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Pharmaceutical and pharmacokinetic profiling of methotrexate for cancer therapeutics

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ABSTRACT :

Cancer known as a major contributor to morbidity and mortality worldwide, with carcinoma accounting for the majority of cases. Head and neck squamous cell carcinoma (HNSCC), a prevalent subtype, is driven by complex genetic and epigenetic alterations affecting key regulatory pathways. Methotrexate (MTX), a key agent in cancer therapy due to its strong efficacy and specific action as a folate antagonist. This study examines the comprehensive pharmacological characteristics of MTX, including its mechanism of action, pharmacokinetic profile, potential drug interactions, toxicity, and contraindications. In addition, detailed preformulation studies were carried out to analyse the physicochemical and micromeritic properties of MTX, providing essential data for its formulation development. These include organoleptic analysis, solubility, stability, hygroscopicity, partition coefficient, and FTIR compatibility studies. The results support the suitability of methotrexate for formulation development and highlight considerations for optimizing its therapeutic potential.

Keywords: Cancer, Carcinoma, Methotrexate, Antimetabolites, Preformulation Studies.

INTRODUCTION

One of the most dreaded illnesses of the 20th century, cancer is continuing to spread and is becoming more common in the 21st century. Every fourth person has a lifetime risk of developing cancer, which is a concerning situation [(1)].

Cancer is a complex series of diseases characterized by an uncontrolled proliferation of cells into masses, referred to as malignant neoplasm or malignant tumours. When some tumour cells spread to different areas of the body from the original tumour site, this is known as metastasis [(2)].
There are various forms of cancer, such as leukemia, lymphoma, sarcoma, and carcinoma [(2)].The choice of an optimal cancer therapy depends on several factors, including the type of malignancies, stage of cancer, age of patients, dose of medicines, and health of patients . The treatment strategies include surgery, radiation, immunotherapy, targeted delivery and chemotherapy [(3)].

CARCINOMA

Eighty to ninety percent of all cancer diagnoses are carcinomas, making them the most prevalent type of cancer. Carcinoma develops in the epithelial tissue that lines your skin, internal organs, and bodily passages. Tumours that develop on your skin or in your lungs, breasts, prostate, colon, kidneys, pancreas, etc., are known as carcinomas. Carcinoma were classified into adenocarcinoma, basal cell carcinoma (BCC), and squamous cell carcinoma (SCC) [(4)].

1.1 HEAD AND NECK CANCER

Squamous cell carcinomas (SCC) account for 90% of head and neck cancers, which are defined as cancers that develop in the mucosal surfaces of the oral cavity, pharynx, and larynx. Human papillomavirus (HPV) infection, poor diet, and alcohol and tobacco use are risk factors linked to head and neck squamous cell carcinoma [(5)].

1.2 PATHOPHYSIOLOGY OF HEAD AND NECK CANCER

Head and neck squamous cell carcinoma (HNSCC) is driven by genetic and epigenetic changes affecting key oncogenes and tumour suppressors. The p53 tumour suppressor gene is mutated, leading to impaired DNA repair and apoptosis. In HPV-positive tumours, the HPV16 E6 protein also contributes to p53 inactivation by promoting its degradation. The epidermal growth factor receptor variant III (EGFRvIII), a mutant or overexpressed form of EGFR, triggers downstream signalling through the PI3K/Akt and MAPK pathways. This promotes uncontrolled growth and survival. The transcription factor

STAT3 is frequently persistently active in HNSCC, enhancing tumour angiogenesis, proliferation, and resistance to cell death. Additionally, c-Met, a receptor activated by hepatocyte growth factor (HGF), drives invasion and blood vessel formation through the Akt and MEK signalling pathways.

By activating MAPK and phosphoinositol-3-kinase (PI3K), the insulin-like growth factor-1 receptor (IGF-1R) facilitates the growth and spread of tumours and can dimerize with EGFR. Downstream of PI3K/Akt, mammalian target of rapamycin (mTOR) controls protein synthesis and is frequently active in HNSCC. STAT3-upregulated vascular endothelial growth factor (VEGF) signalling promotes angiogenesis, while stromal and tumour cells aid in vascular remodelling [(5)].

2 . METHOTREXATE [MTX]

- Antimetabolites were one of the most effective widely used class of anticancer drugs. As the earliest rationally designed agents targeting DNA and RNA synthesis, they are considered the first generation of targeted cancer therapies [(6)].
- Methotrexate, an antimetabolite act as a folate antagonist and was originally developed for cancer treatment [(7)].

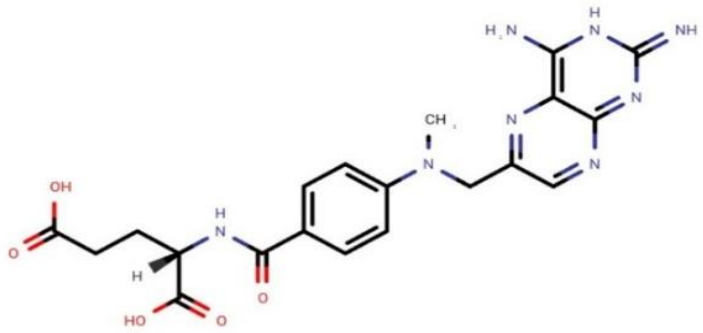

Fig 1 :Structure of methotrexate

2.1 . MECHANISM OF ACTION

Methotrexate is a folic acid antagonist. In liver, it is converted to polyglutamate which then bind to polyglutamate and inhibits the formation of tetrahydrofolate (THF). The formation of THF is prevented by the binding of polyglutamate to DHFR. THF is a coenzyme required for the several reactions in DNA, RNA and protein synthesis. Since the shortage inhibits protein synthesis, cells that proliferate quickly are most impacted. It is most effective against cells actively dividing during the S phase of the cell cycle, where DNA replication takes place [(9)].

2.2 . SIDE EFFECTS

Side effects include bone marrow suppression, nausea, vomiting, diarrhoea, alopecia, and dermatitis. As the drug may get precipitated in the renal tubules, nephrotoxicity can be caused. When injected intrathecally, methotrexate can cause myelopathy and encephalopathy [(9)].

2.3 . PHARMACOKINETICS

- **Absorption** :Methotrexate is absorbed quickly and almost complete after the intramuscular injection or on the uptake of low oral dose (≤ 30 mg/m²) while the oral administration in higher doses (> 80 mg/m²) may lead to reduce the absorption due to limited uptake in the gastrointestinal tract.
  In case of oral methotrexate, the gastrointestinal absorption is increased on pretreatment with kanamycin and decreased on pretreatment with oral neomycin. Through a carrier-mediated active type mechanism, methotrexate is transferred across cellular membranes. When the carrier pathway is saturated at large concentrations, passive diffusion becomes more significant.
- **Distribution:** Methotrexate binds to plasma proteins at a moderate level, about 50% and can influence its distribution and clearance. However, the medication does not significantly accumulate in the cerebral fluid due to its strong ionization at physiological pH.
- **Metabolism:** Methotrexate is metabolized by gut microorganisms during absorption. Urine from patients receiving large doses of methotrexate has also been observed to contain trace quantities (<11%) of 7-hydroxymethotrexate. Methotrexate is a more efficient inhibitor of dihydrofolate reductase than the metabolites, with the exception of poly-γ-glutamates**.**
- **Excretion**: Methotrexate is eliminated primarily by renal excretion (about 80 %); the general organic acid transport system actively secretes the medication in the renal tubule. Therefore, concurrent administration of organic acids, such as salicylate, reduces renal clearance of methotrexate. There is a correlation between endogenous creatinine clearance and the renal clearance of methotrexate [(10)].

2.4 . DRUG INTERACTIONS

- Salicylate and Sulfisoxazole may increase the free methotrexate levels in plasma by displacing drug from its binding sites on plasma proteins.
- Probenicid, Salicylate and other weak organic acids inhibits the renal tubular transport.
- Cephalothin and Hydrocortisone blocks the absorption of Methotrexate.
- Disruption of the enterohepatic circulation of Methotrexate by antibiotics [(11)].

2.6 . CONTRAINDICATIONS

The following conditions make methotrexate contraindicated: known sensitivity to MTX; active pulmonary disease; peptic ulcer disease; hepatic, renal, or haematologic dysfunction; overt or laboratory evidence of immunodeficiency; alcoholism, alcoholic liver disease, or other chronic liver disease; pre-existing blood dyscrasias, such as bone marrow hypoplasia, leucopenia, thrombocytopenia, or significant anaemia; breastfeeding [(12)].

2.7. TOXICITY

If a patient shows signs and symptoms of possible MTX poisoning, they should be taken to the hospital and treated there. Reverse barrier nursing should be monitored by treatment professionals. The three major goals of treating severe MTX toxicity are: removing MTX from the bloodstream, administering folinic acid, and treating the organ [(13)].

Leucovorin is extremely effective in avoiding myelosuppression, gastrointestinal toxicity, and neurotoxic consequences following HD-MTX therapy. HD-MTX chemotherapy protocols include instructions for leucovorin treatment timing and dose to prevent cell damage. Leucovorin effectively neutralizes methotrexate effects [(14)].

2.8. AVAILABLE FORMULATION

Table 1: Available formulation of methotrexate [(15)]

Brand name	Dosage form	Route
Biotrexate	Tablet	Oral
Mext	Tablet	Oral
Trexall	Tablet	Oral
Folitrax	Tablet	Oral
Jylamvo	Solution	Oral
Methotrexate injection	Injection	IV ,IM, and Subcutaneous

PREFORMULATION STUDIES

Preformulation studies are intended to provide all relevant information, particularly about the physicochemical, physicomechanical, and biopharmaceutical characteristics of medicinal ingredients, excipients, and packaging materials. Preformulation testing's main goal is to produce data that will help formulators create stable, bioavailable, and mass-producible dosage forms [(16)].

SIGNIFICANCE:

- Developing advanced dosage forms is essential to enhance the safety, stability, and therapeutic performance of pharmaceutical products, ultimately improving patient outcomes.
- To understand the drug's properties for better formulation and quality control.
- To choose the best physical form of the drug for optimal performance and stability.
- Studying the degradation patterns and kinetics of the drug helps predict its shelf life, define proper storage conditions, and ensure long-term effectiveness and safety.
- To act as the initial and essential phase in designing a suitable dosage form for a new drug compound.
- To investigate the compatibility of the drug with excipients used in pharmaceutical formulations [(16)].

3.1. PREFORMULATION STUDY OF METHOTREXATE

A. **Organoleptic properties:** The sample methotrexate was a brown, crystalline, odourless, hygroscopic, yellow to orange powder [(17)].

B. Physical properties

Melting point:

The capillary method is used for the determination of melting point of methotrexate by the use of Thiele's melting point apparatus. Here the temperature at which the sample methotrexate began to melt was recorded. The methotrexate sample were poured into a capillary tube which is sealed at one end and tied in a manner that it remains dipped in a liquid paraffin bath. Then the melting temperature was noted. Methotrexate melts at a temperature of 180-190 °C [18].

Solubility studies:

The solubility is the maximum quantity of a solute that can be dissolved in certain quantity of solution at specified temperature. 10 mg of methotrexate were dissolved in a 10ml of different solvents [chloroform. 0.1N NaOH. Ethanol. ether. 1-2 dichloromethane. water and Phosphate buffer (7.4)] at about room temperature. Then kept for 3 days in the separating funnel and was regularly shaken. Solubility is analysed by using a UV Spectrophotometer.

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Table 1: Solubility of solvents on methotrexate

S/N	Solvents	Solubility
1	0.1 N sodium hydroxide	Highly soluble
2	Chloroform	Insoluble
3	Ethanol	Insoluble
4	Ether	Insoluble
5	1,2-dichloromethane	Insoluble
6	Water	Insoluble
7	Phosphate buffer (pH 7.4)	Soluble

Hygroscopicity

The capacity of a substance to absorb moisture from its surroundings, known as hygroscopicity, significantly influences its stability, texture, and overall product performance. The most common method for measuring water uptake in pharmaceutical powders is gravimetric. For a predetermined amount of time, samples are exposed to different relative humidities while maintaining a constant temperature. A water vapour sorption isotherm is then created by converting the solid's weight change as a function of relative humidity. The nature of methotrexate is hygroscopic [20].

C. Chemical properties:

Partition co-efficient

Partition co efficient was determined by shaking 10 mg of methotrexate sample in a separating funnels having 10 ml of n-octanol and 10ml water. The it was shaken for 2hrs by using the wrist action shaker equilibration. Following the proper dilution, two phases (aqueous and organic phase) were separated, and the amount of medication in the aqueous phases was measured at 300 nm spectrophotometrically. The partition coefficient of the drug was calculated by the formula; [21].

$$\text{Partition coefficient} = \frac{\text{concentration of drug in organic phase}}{\text{concentration of drug in aqueous phase}} \qquad (1)$$

Dissociation constant

Tablets of methotrexate should be dissolved in an appropriate solvent, such as water or buffer solution. Use buffer solutions with established pH values to calibrate the pH meter. Perform potentiometric titration by adding the titrant solution (e.g., NaOH or HCl) to the methotrexate solution while measuring the pH. Record the pH values against the volume of titrant added. Plot the pH values against the volume of titrant added and determine the pK_a value from the inflection point of the curve. Methotrexate has a pK_a value of approximately 4.7 [22].

Stability studies

pH is one of the most important factors affecting the stability of a drug that in turn is a major criterion in determining its suitability for a particular application. N10-methylfolic acid is a byproduct of MTX breakdown at alkaline pH ranges. Hence, it is customary to develop "pH-Rate profiles " for drugs to determine the pH at which they are most susceptible to degradation [23].

The MTX is moderately sensitive to heat. Methotrexate's photostability was evaluated using a forced degradation study under UV light for 20 days, with periodic analysis of MTX content. Significant degradation was only observed with intense artificial light exposure [24].

D. Micromeritic properties:

Angle of repose

The angle of repose, representing the steepest angle between a powder pile and a horizontal surface, was measured using the funnel method. This involved pouring the mixture through a vertically adjustable funnel until a predetermined cone height was reached. The angle of repose (θ) was computed using the following formula once the heap's radius was measured;

$$Tan\ \theta = \frac{h}{r} \qquad (2)$$

Where r = pile's base radius and h = pile's height [25].

Table 2 : Angle of repose

S/N	Angle of repose	Flow property
1	<25	Excellent
2	25-30	Good
3	30-40	Passable
4	>40	Poor

Compressibility index

Compressibility, a measure of how easily a material can be made to flow, was the most straightforward method for determining the powder's flow [25].

$$Compressibility\ index = \frac{Tapped\ density - bulk\ density}{Tapped\ density} \times 100 \qquad (3)$$

Table 3 : Compressibility index

S/N	Compressibility index	Flow property
1	5-11	Excellent
2	12-17	Good
3	18-21	Fair to passable
4	22-34	Poor
5	>35	Very poor

Hausner ratio

It is the ratio of bulk density to tapped density. Cohesion reflecting particle friction is measured using it. Hausner ratio value that are less than 1.25 exhibit good flow property while more than 1.25 exhibit poor flow property [26].

$$Hausner's\ ratio = \frac{Tapped\ density}{bulk\ density} \qquad (4)$$

Bulk density

The blend was poured into a graduated cylinder to determine the apparent bulk density. The bulk density was determined by dividing the mass of the powder by its bulk volume (27).

$$Apparent\ bulk\ density = \frac{weight\ of\ powder}{bulk\ volume} \qquad (5)$$

Tapped density

Using a density device, the measuring cylinder containing a known mass of blend was tapped 100 times. After tappings, the blend's weight and the constant minimum volume occupied in the cylinder were measured [27].

$$Tapped\ density = \frac{weight\ of\ powder}{Tapped\ volume} \qquad (6)$$

3.2 DRUG EXCIPIENT COMPATIBILITY STUDIES

Fourier transform infrared Spectroscopy (FTIR)

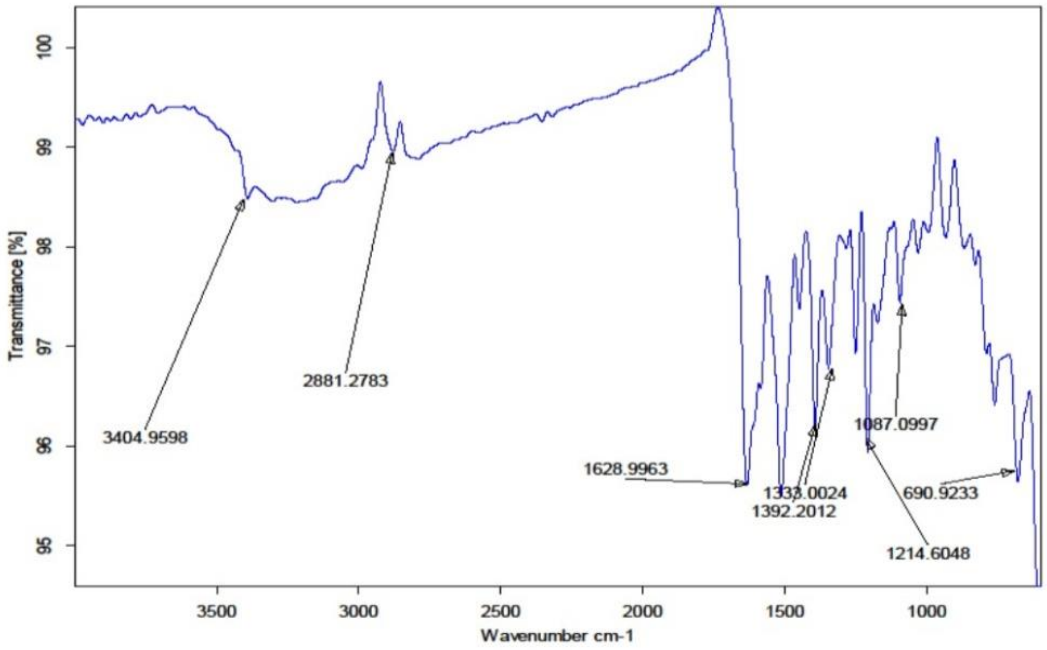

Fig 2 : FTIR Spectra of methotrexate

FTIR spectra was recorded on the pure drug and its 1:1 mixture with excipients by FTIR spectrophotometer, using KBr discs for sample preparation. A hydrostatic press (Kimaya engineers, Mumbai, India) was used to press each sample for five minutes at a pressure of ten tons after it had been gently triturated with KBr in a weight ratio of one to one hundred. With a resolution of 1 cm-1, the disk was scanned from 4000 to 500 cm-1 while in the sample holder [28].

Table 4 : FTIR characterization of methotrexate

Wavelength	Functional group
3404.96	OH/NH Stretch
2881.28	CH stretch
1628.99	C=O stretch
1392.20	Symmetric CH_3 bending
1338.00	CN stretch

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REFERENCES :

1. 1.Mackay J, Jemal A, Lee NC, Parkin DM The Cancer Atlas. 2006 Atlanta American Cancer Society.
2. 2.Shampton JS, Delaney L, Murphy ME, Smith K, Spahn MA. Introduction to cancer. Journal of Continuing Education Topics & Issues. 2007 Apr;9(2).
3. 3.Jones A . Pathology of Cancer: Causes, Pathophysiology, Diagnosis, Prevention and Treatment. J Med Surg Pathol. 2023 ; 08:267.
4. 4.Cleveland Clinic. Carcinoma. [Cited: 31.05.2023].
5. Jonah D klein, Gennifer R Grandis. The molecular pathogenesis of head and neck cancer. Cancer Biol Ther. National library of medicine. 2010 Jan 9;9(1):1-7.

6. 6.Peters GJ. Novel Developments in the Use of Antimetabolites. Nucleosides, Nucleotides & Nucleic Acids. 2014;33(4-6):358-374.

7. 7.Genestier L, Paillot R, Quemeneur L, Izeradjene K, Revillard J. Mechanisms of action of methotrexate. Immunopharmacology. 2000 May;47(2-3):247-257.

8. 8.Lotfi M, Moniruzzaman M, Abdul Mutalib MI, Wilfred CD, Binti Atirahh N, Ghaffour N. Analysis of Multiple Solvation Interactions of Methotrexate and Ammonium Based Ionic Liquids Using COSMO-RS. Procedia Engineering. 2016;148:439-446.

9. 9.Mantra Pharma Inc. Methotrexate Product Monograph. Brossard, Quebec: Mantra Pharma Inc.; 2023 Feb 1.

10. 10.Shen DD, Azarnoff DL. Clinical Pharmacokinetics of Methotrexate. 2012;3:1-13.

11. Bleyer WA. Methotrexate: clinical pharmacology, current status and therapeutic guidelines. Cancer Treatment Reviews 1977; 4: 67-101.

12. 12.Barnhart K, Coutifaris C, Esposito M. The pharmacology of methotrexate. Expert Opin Pharmacother. 2001;2(3):409-417.

13. Niinimäki R, Aarnivala H, Banerjee J, Pokka T, Vepsäläinen K, Harila-Saari A: Reduced dose folinic acid Rescue after rapid high-dose methotrexate clearance is not associated with increased toxicity in a pediatric Cohort. Support Care Cancer. 2022, 30:127-33.

14. 14.Widemann BC, Adamson PC: Understanding and managing methotrexate nephrotoxicity. Oncologist. 2006,11:694-703. 10.1634/theoncologist.11-6-694.

15. 15.Methotrexate : Uses, Interactions, Mechanism of Action. Drug Bank [Internet]. [cited 2023 Feb 20].

16. 16.Kumar D, Dixit G, Dixit K. An overview on preformulation studies. Indo American journal of pharmaceutical science. 2015; 2 (10) : 1399-1407.

17. 17.Lewis ,R.J., Sr (Ed).Hawley's Condensed Chemical Dictionary. 13th edition. New York, John wiley and sons, Inc. 1997., page no. 722.

18. 18.Shukla K, Singh B. To Evaluate the Methotrexate Drug with its Preformulation Method Confirming for the Preparation of Nano-formulation Optimization. World J Pharm Res. 2020;9(6):1882-1889.

19. 19.Fry, Margaret Mary, and Caroline Dacey. "Factors contributing to incidents in medicine Administration. Part 2." British Journal of Nursing, 2007; 16: 11.

20. Ann w. Newman, George zografi , Susan M. Characterisation of the hygroscopic properties of active pharmaceutical ingredients. Journal of Pharmaceutical science. March 2008, 97(3) ,page no.1047-1059.

21. Chaurasia G. A Review on Pharmaceutical Preformulation Studies in Formulation and Development of New Drug Molecules. Int J Pharm Sci Res. 2016;7(6):2313-2320.

22. Zoltan Szakacs, Bela noszal, electrophoresis. Determination of dissociation constant of folic acid, methotrexate and other photolabile pteridines by pressure assisted capillary electrophoresis. 2006 ,27 ; 3399-3409.

23. 23.Sayantan ray, Mathew joy, beswanath , swapankumar ghosh and juice Chakraborty. pH dependant chemical stability and release of methotrexate from a novel nanoceramic carrier, RSC advances, 2015 ; 5; 39482-39494.

24. Bhupendra raj giri, jung suk kim, jong hyuck park, sung giu jin. Improved bioavailability and high photostability of methotrexate by spray dried surface attached solid dispersion with an aqueous medium. 2021,13 (1).

25. Hasan MM, Habib SA, Islam MM, Islam MM, Karim MS, Bekir K, Rashid HA. Pharmaceutical preformulation for product development & analytical techniques use in new dosage form. Int J Pharm Ther. 2017;8(1):16-32.

26. Ofori-Kwakye K, Osei-Yeboah F, Kipo SL. Formulation and quality evaluation of two conventional release tablet formulations. Int J Pharm Sci Rev Res. 2010;4(1):Article 017.

27. Bhardwaj J, Mukhopadhyay S. Design and Evaluation of Methotrexate Loaded Multi-layered Tablet Formulation for Treatment of Colon Cancer. Int J Pharm Sci Res. 2014;5(4):1352-1361.

28. Abbas HH, Flayyih MT. Synthesis and characterization of methotrexate nanoparticles. International Journal of Health Sciences. 2022;6(S6):7255-63.