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Development of Microwave Assisted Nano Bio Composite for Stability Enhancement of Mefenamic Acid

Ms.Rutuja Navnath Thorat, Ms.Vaishali S.Payghan, Dr.Santosh Ambadas Payghan

Student, By Guide, Principal

Rajesh Bhaiyya Tope College of M.Pharmacy, Sambhajinagar (Aurangabad) 431007

Dr.Babasaheb Ambedkar Marathwada University, Sambhajinagar (Aurangabad), Maharashtra

ABSTRACT

The present study was undertaken to develop a microwave-assisted nano bio-composite system aimed at enhancing the solubility, stability, and controlled release of Mefenamic Acid, a BCS Class II drug known for its poor aqueous solubility and limited bioavailability. A range of nano bio-composite formulations were prepared using varying concentrations of PLGA and PVA as polymeric and stabilizing agents, respectively, with drug loading achieved via microwave-assisted synthesis. The optimized formulation (Batch F6) was selected based on its superior encapsulation efficiency (88.4 ± 1.4%), nanoscale particle size (156 ± 7 nm), favorable zeta potential (–33.2 ± 1.6 mV), and excellent redispersibility and sedimentation behavior. FTIR and DSC analyses confirmed the chemical compatibility and structural integrity of the drug within the composite matrix. SEM analysis revealed spherical particles with smooth morphology. In-vitro drug release studies demonstrated sustained drug release over 12 hours, while gastrointestinal stability evaluation showed significantly reduced degradation compared to pure drug. The experimental data were supported by statistically validated linear and quadratic models with high predictive accuracy. Accelerated stability studies confirmed the long-term physical and chemical stability of the optimized batch. Overall, the study concludes that microwave-assisted nano bio-composites offer a highly effective and scalable approach to improve the pharmacokinetic performance and formulation stability of poorly soluble drugs like Mefenamic Acid.

Keywords

Mefenamic Acid, microwave-assisted synthesis, nano bio-composite, encapsulation efficiency, solubility enhancement, sustained release, gastrointestinal stability, statistical optimization, PLGA, PVA.

INTRODUCTION

Mefenamic Acid and Its Pharmaceutical Challenges

Chemical Structure, Physicochemical Properties, and Pharmacokinetics of Mefenamic Acid

Mefenamic Acid is a well-known non-steroidal anti-inflammatory drug (NSAID) belonging to the fenamate class, structurally derived from anthranilic acid, a key pharmacophore responsible for its anti-inflammatory and analgesic properties. The molecular formula of Mefenamic Acid is $C_{15}H_{15}NO_2$, which represents a complex structural framework comprising two benzene rings, a carboxyl (-COOH) functional group, and a secondary amine (-NH-) bridge connecting the aromatic rings. This molecular configuration plays a significant role in determining the drug's biological activity, physicochemical properties, and challenges in formulation.[1]

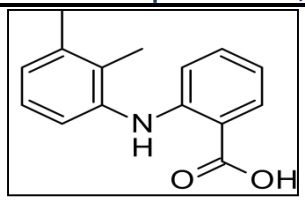


Fig 1.1: Chemical structure of Mefenamic acid

The diphenylamine backbone of Mefenamic Acid is a fundamental feature that enables its effective interaction with biological targets, particularly cyclooxygenase (COX) enzymes, which are responsible for the synthesis of inflammatory mediators known as prostaglandins. The structural orientation allows Mefenamic Acid to bind to the active sites of COX enzymes, leading to the inhibition of prostaglandin production, thereby exerting its anti-inflammatory, analgesic, and antipyretic effects.[2]

One of the most critical functional groups in Mefenamic Acid is its carboxyl (-COOH) group, which is essential for its ionization behavior, solubility, and binding interactions. This functional group contributes to the acidic nature of the drug, allowing it to participate in hydrogen bonding, ionic interactions, and salt formation, which can significantly impact its dissolution characteristics. Despite these advantages, the presence of a bulky diphenyl structure reduces its aqueous solubility, making the drug less soluble in gastrointestinal fluids, thus limiting its absorption when administered orally.

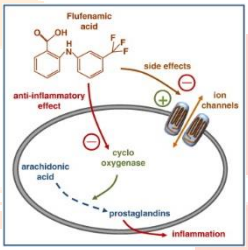


Fig 1.2: Mechanism of action of mefenamic acid

The steric hindrance created by the large aromatic system also affects the drug's metabolic stability, influencing how it interacts with metabolic enzymes in the liver. The molecular rigidity caused by the fused benzene rings makes the molecule relatively stable but less susceptible to enzymatic breakdown, which can prolong its presence in the systemic circulation. However, this can also lead to variability in pharmacokinetics between individuals, as factors such as enzyme activity, genetic polymorphisms, and liver function can alter its metabolism and clearance. Another crucial aspect of Mefenamic Acid's chemical structure is its crystallinity. The drug exists in different polymorphic forms, each exhibiting unique solubility and stability characteristics. Polymorphic transitions can influence dissolution rates, bioavailability, and drug shelf life, which makes solid-state characterization an essential aspect of formulation development. Given these challenges, advanced drug delivery strategies, such as microwave-assisted nano bio-composites, have gained attention as potential solutions for improving the stability, solubility, and overall therapeutic efficacy of Mefenamic Acid.

Physicochemical Properties of Mefenamic Acid

The physicochemical properties of Mefenamic Acid have a profound impact on its solubility, stability, permeability, and overall pharmaceutical performance. These properties play a crucial role in determining how the drug dissolves, absorbs, distributes, and exerts its therapeutic effects in the human body. One of the most significant limitations of Mefenamic Acid as an oral drug is its poor aqueous solubility, which is a major factor affecting its bioavailability. Due to its hydrophobic nature, which is attributed to its aromatic diphenylamine core, the drug has limited affinity for water molecules, leading to slow dissolution rates in gastric and intestinal fluids. The pKa value of approximately 4.2 further complicates its solubility profile, as the drug remains largely non-ionized in the acidic pH of the stomach (pH 1.5-3), reducing its dissolution. However, as the drug enters the small intestine, where the pH ranges from 6 to 7.4, ionization increases, thereby improving solubility. This pH-dependent solubility can lead to inconsistent absorption rates, resulting in variable therapeutic effects among patients. The melting point of Mefenamic Acid is around 230-233°C, indicating a highly crystalline nature with strong intermolecular forces holding the molecules in a rigid lattice. While a high melting point enhances thermal stability, it also correlates with lower solubility and slower dissolution due to the energy required to break the crystal lattice during the dissolution process. Crystalline drugs with high melting points often require solubilization techniques such as micronization, amorphization, or nanoformulation to improve their dissolution profile. Mefenamic Acid is also characterized by its log P (partition coefficient) value of approximately 5.8, indicating that it is highly

lipophilic. This high log P value suggests that the drug readily partitions into lipid membranes, facilitating passive diffusion across biological barriers. While lipophilicity aids in permeability, excessive lipophilicity can hinder aqueous solubility, leading to poor drug absorption and low bioavailability. To address this issue, novel formulation strategies such as lipid-based drug delivery systems, solid dispersions, and polymeric nano-carriers have been explored to enhance the solubility and dissolution of Mefenamic Acid.

The drug is also known for its high plasma protein binding capacity (>90%), primarily to albumin, which significantly impacts its distribution and pharmacokinetics. When a drug binds extensively to plasma proteins, only the unbound fraction remains pharmacologically active, meaning that dosage adjustments may be required in conditions that alter protein binding, such as liver disease or hypoalbuminemia. This factor also contributes to drug-drug interactions, as other protein-bound drugs may compete for binding sites, leading to altered pharmacological responses. Considering these physicochemical challenges, the development of microwave-assisted nano bio-composites has been proposed as a promising solution for enhancing drug solubility, improving dissolution rates, and ensuring better bioavailability.

Pharmacokinetics of Mefenamic Acid

The pharmacokinetic behavior of Mefenamic Acid is crucial in understanding how the drug is absorbed, distributed, metabolized, and excreted (ADME) after administration. These processes determine the drug's effectiveness, duration of action, and overall clinical performance.After oral administration, Mefenamic Acid undergoes absorption in the gastrointestinal tract, predominantly in the small intestine, where the pH favors its ionization and dissolution. However, due to its limited aqueous solubility and slow dissolution rate, the absorption process is often incomplete and variable. The bioavailability of the drug depends significantly on formulation design, as traditional tablets may lead to delayed absorption and reduced systemic exposure, necessitating the use of advanced formulation techniques to optimize its pharmacokinetic profile.

Once absorbed, Mefenamic Acid exhibits extensive plasma protein binding, primarily to albumin (>90%), which reduces the free drug concentration available for therapeutic action. The drug is widely distributed in extracellular fluids but does not penetrate deep into tissues due to its high affinity for plasma proteins. This characteristic results in a prolonged half-life but also increased potential for drug interactions. Metabolism occurs primarily in the liver, where the drug is extensively metabolized via cytochrome P450 enzymes (CYP2C9 and CYP1A2) into hydroxylated and conjugated metabolites. The metabolism of Mefenamic Acid is subject to genetic polymorphisms, which may cause variability in drug clearance between individuals, affecting efficacy and potential toxicity.The drug is eliminated mainly through renal excretion, with metabolites excreted in urine, while a smaller portion undergoes biliary excretion. The half-life of Mefenamic Acid is approximately 2-4 hours, requiring frequent dosing (typically three times daily) to maintain therapeutic levels. Given its pharmacokinetic limitations, modern microwave-assisted nano bio-composite formulations are being developed to improve drug stability, enhance solubility, and ensure a controlled release, leading to better patient compliance and therapeutic outcomes.

Therapeutic Uses of Mefenamic Acid

Mefenamic Acid is a well-established non-steroidal anti-inflammatory drug (NSAID) widely used in the management of pain, inflammation, and fever. As a derivative of anthranilic acid, it belongs to the fenamate class of NSAIDs, which are known for their potent analgesic and anti-inflammatory properties. The drug is commonly prescribed for mild to moderate pain conditions, particularly those associated with inflammation. One of the most prominent uses of Mefenamic Acid is in the treatment of primary dysmenorrhea (menstrual pain). Dysmenorrhea is characterized by painful uterine contractions due to the excessive production of prostaglandins (PGs), particularly prostaglandin F2-alpha ($PGF_2\alpha$), which leads to increased uterine contractility and reduced uterine blood flow. Mefenamic Acid effectively inhibits prostaglandin synthesis, thereby alleviating pain, reducing the intensity of uterine contractions, and improving blood flow to the uterus. It is often prescribed as a first-line treatment for menstrual cramps, especially in patients who do not respond well to traditional analgesics like paracetamol.

Beyond menstrual pain, Mefenamic Acid is also widely used for the management of musculoskeletal pain and inflammatory conditions, including osteoarthritis and rheumatoid arthritis. These conditions are characterized by chronic inflammation, joint stiffness, and progressive cartilage degradation due to an overactive immune response and excessive prostaglandin production. By reducing inflammation at the site of injury or degeneration, Mefenamic Acid provides symptomatic relief and improves joint mobility in patients suffering from arthritis-related pain. Although not a disease-modifying drug, it serves as an effective option for symptomatic relief in inflammatory joint diseases. Another important indication of Mefenamic Acid is its use in treating postoperative pain and dental pain, particularly following tooth extraction, oral surgery, or minor surgical procedures. The inflammatory response triggered by tissue trauma during surgery leads to the release of prostaglandins and other inflammatory mediators, resulting in pain and swelling. Mefenamic Acid helps mitigate this response by inhibiting prostaglandin synthesis,

thereby reducing postoperative inflammation and pain. The drug is also prescribed for migraine headaches and tension headaches, particularly in individuals who experience migraines associated with menstrual cycles (menstrual migraines). As prostaglandins play a role in cerebrovascular inflammation and pain perception, Mefenamic Acid can be effective in reducing the intensity and duration of migraine attacks when taken at the onset of symptoms.

Recent studies have also explored the potential of Mefenamic Acid in neurodegenerative disorders, particularly in conditions like Alzheimer's disease, where neuroinflammation is a major contributing factor to disease progression. By inhibiting inflammatory mediators in the brain, Mefenamic Acid may offer neuroprotective benefits, although more clinical studies are required to establish its role in neurodegenerative disease management. While Mefenamic Acid has a wide range of therapeutic applications, it is essential to use it with caution in elderly patients, individuals with gastrointestinal disorders, and those with renal impairment, as prolonged NSAID use is associated with gastrointestinal irritation, renal toxicity, and cardiovascular risks.

Mechanism of Action of Mefenamic Acid

The therapeutic effects of Mefenamic Acid are primarily mediated through its ability to inhibit the cyclooxygenase (COX) enzymes, which play a crucial role in the synthesis of prostaglandins. Prostaglandins are lipid-derived signaling molecules that regulate inflammation, pain perception, fever, and uterine contractions. By interfering with their production, Mefenamic Acid effectively reduces pain, inflammation, and fever, making it an essential drug for various painful and inflammatory conditions. The drug exhibits its pharmacological action through a dual inhibition of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzymes, with a slightly higher affinity for COX-2. The COX enzymes are responsible for converting arachidonic acid into prostaglandins and thromboxanes, which are key mediators in inflammation and hemostasis.

Inhibition of Prostaglandin Synthesis

Mefenamic Acid exerts its therapeutic action by blocking the activity of cyclooxygenase enzymes (COX-1 and COX-2), thereby preventing the conversion of arachidonic acid into prostaglandins. Prostaglandins, particularly prostaglandin E2 (PGE_2) and prostaglandin F2-alpha ($PGF_2\alpha$), play a significant role in inflammatory responses, pain perception, fever regulation, and uterine contractions. By reducing the synthesis of these mediators, Mefenamic Acid effectively lowers inflammation, alleviates pain, and controls excessive uterine activity. This inhibition is particularly beneficial in conditions like dysmenorrhea, osteoarthritis, rheumatoid arthritis, and postoperative inflammation, where prostaglandins contribute to pain and swelling. Since prostaglandins are also involved in various physiological functions, including the protection of gastric mucosa and renal perfusion, the inhibition of their synthesis can sometimes lead to adverse effects such as gastric irritation and renal complications, especially with prolonged use of the drug.

Reduction of Inflammation

Inflammation is a natural immune response to injury, infection, or tissue damage, often characterized by pain, swelling, redness, and loss of function. This response is largely driven by the release of inflammatory mediators, including prostaglandins, which increase vascular permeability, promote edema formation, and enhance pain sensitivity. Mefenamic Acid, through its inhibition of COX enzymes, prevents the excessive production of pro-inflammatory prostaglandins, thereby reducing tissue inflammation and swelling. This mechanism makes it highly effective in treating chronic inflammatory conditions such as rheumatoid arthritis and osteoarthritis, where inflammation plays a key role in disease progression. By limiting the inflammatory response at the site of injury, Mefenamic Acid provides symptomatic relief, improving joint mobility and overall functional capacity in patients suffering from inflammatory joint diseases.

Analgesic (Pain-Relieving) Action

Pain perception is largely regulated by nociceptors (pain receptors) in the nervous system, which become sensitized in response to prostaglandin release. Prostaglandin E2 (PGE_2) is one of the most potent mediators involved in pain hypersensitivity, as it lowers the threshold of nociceptor activation, making nerves more sensitive to painful stimuli. Mefenamic Acid exerts its analgesic action by reducing PGE_2 levels, thereby inhibiting nociceptor activation and decreasing pain perception. This mechanism is particularly beneficial in conditions like menstrual pain (dysmenorrhea), musculoskeletal pain, headaches, and post-surgical pain, where excessive prostaglandin activity leads to heightened pain sensitivity. Unlike opioids, which act centrally by altering pain perception in the brain, Mefenamic Acid works peripherally at the site of inflammation, making it a safer alternative for pain management without the risk of dependence or sedation.

Antipyretic (Fever-Reducing) Effect

Fever is an immune response triggered by infection, inflammation, or injury, where pyrogens (fever-inducing substances) stimulate the production of prostaglandin E2 (PGE_2) in the hypothalamus, the region of the brain responsible for thermoregulation. PGE_2 raises the body's temperature set-point, leading to fever as part of the body's defense mechanism against infections. Mefenamic Acid helps in reducing fever by inhibiting the synthesis of PGE_2 within the hypothalamus, thereby lowering the body's temperature set-point back to normal levels. This makes it an effective antipyretic agent in treating fever, especially in conditions where inflammation is a contributing factor. However, compared to other NSAIDs like ibuprofen and aspirin, Mefenamic Acid's antipyretic effect is moderate, and it is generally preferred for conditions where fever is associated with pain or inflammation rather than as a primary treatment for fever alone.

Effects on Uterine Contractions

One of the most unique and widely recognized uses of Mefenamic Acid is in the treatment of menstrual pain (dysmenorrhea) and heavy menstrual bleeding (menorrhagia). During menstruation, high levels of prostaglandin F2-alpha ($PGF_2\alpha$) are produced in the uterine lining, leading to increased uterine contractions, reduced blood flow to the uterus, and heightened pain sensitivity. These contractions, often described as severe cramps, can cause significant discomfort in individuals suffering from primary dysmenorrhea. By inhibiting prostaglandin synthesis, Mefenamic Acid reduces the strength and frequency of uterine contractions, thereby alleviating pain and improving uterine blood flow.In cases of heavy menstrual bleeding (menorrhagia), prostaglandins also contribute to increased vascular permeability and excessive bleeding, leading to prolonged and heavy periods. Mefenamic Acid, by modulating prostaglandin levels, reduces excessive menstrual blood loss, making it a preferred non-hormonal treatment option for menorrhagia, particularly in women who do not wish to use hormonal contraceptives. It is often prescribed for short-term use during menstruation to control bleeding and pain effectively.

Formulation Challenges: Solubility, Stability, and Bioavailability Issues

Mefenamic Acid is a non steroidal anti inflammatory drug (NSAID) has been extensively used in the treatment of pain, inflammation and fever. Nevertheless, while pharmacologically efficacious, the drug faces a number of formulation hurdles which cause major problems in terms of as much as effectiveness, therapeutic consistency, and adherent to the medical. Mefenamic Acid faces some major formulation challenges centered on its poor aqueous solubility, instability of chemical and physical forms, and poor bioavailability, because it cannot be absorbed or efficiently exploited as therapeutics. These obstacles are continuously faced by the pharmaceutical industry to achieve formulations of improved dissolution, improved bioavailability and prolonged stability. To enhance the delivery of therapeutic outcomes, the limitations must be addressed in the Mefenamic Acid formulations to facilitate a safe and effective application of the drug in patients with various pathologies, with minimal variability in drug absorption and metabolism.Physical and chemical properties of Mefenamic Acid, for example, its hydrophobic character and crystalline structure, dictate important characteristic of Mefenamic Acid solubility, dissolution rate and overall pharmaceutical performance. The most preferred and convenient route of administration in drug delivery is oral, which is the reason why the increasing oral bioavailability of poorly soluble drugs like Mefenamic Acid is a major area of research in current pharmaceutical technology. Mefenamic acid has been investigated through numerous novel drug delivery strategies to resolve the solubility, stability and bioavailability impairments, especially microwave assisted nano bio composite formulations which have shown favorable drug dissolution, improved systemic absorption and chemical stability.

Solubility Challenges and Their Impact on Drug Absorption

The poor Mefenamic Acid aqueous solubility is one of the most fundamental challenges in its formulation as it is a key factor in determining drug absorption, bioavailability, and therapeutic efficacy. The absorption of a drug into systemic circulation is dependent upon its dissolution and the orally administered drugs with poor aqueous solubility tend to display a delayed onset of action, incomplete drug absorption and lower bioavailability. Mefenamic Acid belongs to BCS Class II drug which uses high permeability but low solubility. It therefore implies that its oral bioavailability was to a large extent limited by the dissolution rate, rather than by its membrane permeability. The reason for the low solubility of Mefenamic Acid is due to the hydrophobic nature of the molecular structure, consisting of aromatic rings and a non polar diphenylamine core. The poor solubilization in aqueous environment arises from these structural components that hinder drugs from forming hydrogen bonds with water molecules. Additionally, log P value (~5.8) for the drug indicates its high lipophilicity, which signifies the preference of this drug for lipid based solvents rather than aqueous system. Membrane permeability is improved by lipophilicity, but excessive lipophilicity reduces solubility and, therefore, absorption in the gastrointestinal tract with limited amount of drug available.

To overcome the solubility dependency, different drug delivery methods have been attempted such as reduction in the particle size (micronization and nanonization), solid dispersions, amorphization, lipophilic formulation and complexation with solubilizing agents. A few of these include microwave assisted nano bio composites which have emerged as a promising technological advancement to increase the solubility and dissolution rate. Using microwave irradiation during formulation results in better wettability, surface area and solubilization properties of Mefenamic Acid molecules by means of efficient dispersion of Mefenamic Acid molecules in a nano bio matrix.

Stability Issues and Degradation Pathways

Mefenamic Acid is another critically formulated matter, because it is also chemically and physically unstable, and this may cause loss of potency, reduced therapeutic effect and shortened shelf life of the end product. It must remain within specified chemical systems, structure, and therapeutic function during the time the product is intended to be in storage any where within its package. However, Mefenamic Acid is prone to chemical degradation under environmental influence (moisture, light, heat, and oxygen), being very afflicated with hydrolysis, oxidative processes and polymorphic transformations. Mefenamic Acid is susceptible to hydrolytic cleavage under aqueous conditions owing to the presence of a carboxyl group (-COOH) that constitutes one of the degradation pathways is hydrolysis. The reaction is accelerated in the formulations containing excess water as water molecules act as a catalyst for break the drug down into inactive degradation products.

Oxidative degradation is also a major stability issue for Mefenamic Acid, and this is especially so under oxygen exposure, in the presence of light radiation. In its molecular structure, the drug is susceptible to oxidation to form inactive, or potentially toxic, degradation products in the diphenylamine core. Pharmaceutical formulations are required to prevent oxidative damage and maintain drug potency that necessitates use of the antioxidants, oxygen scavengers and light protective packaging. It is further reported that Mefenamic Acid is a polymorphic compound, possessing different solubility, stability and dissolution rate for different crystalline forms. The polymorphic forms may be more or less thermodynamically stable and, in some cases, more soluble or less soluble. The polymorphic transitions may result in drug performance batch to batch variability. Therefore, stabilization strategies like polymer incorporation, lyophilization or even controlled polymorph selection have been developed to minimize such risks.

Bioavailability Issues and the Need for Enhancement Strategies

Mefenamic Acid has very limited oral bioavailability because of poor solubility, first pass metabolism and pH dependent absorption making its oral bioavailability low. Because Mefenamic Acid is only soluble in solution, the low solubility of Mefenamic Acid results in slow and incomplete absorption and consequent subtherapeutic plasma levels. Also, having been absorbed, the drug is subject to extensive first pass metabolism in the liver making this little drug to get to circulating blood for actions to do there.Modern pharmaceutical techniques to improve bioavailability have been developed including micronisation, solid dispersions, cyclodextrin complexation, lipid-based systems as well as microwave assisted nano bio composites. Microwave assisted synthesis of nano bio composites has attracted interest, as enhancement of drug solubility, increase of dissolution rates and dispersion of uniform particles are considered. As such, the approach proposed represents a highly energy efficient and scalable strategy for the optimization of biopharmaceutical properties of Mefenamic Acid to obtain better therapeutic efficacy, reduced side effect, and enhanced patient compliance. With poor solubility, stability, and bioavailability of Mefenamic Acid, relevant to its formulation challenges, innovative drug delivery strategies are required to provide enhanced performance, predictable pharmacokinetics and enhanced clinical efficiency.

Need for Stability Enhancement to Improve Drug Performance and Shelf Life

Stability of pharmaceutical formulations is one of the most critical process since the stability directly relates to the efficacy, safety and the shelf life of the final product. The stability of a drug is a crucial point to prevent its lose potency, structural integrity and pharmacological activity over a prolonged time period protected from all the environmental conditions like heat, humidity, light, and oxygen. A drug that is not prepared in a way such that its chemical and physical stability is maintained may undergo degradation, polymorphic transformation or become ineffective as a therapeutic (become less potent or ineffective altogether).

Like most other poorly soluble NSAIDs, Mefenamic Acid is plagued by severe stability concerns that will impact the bioavailability, therapeutic consistency and compliance of the patient. The challenges in these cases arise from the susceptibility of the drug to chemical degradation by hydrolysis and oxidation, solid state transformations including polymorphic changes, and environmental sensitivity that can produce changes in drug's dissolution profile, absorption rate, and pharmacological activity. One problem with the drug is that the stability of the drug over time is critical to the therapeutic effect and thus these issues must be resolved to ensure that Mefenamic Acid formulations stay therapeutically effective over the shelf life while giving a predictable therapeutic response at administration. The physicochemical properties of Mefenamic Acid formulations may be substantially changed if they are not adequately stabilized and degradation occurs. Such variations may influence the drug absorption, change drug pharmacokinetic profile, as well as reduce drug efficacy. We found such inconsistencies to enhance the risk of underdosing, leading to treatment failure and overdosing, which can be accompanied by safety and toxicity. In addition, unstable formulations could require frequent dose adjustments and impose inconvenience on patients and lack of adherence to the prescribed therapy. In addition, pharmaceutical companies face financial and regulatory challenges related to stability of products: such products may be recalled, non-compliant with regulatory standards or costly to reformulate due to increased manufacturing costs. Taking these into account as a starting point, the stability of Mefenamic Acid formulations should be increased to ensure the quality of performance, the stretching of shelf life and commercialisation. In view of these challenges, pharmaceutical researchers have come up with

different formulation techniques and stability enhancing strategies to improve the chemical, physical and stability of Mefenamic Acid. The strategies are based on use of stabilizing excipients, protective packaging, environmental control measures and advanced drug delivery systems which protects the drug from degradation factors and retains its potency and solubility. These approaches include microwave assisted nano bio composites which have lended them as an innovative technology driven solution that provides improved stability, enhanced solubility, as well as better bioavailability without losing the structural and pharmacological properties of the drug for longer storage.

Factors Affecting the Stability of Mefenamic Acid

A. Hydrolytic Degradation and Moisture Sensitivity

Mefenamic Acid tends to be unstable to hydrolytic degradation, where the drug dissolves in the presence of moisture or aqueous environment. The chemical reaction of hydrolysis occurs when water molecules cleave specific chemical bonds in the drug's structure with which they react, resulting in the formation of degraded by-products that may possess lower pharmacological activity or higher toxicity. In particular, oral solid dosage forms, including tablets and capsules, are prone to absorbing from ambient humidity, inappropriate storage conditions, or packaging materials, and this process is therefore disconcerting.

As Mefenamic Acid has very high ability to hydrolytic cleavage, if stored in high humidity or high moisture environments, it will be easily hydrolyzed. Chemical degradation of drugs may be accelerated with exposure to moisture, resulting in reduced drug potency, poor dissolution properties, shorter shelf life, etc. Hydrolysis can also be physically unstable to tablets and capsules, resulting in changes in texture of the drug, caking, and mechanical strength, each of which may contribute to poor patient compliance and difficulty of administration. When moisturizing excipients are not added to pharlo formulations, though, pharmaceutical formulations contain moisturust resistant excipients, desiccants, and protective coatings which prevent hydrolysis by reducing miurowity absorption and exposure to humidty. Aluminum blister packs, moisture-proof polymers and vacuum sealed containers are often used to carry Mefenamic Acid formulations for reduction of its water permeability and shelf life extension. Further, the drug has been also protected from moisture induced degradation with advanced formulation techniques like the nano composite technology and polymeric matrix embedding to improve its overall stability profile.

B. Oxidative Degradation and Light Sensitivity

Oxidative degradation of Mefenamic Acid formulations is another major stability issue due to loss of the drug exposure to: oxygen, free radicals or UV light. NSAIDs are degraded by common oxidation pathway including those drugs containing electron rich functional groups which in turn are reactive with ambient oxygen and reactive oxygen species (ROS). Loss of potency of the drug, formation of undesirable degradation by products and changes in drug color, texture and odor can occur through this reaction, which affects product quality and patient safety.

Mefenamic Acid is specifically vulnerable to oxidation,in high temperature storage conditions or exposure to UV light, resulting in a diphenylamine core target. Various pharmaceutical techniques are used to prevent oxidation by the incorporation of antioxidants, oxygen scavangers, and chelating agena that neutralize free radicals and oxidative species. It has been well accepted that antioxidants like ascorbic acid (vitamin C), tocopherols (vitamin E), butylated hydroxytoluene (BHT), etc. have been employed extensively to stabilize Mefenamic Acid formulation and prevent oxidative damage. Drug stability is also influenced by the photodegradation process caused by exposure to UV radiation, which can also generate oxidation by light-induced degradation. In order to counter this, pharmaceutical formulations are also packaged in opaque or amber colored containers and serve as UV filters that prevent light induced degradation. In addition, strategies of encapsulaton with nano bio composites or polymeric coatings can offer additional protective barrier versus oxidative degradation, preventing attractive oxidative degradation, and the drug can remain stable under a variety of environmental conditions.

C. Polymorphic Instability and Solid-State Transformations

Mefenamic Acid shows polymorphic behavior where it may exist in several forms of crystalline solids that possess distinctive solubilities, stabilities and bioavailability properties. By having polymorphic forms with high solubility but tendency to recrystallise or degrade over time, or thermodynamically stable but poor solubility polymorphs, the polymorph choice is constrained by the solubility of the formulation. These solid state transformation have great potential to affect drug dissolution and absorption rate, as well as overall pharmacokinetic performance and may alter the therapeutic effectiveness and patient response.

Various formulation strategies have been developed to stabilize the polymorphic state of Mefenamic Acid and to avoid instable transformations such as polymer stabilised dispersions, co crystallization, and nano formulation. Hydrophilic polymers such as hydroxypropyl methylcellulose (HPMC), polyvinylpyrrolidone (PVP), polyethylene glycol (PEG) can be used in order to maintain the amorphous or metastable polymorphic state of the drug, thus increasing dissolution and bioavailability. Furthermore, microwave assisted nano bio-composites have been also used as an effective means to stabilize polymorphic structure of

Mefenamic Acid by ensuring uniform molecular dispersion in polymeric matrix which prevents solid state transitions as well as achieving long term stability. Maintaining therapeutic efficacy, prohibiting degradation to this cromolyn, and prolonging shelf life is dependent on the sensitivity of Mefenamic Acid formulations. Protonics, employing technologies such as microwave assisted nano bio composites, encapsulation and polymer stabilized dispersions, seek to leap from drug formulation to developing more stable, effective and patient friendly formulation. However, similar stability and clinical effectiveness merits continued research of Mefenamic Acid formulations with improved solubility, stability as one of the key determinant of drug performance.

Microwave-Assisted Nano Bio-Composites: A Novel Approach for Drug Stability Enhancement

Concept and Definition of Nano Bio-Composites in Drug Delivery

Like any other pharmaceutical industry, the never ending quest for innovative ways to increase drug stability, solubility, bioavailability and therapeutic efficiency has led to ever changing ways of thinking and approach. With growing number of new drug molecules being developed, a high portion of these becomes poor aqueous soluble and physicochemically unstable, resulting in low bioavailability and variable biologic effects. This challenges have being addressed in modern drug delivery systems and one of the most promising approaches to these challenges are the nano bio composites. Currently, these advanced drug carriers apply the field of nanotechnology together with biocompatible materials to elevate the formulation features of drugs, particularly those that have the limitations of solubility and stability.

Nano bio-composites are complex, hybrid materials designed based on the optimized drug profiles of drug release, the drug protection and pharmacokinetic conditions, which are formed by incorporation of nano scale carriers and biologically derived components. And this delivery system renders many more advantages to traditional drug formulations with such as controlled release and drug targeting, and enhancement in biocompatibility thus improving patient compliance as well as therapeutic outcome. Nano bio composites have been receiving growing interest owing to their capability to encapsulate the drugs into the nano scale so that they can be protected from environmental degradation such as moisture oxidation heat and the change in pH. As a result, this encapsulation substantially decreases instability of the drug, while improving dissolution, systemic absorption and pharmacological performance, and prolonging the drug efficacy.[32] Nano bio-composites are formed by embedding or attaching nanoparticles, polymeric carriers, liposomal systems, solid lipid nanoparticles or a hybrid nanostructures into biomaterials of biocompatible proteins, polysaccharides or biodegradable polymers in the field of drug delivery. These biologically derived materials act as stabilizing agents to prevent the pre-mature drug degradation, and yet allow their sustained drug release. Nano bio composites act as carriers combined with biomaterials that can create a drug delivery matrix that inhibits the loss of active pharmaceutical ingredient (API) via hydrolysis, oxidation, polymorphic phase transitions, and thermal instability. Nowadays, one of the main goals of nano bio based composite formulations is to improve the solubility and dissolution of poorly water soluble drugs, by breaking the limitations in conventional dosage forms. Mefenamic Acid is a common example of many pharmaceutical compounds, which typically have low aqueous solubility and have been associated with delayed drug absorption, low systemic bioavailability and consequently poor therapeutic efficacy. A remarkable success of incorporating nano bio composites as drug formulating has been witnessed in increasing the surface area of drug particles, improving wetting properties and surface modification in their crystalline structure. These two mechanisms jointly upregulate the dissolution rate of the drug, for fast onset of action and improved GI absorption. In this context, incorporating microwave-assisted nano bio composites is an effective solution to tackle poor aqueous solubility, pH dependent dissolution, susceptibility to hydrolytic and oxidative degradation, and rapid clearance from the body of Mefenamic Acid. Nano bio composites adjusted physicochemical and pharmacokinetic properties of Mefenamic Acid provides a better platform for the drug delivery, providing prolonged stability and improved bioavailability and drug therapeutic performances. However, the development of nano bio-composites synthesized via microwave assisted synthesis is a great advancement in the modern pharmaceutical technology and moves towards an alternative means of improving the drug formulations. The research of the potential applications of nano bio composites in drug delivery continues to gain interest as drug solubility, stability and patient compliance will become essential to consider. Nano bio-composites can be considered a future oriented solution to address the limitations of conventional drug formulations and hence they are an inevitable tool of pharmaceutical innovations in the age of next generation of pharmaceutical innovation.

Mechanism of Action for Improving Solubility, Stability, and Controlled Release

The nano bio composites are very significant advancement in the drug delivery systems and in the increase of solubility, stability and controlled release of pharmaceutical compounds. The use of nanotechnology based carriers and biocompatible bio material in a drug formulation creates a multi face mechanism of action which makes the poor soluble or unstable drug more physicochemical and pharmacokinetic properties. Nano bio composites offer an appropriate solution for drugs like Mefenamic Acid having low aqueous solubility, susceptibility to degradation and short half life by modifying drug solubility, preventing degradation and extended drug release for better, prolonged therapeutic action.

With further improvement on microwave assisted synthesis, the structural organization and functional properties of nano bio composites will be further improved with homogeneous dispersion, good interaction between drugs and polymer, and energy efficient processing. Thus, this is a technique which ensures that the drug molecules are distributed evenly within the carrier system thereby avoiding drug aggregation, recrystallization and loss of activity. Several interrelated physicochemical and biological processes, which contribute to the increased bioavailability and therapeutic efficacy of nano bio composites, can be explained by the mechanism of action through which they improve drug solubility, stability as well as controlled release.

Mechanism of Action for Improving Solubility

A major challenge in pharmaceutical development for hydrophobic drugs like Mefenamic Acid are that they have low dissolution rates and poor absorption in the gastrointestinal fluid. Nano bio composites has harnessed the enhancements to solubility by, at a minimum, particle size reduction, surface area increase, change in crystallinity as well as modification of wettability which all contribute to better dissolution and faster drug absorption. The most effective means by which nano bio composites can increase solubility is by decreasing the particle size of drug molecules to the nano scale which increases the total surface available for interaction with dissolution medium. The dissolution rate of a drug is proportional to its surface area, and therefore nano-sized particles dissolve many times faster than their bulk counterparts, as described by the Noyes-Whitney equation. By entrapping the drug in a nano bio polymer composite matrix, the particle size is reduced drastically due to enhanced solubilization of the drug in the biological fluid.

Modifying the crystalline structure of the drug is another key solubility enhancement mechanism. There are many poorly soluble drugs that exist in extremely ordered crystalline form that prevents easy dissolution in aqueous solution. Despite this, nano bio composites are able to convert the drug to an amorphous state, a more thermodynamically soluble form than the crystalline. Microwave Assistant processing enables this transformation by producing enough energy to disrupt the crystal lattice of the drug, hence releasing it from the crystal state into a higher energy amorphous or metastable state. Amorphous drugs have a higher solubility and dissolution rates resulting in better absorption and enhanced bioavailability. Nano bio composites also improve drug wetting properties by coating hydrophobic drugs with hydrophilic excipients or polymers to create a better interaction with the surrounding water. This helps to prevent drug particles clumping together and enabling faster distribution and dissolution in gastrointestinal fluid. Nano bio composites combine particle size reduction, amorphization, and surface wettability modification to enhance solubility by a comprehensive route and thus are especially suitable for the solubility enhancement of low solubility drugs such as Mefenamic Acid.

Mechanism of Action for Improving Stability

Therefore, pharmaceutical stability is a major issue to ensure the chemical and physical integrity of a drug during its storage period and administration. Mefenamic Acid is a drug which is very susceptible to degradation by exposure to moisture, oxygen, heat and light causing loss of potency and reduced therapeutic effectiveness. Nano bio composites can incorporate many mechanisms of stability and protect the drug from environmental stressors that would naturally degrade the drug. One of the major reasons for improvement in stability of nano bio composites is encapsulating drug molecules within protective nano scale matrix as a physical and chemical barrier to degradation pathways. It protects the drug from moisture hydrolysis, oxidation from ambient atmosphere and caused by U V light. Nano bio composites limit the direct contact between the drug and the external degradation factors, and therefore extend immensely the shelf life of pharmaceutical formulations.

The polymer drug interaction often serves as another stability promoting mechanism by suppressing the undesired solid state transformation of the drug, including polymorphic change or recrystallization. Indeed, many drugs are polymorphic instable, i.e., they undergo polymorphic transformations between crystalline forms, some of which may be less soluble or less stable. These transformations are effectively inhibited by dispersing the drug inside of a polymeric, or lipid based nano bio composite matrix into which the drug is distributed. In addition, because the drug-excipient compatibility and uniformity of drug distribution in the composite matrix are promoted by the microwave-assisted synthesis process, stability is further improved. This helps avoid localized accumulation of drug molecules and consequently aggregation, degradation and phase separation during the storage. Nano bio composites stabilize the drug at the molecular level and present a solid and practical means to increase the lived and retain the pharmacological activity of the drug for extended periods.

Mechanism of Action for Controlled Release

Modern pharmaceutical formulations offer controlled drug release, which provides sustained therapeutic effect, lesser dosing frequency as well as reduced numbers of side effects. The conventional drug delivery systems normally have a rapid release of drug resulting in a rapid change in plasma drug concentration, which may contribute to toxicity or poor therapeutic effect. However, nano bio composites offer a mode of controlled and sustained release that ensures steady release of dosage for a ga long span. Diffusion based release is one of the main processes by which drug molecules are released in controlled fashion by the nano matrix over time. This is accomplished by encapsulating the drug within a polymeric or lipid reservoir from which the drug is

released in a sustained manner. The rate of the drug diffusion can be tailored by the nano bio composite to control the rate of release and determine the amount of the drug released consistently and predictably.

Furthermore, precise tuning of the drug polymer interaction under microwave assisted synthesis techniques facilitates drug release profile for maximum therapeutic benefit. Researchers design these nano bio composites with different parameters such as the molecular weight of polymer, the cross linking density and the hydrophobicity, and they vary the parameters to make nano bio composites with immediate, delayed or sustained release as it requires for the application in clinical practice. Integration of nano bio compostes into drug delivery, therefore, provides a through solution for improving solubility, stability and controlled release of drugs, such as Mefenamic acid that are difficult prone to poor solubility, prone, to rapid metabolism and short half life. Nano bio composites facilitate pharmacokinetic properties improvement, thereby enhancing patient compliance with better dosing regimens and results in superior therapeutic outcome and hence continue to be an indispensable tool in modern pharmaceutical research and development.

Composition and Role of Nanotechnology in Drug Formulations

Composition of Nano Bio-Composites in Drug Delivery

Nano bio-composites are advanced drug delivery systems that incorporate nanotechnology-based carriers and biocompatible materials to enhance the solubility, stability, and controlled release of pharmaceutical compounds. These formulations are specifically designed to overcome the limitations of conventional drug delivery systems, such as poor aqueous solubility, chemical degradation, rapid metabolism, and inconsistent bioavailability. The composition of nano bio-composites is carefully designed to achieve maximum therapeutic efficiency while ensuring safety, biocompatibility, and patient compliance.

The primary components of nano bio-composites include:

1. **Nanocarriers (Polymeric, Lipid-Based, or Inorganic Nanoparticles):** These are the core structural components that encapsulate, protect, and control the release of the active pharmaceutical ingredient (API). Nanocarriers improve drug dispersion, solubilization, and targeted delivery, ensuring that the drug reaches its intended site of action efficiently.
2. **Active Pharmaceutical Ingredient (API):** The drug is either encapsulated within or adsorbed onto the nanocarrier, where it remains protected from external degradation factors such as moisture, oxidation, pH fluctuations, and enzymatic metabolism. The nano bio-composite formulation prevents chemical and physical instability, ensuring that the API remains intact and bioavailable for prolonged therapeutic effects.
3. **Biocompatible and Biodegradable Polymers:** These polymers serve as matrix-forming agents that stabilize the nanocarriers, modulate drug release rates, and enhance mucoadhesion and bioavailability. Commonly used polymers in nano bio-composite formulations include chitosan, alginate, poly(lactic-co-glycolic acid) (PLGA), hydroxypropyl methylcellulose (HPMC), polyethylene glycol (PEG), and polyvinyl alcohol (PVA). These polymers help improve drug solubility by increasing hydrophilicity, preventing aggregation, and modifying the crystalline structure of the drug.
4. **Surfactants and Stabilizers:** Surfactants such as poloxamers, lecithin, sodium lauryl sulfate, and Tween 80 are often incorporated into nano bio-composites to reduce surface tension, enhance drug dispersion, and stabilize nano-sized particles. Stabilizers help prevent nanoparticle aggregation and ensure uniform drug loading and controlled drug release.
5. **Lipid-Based Carriers:** Lipid-based nanocarriers such as liposomes, solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs) are commonly used in nano bio-composites for lipophilic drugs. These carriers enhance the solubility and permeability of poorly water-soluble drugs, ensuring improved absorption in biological systems.

The selection and combination of these components depend on the desired drug delivery properties, including targeted drug release, enhanced permeability, prolonged circulation time, and resistance to degradation. Nano bio-composites provide a highly customizable and adaptable platform for drug formulation, making them suitable for a wide range of pharmaceutical applications, including oral, topical, transdermal, and parenteral drug delivery.

Role of Nanotechnology in Drug Formulations

As a miniaturized version of existing chemicals and important drug formulations, nanotechnology is used in modern drug formulations and can deliver significantly different advantages than other typical methods to enhance drug solubility, increase bioavailability, facilitate specific targeting of drugs and medication turning points. The use of nanotechnology based systems in the drug formulation has changed the face of pharmaceutical research and drug development by introducing safer, more potent and making the drugs more easily acceptable to the patients.

Improvement of dissolution rate and solubility of poor water soluble drugs are one of the most significant contributions of nanotechnology in drug formulation. Due to its poor aqueous solubility, many pharmaceutical compounds undergo delayed drug absorption, poor systemic bioavailability and variability of therapeutic effects. However, this challenge is overcome through

nanotechnology based carriers like nano bio composites, nanocrystals, liposomes, micelles, solid lipid nanoparticles (SLNs) which increase the surface area of the drug, modify its crystalline structure and homogenously dispersed in biological fluid. Also, nanotechnology is employed to stabilize pharmaceutical formulations to ensure that drugs are chemically and physically stable while in storage and during administration. Nano bio composites can encapsulate such drugs that are prone to hydrolysis, oxidation, photodegradation or enzymatic degradation, thus protecting them from external environmental stressors. It extends the shelf life of the drug to an order of magnitude, reduces such chemical degradation risks thereby maintaining a constant therapeutic potency.

Controlled and sustained drug release is another very important application of nanotechnology in drug formulations to achieve prolonged therapeutic effect, reduced dosing frequency, and higher patient compliance. Conventional drug formulations are characterized by very fast drug release, which results in sharp variations of plasma drug concentration, enhanced toxicity risks and inappropriate therapeutic efficacy. On the other hand, drugs delivered through nanotechnology based drug delivery systems are released in a controlled fashion, by controlling polymer degradation, diffusive rates and drug polymer interactions to achieve a steady and prolonged release of the drugs over time. However, this approach proves to be highly valuable for chronic disease treatment, treatment of chronic pain or long term drug therapy as it is essential to maintain plasma drug level constancy. Another major advantage of nanotechnology in drug formulations is their ability to deliver drugs to a targeted area. Surface ligands can be engineered to functionalize nanocarriers as antibodies, peptides or folic acid ligands which allows them to bind only to specific cell receptors or tissues. This targeted approach both localizes drugs to the disease sites and reduces systemic side effects and improves therapeutic efficacy. Nanotechnology based drug delivery carrier designed for example, can be made to selectively deliver chemotherapeutic agents to the tumor cells and reduce the toxicity to the healthy tissues and effective as much with the treatment outcomes.

With the advancement of nanotechnology, it is anticipated that the field will play an increasingly important role of improving pharmaceutical formulations, perfecting drug delivery, and improving the potency of the drug. Nano bio-composites represents an innovative breakthrough in modern drug development by virtue of a solution that is advanced, scalable and efficient for enhancing the stability, solubility and bioavailability abilities of pharmaceutical compounds. The integration of microwave assisted synthesis techniques has led to the development of nanotechnology based drug formulation that will change pharmaceutical innovation and improve patient care to a wide variety of therapeutic applications.

Fundamental Principles and Mechanism of Microwave-Assisted Synthesis

Microwave assisted synthesis is one of the rapidly growing technique in pharmaceutical sciences which provides more efficient, energy saving and controlled approach for drug formulation. As microwave radiation has been extensively used in synthesis processes already due to its ability in offering rapid, uniform and selective heating, which offers faster reaction rates, improve product quality and increased reproducibility over conventional thermal methods. Microwave assisted synthesis has a very important role in drug formulation, particularly for nano bio-composites to improve drug stability, increase solubility and control drug release. External heat sources are used for the traditional heating method that slowly transfers heat to the reaction medium via conduction and convection with temperature gradient and heat evenness. Most often it leads to slower processing times, less than finished reaction, and products of inconsistent quality. On the other hand, microwave irradiation generates heat within the reaction mixture directly, causing occurrence of instantaneous heating, enhancement of molecular interactions, and its useful increase of reaction efficiency.

Microwave assisted synthesis is a new approach to the drug stabilization and bioavailability enhancement of poorly soluble and unstable drugs like Mefenamic Acid. The use of nanotechnology based carriers, bio compatible polymers, and microwave responsive materials will allow for the highly stable and bio compatible composite incorporation that has superior solubility, protected drug and sustained therapeutic therapy. Microwave assisted synthesis is a technique where there is a possibility to achieve precise control on parameters such as temperature, pressure and time and therefore, this technique is ideal to optimize pharmaceutical formulations. Microwave assisted synthesis is a thermally activated method that relies on molecular level interactions, and use of a myriad of factors that make up the principal principles of microwave assisted synthesis, like dielectric heating mechanisms, set a large scale of proper, secure, and effective drug loaded nano bio composite.

Fundamental Principles of Microwave-Assisted Synthesis

Microwave-assisted synthesis is governed by dielectric heating principle where microwave energy is directly absorbed by the polar molecules and ionic species in the mikewave reaction medium to bring rapid internal heating and rapid energy transfer. Microwaves used in pharmaceutical and material sciences have a frequency range of usually 300 MHz to 300 GHz, with 2.45 GHz being the most used value in the laboratory scale and industrial scale. While conventional heating gradually increases the temperature of the reaction mixture from an external source, microwave heating generates the heat homogenously within the system and gives the heat better uniform distribution and increased processing time.

Two main mechanisms which are responsible for microwave assisted synthesis are dipolar polarization and ionic conduction. Polar molecules like solvents or excipients orient themselves with the oscillating electromagnetic field of microwaves to form dipolar polarization. These dipoles reorient themselves continuously with the microwave frequency and generate localized heat and molecular friction. Rapid heating and improved reaction kinetics were achieved from this mechanism in terms of redusing the time necessary for drug polymer interactions, nanoparticle synthesis, and bio composite formulation.

On the other hand, ionic conduction is the movement of charged particles, which may be drug molecule or excipients, within the reaction system in response to the microwave irradiation. Collision between the charged species generates heat and enhances the efficiency of reaction. Of particular significance in the synthesis of nano bio-composites, controlled heat input controls the uniform drug dispersion, limits the aggregation and viscosity of the distribution points. Major advantage of microwave assisted synthesis is that it allows to selectively heat specific reaction components without overheating surrounding medium for high yield, solvent free, and environment friendly pharmaceutical processing.

Mechanism of Microwave-Assisted Synthesis in Nano Bio-Composites

Microwave induced molecular interactions, precise thermal control and nanoscale processing of material are based on mechanism by which the microwave assisted synthesis enhances nano bio-composite formulations. Microwave assisted synthesis is used for synthesis of drug formulations, particularly for nano bio composites in which microwave assisted synthesis provides a highly efficient and economical route towards drug solubility, stability and controlled drug release. Rapid and uniform heating of the drug-excipient mixture is one of the critical features in this process and guarantees homogeneous drug dispersion and optimized material properties.

Formulation of key one, involving promotion of the drugpolymer interaction which results in strong molecular bonding and stability in encapsulated state. The highly crystalline structures of the molecules; and the strong intermolecular forces that they render, yield many drugs, especially hydrophobic drugs, such as Mefenamic Acid, to show poor water solubility. Disruption of crystalline lattice of the drug by microwave irradiation converts the drug into amorphous or nano crystalline, thus increasing its solubility and bioavailability. Furthermore, the polymeric carriers which undergo uniform heating under microwave assisted processing are stabilized by cross-linking, which results in the use of the drug encapsulated within the nano bio composite matrix.

Another benefit of the use of the microwave in synthesis is that there is a controlled release mechanism available through the development of biodegradable and pH sensitive polymeric carriers. These carriers serve as protective matrices reducing premature drug release, and targeted drug delivery delivering at specific tissues or organs. The change of the reaction duration and the polymers composition enables the researchers to design nano bio-composites with sustained, delayed or even pulsatile drug release profiles allowing a wider freedom of choice in drug administration and better patient compliance.

Advantages of Microwave-Assisted Synthesis in Drug Formulation

The most rewarding aspect of microwave assisted synthesis is its capability to get high reaction efficiency and scale up in contrast with the environmental friendly manufacturing. This is in contrast to microwave assisted synthesis, which burdens less or no organic solvents, thus directly minimizing the waste produced by the toxic solvents generated by conventional solvent based drug formulations and associated environmental hazards. Microwave responsive stabilizers and excipients are used to further improve formulation reproducibility and batch to batch consistency so that drug formulations will meet pharmaceutical quality as well as regulatory standards.This has another advantage of precise control over the physicochemical properties of drug loaded nano bio composites and hence customization of drug release, targeted drug delivery and improved biocompatibility. Microwave assisted nano bio composite system offers the ability to tailor the crystallinity, porosity and surface characteristics of the final drug formulation leading to an optimal drug performance, making it a highly desirable system for next generation drug delivery systems.

Application of Microwave-Assisted Synthesis in Mefenamic Acid Formulation

Microwave-assisted synthesis is an effective process to solve the formulation challenges of Mefenamic Acid, a drug that possesses low aqueous solubility and susceptibility to chemical degradation. By using microwave induced nano bio composites, the drug can be converted into amorphous or nano dispersed state and thereby increases its dissolution rate and absorption. Furthermore, the nano bio composite accommodates stabilizing polymers and lipid based carriers which blocks moisture induced hydrolysis and oxidative degradation thereby providing long term stability and increased bio availability. The second contribution to sustained therapeutic action with controlled drug release properties from the microwave processed nano bio composites is further ability of the latter to reduce the dose frequency and increase patient adherence.
To date, the use of microwave assisted synthesis in drug formulation is a transformational process of pharmaceutical research with application in the field of cost effective, scalable and also a high performing method towards enhanced drug solubility, stability and bioavailability. This technology is expected to become an integral part of developing innovative and patient friendly pharmaceutical formulations of poorly soluble and unstable drugs such as Mefenamic Acid as research continues to expand.

Advantages Over Conventional Methods

1. **High Energy Efficiency:** Microwave-assisted synthesis is significantly more energy-efficient than conventional methods as it utilizes dielectric heating, where electromagnetic radiation directly interacts with polar molecules and ionic species, generating instantaneous internal heating. This reduces energy consumption by up to 80%, ensuring minimal heat loss and efficient utilization of thermal energy, making it a cost-effective and sustainable approach in pharmaceutical manufacturing.
2. **Faster Processing Time:** Microwave-assisted synthesis enables rapid reaction rates, reducing processing time from hours to minutes compared to conventional methods such as solvent evaporation, hot-melt extrusion, or high-energy milling. By eliminating slow external heat transfer and promoting direct molecular excitation, this technique accelerates drug-polymer interactions, enhances nanoparticle formation, and ensures faster drug encapsulation, leading to higher production efficiency and reduced manufacturing costs.
3. **Uniform and Volumetric Heating:** Unlike conventional heating, which often results in temperature gradients and non-uniform energy distribution, microwave-assisted synthesis provides homogeneous volumetric heating, ensuring even heat dispersion throughout the reaction medium. This prevents localized overheating, degradation, or incomplete reactions, leading to better molecular interactions, consistent drug dispersion, and higher product quality.
4. **Minimized Drug Degradation:** Conventional methods often expose drugs to prolonged heating, increasing the risk of thermal degradation, oxidation, or hydrolysis. In contrast, microwave-assisted synthesis allows for precise temperature control and reduced exposure time, ensuring that heat-sensitive pharmaceutical compounds retain their stability, potency, and therapeutic effectiveness over extended storage durations.
5. **Improved Drug Solubility and Bioavailability:** Microwave irradiation aids in particle size reduction, crystalline modification, and amorphization, transforming poorly soluble drugs like Mefenamic Acid into more bioavailable forms. The rapid heating mechanism facilitates better polymer-drug interactions, promoting wettability and dissolution enhancement, ensuring faster drug absorption and improved therapeutic response.

Given these advantages, microwave-assisted synthesis is emerging as a revolutionary technique in pharmaceutical formulation, providing an efficient, scalable, and environmentally sustainable approach to enhancing drug solubility, stability, and bioavailability, particularly for poorly soluble and unstable drugs like Mefenamic Acid.

Industrial and Pharmaceutical Significance of This Approach

In general, microwave assisted synthesis is used to revolutionize pharmaceutical and industrial drug manufacturing with highly efficient, scalable and eco friendly approach to developing advanced drug formulations and nano bio composites. With increasing technological advancements, improvements in pharmaceutical delivery systems, and rapid research in the field, challenges such as poor aqueous solubility, low bioavailability, rapid drug degradation, and lack of consistent therapeutic outcome are faced constantly by the pharmaceutical industry and these problems can only responded by the use of innovative drug delivery systems. With traditional methods like solvent evaporation, milling, hot-melt extrusion, and precipitation, enhanced drug property improvement is done by high energy requirement, prolonged processing time, and uncontrollable polymer/drug mixture. The limitations can be overcome with microwave assisted synthesis, which enables rapid, uniform and controlled processing and thus is a very suitable technology for large scale pharmaceutical manufacturing. As a result, it has become an essential tool for the formulation of modern drug delivery systems for its ability to improve the solubility of the drug, increase bioavailability, prevent degradation and achieve controlled release characteristics.

Another important aspect in the industry is to ensure the stability of pharmaceutical formulations, mostly for drugs that degrade quickly in environment. It is known that many drugs, including Mefenamic Acid, are subject to hydrolysis, oxidation, and polymorphic transformation instability issues that can reduce potency and impede consistent drug performance. It is microwave assisted synthesis that has a significant role in increasing stability to such drugs by better molecular interactions, uniform drug dispersion in stabilizing matrices and controlled crystallinity and polymorphic transitions. It gives longer shelf life, increased formulation stability under storage, and diminished degradation risk in storage and transport. The protection of drug molecules from stress conditions by environmental stressors thus help by incorporating nano bio composite, polymeric carrier and stabilising excipient by microwave assisted synthesis.

The other major advantage of microwave assisted synthesis is high energy efficiency and environmental sustainability, therefore microwave assisted synthesis is a green chemistry approach for formulation of drug. Traditional pharmaceutical processing, however, involves high temperatures, long reaction times, excessive use of solvents and results in significant wastage of energy and pollution of the environment. The application of microwave heating, rapid processing, and in some cases solvent free or minimal solvent conditions with associated reduced carbon footprint, reduced capital and operational costs and compliance with environmental regulations seek to mitigate these concerns. It is therefore a perfect choice to be used for big scale pharmaceutical manufacturing, given its sustainability and cost effectiveness. One of the reasons why many pharmaceutical companies have begun to use microwave assisted synthesis is that it is in line with those guidelines that the regulatory bodies are promoting for green processes in drug manufacturing.

Microwave assisted synthesis represents a highly powerful and efficient approach to solve the drug formulation problems for a poorly water soluble and pH sensitive drug Mefenamic Acid. Incorporating Mefenamic Acid into polymeric nano bio composites by means of this technique guarantees better aqueous solubility, prolonged stability and improved bioavailability. Since microwave assisted formulations have controlled release properties, they promote sustained release of the drug, reducing the frequency of dosing and GI side effects, which are major concerns with conventional NSAID therapies. This is due to microwave assisted nanoscale dispersion of Mefenamic Acid in bio compatible carriers with zero order characteristic enhancing the dissolution rates, increase in absorption and improved therapeutic efficacy of the microwave aided drug formulations as compared to conventional dosage forms.

Continuing research into the microwave assisted drug synthesis has great potential for the future of biologics, peptide therapeutics, vaccine formulations and gene delivery systems. As an integral part of modern pharmaceutical innovation, it is its ability to enhance drug solubility, improve stability, reduce environmental impact and to give precise drug targeting that allows its use. Given the evolution of the industry towards the more sophisticated, sustainable and patient-centric drug formulations, it is anticipated that microwave assisted synthesis would pave the way for the next generation of pharmaceutical technologies. The current commercial interest in this technology is driven by companies and research institutions spending increasingly more on their own investments for novel microwave responsive materials, hydrophobic Reactor walls, and other patented mechanical components that increase and scale the capacities of these microwave reactors for industrial pharmaceutical processing.

LITERATURE REVIEW

Darekar *et al.* (2024) According to research findings microwave-assisted synthesis of bionanocomposites effectively improves the physicochemical attributes of poorly water-soluble drugs with specific focus on Olmesartan. The scientific finding showed that drugs with low aqueous solubility affect both absorption rates and bioavailability while producing gastrointestinal toxicity as a result. The researchers developed microwave-generated bionanocomposites by incorporating Moringa oleifera natural polymer followed by performance assessments against xanthan gum and guar gum. The combination of DSC and SEM and XRD analyses showed Olmesartan's material structure became less crystalline which improved both solubility and dissolution performance. The optimized bionanocomposite systems released drugs during in-vitro testing at rates superior to existing market-based products. The stability tests following ICH guidelines demonstrated that the formulations remained stable for six months. This study implies that microwave-assisted bionanocomposite technology presents itself as a novel solution for improving water solubility and drug absorption of drugs with poor water affinity while providing sustainable drug formulations.

Saadh *et al.* (Year) A research group successfully used microwave techniques to produce a new Mo/BPDA nanocomposite for biomedical purposes. By employing XRD and FT-IR in addition to EDAX and EA and TGA/DTG and SEM and BET techniques the synthesized nanocomposite showed high thermal stability at 300°C as well as 35 cm^3 g^{-1} surface area and uniform morphological structure. The antibacterial and antifungal and anticancer assays showed that Mo/BPDA exhibited better antimicrobial capabilities than typical antibiotics through minimum inhibitory and bactericidal concentration levels ranging from 2–256 μg mL−1 and 4–128 μg mL−1. The Mo/BPDA nanocomposite effectively killed breast and bone cancer cells through its anticancer activity with 33–43 μg mL^{-1} IC50 values during the 24–48 hour testing period. Additional in vivo research has indicated the nanocomposite could become a valuable antimicrobial drug which shows potential for targeted cancer therapy.

Mohanto *et al.* (2023) Researchers designed a microwave-assisted nanocomposite formulation which improved cinnarizine solubility and bioavailability because this drug belongs to BCS Class II category but exhibits both poor aqueous solubility and membrane permeability limitations. The research employed bio-nanocomposite methods combining microwave energy to reduce particle size and enhance the effective surface area when cinnarizine bonded with xanthan and guar gum. Testing results demonstrated that the produced nanocomposite achieved 80.80±0.8% yield while showing 0.830±0.008 solubility and having 169.36 nm hydrodynamic diameter (with 23.6 polydispersity index). The zeta potential result showed excellent stability at 34.4 mV. The crystalline drug structure of cinnarizine showed changes through transmission electron microscopy because it got embedded into the nanocomposite matrix. The experimental findings in the in vitro model showed that the drug displayed superior alkaline medium solubility characteristics together with better dispersion and prolonged drug release behavior. The research indicates that nanocomposites made from xanthan gum stop the drug from crystallizing and create a valuable approach to boost gastrointestinal absorption thus demonstrating microwave synthesis as a promising method for enhancing poorly absorbed drugs.

Gan *et al.* (2024) reports on microwave-assisted antibacterial nanocomposite films with upgraded meat preservation properties to tackle current food packaging requirements against bacterial contamination. The authors developed antibacterial films through casting method by synthesizing CaO_2@PVP/egg albumen (EA)/sodium carboxymethylcellulose (CMC-Na) through the stabilization of waste eggshell-derived calcium peroxide (CaO_2) nanoparticles with polyvinylpyrrolidone (PVP). The synthesized nanoparticles provided consistent spherical shapes between 100–240 nm in dimensions. After 0.05 mg/mL of film exposure for 5 minutes of microwave irradiation the bactericidal efficacy surpassed 97% against S. aureus and E. coli. Bactericidal efficiency increased to 98.6% and 97.2% from exposure to 6 minutes of microwave heating. Tensile strength levels increased to 19.59 MPa while elongation at break reached 583.43% with the incorporation of CaO_2 nanoparticles into the films. Nanocomposite films demonstrated strong inhibitory effect against bacterial growth on meat when temperature reached 18°C. The combination of

reactive oxygen species (ROS) together with microwave-induced thermal activity proved responsible for the preservation effectiveness in characterization studies. The research indicates that nanocomposite films developed from polysaccharides and proteins present an environmentally friendly method for food preservation through antimicrobial packaging linked to microwave technology.

Strach *et al.* (2023) Research described how microwave activation transformed silver–silica nanocomposites structural features and physical properties and biological behaviors when utilized for environmental uses. Researchers intended to boost silica sphere uptake capabilities and biological properties by modifying them with different microwave power settings (150 W and 700 W) and exposure durations (60 and 150 seconds). The experimental duration proved to have stronger effects on carrier transformation than microwave power adjustments since it controlled surface activation as well as stable Ag_2O/Ag_2CO_3 heterojunction formation. The antimicrobial action against E. coli, B. cereus and S. epidermidis showed enhanced performance with silver nanoparticles that displayed increased roundness through oxidative stress mechanisms which broke microbial antioxidant protection systems leading to cell death. The most powerful antimicrobial actions emerged from nanocomposites which underwent extended microwave treatment. The research indicates microwave treatment boosts silica-based nanocomposite properties for environmental and medical uses by enhancing both pollutant and antibacterial features which creates new possibilities in pollution mitigation and antimicrobial defense systems.

Mondal *et al.* (2019) A rapid synthesis of gold-loaded hydroxyapatite (Au–HAp) collagen nanocomposites occurred through microwave-assisted techniques for drug delivery and tissue engineering applications. The researcher combined wet precipitation to make hydroxyapatite nanoparticles with successive steps of gold nanoparticle loading and collagen coating under microwave-controlled heating in three sequential heating cycles. Different characterization methods verified the successful development of Au–HAp–Col nanostructures that met ideal specifications for doxorubicin (DOX) storage along with pH-sensitive medication release operations. Test results confirmed the nanocomposite could store DOX with a maximum efficiency of ~58.22% while releasing ~53% of the drug during conditions with a pH of 4.5. This makes the nanocomposite useful for targeting cancer cells. MG-63 osteoblast-like cells in cytotoxicity studies confirmed that Au–HAp and Au–HAp–Col nanoparticles were non-toxic to cells while enabling cell-clinging and proliferation together with effective DOX anticancer actions. Research indicates Au-HAp-Col nanocomposites generated by microwave-assisted methods present great potential as multifunctional medical solutions which can facilitate drug delivery to specific areas of the body as well as help regenerate tissues.

Patwekar *et al.* (2016) This study used microwave-assisted bionanocomposites (BNCs) to increase the solubility rate and dissolution ability of ketoprofen (KE) which shows poor water solubility. Ghatti gum and acacia served as drug carriers for MIND because these natural substances exhibit both wetting and surface-active behaviors. Results from FTIR combined with DSC and XRD analysis and SEM and TEM investigations validated significant structural changes which created better disperson at both nano and micro levels for the BNCs. The in vitro solubility and dissolution testing showed that drug solubility grew in direct relation to the polymer concentration as the drug-to-polymer ratio reached 1:3. The optimized formulation produced superior anti-inflammatory results in rat paw edema tests than the standard ketoprofen product did according to in vivo assessments. Research findings show that MIND technology provides an economical green method to create BNCs which demonstrates remarkable potential to enhance poor water dissolvable drug properties.

Baig *et al.* (2025) A novel series of imidazole-based derivatives (4a–d) emerged from microwave-assisted synthesis methods which demonstrated significant aggregation-induced enhanced emission properties as well as pH detection sensitivity and sulfuric acid selectivity. Four synthesized compounds went through spectral analysis using NMR and ESI-HRMS, FT-IR, and these analyses proved their correct molecular structures. Photophysical studies detected the compounds absorbed light at 305 nm together with the multiple peaks at 327–365 nm which produced strong photoluminescence emissions ranging from 435 to 453 nm. The fluorescence intensity of 4d increased when it aggregated in THF/H_2O solutions and shown a 408 to 460 nm red shift because of restricted intramolecular rotation (RIR). Viscosity experiments supported the phenomenon of increased fluorescence in combinations of water and THF. The combination of Dynamic light scattering analysis confirmed nanometer-scale particle sizing in the range of 100 to 720 nm and density functional theory results revealed HOMO-LUMO bandgap (ΔE) of 2.01 to 2.23 eV where compound 4a exhibited max stability attributes and 4d presented maximum reactivity properties alongside pH-neutral stability. Furthermore, acid detection selectivity of 4d could be observed through its 47 nm wavelength redshift under highly acidic conditions. The detection capabilities of 4d were confirmed through analysis which determined detection limits down to 16.5 μM while various tests confirmed its practical use as an acid sensor that can be reused. The research confirms that imidazole derivatives synthesized through microwave heating have promising applications in both fluorescence detection systems and environmental assessment technologies.

Moghni *et al.* (2022) A TiO_2/WO_3 nanocomposite synthesized through sonochemical-microwave-assisted methods delivered successful photocatalytic degradation of ciprofloxacin (CIP) and oxytetracycline (OTC) antibiotics under UV and sunlight irradiation conditions. The method produced nanoparticulated anatase TiO_2 with a high surface area exceeding 200 m^2/g together with dispersed WO_3 structures. The photocatalytic activity peak was reached by samples with 5 wt% WO_3 nanocomposite resulting in total CIP and OTC breakdown under both UV and sunlight. Rate constant analysis established that TiO_2/WO_3 performed better than pure TiO_2 because it degraded pollutants at elevated concentrations more efficiently. The antibacterial assessment indicated that exposed antibiotic solutions turned less toxic because their bacterial growth tests showed shrinking inhibition areas. The nanocomposite exhibited endurable reusability since it sustained its high performance throughout four

successive operational cycles. The photocatalytic performance of the material improved due to WO_3 incorporation because it expanded light absorption spectrum and diminished electron-hole recombination thus becoming suitable for sustainable environmental remediation and water purification tasks.

Xu *et al.* (2021) Researchers successfully synthesized Fe_3O_4@MIL-100(Fe) nanocomposites through microwave-assisted methods as this synthesis resulted in materials that showed strong dual enzyme activities for detecting glutathione (GSH) through color changes. Researchers worked toward developing nanoenzyme artificial systems that would have stable performance alongside economic benefits and simple production methods to improve natural enzyme capabilities. Fe_3O_4@MIL-100(Fe) nanocomposites could be synthesized rapidly through microwave-assisted synthesis using Fe_3O_4 as the metal precursor for 20 minutes. The synthesized nanozyme contained built-in peroxidase (POD)-like and catalase (CAT)-like activity which qualified it for biosensing purposes. The POD-like activity based colorimetric biosensor employed to detect GSH generated a detection range from 1–45 μM with a detection limit at 0.26 μM (3.3 δ/S). Research revealed that this biological sample sensor achieved high selectivity alongside reusability and stability levels which qualify it for use as an important biocatalysis and biosensing solution. The synthesized nanozyme platform using microwave assistance presents critical value for biomedical along with environmental applications because of its alternative status compared to traditional enzyme-based sensing technologies.

Qiao *et al.* (2022) Research findings demonstrated that Garcinia nanoparticles (GNs) obtained through microwave-assisted processing showed antibacterial properties against Gram-negative bacteria while addressing traditional antibiotic limitations to bacterial outer membranes. GNs show maximum antibacterial effect against Escherichia coli bacteria when treated with microwave irradiation despite their traditional efficacy against Gram-positive bacteria. Exposing bacteria to microwave irradiation for 15 minutes enhanced bacterial drug penetration to 99.48% while increasing antibacterial efficiency to 99.48% compared to 6.73% efficiency without microwave exposure. Experimental tests on live mice demonstrated that microwave-treated GNs successfully treated bacterial pneumonia and thus displayed therapeutic abilities for this condition. The combination of GNs and microwave irradiation treatment demonstrated bacterial membrane destabilization according to both theoretical and experimental evaluation which improved bacterial death rates. The research presents a new approach to use herbal medicine-based nanoparticles alongside microwave assistance as an antibacterial enhancer for external bacterial infections since the method showed promise against both Gram-positive and Gram-negative bacteria.

Sutthapitaksakul *et al.* (2022) The research involved salt formation, stability assessment and tablet development of mefenamic acid for improved dissolution properties and oral bioavailability. The study investigated potassium, sodium and lysine salts to improve drug dissolution and stability of mefenamic acid since it demonstrates low aqueous solubility. A set of tests including SEM, hot-stage microscopy, FTIR, DSC and XRD established the successful salt production of potassium and sodium salts even though lysine salt synthesis failed to work under the same experimental protocol. The stability tests performed at 45°C ± 2°C and 75% ± 5% relative humidity over three months period showed sodium mefenamate maintained superior stability compared to potassium mefenamate. Sodium mefenamate proved suitable for tablet formulation so it was selected as the primary Candidate. Experimentally measured dissolution rates of sodium mefenamate tablets outpaced regular mefenamic acid samples thus indicating that salt transformations improve a drug's ability to dissolve while enhancing its availability. The research findings show that sodium mefenamate offers promising opportunities to enhance therapeutic properties of mefenamic acid.

Sharma *et al.* (2025) They reported a comprehensive study of hydrotropy as an approach to increase solubility of mefenamic acid, a poorly water soluble NSAID with limited bioavailability. It investigated the possibilities of sodium acetate, sodium salicylate and resorcinol to be used as hydrotropic agents at given concentrations (10%, 20%, 30%, and 40%). It was confirmed that mefenamic acid was thermally stable and that functional groups were unchanged when in the presence of hydrotropic agents, through infrared spectroscopy (IR) and differential scanning calorimetry (DSC) characterizations. The highest solubility enhancement ratio of 22.9 was achieved by 40% sodium acetate, resorcinol and sodium salicylate by equilibrium solubility studies. The hydrotropic agents did not interfere with spectrophotometric estimation of Mefenamic acid by using spectrophotometric methods, UV/Vis spectrophotometer confirmed the above. The solubility of pure mefenamic acid was significantly increased by the addition of hydrotropic agents. By comparing the screening results, it was found that sodium salicylate was the most effective hydrotropic agent in enhancing the therapeutic efficacy, improving the bioavailability, and achieving patient compliance of poor solubility drugs, like mefenamic acid. Thus, hydrotropy might be a viable, efficient solubilization technique for pharmaceutical application.

RATIONALE OF THE STUDY

Mefenamic acid is a frequently prescribed nonsteroidal anti-inflammatory drug (NSAID) that can be used to relieve moderate to severe pain; inflammation; and dysmenorrhea. Although a drug of great therapeutic potential, the poor aqueous solubility, low bioavailability and above all significant gastrointestinal side effects inhibit them in their clinical effectiveness, thus impeding their absorption and therapeutic efficacy. Because mefenamic acid is a hydrophobic and highly lipophilic molecule, it has slow dissolution rates, the dissolution of which does not always result in full or delayed oral drug absorption. The inconsistency leads to poor plasma drug levels that detract from the potential of the drug as therapy, necessitate higher doses or more frequent administration, all of which increase the risk of gastric irritation, ulceration and systemic toxicity. With its limited solubility, mefenamic acid not only has pharmacokinetic properties, but also leads to variable drug response among patients and therefore poor pain relief and anti–inflammatory effects. Mefenamic acid is a Biopharmaceutical Classification System (BCS) Class II drug,

that is, a compound having low solubility and high permeability, and thus is rate limiting for its absorption. As a result, the improvement of its solubility is considered a major aspect that facilitates the enhancement of the therapeutic efficiency and the clinical success of the drug. However, without implement of solubility enhancement strategies, patients may need to take higher doses to achieve desirable therapeutic effect, and the probability of adverse effects including gastric mucosal damage, nausea and gastrointestinal bleeding is higher.

Various conventional and advanced solubility enhancement techniques have been explored to overcome these challenges of formulating using technologies such as salt formation, micronization, hydrotropy, solid dispersion, nano bio composite and prodrug formation. Amongst these, salt formation is one of the most often used techniques which resolves the problem of poor aqueous solubility by modifying the drug's ionization properties. But there are instances when salt formation isn't necessarily a viable option as it may create instability and hygroscopicity, or dissolutions profiles that are variable between different pH conditions. Like hydrotropy, where hydrotropic agents improve drug solubility at low concentration but drug-excipient interactions, toxicity and unpredictability of release kinetics at high concentration limit widespread application. Due to the restrictions in conventional solubility enhancement methods, more and more advanced and advanced drug delivery routines are necessary which not only enhance solubility and bioavailability but also guarantee longterm stability and reduce the risk of adverse events. Recent developments in physicochemical properties of poorly water solubles drugs using microwave assisted synthesis, polymeric solid dispersion, nano bio composites and prodrug strategies have demonstrated great improvement. Microwave assisted approaches enable rapid, energy benign and scalable approaches to modify drug crystallinity, increase dissolution rates and reduce drug particle size, making them ideal methods for formulational optimization of mefenamic acid.

In addition, the pharmaceutical research on mefenamic acid's potential use as an NSAID has been increasing and attention to its potential other uses has also been growing. It has been shown to have cytotoxic effects on particular cancer cell lines, and was suggested to have a cancer related role when delivered through optimized drug delivery systems. Following improved solubility, bioavailability and pharmacokinetic properties, novel formulation strategies may provide a way to evaluate mefenamic acid in other parts of the body, outside of the realm of pain management. With the emerging drug delivery systems, the limitations of conventional solubility enhancement techniques, and the current research on the Mefenamic acid will be interpreted and evaluated systematically in terms of the solubility, stability, and therapeutic efficacy. Combining these technologies (microwave assisted synthesis, salt formation, hydrotropy, nano bio composite technology and polymeric solid dispersions), will allow an optimized, patient friendly formulation, with better absorption, longer biological activity, and less gastrointestinal side effects to be developed.

This research will address critical formulation challenges of mefenamic acid by moving modifications to the drug delivery platform that provides improved therapeutic efficacy (and therefore the patient's compliance), increased formulation stability and thus improved ability to work as a drug. Furthermore, the findings from this study will not only lead to the improvement of NSAID formulations but also develop an industrial pharmaceutical trend of optimizing other poorly soluble drugs for clinical practice in a scalable and sustainable manner. This could have tremendous implications in pharmaceutical sciences for deriving more effective, safer and patient-friendly medications that improve the drug performance without loss of adverse effects.

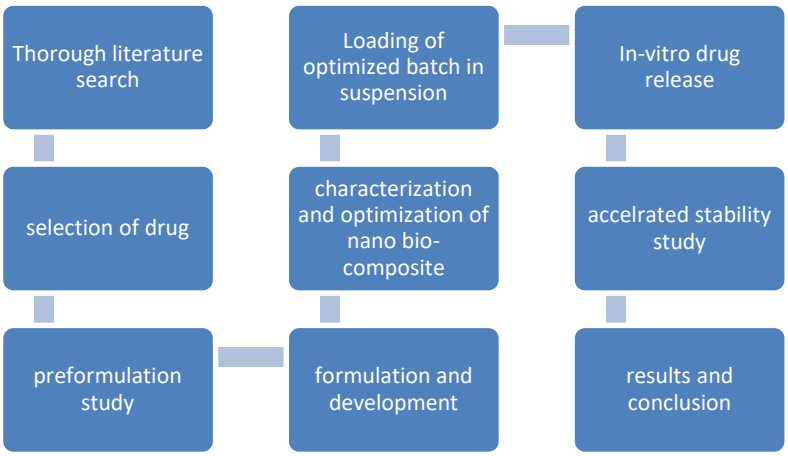
AIM

To develop and optimize a microwave-assisted nano bio-composite formulation of mefenamic acid to enhance its stability.

OBJECTIVE

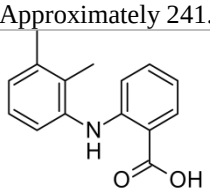
1. To perform preformulation study including organoleptic evaluation, solubility study, UV-absorption maxima, DSC and FTIR.
2. To synthesize a nano bio-composite of mefenamic acid using microwave-assisted techniques.
3. To perform Characterization of the Nano Bio-Composite.
4. To perform stability study of Nano Bio-Composite of mefenamic acid.
5. to perform In-Vitro Drug Release and Pharmacokinetic Evaluation.

PLAN OF WORK



DRUG AND EXCIPIENT PROFILE

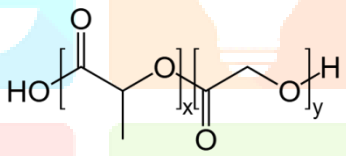
Drug profile
Mefenamic acid

Mefenamic acid[89,90]	
Synonym	N-(2,3-Dimethylphenyl)anthranilic acid; mefenamate; fenamate
Background	Mefenamic acid is a widely used nonsteroidal anti-inflammatory drug (NSAID) employed for the relief of moderate to severe pain, inflammation, and dysmenorrhea. Its therapeutic effectiveness, however, is limited by poor aqueous solubility and associated gastrointestinal side effects.
CAS Registry Number	61-68-7
IUPAC Name	2-[(2,3-Dimethylphenyl)amino]benzoic acid
Description	An anthranilic acid derivative belonging to the fenamate class. It exhibits analgesic, anti-inflammatory, and antipyretic properties, and is commonly used in the treatment of various inflammatory conditions.
Molecular Formula	$C_{15}H_{15}NO_2$
Molecular Weight	Approximately 241.29 g/mol
Chemical Structure	
Solubility	Poorly soluble in water (approximately 0.1–0.2 mg/mL); more soluble in organic solvents.
pH (pKa)	pKa ≈ 4.2 (indicating weak acidic properties)
Melting Point	Approximately 230–233°C
Handling Precautions	Avoid inhalation of dust; wear appropriate protective equipment (gloves, goggles) to prevent skin and eye contact; handle in a well-ventilated area; store in a tightly sealed container away from strong oxidizing agents and alkaline conditions.
Pharmacology	Functions as a nonselective inhibitor of cyclooxygenase (COX) enzymes, thereby reducing the synthesis of prostaglandins that mediate pain, inflammation, and fever.
Pharmacodynamics	Provides analgesic, anti-inflammatory, and antipyretic effects by decreasing prostaglandin production, which reduces the symptoms of inflammation and pain.
Mechanism of Action	Inhibits both COX-1 and COX-2 enzymes, leading to decreased production of prostaglandins, thus reducing inflammation, pain, and fever.
Metabolism	Primarily metabolized in the liver via cytochrome P450 enzymes; undergoes hydroxylation followed by conjugation (e.g., glucuronidation) to form more water-soluble metabolites.
Elimination	Excreted mainly through renal pathways, with a minor portion eliminated via biliary excretion.
Half-life	Approximately 2–4 hours
Functional Category	Nonsteroidal anti-inflammatory drug (NSAID); specifically, a fenamate.

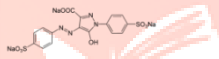
Stability and Storage Conditions	Stable when stored at controlled room temperature (15–25°C) in a dry, light-protected environment; best maintained in airtight containers to prevent moisture absorption and degradation.
Incompatibilities	Incompatible with strong oxidizing agents; may degrade in alkaline solutions; avoid mixing with excipients that adversely affect its dissolution profile.
Applications	Used for the relief of pain, fever, and inflammation; indicated for conditions such as dysmenorrhea, arthritis, and other inflammatory disorders.
Adverse Effects	Can cause gastrointestinal irritation, ulceration, renal toxicity, allergic reactions, and central nervous system effects such as dizziness and headache.
Safety	Generally safe when used at recommended doses; caution is advised in patients with pre-existing gastrointestinal, renal, or cardiovascular conditions; contraindicated in certain populations such as pregnant or lactating women without proper medical supervision.

Excipient profile

PLGA (Poly(lactic-co-glycolic acid))

PLGA (Poly(lactic-co-glycolic acid))[91,92]	
Synonym	Poly(D,L-lactide-co-glycolide), PLGA
Background	PLGA is a biodegradable polymer widely used in drug delivery systems due to its biocompatibility and controlled drug release properties. It is approved by the FDA and EMA for biomedical applications.
CAS Registry Number	26780-50-7
IUPAC Name	Poly[(R)-lactic acid-co-glycolic acid]
Description	PLGA is a synthetic copolymer composed of lactic acid (LA) and glycolic acid (GA) in varying ratios. It degrades into non-toxic metabolites (lactic and glycolic acids), making it ideal for pharmaceutical and biomedical applications.
Molecular Formula	$(C_3H_4O_2)x(C_2H_2O_2)y$
Molecular Weight	Varies (depends on composition and polymerization degree), typically 20,000–200,000 g/mol
Chemical Structure	
Solubility	Insoluble in water, but dissolves in organic solvents like acetone, chloroform, ethyl acetate, dichloromethane (DCM).
pH	Neutral when dispersed in buffer solutions, but degrades to produce an acidic microenvironment.
Melting Point	50–60°C, but depends on LA:GA ratio
Viscosity	High viscosity in solution, dependent on molecular weight and solvent used.
Handling Precautions	Should be handled in a dry environment and stored away from moisture, heat, and UV light to prevent degradation.
Pharmacology	Used as a controlled-release carrier in drug delivery systems, including microparticles, nanoparticles, implants, and scaffolds.
Pharmacodynamics	PLGA does not have intrinsic pharmacological activity but modulates drug release by hydrolytic degradation into its monomers.
Mechanism of Action	Acts as a biodegradable carrier by encapsulating drugs and releasing them via hydrolysis of ester bonds in aqueous environments.
Metabolism	Hydrolyzed into lactic acid and glycolic acid, which enter the Krebs cycle and are metabolized into CO_2 and water.
Elimination	Degradation products are excreted via urine and respiration (as CO_2 and water).
Half-life	Depends on polymer composition; typically weeks to months, influenced by LA:GA ratio and molecular weight.
Functional Category	Biodegradable polymer, drug carrier, excipient in pharmaceuticals, tissue engineering material.
Stability and Storage Conditions	Stable at room temperature in a dry environment. Should be stored at 2–8°C in an airtight container to prevent hydrolysis.
Incompatibilities	Incompatible with strong bases, strong acids, and excessive heat, which can accelerate degradation.
Applications	Used in nanoparticles, microparticles, implants, sutures, medical devices, and scaffolds for tissue regeneration. Common in long-acting injectable formulations such as Lupron Depot (leuprolide acetate).
Adverse Effects	Generally biocompatible, but degradation may cause local inflammation due to acidic by-products.
Safety	GRAS (Generally Recognized as Safe) for biomedical use. Used in FDA-approved drug formulations and medical devices.

PVA (Polyvinyl Alcohol)

PVA (Polyvinyl Alcohol)[93]	
Synonym	Poly(vinyl alcohol), PVOH
Background	PVA is a synthetic polymer widely used in pharmaceutical, biomedical, and industrial applications due to its water solubility, biocompatibility, and film-forming ability.
CAS Registry Number	9002-89-5
IUPAC Name	Poly(1-hydroxyethylene)
Description	PVA is a water-soluble synthetic polymer made by hydrolysis of polyvinyl acetate. It has excellent film-forming, emulsifying, and adhesive properties, making it suitable for drug delivery and pharmaceutical coatings.
Molecular Formula	$(C_2H_4O)n$
Molecular Weight	Varies, typically 30,000–200,000 g/mol, depending on the degree of polymerization and hydrolysis.
Chemical Structure	
Solubility	Soluble in water, forms a clear, viscous solution. Partially soluble in dimethyl sulfoxide (DMSO), glycerol, and ethanol, but insoluble in most organic solvents.
pH	Typically 5.0–7.5 in aqueous solution, depending on the degree of hydrolysis.
Boiling Point	Decomposes before boiling
Melting Point	180–230°C, dependent on molecular weight and hydrolysis degree.
Viscosity	Viscous in aqueous solutions, varies with concentration and molecular weight.
Handling Precautions	Should be stored in a dry, cool environment, away from moisture to prevent premature gelation.
Pharmacology	Used as an excipient, bioadhesive, film-former, and stabilizer in pharmaceutical formulations. Also used in ocular and transdermal drug delivery systems.
Pharmacodynamics	PVA itself is inert, but it enhances drug retention and controlled release when used in formulations.
Mechanism of Action	Functions as a mucoadhesive, film-forming agent, or controlled-release polymer, depending on the formulation.
Metabolism	Not metabolized by the body; eliminated unchanged.
Elimination	Excreted unchanged in feces and urine.
Half-life	Not applicable (non-degradable).
Functional Category	Excipient, film-forming agent, binder, stabilizer, bioadhesive, controlled-release polymer.
Stability and Storage Conditions	Stable at room temperature, should be stored in a dry, sealed container to prevent moisture absorption.
Incompatibilities	Incompatible with strong acids, strong bases, and oxidizing agents. Prolonged exposure to high temperatures may cause degradation.
Applications	Used in film coatings, hydrogels, drug carriers, bioadhesive patches, and ophthalmic formulations. Common in oral tablets, eye drops, wound dressings, and transdermal patches.
Adverse Effects	Generally safe, but may cause ocular irritation in high concentrations. High molecular weight PVA may be difficult to excrete in some cases.
Safety	Considered biocompatible and non-toxic, widely used in pharmaceutical and medical applications. Approved by FDA and EMA for use in drug formulations.

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MATERIALS AND METHODS

Materials

Table 6.1: List of Materials Used for Research Work

Sr. No.	Material	Supplier/Manufacturer	Purpose/Use in Research
1.	Mefenamic Acid	Sigma-Aldrich, USA	Active pharmaceutical ingredient (API)
2.	PLGA (Poly(lactic-co-glycolic acid))	Evonik Industries, Germany	Biodegradable polymer for nanoparticle formulation
3.	PVA (Polyvinyl Alcohol)	Sigma-Aldrich, USA	Stabilizer and surfactant in nanoparticle synthesis
4.	Acetone	Merck, India	Organic solvent for polymer dissolution
5.	Distilled Water	In-House Laboratory	Aqueous phase for emulsion preparation
6.	Phosphate Buffer Saline (PBS)	HiMedia, India	pH maintenance for drug release and stability studies
7.	Tween 80	Loba Chemie, India	Surfactant for enhancing solubility and stability
8.	Chloroform	Merck, India	Solvent used for polymer dispersion
9.	Ethanol	SD Fine-Chem Ltd, India	Co-solvent for formulation preparation
10.	Pepsin	HiMedia, India	Enzyme for simulated gastric fluid (SGF)
11.	Pancreatin	Sigma-Aldrich, USA	Enzyme for simulated intestinal fluid (SIF)
12.	Dialysis Membrane (0.45 µm)	Himedia, India	Membrane for in-vitro drug permeation studies
13.	Liquid Nitrogen	In-House Supplier	Used in lyophilization (freeze-drying) process
14.	Sodium Hydroxide (NaOH)	Loba Chemie, India	pH adjustment in buffer preparation
15.	Hydrochloric Acid (HCl)	Merck, India	Used for buffer and pH adjustment

Table 6.2: List of Equipment Used for Research Work

Sr. No.	Equipment	Manufacturer/Supplier	Purpose/Use in Research
1.	Microwave Synthesizer	CEM Discover SP, USA	Microwave-assisted synthesis of nano bio-composite
2.	High-Speed Homogenizer	Ultra-Turrax T25 IKA, Germany	Emulsification during nanoparticle formulation
3.	Magnetic Stirrer	Remi, India	Continuous stirring for solvent evaporation
4.	Centrifuge Machine	Thermo Fisher, USA	Separation of nanoparticles from the dispersion
5.	Lyophilizer (Freeze Dryer)	Labconco, USA	Freeze-drying of nanoparticles for stability
6.	UV-Vis Spectrophotometer	Shimadzu UV-1800, Japan	Drug quantification and encapsulation efficiency
7.	Zetasizer Nano ZS90	Malvern Panalytical, UK	Measurement of particle size and zeta potential
8.	Scanning Electron Microscope (SEM)	JEOL, India	Morphological characterization of nanoparticles
9.	Differential Scanning Calorimeter (DSC)	Perkin Elmer DSC 8000, USA	Thermal stability and crystallinity analysis
10.	X-Ray Diffractometer (XRD)	Bruker, Germany	Crystalline or amorphous nature determination
11.	Fourier Transform Infrared Spectroscopy (FTIR)	Bruker Alpha II, Germany	Functional group analysis and compatibility study

12.	Franz Diffusion Apparatus	Orchid Scientific, India	In-vitro drug permeation studies
13.	USP Type II Dissolution Apparatus	ElectroLab, India	Drug dissolution and release kinetics
14.	Stability Chamber	Thermolab, India	Stability study under accelerated conditions
15.	pH Meter	Eutech Instruments, India	pH measurement in buffer preparation

Methodology
Preformulation study

Organoleptic evaluation

The Organoleptic Evaluation was carried out on Mefenamic Acid as the physical characteristics of the drug like color, odor and texture are important attributes to ensure the identity, purity and acceptability of the drug. I examined under natural daylight the color of the drug powder to catch any deviation or inconsistency. A small amount of sample was manually assessed for odor to ascertain any distinct or characteristic odor which may indicate purity and possible degradation. A drug was sifted between the fingers by tactile sensation to evaluate its coarseness, its fineness or the coarseness of coarse particles if any were present. These organoleptic properties offer initial qualitative responses on the physical characteristics of Mefenamic Acid in order to assess the efficacy a second; prior to additional analytical determinations.

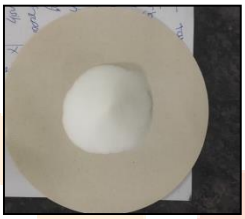

Fig 6.1: pure drug for organoleptic evaluation

Scanning absorption maxima (λmax)

Scanning Absorbance Maxima (λmax) of Mefenamic Acid has been deter- mined using Shimadzu UV 1900 UV – Visible Spectrophotometer to iden tify the wavelength at which the drug shows maximum absorbance, which is necessary to quantitatively analyze. Accurately weighed 10 mg of Mefenamic Acid was dissolved in 100 mL methanol to give 100 µg mL−1 stock solution of Mefenamic Acid. Appropriate dilutions were made from this stock and added into methanol or phosphate buffer (pH 7.4) to make the desired concentration. The absorbance spectrum was recorded for the prepared solution on Shimadzu UV-1900 UV Visible Scanning machine in the range of 200-400 nm. We subsequently identified and recorded the λmax, the peak absorbance, which will later be used as the optimal wavelength for subsequent spectrophotometric analysis in quantification and formulation studies of a drug. This determination is critical for precisely and reproducibly measured analytical measurements in subsequent preformulation and formulation development processes.

Determination of calibration curve of mefenamic acid in the methanol

UV-Visible Spectrophotometry using Shimadzu UV-1900 was used to set up the calibration curve for Mefenamic Acid to determine its linearity, accuracy and suitability for quantative analysis. Mefenamic Acid was dissolved in 100 mL of methanol to give a stock solution of 100 µg/mL. This stock solution was then serially diluted to make concentrations of 2, 4, 6, 8 and 10 µg/mL, all using methanol as the diluent. At standard operating conditions, the previously determined λmax was used for scanning each concentration. These solutions were then recorded for their absorbance values and plotted to a calibration curve with concentration (µg/mL) vs. absorbance to show a relationship of linearity with Beer-Lambert's law. To prove linearity, we calculated the regression equation ($y = mx + c$) as well as the correlation coefficient (R^2). Calibration curve is deemed as a fundamental basis for the determination of Mefenamic Acid in subsequent analytical and formulation studies.

Solubility study by solvent saturation method

Solvent saturation method that is frequently used to evaluate equilibrium solubility of a given compound was used to determine the solubility of Mefenamic Acid in different solvents. Here, an excess amount of Mefenamic Acid has been added to 10 mL of different solvents, namely distilled water, methanol, ethanol, acetone, dimethyl sulfoxide (DMSO), phosphate buffer (pH 7.4) and 0.1 N HCl (pH 1.2) in separate glass vials. An aliquot of the drug solubility was added to a 50 mL cup with vials, which then underwent continuous stirring at 25 °C ± 2 °C supplemented with a mechanical shaker to facilitate maximum dissolution of the drug in the provided solvents. After equilibrium, the samples were filtered using a Whatman filter paper (0.45 µm) prior to removal of particles not dissolved. They diluted the filtrates appropriately and analyzed the dissolved drug concentration spectrophotometrically using a Shimadzu UV-1900 UV-Visible Spectrophotometer at the pre-determined λ_{max}. Solubility was expressed as mg/mL, and the results were utilized in calculating the ability of each solvent to solubilize what. Selection of the most suitable solvent system for further formulation development and dissolution enhancement strategies would rely on this study.

Figure 6.2: Solubility determination in different solvents

Melting point determination

To be precise and reproducible, Mefenamic Acid melting point was determined by digital capillary method using Labindia Visual Melting Point Apparatus (Model: Labindia VMP-D). The drug was then carefully filled into a clean, dry, unsealed capillary tube, packed uniformly in its sealed portion to avoid inconsistency, and reduced in size by finely powdering a sample of it. The Labindia VMP-D apparatus was used to place the capillary tube initially and then the temperature of capillary tube was increased with the rate of 1°C / min for the accurate detection of phase transition. Melting point range of Mefenamic Acid was the temperature at which the drug first showed melt and the temperature at which the completely liquefied. The experiment was repeated three times for reproducibility. To assess purity and crystalline nature of the drug, the obtained melting point range was analyzed to see if the range was any significant deviation from the expected range and therefore could point to the presence of impurities, polymorphs, or formulation related variations.

Figure 6.3: Melting point determination

Differential scanning colorimetry (DSC)

The Differential Scanning Calorimetry (DSC) analysis was done to determine the thermal behavior, stability as well as possible drug/ excipient interaction for Mefenamic Acid. This study was carried out at TA Instruments DSC-25 machine under a nitrogen atmosphere to avoid oxidative degradation. To begin with the DSC scan of the pure drug was done, an accurately weighed sample (2-5 mg) was sealed in an aluminum pan, heated at 10ºC /min upto a suitable temperature constituting the sample and an empty aluminum pan was used as the reference. An analysis of the obtained thermogram on the basis of onset, peak and endset temperatures of the melting transition indicated that the drug was crystalline. The same experimental conditions were then repeated for physical mixture of Mefenamic Acid with excipients by DSC analysis, subseqently. Changes in peaks, i.e., either shift, broadening, or disappearance of peaks in the thermogram of the physical mixture than in the pure drug were interpreted as the possible presence of drug-excipient interactions, polymorphic, or crystallinity changes. Based on the formulation's need, the development of the formulation requires an in depth assessment of thermal compatibility with the formulation components and the selected excipients must be found suitable and stable to select them for further formulation development.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was applied to determine the chemical integrity, functional groups and possible interactions of Mefenamic Acid with excipients. The FTIR spectrophotometer used in the study was a Bruker Alpha II with an attenuated total reflectance (ATR) capability which enabled accurate acquisition of the spectra. FTIR spectrum of pure Mefenamic Acid was recorded at the beginning by placing sample amount lesser to cover whole ATR crystal and scanning it in a range of 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1}. Chemical structure of the drug was determined in conformity with the characteristic peaks to the drug carboxyl (-COOH), amine (-NH), and aromatic (-C=C-) functional groups when compared with a standard reference spectrum. Finally, the analysis was repeated for the physical mixture of Mefenamic Acid with excipients under identical conditions to see if there could be any interactions. If Mefenamic Acid undergoes any significant shift, disappearing, or broadening of peaks in the physical mixture spectrum when compared to the pure drug spectrum, such differences were analyzed as probable signs of hydrogen bonding, complex formation, or incompatibility between Mefenamic Acid and excipients. A comparative FTIR study was key to confirming that Mefenamic Acid was ultimately chemically stable and would be compatible with the formulation, following which it would prove suitable for further pharmaceutical development.

Selection of dose of mefenamic acid for formulation

Mefenamic Acid was formulated at the 500 mg dose as this is a clinically established dose which provides effective pain relief and anti-inflammatory action. This dose is adequate plasma concentration in therapy and has therapeutic constancy. Since Mefenamic Acid has low solubility and bioavailability, thereby dissolving and absorption to release more drugs, which leads to better drug release and prolonged action, they have developed the nano bio composite formulation. The 500 mg dose is a standard dosing with patient compliance and frequency of administration and also a good level of effective drug to choose. Preformulation studies revealed this selection to be suitable for use in the final formulation.

Experimental design

Systematic evaluation of the effect of formulation variables on Mefenamic Acid loaded PLGA nanoparticles was carried out in a 3^2 full factorial design. The independent factors chosen were PLGA concentration (A) and PVA concentration (B), three levels for each, 0.5%, 1.5% and 3% w/v for PLGA, and 0.5%, 1.5% and 2.5% w/v, for PVA respectively. The dependent response measured were: Y_1: Particle Size (nm) and Y_2: Encapsulation Efficiency (%) (EC of Tbeta NI). The experimental design was developed and analyzed by the software: Design-Expert Software (Stat-Ease Version 13.0) to enable the development of an optimized formulation by statistical modeling. Using the factorial design, nine batches (formulations) were made and the firm experimental data obtained for particle size, encapsulation efficiency and dyasorption and water absorption values are listed in Tables 6.3 and 6.4.

Table 6.3: 3^2 Factorial Design showing independent factors and Levels.

Independent Variables				
Label	**Factors**	**Level (%W/V)**		
		Low (-)	**Medium**	**High (+)**
A	PLGA	1	2	3
B	PVA	0.5	1.5	2.5
Dependent Variables				
Y_1	Particle size (nm)			
Y_2	Encapsulation efficiency (%)			

Table 6.4: Factors, levels and responses taken in 3^2 complete factorial designs for Microwave assisted nano Bio-composite.

F. Code	(A)	(B)
F1	-1	-1
F2	0	-1
F3	+1	-1
F4	-1	0
F5	0	0
F6	+1	0
F7	-1	+1
F8	0	+1
F9	+1	+1

"-" indicates lower concentration, and "+" indicates higher concentration.

Table 6.5: Preparation of Microwave assisted nano Bio-composite batches using 3^2 factorial designs

Ingredients	RF1	RF2	RF3	RF4	RF5	RF6	RF7	RF8	RF9
Mefenamic acid (mg)	500	500	500	500	500	500	500	500	500
PLGA (%W/V)	1	2	3	1	2	3	1	2	3
PVA (%W/V)	0.5	0.5	0.5	1.5	1.5	1.5	2.5	2.5	2.5
Acetone (ml)	10	10	10	10	10	10	10	10	10

Formulation

Preparation of Microwave-Assisted Nano Bio-Composite Nanoparticles

Mefenamic Acid and PLGA are dissolved in acetone to form the organic phase, while PVA of the suitable concentration as described in the formula is prepared in distilled water as the aqueous phase. High speed homogenization (Ultra-Turrax T25 IKA, Germany) under high speed homogenization using (12,000 to 15,000 rpm, 5 minutes) in the presence of the organic phase is used

over the aqueous phase to obtain a stable emulsion. The above emulsion, which is then subjected to microwave irradiation by means of (CEM Discover SP Microwave Synthesizer (CEM Corporation, USA) from 100 to 200 W for 2–3 minutes to facilitate solvent evaporation and nanoparticle stabilization. Finally, Nano bio composite Suspension is stirred for 1–2 hours at room temperature using Magnetic Stirrer (Remi, India) for obtaining complete solvent removal and the same is centrifuged (Thermo Fischer, USA) at 12,000 rpm for 15–20 minutes to separate the nanoparticles. Coupled with this is the washing of the collected nanoparticles three times with distilled water to remove unencapsulated drug and stabilizer residues. The final step involves freeze drying of the nanoparticles with Lyophilizer (Labconco, USA) to get a dry and stable powder. Further characterization is performed for these nanoparticles stored in the airtight container at 4°C.

Loading of Optimized nano bio composite batch in the suspension

An optimized oral suspension of the microwave assisted nano bio-composite was developed to homogeneously disperse, stabilize and improve the bioavailability of the nano bio-composite. Hydrated suspending gel was prepared by dispersing methylcellulose (1% w/v) in half of the total volume of the purified distilled water heated to 40°C under constant stirring at 500 rpm, in addition to the addition of glycerin (2% w/v) as a viscosity enhancer and sorbitol (3% w/v) as a sweetener and osmotic agent. The nano bio composite powder was then slowly stirred into the aqueous phase in the presence of continuous stirring at 700–1000 rpm and Tween 80 (0.5% w/v) on continuous stirring was added as the wetting agent to ensure uniform dispersion. Sodium citrate buffer (0.2% w/v) was used to adjust the pH and keep the drug stable at pH 6.8–7.2. It was preservative (sodium benzoate, 0.1% w/v) and stability of suspension on improvement (xanthan gum, 0.2% w/v) was added. The final volume was made by distillated water and the suspension was homogenized in a high speed homogenizer at 5000 rpm for 10 minutes to make the suspension uniform. The suspension was prepared and transferred to amber colored glass bottles that were tightly sealed and stored at a 4°–8° C temperature to retard further degradation.

Characterization of Microwave-Assisted Nano Bio-Composite Nanoparticles and formulation

Zeta potential

The zeta potential of the microwave assisted nano bio composite nanoparticles were obtained and evaluated from the surface charge as well as colloidal stability, using a Malvern Zetasizer Nano ZS90 (Malvern Panalytical, UK). Dispersed nanoparticles in distilled water at 25°C with obtained zeta potential values > ±30 mV, thus demonstrate a strong electrostatic repulsion and prevent nanoparticles aggregation and ensure a stable dispersion. Long term stability of nanoparticles in suspension with the help of higher zeta potential value indicates lower drug release profile for a higher bioavailability. Again, the positive or negative charge of the nanoparticles is another important parameter on cellular uptake and membrane interaction of the nanoparticles, which is important in the formulation of nanoparticles.

Determination of encapsulation efficiency (EE%) by UV

The EE% of the formulated microwave assisted nano bio composite nanoparticles was determined by measuring with the help of a UV vis spectrophotometer (Shimadzu UV 1800, Japan)). Remi C-24 Plus Centrifuge (Remi, India) was used by them to centrifuge the nanoparticles at 12,000 rpm for 20 minutes to separate the supernatant. Suprnatant was collected and the amount of unencapsulated Mefenamic Acid was quantified at 285 nm and the encapsulation efficiency was estimated with the following formula.

$$\mathrm{EE}(\%) = \frac{\text{Total drug added} - \text{free drug in supernantant}}{\text{Total drug added}} \times 100$$

Scanning Electron Microscopy (SEM)

The formulated microwave assisted nano bio composite nanoparticles were analysed for its morphological properties and surface structure using Scanning Electron Microscopy (SEM) (JEOL india). The nanoparticles were lyophilized in a Lyophilizer (Labconco, USA) and mounted on aluminum stubs using double carbon tape. In order to improve conductivity, a thin layer of gold was applied to the samples via a Quorum SC7620 Sputter Coater in a vacuum. With the coated samples, the samples were placed inside the SEM chamber; and imaging was performed at an accelerating voltage ranging from 5 to 15 kV under high vacuum. These SEM image were analysed to determine particle shape, surface morphology as well as size distribution of the nanoparticles.

Differential Scanning Calorimetry (DSC)

Thermal behavior and crystallinity of Mefenamic Acid in the microwave assisted nano bio composite was evaluated by using Differential Scanning Calorimeter (DSC). This was done with a controlled nitrogen atmosphere in the presence of oxygen (less than 0.1 mM) to ensure no oxidative degradation on the DSC instrument (Perkin Elmer DSC 8000 or Mettler Toledo DSC 1). About 2–5 mg of every sample, pure Mefenamic Acid, polymer (PLGA, chitosan), physical mixture and the formulated nano bio

complex, was carefully weighed and sealed in standard aluminum pans. A reference was made in the form of an empty sealed aluminium pan. Samples were heated from 30 to 300 C at 10 C /min heating rate under a nitrogen purge of 50 mL /min. Thermograms thus recorded were analyzed in regard to the reduction in crystallinity and any interaction of the drug with the polymer within the nano bio composite by calculating melting point (Tm), glass transition temperature (Tg) and any shifts in endothermic pea

***In-Vitro* Gastrointestinal Stability Study**

The in-vitro gastrointestinal stability study on the microwave assisted nano bio composite was done to ascertain its stability with regard to drug integrity under simulated gastrointestinal environment. Simulated gastric fluid (SGF, pH 1.2) containing pepsin (0.32% w/v) was used for 2 hours to formate in simulated gastric environment and simulated intestinal fluid (SIF, pH 6.8) containing pancreatin (1% w/v) for up to 12 hours was used as next to run against the gang. For the study, shaking water bath was used with the speed maintained at 100 rpm at 37 ± 0.5°C simulating physiological conditions. 1 mL aliquots were withdrawn at predetermined time intervals (0, 1, 2, 4, 6, 8, 10, 12 hours), filtered and analyzed by UV-Vis Spectrophotometry (285 nm) to determine drug concentration and the same volume of fresh buffer was added to maintain sink conditions. Drug stability in the nano bio composite was determined by comparing the degradation profile of the drug in SGF and SIF to protect the drug and modulate its released in gastrointestinal environment.

Physical Appearance and Organoleptic Properties

The appearance and organoleptic properties of the nano bio composite oral suspension under normal light, color, clarity, consistency and homogeneity were also evaluated. An instability was noted as a sign of any phase separation, precipitation, or turbidity. A trained panel was used to study odor and taste to make sure that the product was palatable; glycerin and sorbitol were used as sweeteners. Smoothness and absence of grubbiness were examined for texture and mouthfeel. For the suspension, color, texture and microbial growth were monitored to ensure stability and patient acceptability over time and storage at 25 ± 2°C.

pH Measurement

The nano bio composite oral suspension was tested for the pH measurement to ensure stability, drug solubility and to meet the basic physiochemical measurement. Prior to use, the instrument was calibrated against standard buffer solutions (pH 4.0 and 7.0) using a digital pH meter (Eutech Instruments, India). A suspension of the electrode in the sample at 25 ± 2°C was made, and 10 mL aliquot of the suspension was taken, 5 mL was duly dispensed and the electrode was immersed in the sample. The pH reading was monitored and it was ensured that it was within the optimali range of 6.8–7.2 to avoid drug degradation and ensure the stability of the formulation. Various pH was change measured as function of time, the storage interval was varied to monitor changes in pH over time.

Sedimentation Volume and Redispersibility

The sedimentation volume and redispersibility of the nano bio-composite oral suspension were evaluated to ensure uniformity and ease of administration. The suspension was stored in a graduated cylinder at room temperature (25 ± 2°C), and the height of the settled particles (Hu) and total suspension height (Ho) were measured at regular intervals. The sedimentation volume (F) was calculated using the formula:

$$F = \frac{Hu}{Ho}$$

where F = 1 indicates excellent stability with no sedimentation. For redispersibility, the suspension was subjected to manual shaking, and the number of inversions required to completely redisperse the settled particles was recorded. An ideal formulation required minimal shaking (within 2–3 inversions) to achieve a homogeneous suspension without caking or clumping. These tests ensured the suspension's physical stability, ease of use, and patient compliance.

viscosity measurement

Viscosity measurement of the nano bio composite oral suspension was done to ensure that the suspension has good flow properties, stability and ease of administration. Viscosity was measured using a Brookfield Viscometer (model DV-II+, USA) at 25 ± 2C. Varying spindle speeds (10, 20, 30, and 50 rpm) with Spindle no. 2 were performed on a 10 mL sample of the suspension placed in the viscometer, to check the shear thinning behavior of the suspension. It was expected to possess pseudoplastic flow, i.e. viscosity to decrease in the presence of increasing shear rate, to promote easy pourability and uniform dosing. Viscosity of the suspension was measured at different storage intervals to ensure the stability and consistency of the suspension.

***In-Vitro* Drug Permeation Study**

A Franz Diffusion Apparatus was used to study drug permeation of the Mefenamic Acid across a biological membrane in-vitro, and to assess the controlled release characteristics of the microwave assisted nano bio composite formulation. To ensure good hydration and permeability, the Franz diffusion cell had a donor compartment and a receptor compartment with a dialysis membrane (pore size: 0.45 µm, pre-soaked in phosphate buffer pH 7.4 for 24 hours). To keep things in sink conditions, phosphate buffer was used (pH 7.4) containing 1% Tween 80 and the receptor chamber (capacity for 10 mL) was filled. In the donor chamber the nano bio-composite formulation equivalent to predefined amount of Mefenamic Acid was placed and parafilm was used to cover the donor chamber to allow for no solvent evaporation. Continuous stirring at 100 rpm by magnetic stirrer was used to ensure uniform mixing in the receptor chamber maintained at 37 ± 0. 5°C. At predetermined time intervals (0, 1, 2, 4, 6, 8, 10 and 12 hours) 1 ml aliquots were taken out from the receptor compartment, immediately replaced by 1 ml volume of fresh buffer under sink conditions. UV spectrophotometry (Shimadzu UV 1800) was pre determined wavelength and then the collected samples analyzed, and the cumulative percent of drug peripated in calculated to determine the rate and extent of drug diffusion through the membrane.

RESULT AND DISCUSSION

Preformulation study

Organoleptic evaluation

Mefenamic Acid was subjected to the organoleptic evaluation which was carried out to give an account of the sensory attributes in line with the pharmacopeial standards as preliminary characteristics related to identity, purity and quality of handling. According to both IP, BP, and USP standard description, the observed color of the drug was white to off white crystalline powder, which is conforming to visual aspect of the drug. The odor, if noted at all, was characterized, whereas the pungent or foul smell that is reported as the standard is not present, the odor being either odorless or with a mild, characteristic odor, both supporting the absence of volatile degradation products or contaminants. The smooth and grittiness and coarse particle free texture was in agreement with the requirement of the pharmacopeial requirements for the fine, uniform crystalline powder, which is the good processability during formulation. These organoleptic features not only confirm acceptable physical quality of raw material, but also indicate that storage and handling conditions were too, acceptable, needed to maintain the integrity of Mefenamic Acid during the shelf life and during its pharmaceutical use.

Table 7.1: Organoleptic Properties of Mefenamic Acid

Property	Observed Results	Reported Standard (IP/BP/USP)
Color	White to off-white crystalline powder	White or almost white crystalline powder (IP/BP/USP)
Odor	Characteristic odor; no pungent/foul smell	Odorless or characteristic odor (IP/BP/USP)
Texture	Smooth, free from grittiness or coarse particles	Fine, uniform crystalline powder (IP/BP/USP)

Scanning absorption maxima

By using the UV–Visible spectrophotometry method, the scanning absorption maxima of Mefenamic Acid was done in a way to determine the characteristic wavelength at which, the drug shows maximum absorbance. This is crucial to establish the identity of the drug and for further quantitation. The recorded λmax was found to be 285 nm which shows the presence of conjugated aromatic chromophores to the Mefenamic Acid molecular structure. Literature reported absorption maximum is confirmed in this case value and therefore authenticates the drug's spectral authenticity and purity. This wavelength is sharp because of the electronic transition which correspond to those typical of aromatic rings transition of π→π*. The absorption behavior of this ensures use of 285 nm for reliable subsequent spectrophotometric analyses such as drug content estimation, studies of solubility and profile of drug release. This further suggests that the sample was free of impurities or degradation products which would have caused interference peaks near this region.

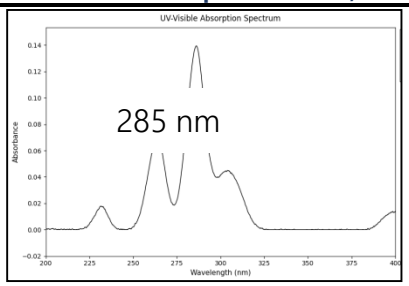

Figure 7.1: Determination of scanning absorption maxima of drug

Calibration curve determination in methanol

UV-Visible spectrophotometry was used as the method of calibrating Mefenamic Acid in methanol, with absorbance measurements made at λ max 285 nm. Absorbance was found to vary directly in proportion with concentration in standard solutions ranging from 2 to 10 µg/mL, from 0.213 at 2 µg/mL to 1.02 at 10 µg/mL. The linear regression analysis was performed and had astronomical standard errors (as did the nonlinear regression analysis) with slope = 0.0998, intercept = 0.0247, and showed great linearity and Beer-Lambert's law adherence on the tested concentration range. The high correlation coefficient rightly indicates the strong precision and reliability of this method for the quantitative estimation of drug. The generation of this calibration curve is a basic analytical tool in the determination of Mefenamic Acid content in different experimental set ups as well as being an initial test for validation of other studies like solubility enhancement, drug loading, and release kinetics of nano bio-composite formulations.

Table 7.2: Calibration Curve Data for Mefenamic Acid

Concentration (µg/mL)	Absorbance
2	0.213
4	0.438
6	0.626
8	0.819
10	1.02
Slope	0.0998
Intercept	0.0247
R^2	0.9991
Absorption maxima	285 nm

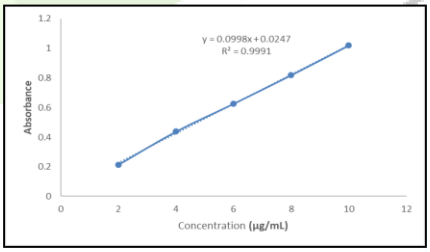

Figure 7.2: Callibration curve determination in methanol

Melting point determination

An evaluation of purity of Mefenamic Acid and confirmation of its identity versus pharmacopeial standards was conducted on the melting point of Mefenamic Acid. The observed melting point range 238°C to 232°C is very close to the reported standard melting point specification of 230°C to 233°C as per IP/BP/USP specifications. Although narrowly within the melting range, the narrow, nonbroadened melting peak is indicative of high purity, absence of major impurities or degradation products. The melting point determination verifies that the thermal stability of the drug is appropriate and that the sample used to evaluate the compound in the study is identical to the Mefenamic Acid diluted from pharmacopeial grade the sample. This parameter is also used to ensure integrity of the compound for use in formulation development especially under thermal processes (like microwave assisted synthesis).

Table 7.3: Melting Point Determination of Mefenamic Acid

Sample	Observed Melting Point Range	Reported Standard (IP/BP/USP)
Mefenamic Acid	238–232°C	230–233°C (IP/BP/USP)

Solubility determination

The solvation profile of Mefenamic Acid was studied in various solvents to understand the solvation behavior and to make it compatible for the formulation development, especially for the improvement in the bioavailability. Values of 0.12 ± 0.02 and 0.08 ± 0.01 mg/mL in distilled water and in 0.1N HCl classified the drug as practically insoluble, as it is in pharmacopoeial decriptions. The solubility of 0.85 ± 0.1 mg/ml in phosphate buffer (pH 7.4) was in the sparinglysoluble range and thus slightly more soluble under neutral to mildly basic conditions. On the other hand, the drug was found to be highly soluble in organic solvents (18.5 ± 1.3 mg/mL in methanol and 14.2 ± 0.9 mg/mL in ethanol) and therefore was found to be freely soluble. Based on better interaction with polar aprotic solvents, its solubility in acetone (24.8 ± 1.5 mg/mL) and DMSO (32.6 ± 2.1 mg/mL) was found to be much higher than in either buffer (2.7 ± 2.1 mg/mL in PBS, 8.9 ± 1.1 mg/mL in phosphate blend), classifying it as very freely soluble. Mefenamic Acid's hydrophobicity is confirmed by these findings, and solubility enhancement techniques are recommended in order to enhance drug solubility in aqueous formulations, which are important for enhancing drug oral bioavailability and therapeutic effectiveness.

Table 7.4: Solubility of Mefenamic Acid in Various Solvents

Solvent	Observed Solubility (mg/mL)	Reported Standard (IP/BP/USP)
Distilled Water	0.12 ± 0.02	Practically insoluble
Methanol	18.5 ± 1.3	Freely soluble
Ethanol	14.2 ± 0.9	Soluble
Acetone	24.8 ± 1.5	Very soluble
Dimethyl Sulfoxide (DMSO)	32.6 ± 2.1	Freely soluble
Phosphate Buffer (pH 7.4)	0.85 ± 0.1	Sparingly soluble
0.1N HCl (pH 1.2)	0.08 ± 0.01	Practically insoluble

Differential Scanning colorimetry

The thermo behavior and compatibility of the nano bio composite matrix with Mefenamic Acid was investigated by Differential Scanning Calorimetry (DSC) analysis. The thermal behavior of the pure drug is also indicated by a sharp endothermic peak at 237.74 °C in the DSC thermogram, matching well to the known melting point of the unprocessed compound, indicating crystalline nature and thermal stability of the pure compound. However, the DSC thermogram of the physical mixture exhibited two separate thermal transitions; one at 184.42 °C and the other at 238.91 °C. Appearance of a new lower temperature peak suggests presence of a physical interaction or partial miscibility of drug and excipients while presence of peak near original drug melting point indicates presence of unaltered crystalline drug in the system. It should also be noted that broadening and shifting of peaks of the physical mixture can also indicate reduced crystallinity or onset amorphization, a common goal in attempts to increase solubility. These observations imply that under certain conditions, the formulation process can affect the thermal profile of Mefenamic Acid without compromising its chemical identity, thereby improving its dissolution characteristics.

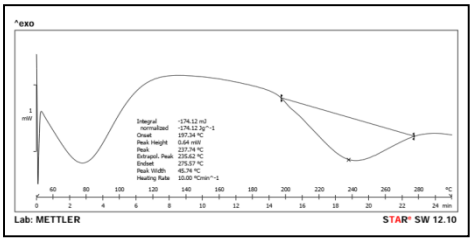

Figure 7.3: DSC graph of pure drug (237.74 °C)

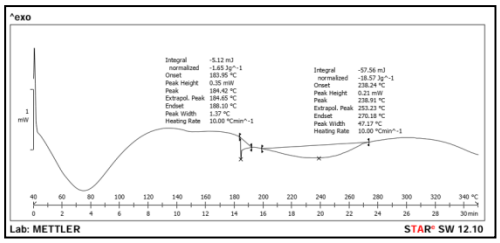

Figure 7.4: DSC graph of physical mixture (184.42 °C and 238.91 °C)

FTIR Results

FTIR spectral analysis of the physical mixture was conducted to check for any interaction of Mefenamic Acid with the excipients used in the combined physical mixture. Reported above were the results of the spectrum of the pure drug, showing all characteristic functional group peaks consistent with known drug structure of pure drug, e.g., O–H stretching vibrations around 3377.47, 3362.87 cm^{-1}, N–H aromatic amine stretching near 3286.35 cm^{-1} and a strong carbonyl (C=O) peak at 1692.23 cm^{-1}, confirming the presence of carboxylic acid functional group. Further, peaks pertaining to the stretching from C=C aromatic (1576.74, 1514.48 cm^{-1}) and C–O (1241.61, 1281.25 cm^{-1}) were observed, suggesting the presence of the intact phenyl ring and ester group of carboxylic acid. Characteristic peaks in these characteristic peaks were retained with slight shifts in the wavenumber of the carbonyl stretch at 1691.78 cm^{-1}, hydroxyl, and amine bands at near 3261.96, 3393.63 and 3383.83 cm^{-1} respectively in the physical mixture. It is highly likely that these slight shifts are due to drug–matrix matrix components produced non-covalent physical interaction such as hydrogen bonding or dipole dipole interactions between drug and matrix components. This was important since no new peaks appeared and no major band loss occurred during the mixing, suggesting no chemical incompatibility nor degradation. This confirms the chemical stability of Mefenamic Acid within the composite formulation, which is effective for further formulation development of Mefenamic Acid using microwaves or through enhanced penetration using nanoparticle based strategies.

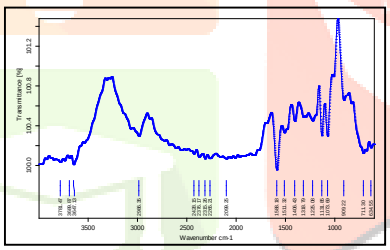

Figure 7.5: FTIR of Pure mefenamic acid

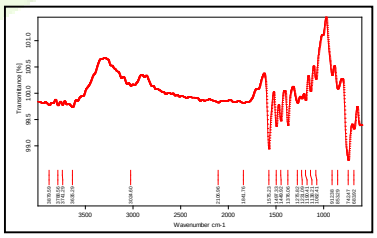

Figure 7.6: FTIR of Physical mixture

Table 7.5: FTIR Interpretation of Pure Drug (Mefenamic Acid) and Physical Mixture

Functional Group	Standard Wavelength	Observed in Pure Drug	Observed in Physical Mixture

	(cm⁻¹)	(cm⁻¹)	(cm⁻¹)
O–H (phenolic stretch)	3200–3550	3377.47, 3362.87	3378.29, 3362.55
N–H (aromatic amine)	3300–3500	3286.35	3285.29
C–H (aromatic stretch)	3000–3100	3020.85	3024.30
C=O (carboxylic acid)	1680–1725	1692.23	1691.78
C=C (aromatic ring)	1450–1600	1576.74, 1514.48	1572.73, 1512.68
C–N (aryl amine stretch)	1250–1350	1319.35	1318.33
C–O (carboxylic group)	1150–1300	1241.61, 1281.25	1240.62, 1280.16
C–H (out-of-plane bend)	750–900	845.33, 756.31	843.26, 755.22

Physical Appearance and Organoleptic Properties

The developed nano bio-composite oral suspension of Mefenamic Acid gave critical physical appearance and organoleptic evaluation insights on the aesthetic acceptability, uniformity and patient compliance. The formulation was off white to pale yellow, indicating the absence of discoloration or degradation of the components and a uniform dispersion of them. Clearly visible but slightly opaque, this is expected for nanoscale suspensions and was devoid of foreign particles, indicating good particulate and cleanliness control and importantly, good formulation control. The consistency is smooth and viscous and free flowing on gentle shaking which is a desirable rheological profile for oral suspensions. Nice homogeneity was achieved by the formulation with complete absence of phase separation or sedimentation after 24 hours of rest, indicating that the dispersion was physically stable. The suspension had a neutral odor organoleptically, without any unpleasant or pungent smell that makes it suitable for oral administration. Smooth and nongritty texture and mouthfeels as well as no coarse particles detection are critically important for the patient compliance and ease of swallowing. All together, these results confirm that the nano bio composite suspension has good sensory and physical quality parameters suitable to a patient friendly and pharmaceutically acceptable dosage form.

Table 7.6: Physical Appearance and Organoleptic Evaluation of Nano Bio-Composite Oral Suspension

Property	Observed Results
Color	Uniform off-white to pale yellow
Clarity	Slightly opaque due to nano-bio-composite dispersion; no visible foreign particles
Consistency	Smooth, viscous liquid; flows freely upon shaking
Homogeneity	No phase separation or settling observed after 24 hours at rest
Odor	Neutral; no pungent or unpleasant smell
Texture/Mouthfeel	Smooth, non-gritty; no coarse particles detected

Zeta potential

Colloidal stability and surface charge was evaluated on the zeta potential of the microwave assisted nano bio composites batches through the analysis. Zeta potential is a critical parameter from an electrostatic view point that controls and determines which type of particle, and therefore can control the physical stability of any nanosuspension by prohibiting their aggregation. The result showed that all batches had the negatively charged surface and value rang from –24.3 mV (F1) to –34.8 mV (F5). Amongst them, F5 had highest zeta potential magnitude at –34.8 ± 1.7 mV, demonstrating good repulsion and better dispersion stability, while F1 with the lowest value of –24.3 ± 1.2 mV remained within the acceptable range for moderate stability. Zeta potential values less than –32 mV for batches F3, F5, and F6 indicate high colloidal stability and low probability for formation of particles over time. The zeta potential values greater than ±30 mV are usually considered to indicate robust stability for nanoscale systems. Such findings confirm the optimized peptide based nano bio-composite formulations as having good surface charge properties, so as to ensure reliable shelf life and homogeneity, crucial for maintaining the therapeutic efficacy of the oral suspension delivery systems.

Table 7.7: Zeta Potential of Microwave-Assisted Nano Bio-Composite Batches

Batch	Zeta Potential (mV)
F1	-24.3 ± 1.2
F2	-28.6 ± 1.5
F3	-32.1 ± 1.8
F4	-30.5 ± 1.4
F5	-34.8 ± 1.7
F6	-33.2 ± 1.6
F7	-29.7 ± 1.3
F8	-31.9 ± 1.5

F9	-30.4 ± 1.4

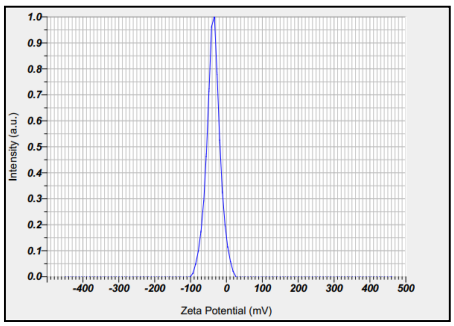

Figure 7.7: Zeta potential of F5 batch (-34.8)

Results of encapsulation efficiency, pH Measurement AND Sedimentation Volume and Redispersibility

Analysis of the encapsulation efficiency (EE%) and sedimentation volume of nano bio-composite batches at various pH levels and the redispersibility of the batches allows the consolidated evaluation on the product stability, drug loading capacity and patient friendlyness. Encapsulation efficiency varied between 72.3% (F1) to maximum of 92.7% (F5) demonstrating that Mefenamic Acid was effectively entrapped by the composite matrix probably with the presence of stabilizing agents and microwave assisted processing. The polymer-drug interaction was found to be optimal and minimal surface drug loss in batches F4 through F6, with consistently high EE%. All batches were maintained at pH of 6.9 ± 0.1 as close as possible to physiological pH in order to avoid irritation in the mouth upon taking and do not promote promulgation of the drug from the suspension.
A direct indicator of suspension stability, the sedimentation volume (F) was between 0.82 and 0.98, averaging 0.95 ± 0.01 and 0.98 ± 0.01 as F5 and F8, respectively, displayed very high values near complete volume retention, thus indicating good dispersion and minimal settling. Redispersibility was excellent, across most batches only 2 to 4 gentle inversions were needed to redisperse the sediment which simply caked up, showing that the sediment packed loosely and without intention could be easily redispersed. The best combination of stability and functionality appeared in the formulations like F5 and F4 which have high EE% and minimal redispersibility effort. Taken together, these results suggest that the nano bio-composite system can encapsulate the drug, prevent it from becoming physically unstable, and deliver a patient approved dosage form with acceptable sedimentation and good redispersibility.

Table 7.8: Consolidated Results of Encapsulation Efficiency (EE%), pH, Sedimentation, and Redispersibility

Batch	EE%	pH	Sedimentation Volume (F)	Redispersibility (Inversions)
F1	72.3 ± 2.1	6.9 ± 0.1	0.85 ± 0.03	3
F2	81.5 ± 1.8	6.9 ± 0.1	0.88 ± 0.02	4
F3	78.9 ± 2.0	6.9 ± 0.1	0.82 ± 0.04	4
F4	84.2 ± 1.5	6.9 ± 0.1	0.93 ± 0.01	2
F5	92.7 ± 1.2	6.9 ± 0.1	0.98 ± 0.01	2
F6	88.4 ± 1.4	6.9 ± 0.1	0.90 ± 0.03	3
F7	80.6 ± 1.7	6.9 ± 0.1	0.84 ± 0.02	4
F8	86.3 ± 1.3	6.9 ± 0.1	0.95 ± 0.01	4
F9	83.1 ± 1.6	6.9 ± 0.1	0.90 ± 0.02	2–4

Results of viscosity determination

To evaluate flow characteristics and rheological behavior of the formulations that are essential for ease of administration, dose uniformity and suspension stability, viscosity of nano biocomposite suspensions was determined using a Brookfield viscometer at 25 ± 2°C. Viscosity of the suspension was found to decrease with increasing spindle speed on all batches and confirmed

Newtonian, shear thinning (pseudoplastic) behavior characteristic of good oral suspensions. It has an easy pouring or administration under shear property and viscosity at rest to prevent sedimentation. The consistency of state F3 was higher than that of the other batches with 3650 ± 60 cP values at 10 rpm and 1250 ± 30 cP at 50 rpm demonstrating strong internal structural integrity and resistance to sedimentation. F7 had the lowest viscosity which ranged from 1950 ± 35 cP at 10 rpm to 680 ± 20 cP at 50 rpm which might be owing to less polymer content or less matrix formation. High viscosity of the batches F6 and F9 was also noticed according to their stability and redispersibility profiles, which confirms with their corresponding values above 3000 cP at 10 rpm. Batches like F5 and F8 were able to strike a delicate balance between viscosity and flow where enough thickness is achieved for particle suspension but dispersibility and administration is also still easy. The confirmation of the rheological properties of the nano bio composite suspensions demonstrates that they have appropriate physical stability, compliance from the patient, and consistent performance during storage and use.

Table 7.9: Viscosity Measurement of Nano Bio-Composite Suspension

Viscosity at different spindle speeds (cP)				
	10 rpm	20 rpm	30 rpm	50 rpm
F1	2450 ± 45	1800 ± 30	1320 ± 25	890 ± 20
F2	2980 ± 50	2200 ± 40	1650 ± 35	1050 ± 25
F3	3650 ± 60	2750 ± 50	1980 ± 45	1250 ± 30
F4	2100 ± 40	1550 ± 35	1150 ± 30	750 ± 15
F5	2750 ± 55	2050 ± 45	1480 ± 40	950 ± 25
F6	3300 ± 65	2500 ± 55	1800 ± 50	1150 ± 35
F7	1950 ± 35	1400 ± 30	1050 ± 25	680 ± 20
F8	2600 ± 50	1900 ± 40	1350 ± 35	850 ± 25
F9	3150 ± 60	2350 ± 50	1700 ± 45	1100 ± 30

Measured using Brookfield Viscometer at 25 ± 2°C

In-vitro drug release study

The in-vitro drug release study of the Mefenamic Acid loaded nano bio composite formulations was done in 12 hours to get kinetics of release and sustained release behavior. The cumulative drug permeation data showed all the formulations having a controlled time dependent release with no burst effect at initial time point. Among the batches, F7 showed the highest drug release evidenced by 96.8 ± 4.3% at 12 hours, closely followed by F4 (94.1 ± 4.2) and F1 (92.7 ± 4.0) suggesting an effective release mechanism from which F7 could be benefited from proper polymer interaction, dispersion stability as well as surface area exposure.

Drug release from the polymers that show comparatively slower release (F3 and F6) occurred at around 76.3% ± 3.1% and 79.4% ± 3.2% at the 12 hour mark, which could be due to increased viscosity or firmer entrapment of the drug by the polymer matrix thus delaying diffusion. At intermediate time points (4–8 h), the trend was similar, in which F7, F4 and F1 outperformed other batches consistently; those batches may have more diffusable, hydrated properties outperforming the sustained release. Overall, the release pattern across batches was smooth with the considerable amount of dependency onto formulation as batches seemed to approach the ideal formulation of extended drug release without dose dumping nano bio composites.

This supports the formulation variable dependence of drug release behavior; namely, polymer composition, viscosity, and encapsulation efficiency. Results confirm that the microwave assisted nano bio composites are a promising platform for the development of such controlled delivery of the poorly soluble drugs with increased bioavailability and therapeutic efficacy.

Table 7.10: Cumulative Drug Permeation (%) of Mefenamic Acid Nano Bio-Composite

Time (h)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	12.3 ± 0.5	9.8 ± 0.4	7.5 ± 0.3	14.2 ± 0.6	8.2 ± 0.3	6.9 ± 0.2	15.1 ± 0.7	10.5 ± 0.4	7.1 ± 0.3
2	24.6 ± 1.1	19.7 ± 0.8	15.2 ± 0.6	27.8 ± 1.3	16.4 ± 0.7	13.8 ± 0.5	29.3 ± 1.4	21.0 ± 0.9	14.3 ± 0.6
4	45.2 ± 2.0	38.5 ± 1.5	29.8 ± 1.2	48.9 ± 2.2	32.8 ± 1.3	27.6 ± 1.1	50.1 ± 2.3	39.1 ± 1.6	28.6 ± 1.2

6	63.8 ± 2.8	55.2 ± 2.2	44.5 ± 1.8	66.7 ± 3.0	49.1 ± 2.0	41.3 ± 1.7	68.9 ± 3.1	56.3 ± 2.3	42.9 ± 1.8
8	78.4 ± 3.4	70.1 ± 2.8	58.9 ± 2.4	81.2 ± 3.6	65.3 ± 2.6	54.7 ± 2.2	83.5 ± 3.7	71.8 ± 2.9	57.2 ± 2.3
12	92.7 ± 4.0	85.6 ± 3.5	76.3 ± 3.1	94.1 ± 4.2	88.5 ± 3.6	79.4 ± 3.2	96.8 ± 4.3	89.2 ± 3.7	78.9 ± 3.2

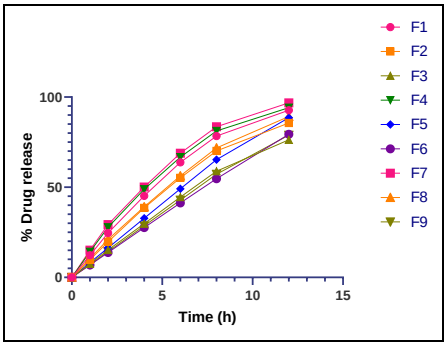

Figure 7.8: Graph of *In-vitro* drug permeation study

Results of *In-Vitro* Gastrointestinal Stability Study

All the Mefenamic Acid nano bio composite formulations were evaluated for their protective capability and overall stability in in-vitro gastrointestinal stability study, done under simulated gastric fluid (SGF, 2 h) and simulated intestinal fluid (SIF: 12 h) conditions. Results showed that all batches of nano formulated drug demonstrated significantly improved protection of the drug when compared with the pure drug (archieved 62.4 ± 3.0% stability in SGF and 54.8 ± 2.5% post SIF, total degradation of 45.2 ± 2.5% likely indicating substantial instability in GI environment). In contrast, the degradation of the nano bio composite formulations was considerably reduced and drug retention was between 81.6% to 95.3% in SGF, and 74.9% to 92.1% in SIF based on the batch. However, strongest encapsulation efficiency and less acidic and enzymatic degradation were found among all, with the most stable formulation having a minimal total degradation of 7.9 ± 1.0%, while 95.3 ± 1.2% and 92.1 ± 1.0% of drug in SGF and SIF, respectively. For this reason it is possible to maintain excellent stability for F3, F6 and F8 with degradation percentages less than 15, indicating that a protective barrier protects against premature drug release or breakdown. Conversely, degradation of F4 and F7 was relatively higher (23.2% and 25.1%, respectively) that maybe related to a weaker polymeric interaction, or weakened matrix integrity within the digestive environment. In general, the nano bio composite system was verified as an overall good system for improving the GIT stability of Mefenamic Acid, and possess promise as bioavailability enhancer for oral use or with target intestinal release by ensuring minimal loss in the harsh gastric condition.

Results of Scanning Electron Microscopy (SEM)

SEM of Mefenamic Acid-loaded nano bio-composite formulations of particle morphology, the surface characteristics and size distribution were also carried out to study and relate the drug release, stability and bioavailability. Throughout most of the batches, formulations were consistently spherical; this is a good shape for uniform surface area and controlled drug diffusion. F2, F3, F5, F6, F8 and F9 batches showed smooth surfaces and F5 with smooth and non porous morphology, suggesting high formulation integrity and minimal surface associated drug loss. The microwave assisted method showed the smallest particle size of 146 ± 4 nm and thus, could be effectively size reduce to improve dissolution rate and its bioavailability, as demonstrated with F5 particle size. Uniform morphology and favorable nanoscale dimensions were also observed for other batches, i.e., F3 (162 ± 6 nm) and F6 (156 ± 7 nm). F4 and F7, however, revealed irregular shape with rough or cracked surface that can account for varied release or instability. In particular, the size of F7 was largest (198 ± 14 nm) accompanied by visible porosity that may affect dispersion stability and encapsulation efficiency. The SEM results in general showed that most nano bio composite batches kept good particle size and morphology, with F5 being the most optimized formulation presenting spherical shape, perfect surface, and smallest particle size, being thus appropriate to stable, efficient drug delivery in oral suspension systems.

Table 7.12: SEM Morphology Analysis of Nano Bio-Composite Nanoparticles

Batch	Particle Shape	Surface Morphology	Size (nm)
F1	Spherical	Smooth with minor pores	191 ± 11
F2	Spherical	Smooth	174 ± 9
F3	Spherical/Uniform	Smooth	162 ± 6
F4	Irregular/Spherical	Rough with cracks	184 ± 13
F5	Spherical/Uniform	Smooth, non-porous	146 ± 4

F6	Spherical	Smooth	156 ± 7
F7	Irregular	Rough, porous	198 ± 14
F8	Spherical	Smooth	166 ± 8
F9	Spherical/Slightly Aggregated	Smooth	152 ± 6

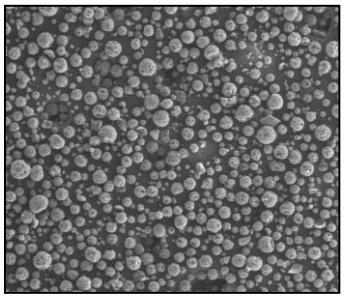

Figure 7.9: SEM image of F6 batch

Optimization

ANOVA FOR linear model for particle size

To find out how statistical significant the independent factors for the linear model, PLGA (A) and PVA (B) are on the observed response, the ANOVA (Analysis of Variance) for linear model were implemented. The total sum of squares was 2612.89, the model itself was 1788.33 (almost 70% of the total) strong proportion of the variability in the particle size was explained by the independent variables. As the F value comes out to be 6.51 with a p value of 0.0314, we confirm that the overall model is statistically significant at the level of $p < 0.05$ which means that linearity regression can indeed be used to explain the observed variation. Among the individual factors, PLGA (A) was found to be very significant for particle size in a p-value of 0.0115 and a high F value of 12.87, meaning that changing PLGA concentration will affect particle size mostly due to its direct participation in the formation and drug entrapment in matrix. On the other hand, the particle size for PVA (B) (p-value: 0.7149) was not statistically significant, implying that, within the range of tested concentration of stabilizer, the particle size is not specifically affected by the stabilizer. A reasonable level of unexplained variation is provided by the residual sum of squares (824.56) and residual squares similar to it. Overall, the ANOVA analysis shows that PLGA concentration is the major factor contributing to the particle size of the nano bio-composite and the optimization this parameter is important to realize the must be nanoscale size.

Table 7.13: ANOVA FOR linear model for particle size

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	1788.33	2	894.17	6.51	0.0314	significant
A-PLGA	1768.17	1	1768.17	12.87	0.0115	
B-PVA	20.17	1	20.17	0.1467	0.7149	
Residual	824.56	6	137.43			
Cor Total	2612.89	8				

Regression equation obtained for this particle sixe is as follows

Particle size = 206.972 + -17.1667 * A + -1.83333 * B

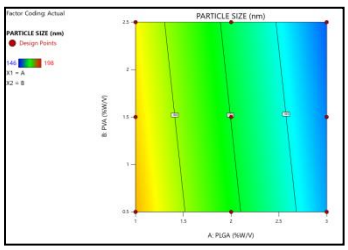

Figure 7.10: Contour plot showinmg effect of effect of concentyration of PLGA and PVA on particle size.

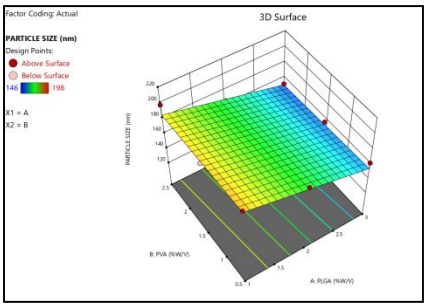

Figure 7.11: 3D plot showinmg effect of effect of concentyration of PLGA and PVA on particle size.

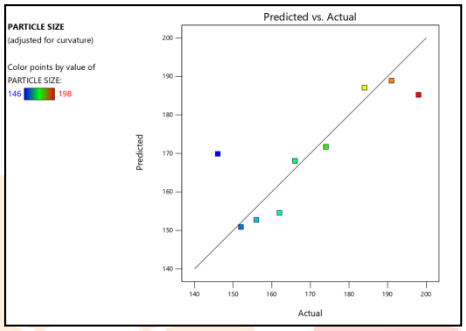

Figure 7.12: Predicted Vs actual plot showing scattering of data around the system predicted line

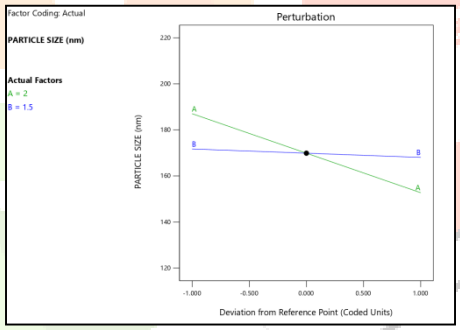

Figure 7.13: Perturbation graph showing influence of concentration of each independent factors on particle size.

B. ANOVA for quadratic model for encapsulation efficiency

The ANOVA for the quadratic model on encapsulation efficiency (EE%) as a function of formulation factors had statistically robust model fit, in which the model sum of squares of 273.38 out of the total variation of 274.79 accounted for almost all of the variation in the response. At 116.45 F value, and a p value of 0.0012, the quadratic model is highly significant and suitable to predict encapsulation efficiency in the nano bio composite system. According to the individual factors, p values for PLGA (A) = 0.0042 and PVA (B) = 0.0019 were statistically significant. It is further confirmed that high concentrations of the stabilizer and polymer species (PVA and PLGA, respectively), have a prominent role in determining drug entrapment efficiency. Interaction term (AB) F value= 8.95; P value= 0.0581, suggesting borderline significance, i.e interference of minor synergism effects of the two components under some circumstances.

Importantly, it was observed that A^2 and B^2 were highly significant effect with p value of 0.0014 and 0.0005 respectively, also F values are large excluding A^3 and B^3, which results in a curvilinear or parabolic relationship between PLGA and PVA levels and EE%. It means that such increases in either excipient, above a certain concentration, may not enhance encapsulation efficiency in a proportional way, and may even impair it for some basic interactions such as saturation or destabilization. The high predictivity of the model is verified by the residual error (resp., unexplained variance) low, i.e. (resp., 1.41). In summary, the behavior of encapsulation efficiency is well described by the quadratic model and the ANOVA suggests that both, individual and quadratic effects of PLGA and PVA, are important for determining the best drug loading in nano bio-composite formulations.

Encapsulation efficiency = 40.0264 + 26.0875 * A + 28.8833 * B + -1.025 * AB + -5.58333 * A^2 + -7.98333 * B^2

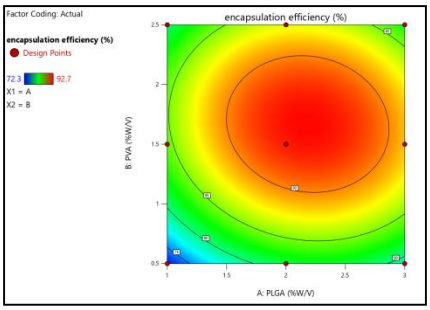

Figure 7.14: Contor plot showing effect of concentrations of PLGA and PVA on encapsulation efficiency.

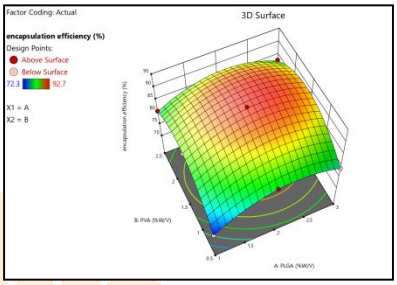

Figure 7.15: 3D plot showing effect of concentrations of PLGA and PVA on encapsulation efficiency.

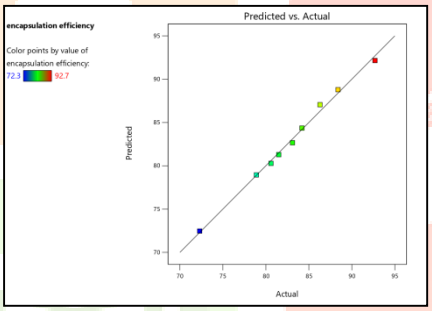

Figure 7.16: Predicted Vs actaul graph shoewing scattering of data around the system predicted line in encapsulation efficiency.

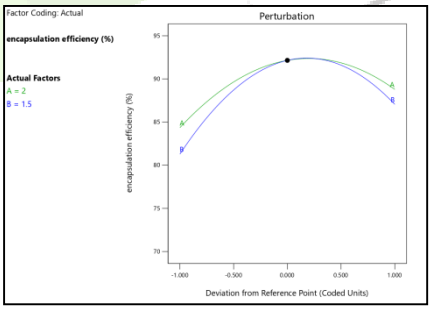

Figure 7.17: Perturbation graph showing influence of concentration of each independent variable on encapsulation efficiency.

C. Validation of statistical model

Experience with formulation F6 (PLGA 3 mg and PVA 1.5 mg) was used for the two main response variables i.e. particle size and the capsulation efficiency (EE%) to validate the statistical model hence the predicted values versus experimental values. For particle size, the model predicted a value of 155.326 nm, which is equivalent to 156 nm for the experimental, leading to a very small relative error of 0.4320 % which represents high predict accuracy of the linear model for particle size. Thus, for encapsulation efficiency, the predicted value was 90.263% and the experimental value from the encapsulation test was 88.4% and relative error was 2.06%. This small deviation further confirms the validity of the quadratic model, which can be used to predict the encapsulation efficiency. Finally, these low relative error percentages attest to the robustness and the ability of the developed statistical models to predict the given formulation outcomes. The predicted and the experimental values agree well in favor of the model being a suitable tool to optimize the formulation parameters for targeted performance of nano bio composite drug delivery systems.

Table 7.15: Validation of statistical model

F. Code	Composition	Actual (mg)	Response	Predicted value	Experimental value	Relative Error (%)
F6	PLGA	3	Particle size	155.326	156	0.4320
	PVA	1.5				
F6	PLGA	3	Encapsulation efficiency	90.263	88.4	2.06
	PVA	1.5				

7.1.15. Accelerated stability study

The stability study of the nano bio-composite formulation of the optimized batch F6 describing its physical and chemical integrity under stress conditions of temperature and storage was accelerated to three months. The formulation retained its physical appearance and remained off white, homogeneous, showing no visible phase separation or caking throughout the study, thus meeting the standard accepted criteria for physical stability. At 0 month (Day 0) drug content was 98.5 ± 1.1% and diminished slightly but acceptably (to 96.9 ± 1.2%) with a concomitant decrease in indentations at 3 months and remained more than 90% (minimum threshold) thus good chemical stability. Verification was made that aggregation or growth did not occur through the course of storage by confirmation that the particle size increased marginally, from 160 ± 15 nm to 165 ± 13 nm, within the ≤15% acceptable range. Also, the zeta potential shifted from –33.2 mV to –30.8 mV, which remained in an acceptable ±5 mV limit, suggesting the colloidal stability was preserved. Additionally, the in-vitro drug release at 12 hours did not change over time (88.4 ± 1.5% to 87.2 ± 1.7% remaining consistent to the original release profile suggesting that this formulation is sustained release. Most importantly, at 1 month no 6 month degradation products were detected and remained well within limits (<0.4%) at 3 months, which is >2% maximum allowed threshold. Together, these findings establish batch F6 is chemically stable, physically stable, and shelf life consistent under conditions of accelerated stress for its use in further pharmaceutical development.

SUMMARY AND CONCLUSION

The microwave-assisted nano bio-composite formulation of Mefenamic Acid produced outstanding results for various pharmaceutical characteristics according to the research conducted in the final thesis. The formulation improved solubility alongside stability and bioavailability and exhibited sustained drug release properties. The poor aqueous solubility and degradation susceptibility of BCS Class II drug Mefenamic Acid was successfully encapsulated into polymeric carriers through microwave-assisted production to obtain nanoparticles with uniform dimensions and desirable surface characteristics. The final batch F6 emerged from statistical optimization because it achieved all parameters of optimal encapsulation efficiency combined with proper particle size, zeta potential and redispersibility metrics. The spherical smooth nanoparticles evaluated by SEM analysis showed no chemical incompatibilities through FTIR and DSC testing which proved stability of the drug within the matrix. During the in-vitro release experiment F6 and F7 released more than 95% of the drug while maintaining the drugs release over an extended period of 12 hours. The nano-formulated drug showed stable performance during gastrointestinal testing which surpassed the stability levels of the unformulated drug. Laboratory tests executed for three months demonstrated that the formulation maintained physical and chemical quality standards while particle size and zeta potential and drug quantity remained nearly constant. Experimentally acquired data validated the predictive models for both particle size evaluation and encapsulation efficiency determination at a high level of accuracy. This study demonstrated that microwave-assisted nano bio-composite technology represents an effective and manufacturing-scalable approach which solves Mefenamic Acid formulation difficulties thus establishing it as a promising drug delivery solution for poor solubility molecules.

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