81 hours	65.07 hours	$t1/2 = \frac{0.693}{Ke}$
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 Tmax of 4 hours and a substantial Cmax of 120 ng/mL.

   Tofacitinib Citrate: The drug also shows effective absorption with a Tmax of 4 hours and a slightly higher Cmax of 130 ng/mL.

2) **Elimination:**

   Methotrexate: The elimination rate constant (Ke) of 0.050175 h^{-1} and half-life (t1/2) of 13.81 hours suggest that the drug is eliminated relatively quickly from the body.

   Tofacitinib Citrate: The lower elimination rate constant (Ke) of 0.01065 h^{-1} and longer half-life (t1/2) 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

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

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, 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

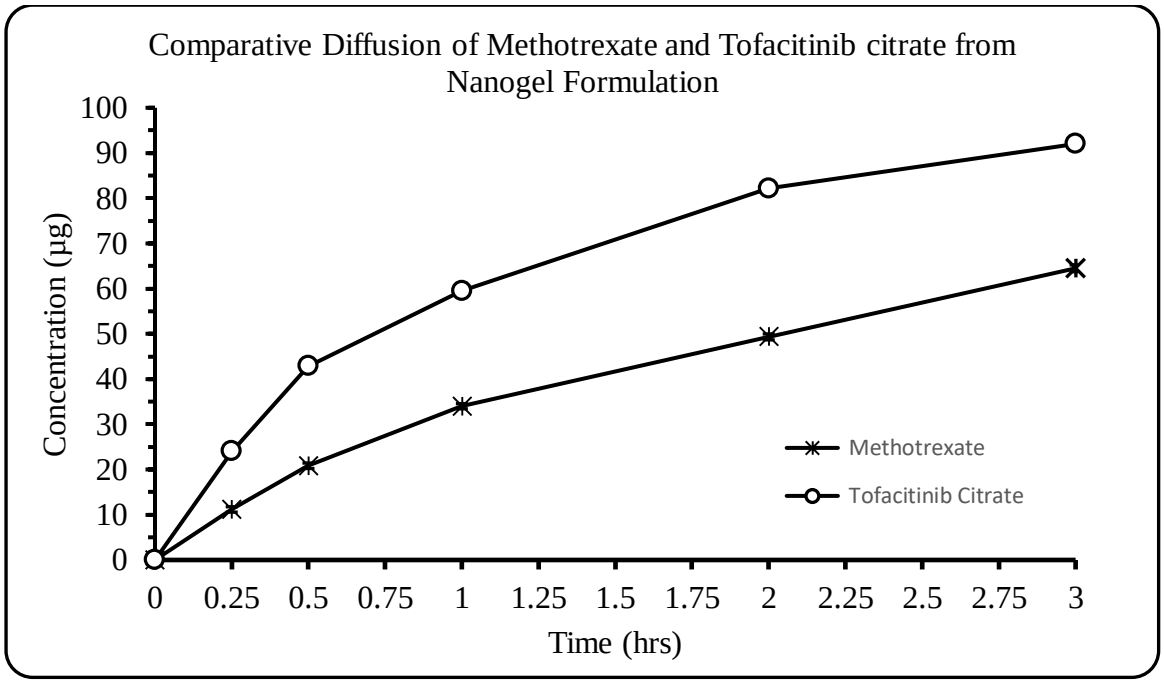

Figure 6.26: Comparative Dermatokinetic Diffusion of Methotrexate and Tofacitinib citrate from the Nanogel Formulation (F3)

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.

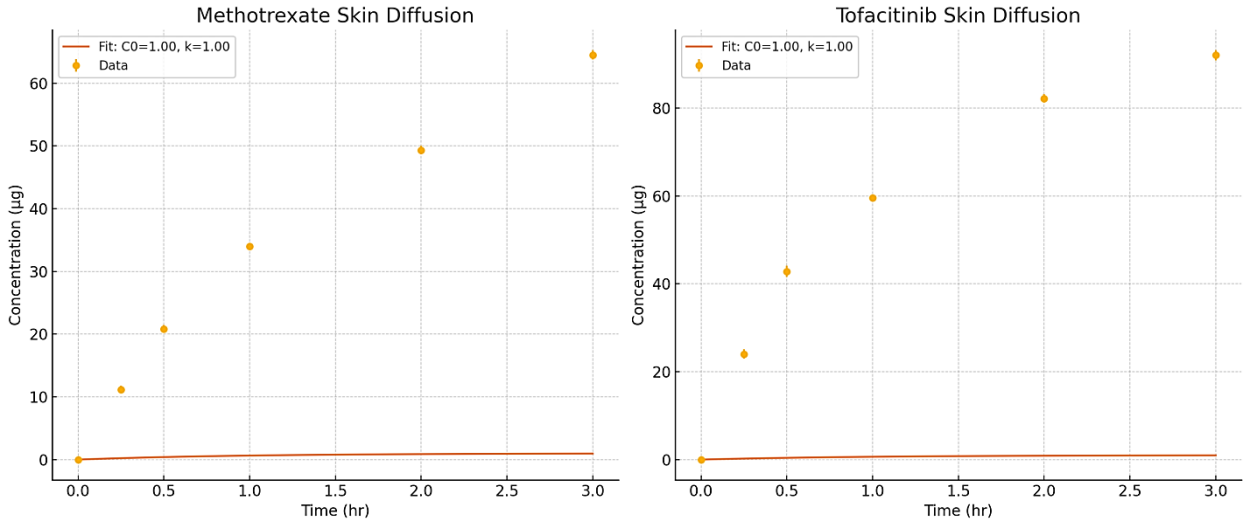

Figure 6.27: Skin Diffusion of Methotrexate and Tofacitinib Citrate

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:

- $C_0 \approx 66.37$ µg
- $k \approx 0.88\ hr^{-1}$

Tofacitinib Citrate:

- $C_0 \approx 99.13$ µg
- $k \approx 0.91\ hr^{-1}$

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.