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To study the solubility of tofacitinib citrate

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Preformulation studies on tofacitinib citrate, a small molecule JAK3 specific inhibitor, have not been previously reported in literature. We therefore conducted several preformulation studies on tofacitinib citrate, and its free base, to better understand factors that affect its solubility, stability, and solid-state characteristics. Further, the results of the preformulation studies helped facilitate the development of a nebulized formulation of tofacitinib citrate for inhalational delivery to house dust mite allergen-challenged, BALB/c mice as a potential treatment for eosinophilic asthma. The preformulation results indicated tofacitinib having a basic p*Ka* of 5.2, with its stability dependent on pH, ionic strength, and temperature. Degradation of tofacitinib follows apparent first-order kinetics. In order to maximize stability of the drug, ionic strength and temperature should be minimized, with an optimal range pH between 2.0 and 5.0. Additionally, our findings demonstrate that tofacitinib citrate can successfully be nebulized at a suitable droplet size for inhalation (1.2 ± 0.2 μm MMAD) through a nose-only chamber. Animals dosed with tofacitinib citrate demonstrated marked reductions in BAL eosinophils and total protein concentrations following HDM challenge. These data suggest that tofacitinib citrate represents the potential to be an effective therapy for eosinophilic asthma.

Keyword – tofacitib citrate, solubility, ph determination

1. Introduction

Two important factors in the entire development process are drug solubility and bioavailability. Poor water solubility has been attributed to almost half of the 150,000 new molecular entities (NMEs) synthesized annually by pharmaceutical companies, and is also claimed to reduce the performance of more than 10% of successfully marketed drugs.

Drug solubility is an important parameter for both oral and intravenous administration. Fororal drug delivery, aqueous solubility in various pH media is one of the most important properties of the drug substance which can significantly affect the drug absorption and subsequently the bioavailability

A careful selection of the buffer system and its concentration is essential for pH solubility screening. The type of the buffer salt and its concentration can have a significant impact onthe solubility of the drug substance. For instance, a pH 4.5 buffer can be prepared using either acetate or citrate salt. These two buffers at identical concentrations my show a difference inthe solubility of the drug substance attributed to the structural properties of the drug substance and/or the salt form. The concentration of the buffer system also must be selected carefully.

A very high buffer concentration may lead to erroneously high solubility number but may not be feasible for i.v. administration.

Various techniques are available for solubility determination of drug substance. The oldest and the most commonly used solubility measurement technique was developed by Higuchi and Connors. Saturation solubility of the drug substance can be carried out by adding aliquots of drug substance to a small volume of the solvent (usually 2–4 mL). The mixture is then allowed to equilibrate by shaking or rotating at ambient temperature for a period of at least 24 h or until un-dissolved particles are observed at the bottom of the vial/tube. The mixtures are centrifuged and/or filtered, the supernatant withdrawn and analyzed for assay. Most researchers like to carry out the saturation solubility study at ambient temperature, whereas some like to perform the study at 37 °C.

In early drug development, a quick and robust tool is required to analyze the solubility of the drug substance. For this reason several high-throughput techniques are

available for solubility screening. These techniques can assist the pre-formulation group to successfully screen thousands of compounds in a short period of time. A list of these techniques are presented.

Limitations:

1.Low Aqueous Solubility
Tofacitinib citrate has limited solubility in water, which can lead to poor absorption in the gastrointestinal tract when administered orally.

2.Variable Bioavailability
Due to its solubility issues, the bioavailability of tofacitinib citrate can be inconsistent, potentially leading to variability in therapeutic outcomes.

3.Formulation Challenges
Developing an effective drug formulation for tofacitinib citrate can be challenging. Techniques such as the use of solubilizing agents, nanoparticles, or solid dispersions might be necessary to improve its solubility and absorption.

4.Food Effects
The presence of food in the stomach can affect the solubility and, consequently, the absorption of tofacitinib citrate, which can complicate dosing regimens.

5.Dosage Form Limitations
The choice of dosage form (e.g., tablet, capsule) may be limited due to the solubility characteristics of tofacitinib citrate, impacting patient compliance and convenience.

6.Potential for Precipitation
In the gastrointestinal tract, the drug may precipitate out of solution, reducing the effective dose that is absorbed into the bloodstream.

Applications

1.Formulation Development
Enhancing the solubility of tofacitinib citrate can lead to the development of more effective oral dosage forms. Techniques such as solid dispersions, inclusion complexes with cyclodextrins, and the use of solubilizing agents can be employed to improve solubility.

2.Bioavailability Improvement
Increased solubility can enhance the bioavailability of tofacitinib citrate, ensuring that a higher percentage of the administered dose reaches systemic circulation. This can lead to moreconsistent therapeutic outcomes.

3.Dosing Efficiency
Improved solubility can reduce the required dose needed to achieve therapeutic effects, potentially minimizing side effects and improving patient compliance.

4.Route of Administration
Solubility considerations can influence the choice of administration routes. For instance, improved solubility may allow for alternative routes such as sublingual or buccal administration, which can bypass first-pass metabolism and improve bioavailability.

5.Combination Therapies
Understanding solubility can aid in developing combination therapies where tofacitinib citrate is co-administered with other drugs. This ensures compatibility and maximizes the therapeuticefficacy of both drugs.

6.Food Effect Mitigation
Formulations designed to improve solubility can help mitigate the effects of food on drug absorption, leading to more predictable pharmacokinetics.

7.Personalized Medicine
Solubility optimization can support the development of personalized medicine approaches, where dosages are tailored to individual patient needs based on their specific absorption characteristics.

8.Regulatory Approval
Demonstrating improved solubility and consistent bioavailability can facilitate regulatory approval processes by providing robust data on the drug's efficacy and safety.

How to study the solubility

1.Preparation of Saturation Solutions:
Weighing: Accurately weigh an excess amount of tofacitinib citrate.
Solvent Selection: Choose various solvents, such as water, buffer solutions of different pH, organic solvents (e.g., ethanol, methanol), and biorelevant media (e.g., simulated gastric fluid, simulated intestinal fluid).
Mixing: Add the solvent to the weighed drug and mix thoroughly to ensure the drug is in excess.

2.Equilibration:
Incubation: Place the mixtures in a constant temperature bath or shaker and maintain them at a controlled temperature (typically 37°C for physiological relevance) for a specified period (usually 24-48 hours) to reach equilibrium.
Agitation: Agitate the mixtures continuously during the incubation period to ensure proper mixing and

dissolution.

3.Filtration:
After equilibration, filter the mixtures to separate the undissolved drug from the saturated solution. Use a suitable filter (e.g., 0.45 µm or 0.22 µm membrane filter).

4.Analysis:
Concentration Determination: Analyze the filtered solutions to determine the concentration of dissolved tofacitinib citrate. Common analytical techniques include:
High-Performance Liquid Chromatography (HPLC): HPLC is a preferred method for its precision and accuracy. Use a suitable mobile phase, column, and detection wavelength.
UV-Visible Spectroscopy: This method can be used if the drug has a strong absorbance in the UV or visible range.
Mass Spectrometry (MS): For more precise quantification and to confirm the presence of thedrug.

5.Data Analysis:
Solubility Calculation: Calculate the solubility of tofacitinib citrate from the concentrationdata obtained from the analytical methods.
pH Solubility Profile: Plot the solubility data against different pH values to understand thesolubility behavior in various pH conditions.

6.Characterization:
Solid-State Characterization: Use techniques like X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC), and Fourier-transform infrared spectroscopy (FTIR) to confirm the physical state and purity of the drug before and after solubility studies.

7.Comparative Studies:
Compare the solubility of tofacitinib citrate with other salts or formulations to explore ways to enhance its solubility

Measuring solubility

In pharmaceutical practice, one important task is to measure the solubility of solids in liquids. The following precautions serve as guidelines when running solubility tests:

• The solvent and the solute must be pure. • The sample is removed for analysis after confirmation of saturation.

• Sample separation from saturated solution with un-dissolved solute must be reliable and satisfactory.

• The method used to analyse the solution must be reliable and reproducible. • Temperature must be adequately controlled. Traditionally, the equilibrium solubility at a given pH andtemperature is determined by the shake flask method. According to this method the compound is added in surplus to a certain medium and shaken at a predetermined time, usually 24h or longer. The saturation is confirmed by observation of the presence of un-dissolved material. Saturation can also be reached if the solvent and excess solute is heated and then allowed to cool to the given temperature. Some solutions can hold a certain amount of excess solute in the solvent, commonly called a supersaturated solution.

This often occurs when the saturated solution is cooled slowly. Super saturation for salts can be avoided by slow cooling and continuous shaking of the sample during cool down. After filtration of the slurry a sample for analysis can be taken. Both filtration and analysis should be performed under the same temperature as the solubility determination and under conditions to minimize loss of volatile components. Often the sample is diluted to prevent crystallization.

The amount of solute contained in the sample is determined by an appropriate method affected by the nature of the solute/solvent and by the concentration. Common methods are;

Ultraviolet /visible spectroscopyChromatographic methods Gravimetric/volumetric

The shake-flask method is the most accurate method to determine solubility but it is time consuming. Due to the growing need to determine solubility faster new devices8,9 and automated methods10,11 have been developed.

4. PLAN OF WORK:

1) Literature Survey
2) Selection of Tofacitinib citrate API
3) Compatibility study of Tofacitinib citrate

- Solubility study

4) Identification of Tofacitinib citrate API: solubility
5) Evaluation Of Tofacitinib citrate

- Appearance
- IUPAC Name
- Colour
- Molecular formula
- Melting point
- BSC class
- Dissolution constant
- Half life
- Solubility
- Dose
- Percentage of compound present

6) Conclusion

5. MATERIAL AND METHOD:-

Sr.no.	Properties	Tofacitinib Citrate
	UPAC Name	-[(3R,4R)-4-Methyl-3-methyl(7H-pyrrolo[2,3-]pyrimidin-4-l)amino]piperidin-1-yl]-3-xopropanenitrile
	Appearance	White to off white powder
	Colour	
	Molecular formula	C22H28N6O
	Molecular weight	12.377 g/mol
	Melting point	98-202° C (dec.)
	Log p	.15
	Category	anus kinase (JAK) inhibitor
	Dissociation constant	.46
10	BSC class	Class III (high aqueous olubility and moderate ermeability)
11	Half life	hours.
12	Synonyms	CP-690550, Xeljanz, Tasocitinib, (3R,4R)-4-Methyl-3-(methyl-7H-yrrolo[2,3-d]pyrimidin-4-lamino)-ß-oxo-1-iperidinepropanenitrile citrate alt
13	Bioavailability	74% oral absorption (absolute ioavailability), with peak lasma concentrations (T max) chieved in 0.5-1 hour.
14	Solubility	Freely soluble in N,N-Dimethylacetamide, slightly oluble in water, and very lightly soluble in ethanol 99.5% ethanol)

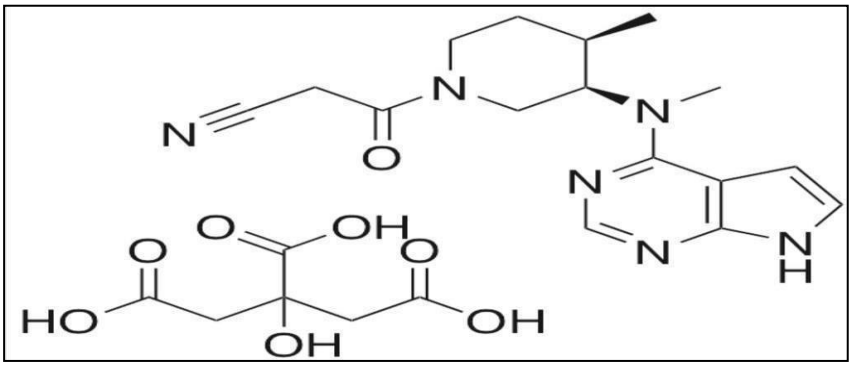


Fig.1.11:- Tofacitinib Citrate

Background:-

Tofacitinib is an inhibitor of Janus kinases, a group of intracellular enzymes involved in signalling pathways that affect hematopoiesis and immune cell function. It is approved by the FDA for treatment of moderate to severe rheumatoid arthritis that responds inadequately to methotrexate or in those who are intolerant to methotrexate. Besides rheumatoid arthritis, tofacitinib has also been studied in clinical trials for the prevention of organ transplant rejection,and is currently under investigation for the treatment of psoriasis. Known adverse effects include nausea and headache as well as more serious immunologic and hematological adverse effects. Tofacitinib is marketed under the brand name Xeljanz by Pfizer.

Indication

Tofacitinib is indicated for the treatment of adult patients with moderately-to-severely active rheumatoid arthritis (RA), active psoriatic arthritis, active ankylosing spondylitis, or moderately-to-severely active ulcerative colitis who have had an inadequate response or intolerance to one or more TNF blockers.5 It is also indicated as an oral solution in patients ≥2years of age for the treatment of polyarticular course juvenile idiopathic arthritis who have hadan inadequate response or intolerance to one or more TNF blockers.

Tofacitinib is not recommended to be used in combination with other biologic disease- modifying anti-rheumatic drugs (DMARDs) or potent immunosuppressive agents such as azathioprine or cyclosporine.

Pharmacodynamics

Tofacitinib targets inflammation present in rheumatoid arthritis by inhibiting the janus kinases involved in the inflammatory response pathway. In placebo controlled trials of rheumatoid arthritis patients receiving 5mg or 10mg of tofacitinib twice daily, higher ACR20 responses were observed within 2 weeks in some patients (with ACR20 being defined as a minimum 20%reduction in joint pain or tenderness and 20% reduction in arthritis pain, patient disability, inflammatory markers, or global assessments of arthritis by patients or by doctors, according to the American College of Rheumatology (ACR) response criteria list), and improvements in physical functioning greater than placebo were also noted. Common known adverse effects of tofacitinib include headaches, diarrhea, nausea, nasopharyngitis and upper respiratory tract infection. More serious immunologic and hematological adverse effects have also been noted resulting in lymphopenia, neutropenia, anemia, and increased risk of cancer and infection.

Before initiations of tofacitinib patients should be tested for latent infections of tuberculosis, and should be closely monitored for signs and symptoms of infection(fungal, viral, bacterial, or mycobacterial) during therapy. Therapy is not to be started in the presence of active infection, systemic or localized, and is to be interrupted if a serious infectionoccurs. Tofacitinib has been associated with an increased risk of lymphomas, such as Epstein- Barr virus associated lymphomas, and other malignancies (including lung, breast, gastric, and colorectal cancers). It is recommended to monitor lymphocytes, neutrophils, hemoglobin, liverenzymes, and lipids.

Tofacitinib use is associated with a rapid decrease in C-reactive protein (CRP), dose dependent decreases in

natural killer cells, and dose dependent increases in B cells.Depression in C-reactive protein levels continue after 2 weeks of tofacitinib discontinuation and suggest that pharmacodynamic activity last longer than pharmacokinetic half life

Fig.1.12: Tofacitinib citrate

API Powder Mechanism of action

Rheumatoid arthritis is an autoimmune disease characterized by a dysregulation of pro-inflammatory cytokines including IL7, IL15, IL21, IL6, IFN-alpha, and IFN-beta. (3) Cytokines signalling results in tissue inflammation and joint damage by stimulating the recruitment and activation of immune cells via the janus kinase signalling pathway.

Tofacitinib is a partial and reversible janus kinase (JAK) inihibitor that will prevent the body from responding to cytokine signals. By inhibiting JAKs, tofacitinib prevents the phosphorylation and activation of STATs. The JAK-STAT signalling pathway is involved in the transcription of cells involved in hematopoiesis, and immune cell function. Tofacitinib works therapeutically by inhibiting the JAK-STAT pathway to decrease the inflammatory response. However, there is evidence to suggest that it may also achieve efficacy via other pathways as well.

Absorption

74% oral absorption (absolute bioavailability), with peak plasma concentrations (T max)achieved in 0.5-1 hour. Administration with fatty meals does not alter AUC but reduces Cmax by 32%.

Toxicity

Minimum lethal dose in rat: 500 mg/kg. Maximum asymptomatic dose in non human primate: 40 mg/kg. Lymphatic, immune system, bone marrow and erythroid cell toxicity was seen in animal studies involving rate and monkeys. Doses used in these studies ranged from 1mg/kg/day to 10mg/kg/day, over a duration of 6 weeks to 6 months. Lymphopenia, neutropenia, and anemia is seen in human subjects and may call for an interruption or discontinuation of therapy if severe.

Reduced female fertility in rats was seen at exposures 17 times the maximum recommended human dose. Fertility may be impaired in human females and harm may be caused to unborn child. Carcinogenic potential is seen, however evidence for dose dependency is lacking. Because the janus kinase pathway plays a role in stimulating the production of red blood cells and is involved in immune cell function, inhibition of this pathway leads to increased risk of anemia, neutropenia, lymphopenia, cancer and infection.Lymphopenia, neutropenia, and anemia in human subjects may call for an interruption or discontinuation of therapy if severe. Role of JAK inhibition in the development of gastrointestinal perforation is not known.

Volume of distribution

Vd= 87L after intravenous administration. Distribution is equal between red blood cells and plasma.

Protein binding

40%, mostly bound to albumin.

Metabolism

Metabolized in the liver by CYP3A4 and CYP2C19. Metabolites produced are inactive.

Route of elimination

70% metabolized in the liver by CYP3A4 (major) and CYP2C19 (minor). Metabolites produced are inactive. 30% renally eliminated as unchanged drug.

Materials:

1.Tofacitinib Citrate: Pharmaceutical-grade sample of tofacitinib citrate.

2.Solvents: Various solvents such as water, organic solvents (e.g., ethanol, methanol, acetone), and sometimes co-solvents or surfactants depending onthe expected solubility characteristics.

3.Analytical Balances: For accurately weighing the drug sample.

4.Vessels: Glass vials or flasks for preparing solvents and samples.

5.Stirrers or Shakers: Equipment for agitation or stirring to facilitate dissolution.

6.Filtration Equipment: Filters (e.g., syringe filters) for separating undissolved particles from the solvent.

7.Analytical Instruments: Analytical balance, UV spectrophotometer, HPLC (High- Performance Liquid Chromatography), or other suitable instruments for quantifying drugconcentration.

Methods:

1.Preparation of Solvents: Prepare solvents of different pH (if required) and at various temperatures (e.g., room temperature, physiological temperature) depending on the studyobjectives.

2.Saturation Solubility Determination:

Equilibrium Method: Add excess tofacitinib citrate to a known volume of solvent in a sealed container. Allow the mixture to equilibrate with periodic shaking or stirring until equilibriumis reached.

3.Sample Collection: Filter the saturated solution to remove any undissolved particles. Collect the filtrate for analysis.

4.Analysis:

UV Spectrophotometry: Measure the absorbance of the filtrate at a specific wavelength corresponding to tofacitinib citrate.

HPLC: Quantify the concentration of tofacitinib citrate using a validated HPLC method, if more precise quantification is needed.

5.Calculation:

Calculate the concentration of tofacitinib citrate in the solvent using the calibration curve obtained from standard solutions of known concentrations.

Express solubility in terms of mg/mL or other suitable units.

6.Data Interpretation: Record and analyze the solubility data, considering factors such as pH, temperature, and

solvent type.

7.Validation: Validate the method for accuracy, precision, specificity, and linearity according to pharmaceutical standards.

8.Reporting: Present results clearly, including conditions under which solubility was determined, any deviations from standard procedures, and any special considerations.

6. Result and discussion

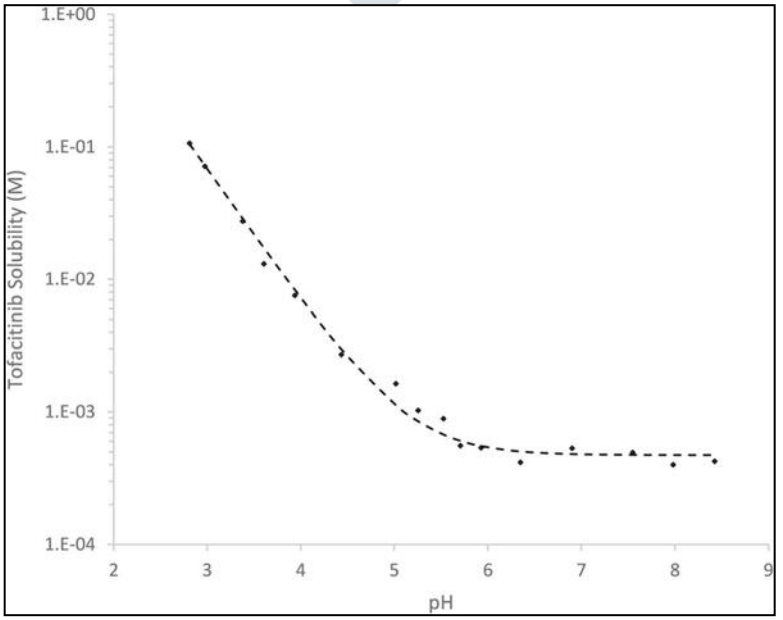
Parameter cheking in the studies of tofacitinib citrate

1.Solubility study

Solubilization studies were carried out using tofacitinib citrate. The effect of pH or cosolvent on the solubility of tofacitinib was determined using a universal buffer. Samples were prepared using an excess of tofacitinib citrate and adjusted for pH as needed using sodium hydroxide and hydrochloric acid. Samples were monitored for equilibration by HPLC. Following equilibration at room temperature, samples were filtered using a 0.22-µm PTFE filter and assayed by HPLC. The effect of pH was evaluated over a range from 2.8 to 8.5 and the effect of cosolvent was evaluated at pH 7 over a range of 2.5% to 10% *v*/*v* of either ethanol of propylene glycol.

2.Ph Solubility Profile

The effects of pH solubilization on tofacitinib are presented in Fig. 1. Tofacitinib solubilization was evaluated from pH 2.8 to pH 8.5 and equilibrated at room temperature for 7 days. Tofacitinib solubility was shown to increase exponentially with decreasing pH below its pK_a. The pK_a was determined to be 5.2 by best fit by residual sum squares (RSS) of a theoretical base using the Henderson-Hasselbalch equation, with the total amount of drug in solution being the sum of the ionized and unionized form (assuming the unionized concentration remains constant). This value was in good agreement with the predicted value calculated using the ACD/Labs PhysChem software version 7.0. The intrinsic solubility was determined to be 147 µg/mL. Additionally, tofacitinib was evaluated at pH 2.2 and pH 3.5 where its solubility was determined to be 5.2 mg/mL and 1.8 mg/mL respectively.



7. Conclusion

Tofacitinib has been shown to undergo apparent first-order degradation *via* base catalysis in aqueous conditions. The stability of tofacitinib is dependent on pH, ionic strength, and temperature, with maximum stability achieved under acidic conditions (below pH 5.0), at low temperatures and ionic strengths. Solubility of tofacitinib can be manipulated with pH, with its solubility increasing below its pKa of 5.2. Solid-state characterization has determined tofacitinib and its salt form, tofacitinib citrate, to be crystalline solids. When formulated as an aerosol for inhalational delivery to HDM-challenged, BALB/c mice, tofacitinib citrate was able to decrease eosinophils in BAL fluid, as well as decreasing total protein concentration.

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