SYNTHESIS AND CHARACTERIZATION OF MEFENAMIC ACID HYDRAZIDE DERIVATIVE

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Abstract- NSAIDs such as mefenamic acid are medications that relieve pain, minimize inflammation and lower fever by preventing the production of prostaglandins which are, chemical messengers that contribute to inflammation and pain. This research focuses on mefenamic acid hydrazide synthesis for Alzheimer disease. The symptoms of Alzheimer's disease include the breakdown of acetylcholine-producing neurons and a decrease in acetylcholine levels in the brain, which is attributed to the activity of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), It mainly affects cognitive function, memory, and behavior. NSAIDs have been shown to inhibit the activity of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), which are enzymes that break down acetylcholine in the brain. AChE and BuChE inhibition can lead to increased levels of acetylcholine. The mefenamic acid hydrazide derivative is synthesized in two steps, the reaction of mefenamic acid with thionyl chloride in toluene under reflux at temperature of 60 to 70 °C for 15 to 20 hour is a first step of the reaction. After confirming our mefenamic acid chloride synthesis with the help of repeated TLC, mefenamic acid chloride is treated with benzoyl hydrazine under reflux to get hydrazide derivative of mefenamic acid. The synthesis of final hydrazide derivative of mefenamic acid is confirmed by the characterization techniques which include Fourier transform infrared spectroscopy and UV-Visible spectrophotometer.

Index Terms- Synthesis, Characterization, Mefenamic Acid Hydrazide Derivative, Drug Development, Pharmaceutical Chemistry

I. INTRODUCTION

The first non-steroidal anti-inflammatory medicine was created when German researchers were able to extract salicylate from willow bark in 1829. More than 130 years later, in the 1960s, indomethacin, the following NSAID, was developed. Although non-steroidal anti-inflammatory medications (NSAIDs) have a long history, John Vane was the first to completely understand their mechanism of action in 1971, By preventing the enzymatic generation of prostaglandins, aspirin and prostaglandin E have been shown by Sir John Vane to shield the stomach mucosa from acid injury and preserve appropriate blood circulation. [1] NSAID drugs are a broad category of medications that relieve inflammation, pain, and fever. They have a similar mode of action, which includes blocking the enzymes involved in producing prostanoids, particularly cyclooxygenases while having very different chemical compositions (COX). Yet, due to their diversity, they may also have extra consequences that affect how effective their treatments are [2] NSAIDs are classified into subgroups based on their underlying chemical structure. A large percentage are low pKa organic acids, and the pharmacological and pharmacokinetic properties are governed by their acidic nature. This rule is not implemented on diaryl heterocyclic chemical compounds, Acetaminophen, and derivatives of pyrazolic, which are typically left out of the NSAID category due to their weak anti-inflammatory effects [3]. NSAIDs' therapeutic effects are principally brought about by their reduction of prostaglandin synthesis. PGHS-1 and PGHS-2, also recognized as COX-1 and COX-2 are enzymes that produce prostanoids. Two separate processes are catalyzed by PGHSs at two physically independent but functionally connected regions. Arachidonic acid undergoes cyclooxygenase-mediated bisoxygenation to produce PGG2, which is then by a peroxidase reaction changed into PGH2. These unstable intermediate compounds are quickly transformed into several prostacyclins, prostaglandins, and thromboxanes by particular synthases. The presence of the substrate arachidonic acid is the major key factor that limits the creation of prostanoids and that restriction often establishes a minimal measure of basal prostanoid synthesis [9]. Yet, when enzyme phospholipase A2 has been active and arachidonic acid (ARA) is liberated from the phospholipids, this method of production is considerably boosted. Inflammation, pain, and fever are all significantly influenced by this arachidonic acid process. PGE2 and prostacyclin (PGI2) enhance local circulation, vascular permeability, and leukocyte infiltration, which in turn increases prostanoid synthesis in inflamed tissues [25]. The COX pathway offers fresh treatment approaches in the field of anti-inflammation. In 1971, Vane, Ferreira, and Smith et al. published ground-breaking research connecting NSAIDs' ability to reduce infection to the suppression of cyclooxygenase and the formation of PG. Non-Steroidal anti-inflammatory medications which have therapeutic value in the treatment of pain, fever, and inflammation, are currently the most often recommended category of medicine [10]. NSAIDs that are now on the market Without a doubt, aspirin (acetylsalicylic acid) is the most significant NSAID for the treatment of inflammation. In actuality, aspirin is the NSAID that is used the most frequently worldwide and serves as the excellent quality by which fresh

anti-inflammatory medications are assessed. Acetylsalicylic acid medicine has been used for a very large time and it is generally accessible without the need for medication. Because of its inexpensiveness and stability, aspirin is the preferred medication for decreasing inflammation, moderate to mild pain, and fever. Aspirin is beneficial in the protection of heart attack and stroke because it decreases platelet aggregation and it has well-recognized analgesic, anti-inflammatory, and anti-fever properties. Moreover, a variety of epidemiological investigations have shown that large term aspirin use at low medication usage may be a good alternative for treating stroke. [2] The use of NSAIDs is undoubtedly on the rise. The population is ageing, which is accompanied by an increase in osteoarthritis, and the accessibility of OTC medications is a factor in this rise. In the United States alone, non-steroidal anti-inflammatory medicines reported 30 billion over-the-counter (OTC) pills and 70 million medications written each year. NSAIDs are filled with paradoxes that offer serious problems for the medical profession despite the fact that we are all familiar with these treatments [31]. NSAIDs are some of the most well-established medications on the market, yet new formulations are always being released. While certain baby formulae may be purchased over the counter and used to treat feverish newborns, others need to be prescribed and are a major source of iatrogenic responses, hospitalizations, and fatalities. Doctors must decide between giving older, less costly NSAIDs and newer, perhaps healthier ones. [23] NSAIDs, which include naproxen, indomethacin, aspirin, ibuprofen, flurbiprofen, and diclofenac, are now advised for conditions like pain, short-term fever, and inflammation. To comprehend the kinetics and structure-activity connections of these medications, scientists have put in a lot of effort. Computer-aided drug design (CADD) is one of the most effective tools in this regard. It makes it possible for us to look for and comprehend ligand interactions with potential protein targets. [3]

The disease Alzheimer

Worldwide recognized AD is an illness that shows causes include cell degeneration in the brain and is the leading risk of illness, specified by deterioration in autonomy and thinking in everyday tasks [30]. AD is believed to be a serious illness, with two primary ideas.

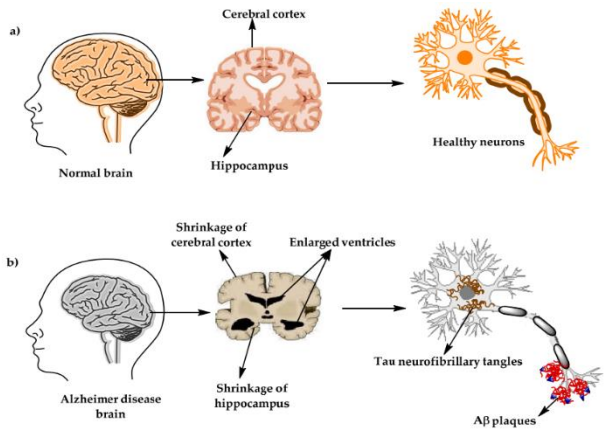


Figure 1 *The physiologic composition of the brain part (normal brain function and one with Alzheimer's disease)*

Cholinergic and amyloid theories have been postulated as causes of AD. The part of the brain of his initial sufferer, who passed away from memory problems and personality problems, Alois Alzheimer found amyloid protein plaques and a significant decrease of neurons [11]. He classified the illness as a terrible cerebral cortex illness. Emil Kraepelin used the name " Alzheimer's disease (AD)" for the initial time to describe this health condition in his psychiatric book's 8th edition. [4] AD and other brain diseases, infections, anomalies in the respiratory and circulatory systems that restrict oxygen delivery to the brain part, dietary deficiencies, vitamins such as vitamin B12 deficiencies, issues like tumors, and other conditions can all result in progressive cognitive function loss. [4]

Globally, there are around Fifty million people who have Alzheimer's disease, and by 2050, that number is projected to increase by a factor of four every five years, to 152 million. AD is a burden that affects people, nations, and financial situations with annual costs of approximately to reach trillion dollars worldwide [12]. While there is presently no treatment for Alzheimer's, there are medications that can lessen its effects.

Several risk factors have been discovered as part of the complicated condition known as Alzheimer's disease. (Fig. 2), includes advancing age, genetic predispositions, brain injuries, vascular conditions, infectious diseases, and environmental factors [13]. The primary cause of the pathologic small changes associated with AD (Neurofibrillary tangles, and synaptic damage) is yet unknown [24]. Two theories are believed to be the most significant among those that have been put forth as causes of AD. Some contend that cholinergic dysfunction is a significant contributor to the disease, while others contend that a change in the protein of amyloid synthesis is the disease's main generating element. To date, no recognized theories explain the origination and development of a disease of Alzheimer's disease.[4]

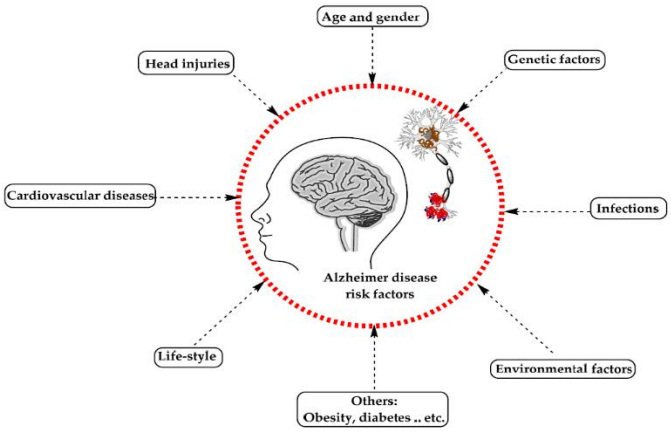


Figure 2 *Hazard elements for AD*

The enzyme choline acetyltransferase (ChAT), which has to generate acetylcholine, was implicated in the era of the 1970s in corticospinal and cholinergic nerve terminal abnormalities (ACh). ACh plays a crucial part in intellectual capabilities, as a result, the cholinergic theory of AD was proposed [14]. Cholinergic neurons synthesize acetylcholine (ACh) through a process involving the enzyme choline acetyltransferase (ChAT) within their cytoplasm [29]. ChAT facilitates the conversion of choline and acetyl coenzyme A into ACh. Once produced, ACh

is transported to the synaptic vesicles by the vesicular acetylcholine transporter (VAChT) (Fig. 3) [4]

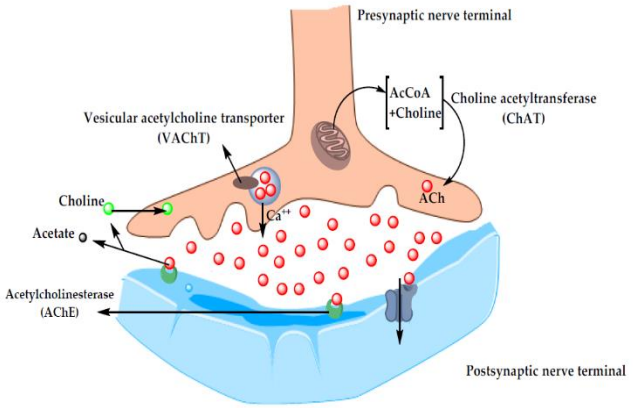


Figure 3 *The process through which presynaptic and postsynaptic nerve terminals create and transfer acetylcholine*

ACh performs a key contribution to the brain's physiological systems related to learning, memory, attention, and other crucial activities. Memory loss and altered cognitive function were shown to be caused by degeneration of the cholinergic neurons, which occurs in AD [28]. A decrease in choline absorption and a release of ACh are thought to be the effects of B-amyloid on cholinergic neurotransmission. Studies have demonstrated a link between the development of amyloid fibrils and cholinergic synaptic loss caused by the neurotoxicity of A oligomers and connections between A peptide and Acetylcholinesterase (AChE.) [27]. Moreover, the factors that play role in the development of Alzheimer's disease, include the loss of muscarinic (M2) Ach receptors and nicotinic acetylcholine Ach receptors, which are found on cholinergic nerve endings, and the shortage in EAA synaptic transmission, where L-glutamic acid accumulation and Aspartic acid consumption importantly decrease in many areas of cortical [16]. The use of scopolamine, a cholinergic receptor antagonist that has been found to create amnesia, is another. This impact can be mitigated by using drugs that increase acetylcholine production. Presynaptic cholinergic indicators have diminished in the cerebral cortex as a result of the severe neurodegeneration that has affected the NBM, the origin of cortical cholinergic neural innervation. As a result, these three concepts form the foundation of the cholinergic hypothesis: how cholinergic antagonists, as opposed to agonists, which have the opposite effect, contribute to memory loss. [4]

MA Drug It is an anthranilic acid derivative. Its physicochemical characteristics, particularly the exceedingly poor water solubility (1 mg mL-1) and unique adhering qualities provide significant difficulties in the formulation and production of dosage forms. Hence, MA's biopharmaceutical efficacy was greatly improved by making considerable efforts to strengthen its dissolving properties. Mefenamic acid has a brief biological half-life of 2 hours [17]. Typically, it comes in the form of tablets, capsules, and suspensions. This medication can be used in doses of 250 mg three times each day. Based on the biopharmaceutical classification system, MA is categorized as class II due to its poor solubility over the range of pH 1.2-7.5. It has poor aqueous solubility yet is intrinsically highly permeable through biological membranes [26]. Numerous studies have been conducted to increase the low solubility or bioavailability due to this, including micronization and solid dispersion [22]. MA is frequently used up to treat mild to medium pain, such as osteoarthritis, rheumatoid arthritis, headaches, tooth discomfort, dysmenorrhea, and other conditions affecting the joints [15]. However, it can have adverse impacts, including stomach problems like Gastric ulcers and gastrointestinal haemorrhage. [5] The chemical structure of MA is presented in (Fig. 4). The chemical name for MA 2- [(2,3- dimethyl phenyl) amino] benzoic acid.

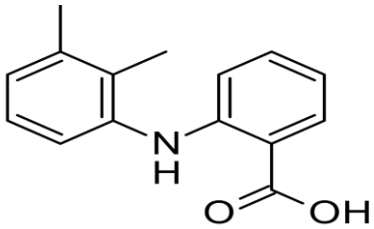


Figure 4 *Mefenamic Acid Structure*

Medicine such as Dyspen, Dysmen500, Fenamin, Contraflam medicine, Coslan medicine, Dolcin medicine, Dysman medicine, Flipal, Femen, Fenamin medicine, Gandin, Hamitan, and Hostan, are only a few of the brand names used to sell mefenamic acid. The NSAID fenamate family includes MA, an anthranilic acid derivative. It is analgesic, antipyretic, and anti-inflammatory. [6]

Prostaglandin synthetase activity is inhibited by MA's binding to COX 1 and COX 2. These receptors contribute to inflammation and/or prostanoid signaling in activitydependent neuroplasticity, which causes a temporary decrease in pain sensations. [5]

Prostaglandin synthesis is encouraged by the enzyme cyclooxygenase-1 in many organs, including the stomach, kidneys, and areas that are prone to inflammation. Prostaglandins encourage the creation of the stomach's natural protective mucus lining. Moreover, they communicate inside the cells that control inflammation and other processes. [5]

An enzyme called cyclooxygenase-2 encourages the creation of particular chemical messengers called prostaglandins, which are crucial in inflammation. Cox-2 activity is suppressed, and inflammation is decreased [21]. Despite cox-1, which is active throughout the stomach, cox-2 is exclusively active at the site of inflammation. [5]

Mefenamic Acid uses against Diseases

Severe Acute Respiratory Syndrome Coronavirus 2 is responsible for the dangerous pandemic known as COVID-19. MA has been identified as an anti-inflammatory medicine with some antiviral properties. For the treatment of COVID-19, it can be used in concert with other antiviral medications that are being studied [18]. Although its primary function may be to lower fever due to the fact that it is also an antipyretic, its effect as an antiviral and as a supporting drug can be highly useful. According to studies, ribavirin and mefenamic acid work well together to lower viral production in cells infected with the

chikungunya virus with a positive-sense RNA genome. Mefenamic acid, a major NSAID component, has been shown to have significant antiviral activity in vitro and in vivo. It has also been shown that this activity is increased when paired with the popular antiviral medication, RIBA. [7] The combination of the antiviral and anti-inflammatory properties of mefenamic acid resulted in a marked reduction of pathological symptoms. [7]
Mefenamic acid, a frequently used with weak anti-inflammatory effects but a cyclooxygenase-1 and 2 inhibitors, was investigated for its therapeutic potential in Alzheimer's disease. Mefenamic acid inhibits the production of the Swe-APP or APP-CTs in cells of neuronal, as well as the neurotoxicity brought on by the treatment with the amyloid peptide (A)1-42 [20]. We further demonstrate that mefenamic acid decreases nitric oxide generation and release of mitochondrial Cytochrome-C in neurons activated by APP CTs or Swe-APP and A1-42. Moreover, mefenamic acid boosts the antiapoptotic protein's expression [19]. Also, our results demonstrate for the initial time that MA enhanced the ability of learning and memory issues in a rat model A1- 42-infused with the disease of Alzheimer's. Together, results of in vitro and in vivo indicate that mefenamic acid may be utilized to cure the disease of Alzheimer's. [8]

II. METHODOLOGY

Equipment:

In our reaction setup, a variety of essential equipment is employed to ensure precision and control. The magnetic stirrer bar and hot plate work in tandem, providing efficient mixing and controlled heating. Beakers and test tubes, held securely in designated racks and holders, serve as vessels for reactions. A fume hood ensures safety by containing any potentially harmful vapors. Measuring cylinders, pipettes, and analytical balances contribute to accurate measurements. Two-necked round-bottom flasks and condensers facilitate distillation processes, while a water bath ensures temperature control. Additional tools include separating funnels, aluminum foil, spatulas, China dishes, and Petri dishes for various experimental needs. The setup also incorporates filtration with filter papers, pH papers for acidity testing, and tweezers for delicate handling. Furthermore, advanced instruments like UV-Visible Spectrophotometer, FTIR, UV lamp, and TLC plates are employed for detailed analysis. The reaction flask, thermometer, and a comprehensive distillation assembly complete the ensemble, providing a versatile and well-equipped environment for chemical experimentation.

Chemicals:

The reaction involves a carefully selected array of chemicals to drive the desired chemical transformations. Ethanol and distilled water serve as common solvents, providing a suitable medium for the reactions to occur. Thionyl chloride plays a pivotal role, often utilized for various synthetic processes. Mefenamic acid and benzoyl hydrazine are key organic compounds, acting as starting materials or reagents in the reaction scheme. Dimethylformamide (DMF) and pyridine contribute as solvents and reaction facilitators, aiding in the dissolution and interaction of the reactants. Toluene, chloroform, and petroleum ether, each with distinct properties, are employed for solvent extraction or as reaction media. These chemicals are chosen for their compatibility with the reaction conditions and their ability to support specific chemical transformations. The thoughtful selection of these compounds reflects the precision and control exercised in the experimental setup to achieve the desired outcomes.

For Solubility Test Solvents:

• Ethanol • Diethyl Ether • Chloroform • Toluene • Acetone • Water

The calculation for Mefenamic Acid Chloride

Mefenamic Acid (MA):

0.00277 moles → 2.77 millimoles
Mass of mefenamic acid = moles × Molar mass
= 0.00277 moles × 241.285
= 0.668 grams (668 mg)

$SOCl_2$:
11.11 millimoles = 0.0111 moles
Moles = $m/(M.mass)$
Mass = Moles × M. mass
Mass = 0.011 × 118.97
= 1.320 g

For conversion into volume:
Density = $mass/Volume$
Volume = $mass/density$ ∵
Density = 1.65
= $1.320/1.64$ ⇨ 0.8048 mL
= 0.8048 × 1000 ⇨ 805 µL

Calculation of Mefenamic Acid Hydrazide:

As Mefenamic acid chloride used for the reaction is 1 mole, the product is also 1 mole, so 0.00277 moles of Benzoyl Hydrazine is required.
Mass of Benzoyl Hydrazine = moles × Molar mass
Mass = 0.00277 × 136.5 = 0.3771 g

Overall reaction

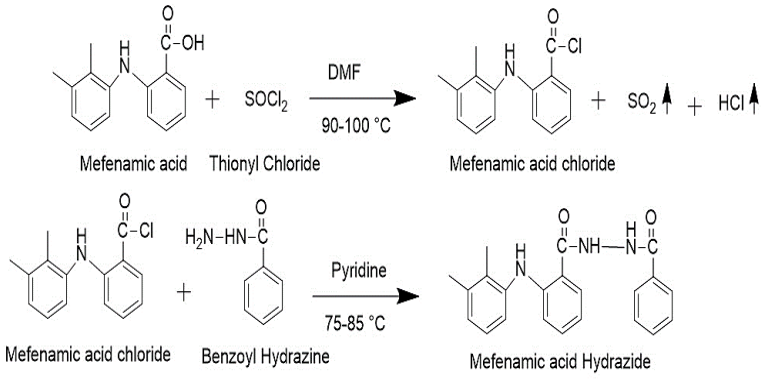


Figure 5 *Two-step reaction for MA hydrazide synthesis*

Synthesis of Mefenamic Acid Chloride
MA chloride, also known as mefenamoyl chloride, can be synthesized by the reaction of mefenamic acid with thionyl chloride (SOCl2) in anhydrous conditions. In the first step, we weigh about 668 mg (0.00277 moles) of mefenamic acid with the

help of analytical balance, then we insert it in the reaction flask which is settled on a Magnetic Hot plate. After this step, we added 15-20 mL of toluene to a reaction flask. The reaction is carried on reflux for 20 to 30 minutes for the complete dissolution of Mefenamic acid into toluene. After making a clear solution of it, we added 805 microliters (0.0111 moles) of Thionyl Chloride dropwise in a solution of Mefenamic acid which is present in the reaction flask while stirring under anhydrous condition, this reaction is exothermic. We settled this reaction on reflux for 15 to 20 hours at a temperature of 60 to 70 °C and keep this reaction continue until mefenamic acid chloride is synthesized. We also added 1 to 2 drops of DMC as a catalyst during this reaction which make our synthesis feasible. During the whole process of synthesis, we check our reaction completion till our desired product is with the help of taking repeated TLC of the reaction mixture. After several hours, the spot of the product on TLC plate confirmed that our desired product Mefenamic acid Chloride is synthesized. The reaction mechanism involves the transformation of the R−COOH group in MA to an acyl chloride by reaction with thionyl chloride. The reaction proceeds through an intermediate where the -OH group of the carboxylic acid is first converted to a -OSO2Cl group. This intermediate then reacts with a molecule of thionyl chloride to form acyl chloride.

Mechanism of Synthesis of Mefenamic Acid Chloride

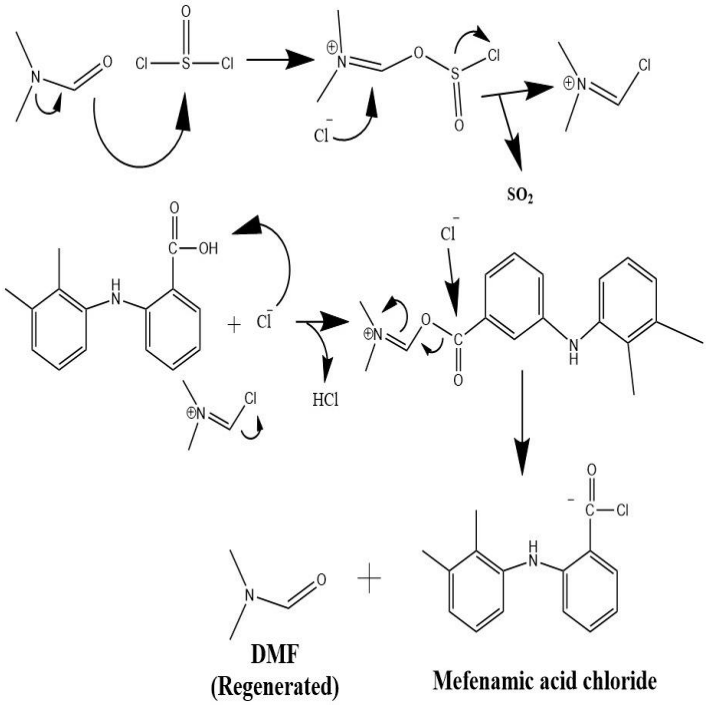


Figure 6 *Mechanism of synthesis of MA chloride*

Synthesis of Mefenamic Acid Hydrazide Derivative

In the solution of Mefenamic acid chloride in toluene, 0.3771 g (0.00277 moles) of Benzoyl hydrazine is added while stirring under anhydrous conditions. We Continued stirring the mixture for 10 to 15 hours at 60 to 70 °C or at a slightly elevated temperature until the reaction is complete. After several hours, the spot of the product on the TLC plate confirmed that desired product Mefenamic acid hydrazide derivative is synthesized. We removed the solvent and excess reagents by evaporation and collected them for next use. We dried our product and collect it for further characterization techniques. The reaction mechanism involves the attack of the nucleophile (benzoyl hydrazine) on the electrophilic acyl chloride (mefenamic acid chloride) to form an intermediate that then undergoes a rearrangement to form the corresponding hydrazide derivative.

Mechanism of Synthesis of Mefenamic Acid Hydrazide

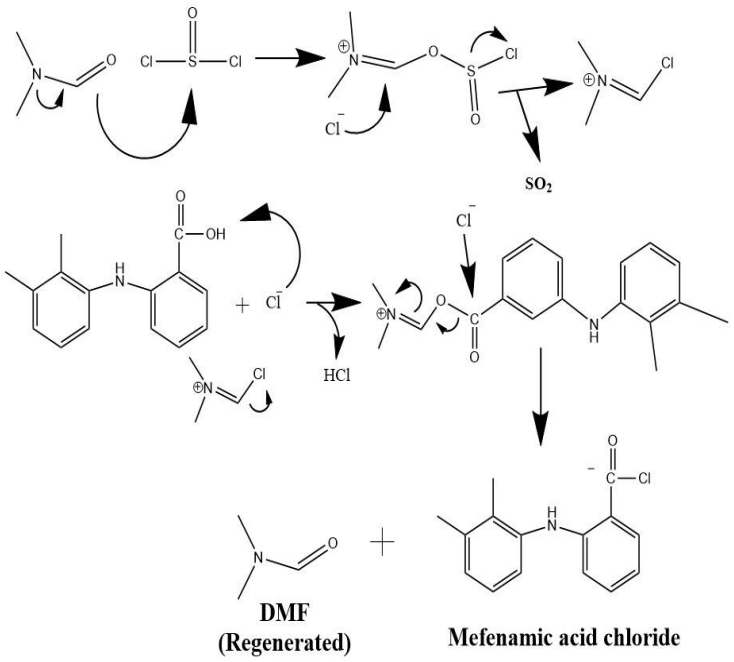


Figure 7 *Mechanism of synthesis of MA chloride*

III. RESULTS AND DISCUSSION

FTIR and their interpretation

The product was characterized by FTIR and the results are mentioned in the (Fig. 7 and Fig. 8).

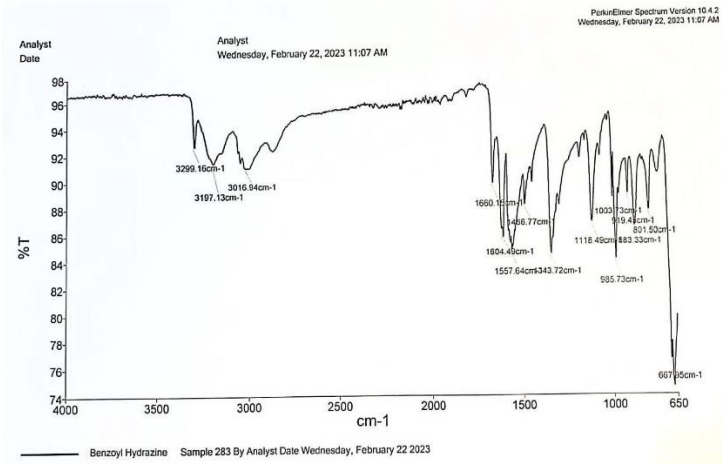


Figure 8 *IR Spectrum of Benzoyl Hydrazine*

Table 1: Interpretation of FTIR Spectrum of Benzoyl hydrazine		
Serial Number	**Functional group present**	**Frequency (band cm^{-1})**
1	NH_2	3299.16 – 3197.13 cm^{-1}
2	Amide group	1660.15 cm^{-1}
3	Aromatic C=C group	1604.49 cm^{-1}

IR spectrum of mefenamic acid hydrazide

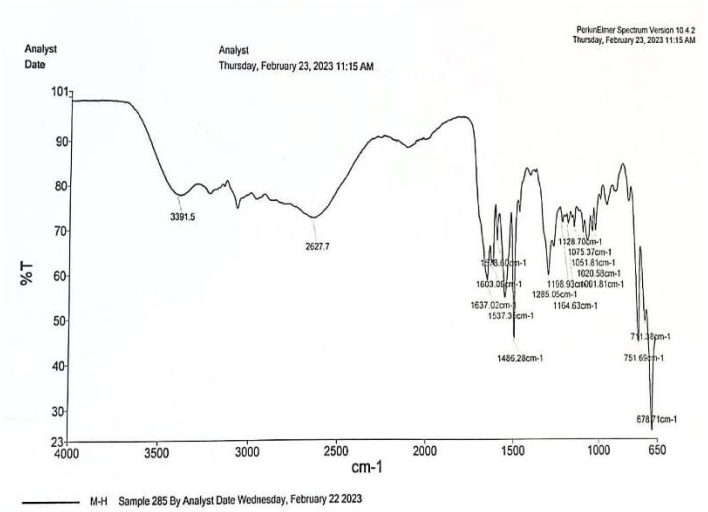


***Figure 9** IR Spectrum of Mefenamic acid Hydrazide*

Table 2: Interpretation FTIR Spectrum of Mefenamic acid hydrazide		
Serial Number	**Functional group present**	**Frequency (band cm^{-1})**
1	Amine group	3391.5 cm^{-1}
2	Amide group	1637.02 cm^{-1}
3	Aromatic C=C group	1603 cm^{-1}

The -NH2 sharp peak which was found in the IR spectrum of benzoyl hydrazine in the range of 3299.16 – 3197.13 cm-1 is present in the mefenamic acid hydrazide IR spectrum in the range of 3391.5 cm-1 and is responsible for the formation of Mefenamic acid hydrazide. Because the amide group of mefenamic hydrazide appears in a broad peak in the 1637.02 cm-1 range. The change in stretching frequency of wavenumber (cm-1) indicates the formation of hydrazide. However, there is no favorable alteration in the C=C mode of the aromatic benzene ring of benzoyl hydrazine and mefenamic hydrazide spectrum. The presence of the peak of the -CONH group in the IR spectrum of mefenamic hydrazide at the frequency of 3391.5 cm-1 confirms that the -NHNH2 group of benzoyl hydrazine are in resonance with mefenamic acid chloride and form mefenamic hydrazide. According to the above discussion, the given IR spectra and after confirming the stretching Frequencies have sharp peak because of resonance on the amide group may indicate the formation of mefenamic acid hydrazide.

UV-Visible Spectrum and their interpretation

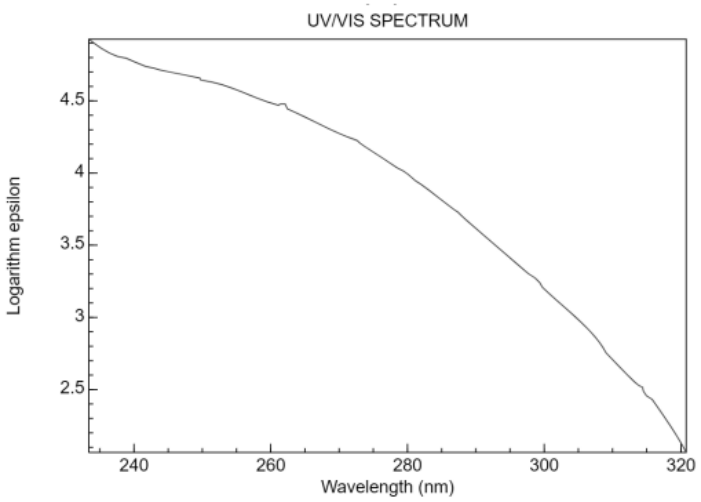


***Figure 10** UV-Visible spectrum of Benzoyl hydrazine*

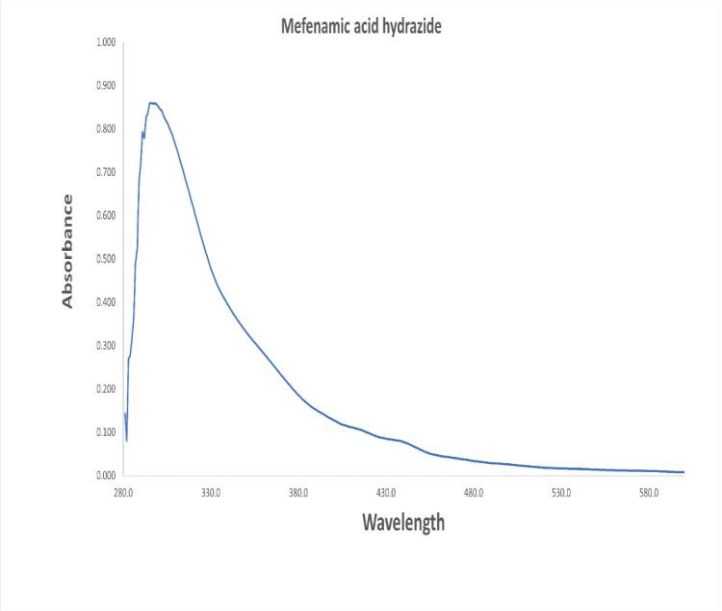


***Figure 11** UV-Visible spectrum of Mefenamic acid hydrazide*

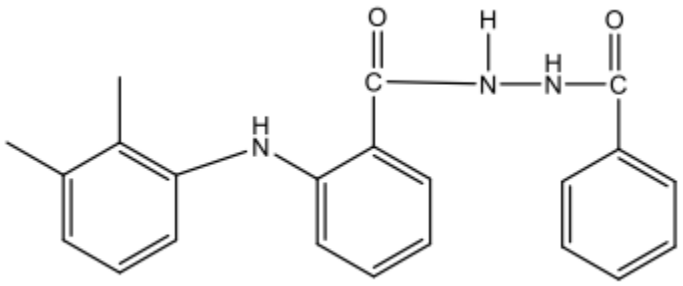
Table 3: Interpretation UV-Visible Spectrum		
Serial Number	**Benzoyl hydrazine Lamda max**	**Mefenamic acid hydrazide Lamda max**
1	231 nm	300 nm

The UV-Visible spectrum of the mefenamic acid hydrazide derivative typically shows absorption bands in the UV-Visible range of 200-400 nm, which are attributed to the presence of the chromophoric groups in the molecule. The spectrum may also show additional peaks or shifts compared to the parent compound, indicating changes in the electronic structure of the molecule due to the introduction of the hydrazide group. The Lamda maximum of Mefenamic acid hydrazide is 300 nm which shows strong absorption of 0.851 at this wavelength. The Lamda maximum of Benzoyl hydrazine is 231 nm.

IV. CONCLUSION

Mefenamic acid hydrazide has been prepared by treating Mefenamic acid chloride with benzoyl hydrazine in toluene at a temperature of 60 to 70 °C with continuous stirring at reflux. Repeated TLC during the synthesis also confirmed a single spot of Mefenamic acid hydrazide. The synthesis of mefenamic acid hydrazide is confirmed by the characterization techniques which involved FTIR and UV-Visible spectrophotometer. The UV-Visible spectrum of the mefenamic acid hydrazide derivative typically shows absorption bands in the UV-Visible range of 200-400 nm, which are attributed to the presence of the chromophoric groups in the molecule. The spectrum may also show additional peaks or shifts compared to the parent compound, indicating changes in the electronic structure of the molecule due to the introduction of the hydrazide group The FTIR spectrum confirmed the synthesis of Mefenamic acid hydrazide by comparison with benzoyl hydrazine FTIR spectrum. Their NMR and Biological activities results are still in progress.

The structure of product is:



Mefenamic acid hydrazide

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