Formulation and evaluation of Aceclofenac loaded in-situ gel for the treatment of Periodontitis

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Title of the thesis

FORMULATION AND EVALUATION OF ACECLOFENAC LOADED IN-SITU GEL FOR THE TREATMENT OF PERIODONTITIS

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CERTIFICATE OF APPROVAL

This is to certify that the thesis entitled **"FORMULATION AND EVALUATION OF ACECLOFENAC LOADED IN-SITU GEL FOR THE TREATMENT OF PERIODONTITIS"** submitted by **Souvik Singha**, of Jadavpur University, for the course of **Master of Pharmacy** is absolutely based upon his work under the supervision of **Dr. Kajal Ghosal**, Assistant Professor, Department of Pharmaceutical Technology, Jadavpur University, Kolkata and that neither his thesis nor any part of the thesis has been submitted for any degree/ diploma or any other academic award anywhere before.

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DECLARATION BY THE CANDIDATE

I do hereby declare that the work incorporated in the thesis entitled **"FORMULATION AND EVALUATION OF ACECLOFENAC LOADED IN-SITU GEL FOR THE TREATMENT OF PERIODONTITIS"** has been carried out by me in the Department of Pharmaceutical Technology, Jadavpur University under the supervision of Dr. Kajal Ghosal, Assistant Professor, Department of Pharmaceutical Technology, Jadavpur University, Kolkata – 700032. Neither the thesis nor any part therefore has been submitted for any other degree.

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Dedicated to my Guide and My Family

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Date: SOUVIK SINGHA

PREFACE

The thesis presented here, titled **"FORMULATION AND EVALUATION OF ACECLOFENAC LOADED IN-SITU GEL FOR THE TREATMENT OF PERIODONTITIS"**

- Chapter 1 introduces the fundamental concepts involved in the preparation of *in-situ* gel
- Chapter 2 presents a comprehensive literature survey on the relevant subject matter.
- In Chapter 3, the aims, objectives, and the planned work sequence are outlined.
- Chapter 4 catalogs the materials utilized in the project.
- Chapter 5 encompasses the procedures for preparing drug loaded *in-situ* gel along with a description of the characterization techniques employed.
- Chapter 6 delves into the presentation and discussion of the results.
- Finally, Chapter 7 offers a summary and conclusion of the present work.

Chapter 1

Introduction

1.1 Introduction to NDDS

NDDS is one of the important tools used by the pharmaceutical industry to extend medicinal markets. Conventional dosage forms show fast drug release, high dose and limited availability, instability, first pass metabolism, and changes in plasma drug level. By improving product shelf life, patient compliance, safety, and efficacy, NDDS can reduce above challenges.[1]

The goal of novel drug delivery systems is to continuously deliver drugs into the bloodstream for a prolonged amount of time at kinetics that are reproducible and predictable. Potential benefits of this idea include less drug-related side effects since therapeutic blood levels are maintained, increased patient compliance because dosages are given less frequently, and decreased total dosages of drug. Therefore, a delivery system with both controlled release and sustained release capabilities would increase the therapeutic efficacy even more.[2]

Two requirements should preferably be met by the novel carriers. First, over the course of therapy, it ought to deliver the drug at a rate determined by the body's requirements. Second, it ought to direct the drug's active ingredient to its site of action. None of these can be met by conventional dosage forms, including prolonged-release dosage forms.[3]

In order to provide easier, more regulated, and targeted distribution, a number of drug delivery systems (NDDS) have recently been developed using the latest technology. The unique characteristics of every drug delivery system influence its release mechanism and rate. This is mostly because of the variations in their morphological, chemical, and physical properties, which will ultimately influence their affinities for various kinds of drugs.[4]

NDDS is designed to improve treatment efficacy and patient compliance. Traditional methods of drug delivery suffer from problems such as poor bioavailability, rapid degradation, and systemic side effects. NDDS, which include nanoparticles, liposomes, and transdermal patches, offer targeted delivery, controlled release, and improved stability. These systems can bypass biological barriers, reduce the number of doses and increase the concentration of the drug in the target area. This review presents various NDDS technologies, methods and applications in different medical fields. NDDS has the potential to revolutionize treatment programs and address unmet health needs, paving the way for better and more personalized health care.[5]

NDDS, like nanoparticles, liposomes, ethosomes and phytosomes provide a platform for controlled and targeted delivery of herbal medicines. Nanoparticles can, for example, contain extracts from herbs that protect them from damage and improve their absorption in the body. Liposomes and phytosomes can increase the solubility and bioavailability of insoluble plant compounds, while ethosomes, which are lipid vesicles, allow deep penetration of plant drugs through the skin.

One of the main advantages of NDDS in herbal medicine is their ability to modify the release profile of active compounds, ensuring a sustained and controlled release over time. This may result in long-term therapeutic benefit, less frequent dosing, and fewer side effects. In addition, targeted delivery systems can direct herbal compounds to the site of action, increasing their efficacy and reducing systemic exposure.

NDDSs have been successful in enhancing the therapeutic potential of herbal medicines. For example, encapsulation of curcumin, a compound with anti-inflammatory and anti-cancer properties, in nanoparticles has shown improved bioavailability and therapeutic outcomes in early studies. Similarly, the use of liposomal formulations for quercetin, a flavonoid with antioxidant properties, has improved stability and biological activity. In addition, NDDS can facilitate the development of new dosage forms such as transdermal patches, gels, and aerosols, thus expanding the range of routes of administration of herbal medicines. This can improve patient compliance and comfort, especially for chronic conditions that require long-term treatment.[6]

To conclude, novel drug delivery systems (NDDS) offer significant advantages over traditional dosage forms by providing controlled, targeted, and sustained drug release. These approaches enhance therapeutic efficacy, reduce side effects, and improve patient compliance with frequent, low-dose regimens. NDDS technologies such as nanoparticles, liposomes, phytosomes and ethosomes hold promise for improving the bioavailability and stability of synthetic drugs and herbal medicines. By overcoming the limitations of traditional drug delivery methods, NDDS have the potential to transform treatment pathways and provide more personalized healthcare treatments, ultimately addressing unmet medical needs and improving patient outcomes.

1.2 Introduction to Periodontitis

The bacteria in dental plaque can cause chronic periodontitis, commonly referred to as adult periodontitis. This infectious, inflammatory illness gradually destroys the tissues that support teeth, including the gingival, periodontal ligament, cementum, and alveolar bone. Periodontal disease exhibits a local microbial burden that causes local inflammation and tissue death. It is characterized by periods of aggravation interspersed with periods of remission.[7]

Estimates of the prevalence of periodontitis vary since there is no universally accepted definition. Current research advises examining all areas of the mouth for indications of inflammation, probing depth, and attachment loss at six different locations on each tooth. However, several widely accepted definitions omit inflammation, making it challenging to determine the efficacy of treatment.

Newer studies, especially in areas where people can easily see a dentist, use extensive inspections to obtain improved estimates of periodontal health rather than relying on outdated techniques like the Community Periodontal Index of Treatment Needs.[8]

Periodontitis, an inflammatory disease that affects the supporting structure of the teeth, is affected by several risk factors. Several important risk factors are identified, including poor oral hygiene, which is an important modifiable risk factor. Plaque accumulation, a bacterial biofilm, plays an important role in the initiation and progression of inflammation in the periodontal tissue.

Genetic predisposition is also considered an important risk factor; studies have shown that people with a family history of periodontitis are more likely to develop the disease. Genetic factors affect the host's immune response to pathogens and thus increase tissue damage. Diseases such as diabetes are also closely associated with periodontitis. Patients with diabetes are at risk of periodontal disease due to impaired immune function and poor wound healing, which may lead to poor diabetes control.

Smoking is another important risk factor which is reported. Smokers have a higher chance of developing periodontitis, and smokers are more likely to develop periodontitis than non-smokers. The adverse effects of smoking on the immune response and blood supply to periodontal tissue are well known.[9]

Risk factors can be divided into behavioral, systemic, and genetic factors, each contributing to the onset and progression of the disease.

Behavioral factors, especially oral hygiene, are considered to be the main cause of the formation of plaques that contain pathogenic bacteria that cause inflammation of the disease. Smoking is considered a behavioral risk factor, and there is strong evidence that it not only increases the risk of cardiovascular disease but also worsens stress. Smoking affects the immune response and impairs the healing process, further damaging the periodontal tissue.

Systemic diseases, especially diabetes, have been identified as important risk factors due to their association with periodontal disease. Diabetes can increase periodontal inflammation through mechanisms such as poor immune function and collagen metabolism. On the other hand, severe periodontal disease can worsen glycemic control, creating an inverse association between the two conditions. Other systemic factors, such as obesity and metabolic syndrome, have also been identified, with emerging evidence suggesting their role in increasing the risk of periodontal disease.

In case of genetic predisposition, genetic differences can influence an individual's immune response to periodontal pathogens and thereby affect disease progression.[10]

Periodontitis, a multifaceted and progressive inflammatory disease, can be treated with non-surgical and surgical methods, each of which has a distinct role and effectiveness.[11]

One of the non-surgical method; Scaling and root planing (SRP) is a mechanical operation used to remove calculus and plaque from periodontal pockets and root surfaces. It is a key component of nonsurgical therapy. SRP is useful in decreasing pocket depths and raising clinical attachment levels, especially in mild to moderate cases of periodontitis. By breaking up the biofilm, the

technique lowers the bacteria burden that triggers the inflammatory response.[12]

There are some adjuvant therapy that can improve the results of nonsurgical treatment in addition to SRP. Among them is the application of antimicrobial medicines, which specifically target periodontal infections and might be systemic or local antibiotics. Although scaling and root planing (SRP) and other debridement techniques are the mainstay of treatment, the additional use of antibiotics is often considered to improve treatment outcomes, especially in patients that are aggressive and resistant to treatment. The rationale for using systemic antibiotics, include the ability of drugs to penetrate deep tissue pockets and tissue invasion, the two sites of infection. The most commonly used antibiotics in periodontal therapy are azithromycin, amoxicillin and metronidazole because of their specific antibacterial effects on important bacteria such as *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*. There is also controversy about the use of antibiotics, especially for its potential side effects and bacterial resistance. Systemic antibiotics need to be saved for situations in which standard mechanical therapy is insufficient to control the illness, rather than being given arbitrarily.[13]

Host modulation therapy, is another strategy which tries to reduce tissue loss by changing the inflammatory response of body to infection.

The two important surgical approaches are- osseous surgery, which aims to repair bone to correct periodontal deficiencies, and flap surgery, which allows for larger defects and pocket reduction. There is also guided tissue regeneration (GTR), a process that promotes the regeneration of lost periodontal tissue using a barrier membrane. There is also soft tissue implants that expand gum tissue and cover exposed roots. In advanced cases, when non-surgical methods alone may not be sufficient, the effectiveness of surgical intervention in improving clinical outcomes compared to non-surgical methods is evaluated. Research suggests that although surgical treatment is more invasive, it can be beneficial in maintaining periodontal health in the long term.[14]

A comprehensive strategy is needed for the management of periodontitis that takes into consideration its complex character. In this respect, nonsurgical and surgical modalities come into play to prevent and reverse periodontal damage; each strategy is therefore tailored by dint of a

patient's unique risk factors and disease severity. In this regard, the microbial burden and inflammation are combated by nonsurgical procedures using SRP combined with systemic antibiotics as the first line of defense. However, surgical procedures like GTR, flap surgery, and osseous surgery become mandatory to provide long-term periodontal stability in advanced situations where nonsurgical therapies remain inadequate.

Systemic antibiotics have to be, therefore, precisely weighed against the risk of antibiotic resistance and side effects with their potential benefits in resolving the deep-seated diseases. In this respect, surgical treatment will be more effective than non-surgical treatment because surgical procedures are more invasive and provide much benefit with respect to tissue regeneration and reduction of cysts.

Ultimately, patience, continuous education, habit, attitude, and heredity all play their roles in optimum recovery from the periodontitis. A personalized treatment plan striving to prevent the disease is also vital. Combining nonsurgical and surgical treatments has made it possible for health professionals to achieve good quality of life in patients with chronic diseases.

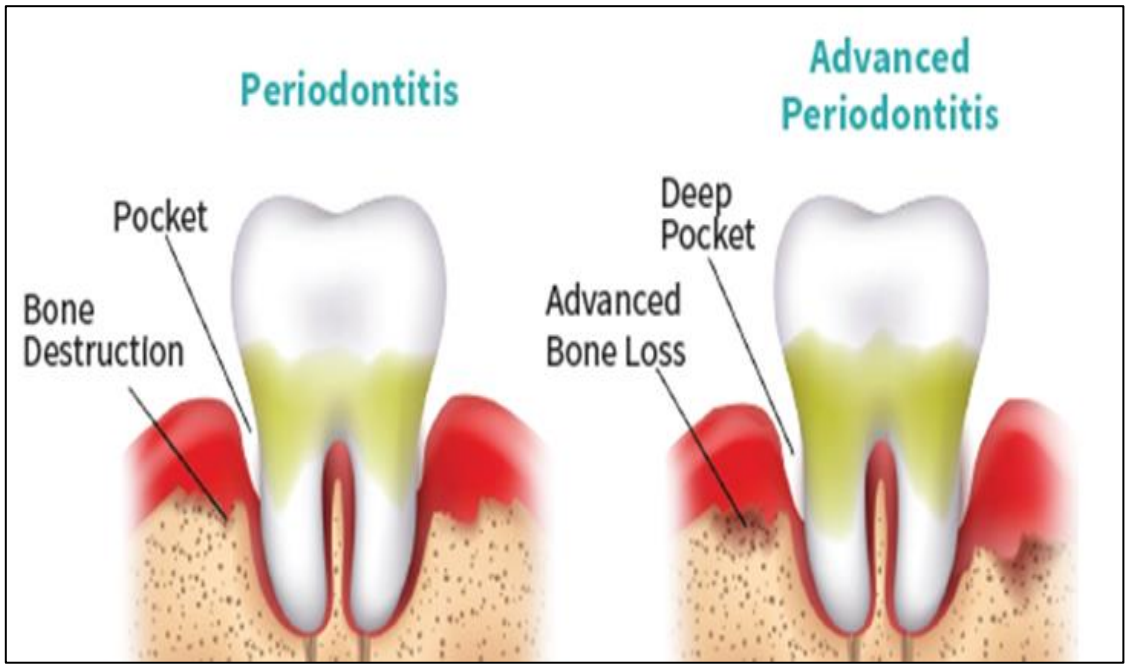


Figure 1: Schematic diagram showing periodontitis.

1.3 Halloysite Nanotube (HNT)

1.3.1 Introduction:

Halloysite nanotubes are naturally occurring aluminosilicate minerals with unique tubular morphology. They consist of alternating alumina (Al_2O_3) and silica (SiO_2) layers, which in turn, are constructed in a concentric manner, much the same as a rolled sheet. With such a structure, HNTs exhibit high surface areas, mechanical strength, and biocompatibility. Potential applications can, therefore, be spanned across various industries, so they become the subject of significant scientific interest.[15]

The synthesis of HNTs normally involves extraction of natural halloysite from clay deposits and subsequent purification procedures to remove impurities. One such widely applied techniques for the production of HNTs is the hydrothermal method, which requires treating the halloysite clay in an autoclave at certain temperature and pressure conditions. It guarantees the preservation of the nanotubular structure and increased quality of the obtained material.[16]

Structurally, HNTs take the form of tubes whose inner diameters are usually from 30 to 100 nm and whose lengths range from 1 to 12 micrometers. The wall composition is composed of alternate layers of alumina and silica, which give them a high aspect ratio and considerable surface area. Their structure lends them to various applications, such as drug delivery, catalysis, and environmental remediation. The unique structure of halloysite nanotubes consists of an alumina-rich octahedral sheet inside and a silica tetrahedral sheet outside, the natural rolling of which forms a tubular morphology. This ultimately endows the HNTs with a large surface area and high capacity for loading and releasing a variety of active molecules with tunable surface chemistry. High aspect ratio, biocompatibility, and mechanical strength further increase the potential of HNTs in a huge number of applications.[17]

One of the most relevant properties of HNTs is their nanocontainer ability. Their hollow cavity could be filled with different active agents, such as drugs, enzymes, or chemical reagents. This

possibility of release control could be achieved by surface chemistry modification of the HNTs or by application of some external stimuli like changes in pH, temperature, or magnetic fields.[18]

Halloysite is mined naturally, but its properties may be further improved using the methods of functionalization. Surface modification is one of the most frequent ways of improving HNT compatibility with different matrices and enhancing performance in specific applications. All this can be achieved using different ways of functionalization, either by silanization, polymer grafting, or introduction of organic/inorganic molecules onto the surface of nanotubes.[19]

Functionalization of halloysite nanotubes improves their properties and compatibility with different materials, hence widening the range of their applications. One of the typical methods is grafting using γ-aminopropyltriethoxysilane (APTES). This process involves the introduction of an amino group onto the surface of the nanotubes, by which an improved dispersion of HNTs in polymer matrices and better interaction with other components are achieved. Therefore, they are effective as a reinforcing phase in composites.[20] Another method uses 2-hydroxybenzoic acid for the solid-phase extraction, which exploits the increased surface area and modified surface chemistry of HNTs to enable it to selectively bind and remove some substances from a mixture.[21] Besides, HNTs can be functionalized through the grafting of hyperbranched (co)polymers via surface-initiated self-condensing vinyl polymerization. Through this procedure, a highly grafted polymer network forms on the surface of nanotubes, which helps enhance their compatibility with the matrix of a polymer for advanced nanocomposite fabrication with fine property tailoring. These foregoing methods all open ways to application-orientation functionalization of HNTs in such topics as drug delivery, environmental remediation, and the development of advanced materials.[22]

Such functionalized HNTs would have improved dispersibility in polymers, better compatibility with biological systems, and enhanced ability for drug or other active agent binding and release. One chooses a functionalization method in view of specific applications and required properties.

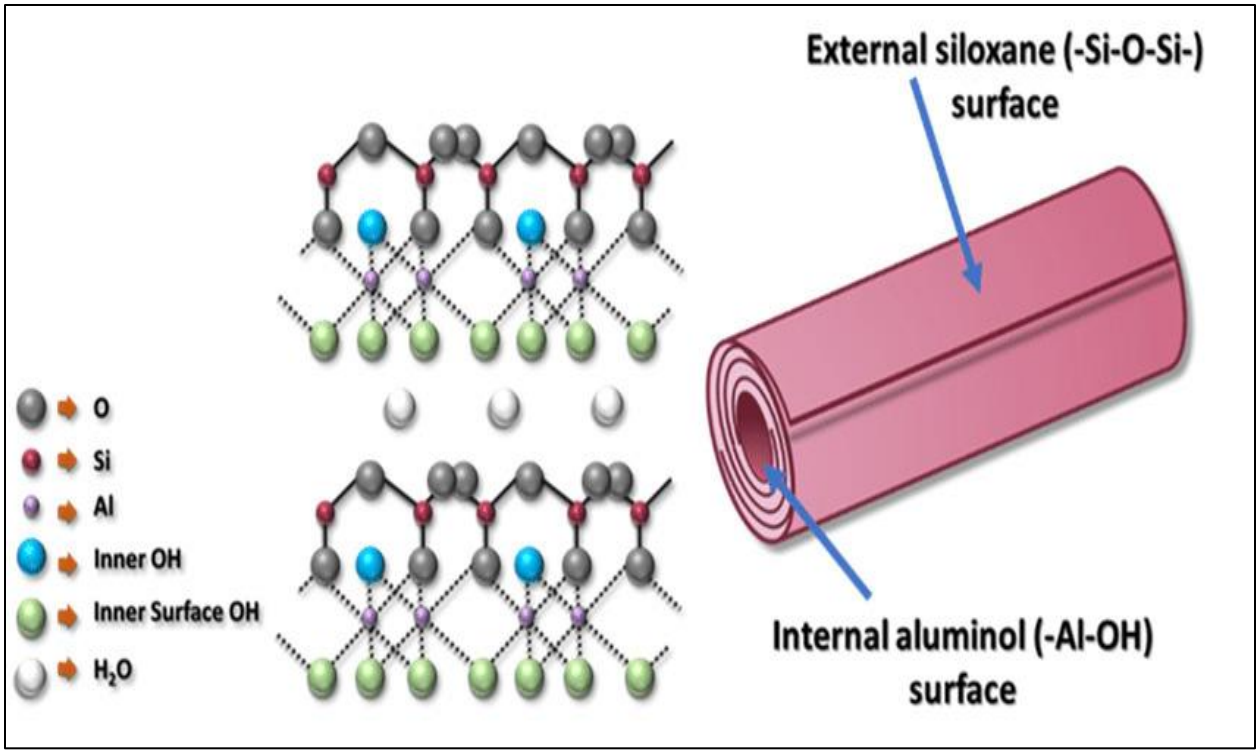


Figure 2: The structure of a Halloysite nanotube

1.3.2 Applications of Halloysite Nanotubes:[22,23]

Reinforcement in Polymer Nanocomposites: HNTs enhance the mechanical, thermal, and barrier performance of polymers; hence, they find applications in packaging, automotive, and construction materials.

Drug Delivery Systems: Controlled and sustained drug release with HNTs improves the stability and bioavailability of drugs.

Environmental Remediation: HNTs prove very effective in removing pollutants—particularly heavy metals and organic compounds—from water and soil.

Catalysis: HNTs act as catalysts or catalyst supports in a wide range of chemical processes, improving their efficiency and selectivity.

Energy Storage: HNTs are being investigated for use in batteries and supercapacitors to improve their performance and increase their capacity.

Flame Retardancy: HNTs improve the thermal stability and flame retardancy of materials; hence, they find applications in safety.

Cosmetic Products: HNTs are used for encapsulation and controlled release of active ingredients in skincare and cosmetic formulations.

Biomedical Applications: Apart from being used as a drug delivery vehicle, HNTs find applications in tissue engineering and the wound healing and biosensors due to its biocompatibility and mechanical properties.

Electronics: Used in making sensors, transistor, and other electronic devices.

Agriculture: Used as fertilizer carrier and pesticide in agriculture, thus allowing controlled release and reducing the impacts on the environment.

Paints and Coatings: HNTs provide enhanced strength, scratch resistance, and ultraviolet protection to paints and coatings.

Food Packaging: HNTs have been studied for their potential in antimicrobial packaging materials that help improve the shelf life of food products.

1.3.3 Advantages of Halloysite Nanotubes:[24,25]

Safe and Natural: Non-toxic, biocompatible, and listed as an EPA 4A material.

Physical Properties: Fine particle size, high surface area, and excellent dispersion.

Chemical Properties: High cation exchange capacity.

Controlled Release: Resulting delivery rates are constant and sustained and there is no initial overdosage.

Triggerable Release: Informed release, such that no release occurs unless triggered, at tunable rates.

Sequestration of Active Agent: Protects the active agent within its lumen during harsh material processing.

Multi-functionality: Can load multiple active agents simultaneously.

Cost Efficiency: Reduces the volume of expensive active agents.

Form Flexibility: Can be done in a variety of forms such as powders, creams, gels, lotions, and sprays.

Better Loading and Adsorption: It provides higher loading rates, faster adsorption, and higher adsorption capacity in comparison to other carriers.

Structural Features: High aspect ratio, which means high porosity and is non-swelling.

Re-generation and Efficacy: Can be regenerated, coupled with enhanced efficacy in applications.

1.4Chitosan

1.4.1 Introduction to Chitosan:

Chitin, the precursor of chitosan, is the most abundant biopolymer in nature after cellulose and is present in a variety of eukaryotic species, including fungus, insects, and crustacea. Chitin (polymer of N-acetyl-D-glucosamine) is known as chitosan when it undergoes deacetylation and the repeating units of the polymer are primarily absent of the acetyl functional group, i.e., as β-1, 4-D-glucosamine. The degree of acetylation (DA) is the N-acetylated repeating units' mole fraction, whereas the degree of deacetylation (DD) is the percentage of the β-1,4-D-glucosamine repeating units in the polysaccharides.[26]

Chitosan, in contrast to chitin, dissolves readily in aqueous solutions of a wide range of organic and inorganic acids. Additionally, because chitosan possesses free primary amino groups that are consistently dispersed throughout its molecular chain, its chemical and biological reactivity is higher than that of chitin. Thus, chitosan's industrial applications as biomedical materials, antimicrobials, food additives, cosmetics, sewage disposal, separators, agricultural materials, and so forth are attracting attention from all over the world. [27]

It was observed that chitosan of different molecular weight and DD play an important role on functional properties (film formation, antimicrobial activity). In both medical and industrial applications, higher molecular weight and DD increase its effectiveness. The presence of reactive amino and hydroxyl groups makes chitosan readily modifiable to suit specific applications.[28]

Chitosan is biocompatible, enzyme degradable and has non-toxic degradation products which are the suitable properties for use in gene therapy, wound dressings, tissue engineering and anti-microbial agents in biomedical field. It is also employed in cosmetic, papermaking, wastewater treatment as well food and feed activities.[29]

Its physiological functions include antioxidant activity, cholesterol reduction, blood pressure lowering and fat absorption inhibition among others. It is so versatile and safe that you can find it present in medical as well as industrial applications.[30]

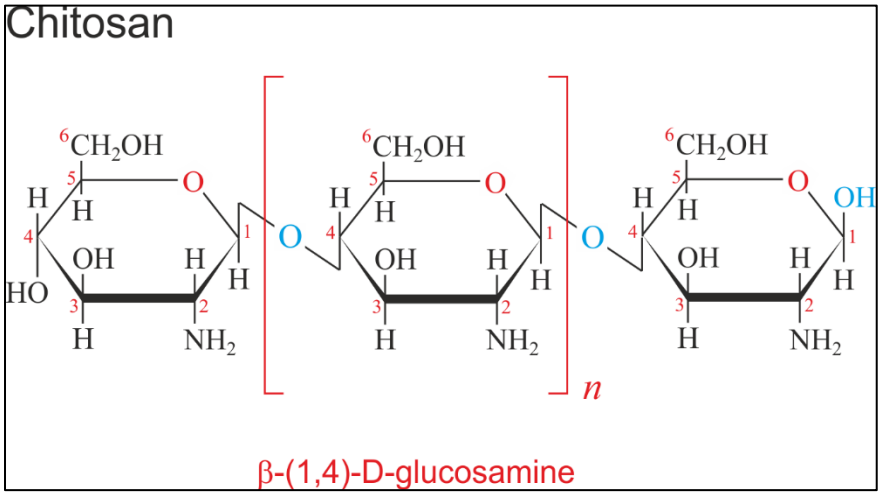


Figure 3- Chemical structure of Chitosan

1.4.2 Application of chitosan

Chitosan is derived from chitin through deacetylation. It is a natural, hydrophilic safe, biocompatible, and biodegradable and non- toxic sugar chain that works well in pharmaceutical technology. [31]

Chitosan has a straight-line structure. It consists of glucosamine units linked by β-(1, 4) bonds. The hydroxyl and amino groups in it can undergo chemical changes. These changes aim to create suitable materials for different uses. [32]

Chitosan dissolves in acidic pH forming gels. It also creates hydrogels when mixed with charged drugs or polyanions, which slow down drug release. Chitosan sticks to biological tissues because of similar ionic interactions with parts of the mucosal membrane. Scientists have developed mucoadhesive formulations to deliver drugs through the eyes, nose, mouth, gut, and vagina. Chitosan helps small polar drugs, including peptides pass through mucous membranes by briefly opening the tight connections between cells.[33]

Interest in chitosan and its derivatives is current within the beverage industries because of its wide range of applications with associated benefits. Chitosan and its derivatives are used in beverages as natural clarifying agents, ensuring clarity and stability by removing impurities, hence reducing turbidity in products. They also act as preservatives, thereby extending the shelf life of beverages through inhibition of the growth of microorganisms and spoilage reduction. Apart from that, these ingredients are utilized in fortifying health-promoting beverages by enhancing their nutrient content and offering antioxidant properties.[34,35]

Because of its polymer structure, researchers have studied chitosan for many types of tiny drug forms. Chitosan might also work to deliver radioactive drugs, genes, and peptides in the future.

1.4.3 Role of Chitosan in Drug Delivery Systems: [36,37]

Colon Targeted Drug Delivery: Chitosan shows promise as a polymer to deliver drugs to the colon because of its biodegradability by colon microorganisms. Research indicates that microspheres coated with chitosan can carry drugs like 5-aminosalicylic acid (5-ASA) to treat ulcerative colitis. These microspheres let out the drug in the colon, which makes it work better.

Mucosal Delivery: Chitosan also works well to deliver drugs through mucous surfaces such as nasal, lung, eye, and vaginal routes. Its sticky properties help drugs stay longer and get absorbed better. Drugs made with chitosan can increase how much medicine gets absorbed without harming body tissues.

Coating Material: People use chitosan as a coating material in drug delivery because it forms good films and sticks well to mucous. It controls how drugs are released, helps them stick to biological surfaces, and allows more drug to be carried.

Ocular Delivery: Chitosan nanoparticles boost how well medicine gets to the eye's mucous membrane. Research shows that drugs like cyclosporin A reach higher levels in eye tissues without much getting into the bloodstream.

Topical Delivery: Gels and mixtures made with chitosan help topical medicines work better and reach where they need to go. Changing the molecular weight and amount of chitosan allows control over how fast the medicine is released, which makes it more effective. Chitosan nano and micro particles are great vehicles for topical delivery of drugs due to their biocompatibility, biodegradability and mucoadhesive properties. They enhance drug penetration through skin, stabilize the drug and control release patterns. This is good for skin diseases and wounds by delivering localized and sustained drug delivery. Chitosan based particles can carry many drugs including antibiotics, anti-inflammatory compounds and anticancer drugs directly to the skin. Using chitosan particles in topical applications can potentially improve treatment outcome and patient compliance compared to conventional topical formulations.

Cosmetics: Chitosan has found several applications in the beauty sector due to its unique qualities. It has good biocompatibility, biodegradability, and non-toxicity, making it perfect for skin contact. Chitosan forms a hydrophilic coating that helps preserve skin moisture and can act as a thickening, emulsion stabilizer, and rheology modifier. Its antibacterial characteristics increase product preservation, allowing for decreased preservative consumption. Additionally, chitosan's film-forming property is beneficial in hair care products, increasing hair structure and texture. Its flexibility is shown in its use in creams, gels, masks, and oral care products.

1.5 *In-situ* gel

1.5.1 Introduction to *in-situ* gel:

In the recent past, *in-situ* gel forming technology has been a popular method for carrying regulated medication delivery. *In-situ* forming polymeric drug delivery methods provide a lot of advantages such as comfort, better patient compliance, convenience of administration and decreased

administration frequency. In an *in-situ* gel to be formed, the pH shift, temperature changes, solvent exchange, or a combination of these stimuli may all be contributing factors. Thus, a variety of methods, such as oral, nasal, and ocular, can be employed for the *in-situ* gelling system design. *In-situ* forming drug delivery systems are formulated with both natural and synthetic polymers. This includes alginic acid, gellan gum, xanthum gum, xyloglucan, pectin, chitosan, poly (DL lactic acid), poly (DL-lactide-co-glycolide), and poly-caprolactone.[38]

Such *in-situ* gel-forming systems are of immense interest in drug delivery due to the unique properties and potential for enhanced therapeutic efficacy. Administration of such systems, which undergo phase transition from liquid to a gel, could be a rather versatile and new methodology in controlled and sustained drug delivery.[39]

In *situ-gel* formation is usually dependent on the physiological conditions that are influenced by temperature, pH, or the presence of particular ions. Upon injectable administration, a liquid formulation undergoes gelling in which, in response to the local physiological environment, changes into a semi-solid or a gel-like structure. This phase change has dual effects because it not only extends the residence time of the drug at the site of action but also releases active therapeutic agent in a sustained manner, thereby obviating frequent dosing.[40]

The most outstanding advantages associated with *in-situ* gels include the potential provision for localized drug delivery. It provides site-specific treatment after administration in diseases where site-specific delivery is very essential, like cancer therapy or ocular treatment. Besides, *in-situ* gels can assure patient compliance because of their minimally invasive nature and ease of administration.[41]

In-situ development of the gel involves proper selection of polymers that respond to physiological stimuli in initiating the gelling process at the site of action. Examples are poloxamers, chitosan, and alginates, which often form part of the ingredients of such formulations, due to their biocompatibility and gelation properties.[42]

In-situ gels as systems that can either be applied as suspensions or solutions and they can quickly turn into a gel when exposed to external stimuli such as abiotic factors like temperature or pH during instillation.[43]

In-situ gel-forming formulations is currently a novel idea to deliver drugs as a liquid dosage form, These formulations cause the gel to form fully on the application site leading to longer life span of the active substance. Poloxamer-containing thermosensitive systems have been studied as a practical dosage form for periodontal pocket administration among in-situ gelling polymers. Among in-situ gelling polymers, it has been demonstrated that poloxamer-containing thermosensitive systems that are liquid at room temperatures but form gels at body temperatures can also be proposed.[44]

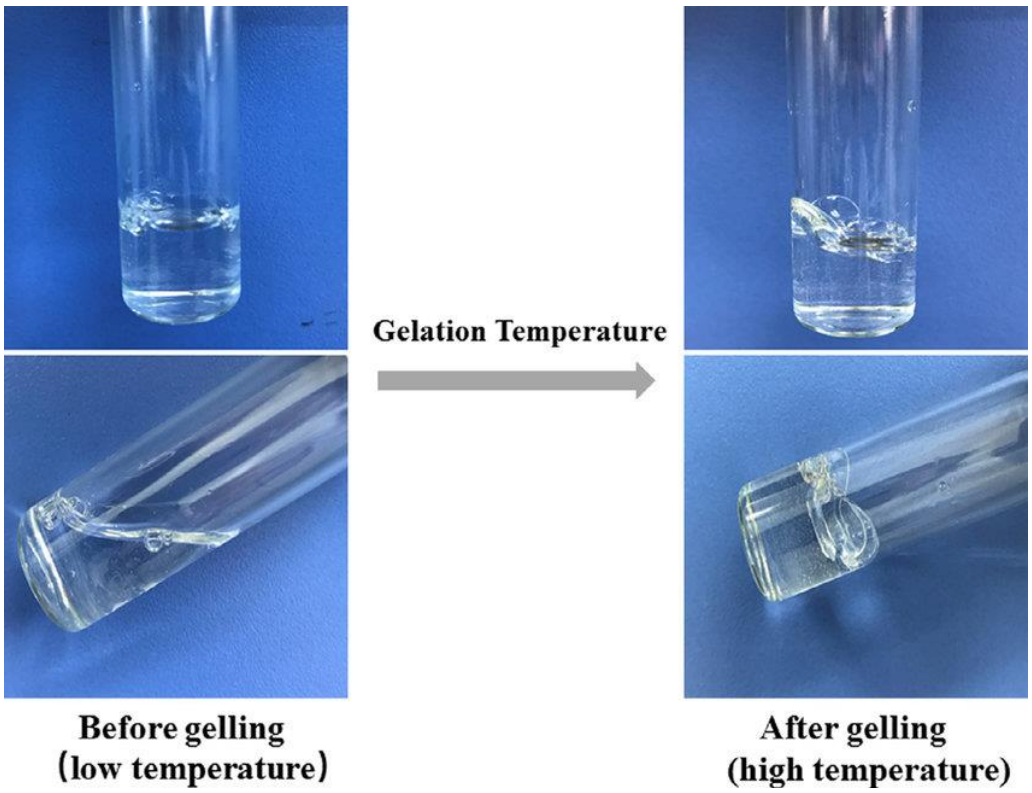


Figure 4: Formation of *in-situ* gel

1.5.2 Applications of *In-situ* gel

In-situ gel has numerous applications and can be employed to the transfer of drugs via different routes.

1. **Oral-delivery:** For the past ten years, in- situ gel systems have been employed in the oral cavity for the localized treatment of oral mucositis. They have shown to be an efficient way of managing pain, reducing inflammation, hastening the healing of wounds, and protecting against bacterial and fungal infections. Anti-inflammatory drugs and antimycotics have also been applied locally using other conventional formulations; nevertheless, the therapy is ineffective because of the drugs' brief half-lives in the oral cavity. The disadvantages of conventional therapy can be efficiently avoided by using a mucoadhesive in- situ gelling system.

   The natural polymers employed for in situ formation of oral drug delivery systems are pectin, xyloglucan, and gellan gum. It has been reported that an oral in situ gelling pectin formulation has the potential for sustained delivery of paracetamol. The key benefit of utilizing pectin in these formulations is that it dissolves easily in water, negating the need for organic solvents in the formulation. It is reported on the use of in situ gelling gellan formulation as a vehicle for theophylline oral administration. Gellan solution with combination of sodium citrate and calcium chloride made up the gellan formulation. When taken orally, the stomach's acidic environment releases calcium ions, which cause gelation of gellan, forming a in-situ gel. Gellan formulations of theophylline showed greater absorption and a sustained drug release profile in rats and rabbits when compared to the commercial sustained release liquid dosage form.[45,46]

2. **Ocular drug delivery:** Conventional drug administration methods sometimes result in poorer bioavailability and much decreased therapeutic efficacy due to the rapid turnover and dynamic nature of tear fluids, which causes fast medication clearance from the eyes. Ocular bioavailability problems have been addressed with ocular in situ gel-based solutions. These methods based on in-situ gels may allow for prolonged drug release, and the residence period of the produced ocular gels may be increased. Natural polymers

including gellan gum, alginic acid, and xyloglucan are the most frequently employed polymers for in- situ gels based ocular administration. Various chemicals, including antibacterial agents, anti-inflammatory agents, and autonomic medications used to alleviate intraocular tension in glaucoma, have been administered locally into the eye. Because of the rapid drugs removal from the eye caused by high tear fluid dynamics and turnover, conventional administration techniques frequently result in low bioavailability and therapeutic response. In order to address issues related to bioavailability, ophthalmic in situ gels was developed.[45,46]

3. **Nasal drug delivery**: A variety of nasal in situ polymeric gels are inserted into the nasal cavity as low-viscosity polymer(s) solutions. When these solutions come in touch with the nasal cavity's mucosal epithelium, they change physically and solidify as in-situ gels. Compared to conventional liquid nasal drops, in situ polymeric gels enable slower and continuous medication release and a longer duration of contact between the medicines in the gel and the absorptive intranasal region. Ion-activated (e.g. gellan gum), pH-triggered (e.g. cellulose acetate phthalate, carbopol) and temperature-dependent (e.g. Pluronics, Tetronics, and polymethacrylates), are the three categories into which in situ gelling systems can be divided. The main benefit of in situ gels over ordinary gels is their ease of administration in terms of precise and reproducible dosage. They also have the advantage of being easily instilled in liquid form, which allows the formulation to stay on the nasal cavity surface for longer because of gelling.[45,46]

4. **Dermal and transedermal drug delivery**: Topical transdermal drug administration has several important advantages over conventional drug delivery, including a lower risk of adverse effects, non-invasive drug delivery, and a decreased frequency of dosing, all of which can increase patient compliance. Many types of in situ gels have lately been investigated for the transdermal delivery of various drugs. The Pluronic F127 in thermally reversible gel was tested as a vehicle for indomethacin percutaneous delivery. According to in-vivo research, a 20% w/w aqueous gel might serve as a useful foundation for topical

medication delivery. Iontophoresis in conjunction with chemical enhancers led to a synergistic increase in insulin permeability.[45,47]

5. **Rectal drug delivery systems**: Despite being the most practical method of drug delivery, oral administration is not feasible from a pharmacological or therapeutic standpoint. In these situations, administering drugs for both local and systemic effects via the rectal route may be a useful substitute. Compared to other parts of the GIT, the environment in the rectum is thought to be quite consistent and stable, and it has modest levels of enzyme activity. However, because of the limited adherence to the rectal membrane and the possibility of dosage form evacuation, irregular medication absorption might pose a problem for the rectal cavity. These can stop dosage form leaks, which happen often when rectal suspensions and enemas are administered. Several kinds of drugs that are manufactured as liquid, semisolid (ointments, creams, and foams), and solid dose forms (suppositories) can be administered via the rectal route. The anti-inflammatory medication acetaminophen was created as a rectal in situ gel using a combination of poloxamer 407, poloxamer F188, and polycarbophil, which are synthetic polymers that form an in situ gelling liquid suppository. This method is thought to be effective and enhances bioavailability. [46,48]

1.5.3 Advantages and disadvantages of *in- situ* gel [49,50,51]

Advantages of *in-situ* gel:

1. Allow sustained and controlled drug release.
2. Convenience of administration of drug.
3. May be given to old patients and patients who are unconscious.
4. Helps in decreasing the wastage of drug.
5. Helps to improve bioavailability of drug.
6. In situ gels can also be designed with bio-adhesive properties to enable non-invasive drug delivery, particularly across mucous membranes.
7. Since natural polymers are used, they offer biocompatibility and biodegradation.
8. Reduce the frequency of doses and toxicity of the drug.

9. The structures of synthetic polymers are often well specified and can be altered to produce acceptable functionality and degradability.

Disadvantages of *in-situ* gel:

1. The drug's sol form is more prone to deterioration.
2. The possibility of stability issues arising from chemical deterioration.
3. Eating and drinking may be restricted for a few hours after taking the medicine.
4. It is possible that the amount and consistency of drug loading into hydrogels will be restricted, especially when it comes to hydrophobic medicines.

1.5.4 SUITABLE DRUG CANDIDATES FOR *IN-SITU* GEL: [52]

1. Limited absorption capacity in the gastrointestinal system, such as in the case of riboflavin and levodopa.

2. Drugs that are primarily absorbed from stomach and upper section of GI tract, e.g., cinnerazine.

3. Drugs that have a localized effect on the stomach, such as antacids and misoprostol

4. Drugs that undergo degradation in the colon include ranitidine HCl and metronidazole.

5. Drugs that affect normal colonic bacteria, e.g., amoxicillin trihydrate

1.5.5 IDEAL CHARACTERISTICS OF POLYMERS USED IN *IN-SITU* GEL:[53]

1. It ought to be biocompatible

2. It should have mucus adhesiveness

3. Ideally, this polymer should be pseudo plastic i.e., have a low rigidity

4. It presents a high tolerance and optical activity

5. Increase the tearing behavior.

1.5.6 Polymers Used in In-Situ Gelling Systems: [50, 54, 55]

Gellan Gum: A deacetylated polysaccharide, secreted from Pseudomonas elodea, gellan gum gels in reaction to temperature or cations. When taken orally, it produces a gel in the stomach's acidic environment, useful for delivering theophylline.

Xyloglucan: Derived from tamarind seeds, this polymer gels thermally. After partial breakdown by β-galactosidase, it forms reversible gels at body temperature, effective for oral, intraperitoneal, ocular, and rectal drug administration.

Alginic Acid: A block copolymer of mannuronic and glucuronic acids, alginic acid gels in the presence of metal ions. It is biodegradable and non-toxic, making it ideal for creating ophthalmic formulations with an extended precorneal residence, which can be used on the eyes for extended drug release.

Chitosan: A biodegradable, thermosensitive polymer from shrimp and crab shells, chitosan forms gels when its aqueous solution's pH surpasses 6.2. It's employed in pH-dependent gel systems without chemical modification, by adding polyol salts.

Carbopol: A pH-dependent polymer that forms low-viscosity gels at alkaline pH. Combined with HPMC, it creates ophthalmic delivery systems for indomethacin and plasmid DNA, leveraging pH-induced precipitation.

Pectin: A polysaccharide with a galacturonic acid backbone, pectin gels in the presence of calcium ions. It’s water-soluble, forming gels in the stomach’s acidic environment, suitable for sustained drug delivery, such as paracetamol.

CHAPTER- 2

Literature Survey

Literature review

2.1 Kajal Ghosal (2020) et al. carried out research which describes the development of bio-nanocomposites using natural halloysite nanotubes (HNTs) and chitosan for sustained release of norfloxacin, an antimicrobial agent. The aim of the study was to increase the therapeutic efficacy and stability of norfloxacin through controlled release.

Halloysite nanotubes were chosen because of their effectiveness as nanochemical carriers. Norfloxacin was incorporated into HNTs by vacuum processing and sonication, and incorporated nanotubes were characterized using techniques such as X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and transmission electron microscopy (TEM). These studies confirmed the success of the injections.

HNTs were then incorporated into the chitosan matrix and the nanocomposites were prepared by solvent casting and freeze drying. Scanning electron microscopy (SEM) showed that the surface of the nanocomposites was compacted and rugged, indicating good integration of loaded HNTs The composites showed good antimicrobial activity and biocompatibility in cytotoxicity studies.

In vitro release studies showed that the HNT/chitosan nanocomposites released norfloxacin consistently and remained stable under various aqueous conditions. These findings suggest that the synthesized bio-nanocomposite could be a promising vehicle for controlled drug delivery, offering increased therapeutic efficacy.[56]

2.2 Alapan Paul (2022) et al. investigated the development and characterization of composites made of halloysite nanotubes (HNTs) and chitosan for drug delivery were prepared by the solvent casting method and their mechanical and thermal chemical extraction properties were investigated. Characterization techniques such as Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and scanning electron microscopy (SEM) have been used to identify HNTs in the chitosan matrix.

Solubility and elimination studies focused on diclofenac, showing that the combination provided a sustained release rate, useful for sustained therapeutic drug concentrations exist for a long time .The biocompatibility and non-toxicity of the formulation were confirmed by cytotoxicity tests, indicating potential safe biomedical applications

Overall, the study highlights the potential of HNT/chitosan blends as effective drug delivery systems due to improved mechanical properties, biocompatibility, and chemical stability.[57]

2.3 Peng Yuan (2015) et al. offered an overview of halloysite nanotubes (HNTs) focusing on their distinct structural and chemical properties. Halloysite is an occurring clay mineral, with a tube structure that provides a large surface area and customizable chemistry.

The study showcases the ranging applications of HNTs in fields such as drug delivery, environmental protection and nanocomposites. Their biocompatibility and ability to control the release of substances make them particularly suitable for purposes. Moreover HNTs are utilized in settings to remove pollutants owing to their adsorption capabilities.

Recent progress has concentrated on enhancing HNTs through functionalization to broaden their application spectrum. These enhancements improve their compatibility with polymers resulting in the creation of nanocomposites with mechanical and thermal characteristics. The paper also addresses opportunities and challenges including the necessity for production methods and further exploration of HNTs potential in novel application domains.

In essence the article underscores the adaptability and promise of HNTs positioning them as a material with potential, for technological advancements.[58]

2.4 H. Nagahama et al. suggested that chitosan which is derived from chitin and gelatin are novel biomaterials. The chitosan/gelatin membranes were made by mixing chitosan hydrogel with gelatin solution with agitation in different ratios as m-1, m-2, m-3, m-4 for gelatin mixture ratio of 0.3,0.5,0.7,1 The chitosan/gelatin membrane were characterized by X-Ray diffraction(XRD), scanning electron microscopy(SEM), transmission electron microscopy(TEM), swelling study, dissolution study, tensile strength determination and thermal study. The surface morphology of the chitosan/gelatin membranes and the chitosan membrane was discovered from the SEM pictures to be relatively smooth. The XRD study indicated that the interaction and compatibility between chitosan and gelatin in the membrane was satisfactory. The reduced peak intensity ratio upon

addition of amorphous gelatin was due to reduced crystallinity of chitosan. The elongation of chitosan/gelatin membranes m-2, where the mixture ratio of gelatin was 0.50 and the stress of the membrane m-1, where the mixture ratio of gelatin was 0.30, were found to be higher than those of the other membranes, in dry condition. Also, the membranes m-2, with a gelatin mixture ratio of 0.50 under wet conditions had greater elongation and stress than the other membranes. These findings demonstrated the usefulness of these membranes for biomedical applications. The swelling study indicated that the swelling ratio of the chitosan/gelatin membranes was almost consistent over the course of 24 h. The chitosan/gelatin membrane, m-2, had a lower thermal stability than the chitosan membrane because it contained amorphous gelatin. The chitosan/gelatin membrane exhibited minimal variation of dissolution ratio, even after heating for 24 hours with water because of strong H-bonding network between chitosan and gelatin which led to their great compatibility. According to cell attachment studies, chitosan/gelatin membranes exhibit high biocompatibility in terms of osteoblastic cell culture. So the novel chitosan/gelatin membrane has some useful application in biomedical field.[59]

2.5 K.E. Crompton (2007) et al. stated that since the adult mammalian central nervous system (CNS) has a limited potential for self-healing, cell replacement therapies are being researched to repair CNS damage. However, to control differentiation and neurite outgrowth, implanted cell growth must be regulated. So, in order to support cells during implantation, suitable scaffolding materials are needed. As cellular scaffolds, hydrogels have a number of benefits, including similar mechanical properties to soft tissue, low interfacial tension, and non-toxic aqueous solvents. Although chitosan is extensively used, lacks compatibility with neurons. A novel chitosan solution was developed that can be injected during surgery without any surgical destruction. If chitosan and chitosan combined with GP (polylysine) are to be employed in the nervous system, they still need to be made more compatible with neurons. Chitosan was covalently bound to optimum concentration of polylysine in order to increase its biocompatibility and affinity for neurons. In order to increase neuron affinity, the study examines cell-hydrogel interactions in various conditions, with the ultimate aim of producing an injectable scaffold that can help repair damaged neuronal connections in the brain through cell replacement therapies. Hydrochloric acid was used to dissolve the chitosan, after which it was filtered, heated with carbon, and then filtered once more. The chitosan was then washed, freeze-dried, and precipitated with potassium hydroxide. Specific molar ratios of PDL, EDAC, and 4-azidoaniline were combined in water and reacted for

4 hours. The chitosan-PDL product underwent washing, freeze-drying, and further experiments and characterisation . This product was used for the following characterisation and experiments: characterization, rheology studies, osmolarity measurement, 2-D cell culture on a film, 3-D cell culture in a gel. For characterization, X-ray photoelectron spectroscopy was done.. Chitosan/GP gelation takes time, affecting its mechanical characteristics. Chitosan concentration had an impact on how stiff the gels were, with lower concentrations being more comparable to the stiffness of brain tissue. The composition chosen by the researchers as being appropriate for brain cell applications is 0.8% chitosan/GP. Neurite outgrowth was similarly inhibited when cells were cultivated on chitosan/GP films, compared to polylysine. Cell survival was substantially decreased. It was discovered that the high osmolarity of chitosan/GP hydrogels may have an impact on neurite outgrowth and cell viability. Cell survival and neurite outgrowth were improved when cells were cultured on 0.5% chitosan/GP, which had an osmolarity that was closer to extracellular fluid. Biocompatibility and neurite outgrowth were not improved by functionalizing chitosan with polylysine. Compared to 2D culture, cell survival was higher when cells were grown in a three-dimensional (3D) gel that was surrounded by the chitosan/GP hydrogel and neurite outgrowth was also decreased. Depending on the dose, adding polylysine to 3D cultures had different impacts on cell survival and neurite outgrowth. Good biocompatibility was demonstrated by the 0.5% chitosan/GP hydrogel, and adding polylysine did not enhance cell survival or neurite outgrowth. In conclusion, chitosan/GP is a promising substrate and scaffold for cortical cells in neural tissue engineering applications, including injectable scaffolds. It exhibits good cell adhesion properties and neuron compatibility at low concentrations, supporting neurons in a 3-dimensional hydrogel environment similar to the extracellular matrix (ECM), and covalent attachment of polylysine to chitosan improves cell survival without affecting neurite outgrowth.[60]

2.6 Maximiano P. Ribeiro, MD (2009) et al. stated that skin lesions are traumatic events that cause various negative effects and despite advances in therapy, infections still pose a significant risk to burn patients.. Wound healing involves a series of complex biological processes, which includes a wide range of mechanisms. However, the regeneration of skin is often imperfect, resulting in the formation of scar tissue. Biocompatible materials on which cells can adhere and proliferate are needed for the replacement of damaged tissues. Cell adhesion and proliferation are greatly influenced by the surface characteristics of the materials, including surface charge, surface free energy, density, and polarity. To replace injured tissues, biocompatible materials are required,

on which cells may adhere and proliferate. According to present study, it is evaluated that chitosan, which can be chemically modified to improve its solubility and expand its applications, can be used in skin tissue engineering and tissue healing. For instance, it has been demonstrated that chitosan-cotton promotes the infiltration of immune cells at the wound site, which speeds up wound healing. Additionally, chitosan has been included into dressings for infected burns and has been utilised to transport bioactive compounds, such as growth factors. To characterize chitosan hydrogel (CH) morphology, scanning electron microscopy, cell proliferation, determination of hydrogel cytotoxicity, animal experiments, histological study, evaluation of the wound size, and statistical analysis was done. The hydrogel's porous structure was discovered by SEM analysis, which suggests that molecules can diffuse easily and that water is retained well. The hydrogel demonstrated good cytocompatibility and supported the growth of fibroblast cells. When compared to the control group in the animal studies, the chitosan hydrogel-treated group showed improved wound healing results. Histological analysis showed that the hydrogel did not cause inflammation or abnormalities in the organs. Epithelial layer thickness increased progressively and all the skin lesions exhibited complete epithelialization after 21days. The study's findings therefore showed the chitosan hydrogel's potential for use in tissue regeneration and wound healing.[61]

2.7 Van Dyke et al. (2005) in their literature review on risk factors for periodontitis have highlighted multiple risk determinants contributing to the initiation and progression of this disease. Overall they have stressed that microbial plaque is the primary etiological agent responsible for periodontitis. Certain bacteria such as Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans are instrumental in the pathogenesis of periodontitis. Host immune response is also an important factor in determining the severity of disease, with genetic predisposition altering susceptibility. Certain genetic polymorphisms predispose a patient to an exaggerated inflammatory response leading to destruction of the surrounding tissue. Specific systemic diseases such as diabetes mellitus increase susceptibility to periodontitis due to impairment in immune response and wound healing. Other general conditions such as stress and malnutrition also increase risk. Socioeconomic variables such as low education levels or income limit access to oral health care services potentially predisposing individuals to increased risk by downmodulating oral hygiene practices. This literature review on host modifying factors provides evidence regarding the multifactorial nature of periodontitis, with microbial, host modifying, environmental and general disease factors orchestrating susceptibility and severity of periodontal

disease. Thus a comprehensive management protocol targeting all these determinants should be adopted if we are interested in successful prevention and control of this condition.[62]

2.8 Legrand et al. (2005) reported the clinical efficacy, safety, and pharmacological profile of aceclofenac, which is a phenylacetic acid derivative non-steroidal anti-inflammatory drug (NSAID). It is mainly used against conditions such as osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis, acting as a dual inhibitor of cyclooxygenase (COX) enzymes COX-1 and COX-2 which in turn dependently reduce prostaglandin synthesis leading to inflammation and pain reduction.

Clinical trials have demonstrated the analgesic and anti-inflammatory efficacy of Aceclofenac, along with a better gastrointestinal tolerance as compared to other NSAIDs. Its anti-inflammatory effect is due to its modulation of the release of inflammatory mediators such as Interleukins & tumor necrosis factor-alpha (TNF-α). Aceclofenac has an enhanced safety profile with minimal chances of gastrointestinal & renal side effects, thereby making it more suitable for chronic inflammatory conditions in long term basis.

Legrand also talks about pharmacokinetics. Aceclofenac is rapidly absorbed and widely distributed, which accounts for its fast acting and prolonged analgesic effect. Based on all of this, the article concludes that Aceclofenac is a good drug candidate for inflammatory pain management, and balance between efficacy and safety is being maintained especially in patients receiving long-term NSAID treatment.[63]

2.9 Harish et al. (2009) explored the development of novel drug delivery systems to improve the treatment of oral fungal infections. Clotrimazole is an antibiotic that can be incorporated into the in-situ gel that is liquid at room temperature but turns into a gel at body temperature and ensures prolonged retention and localized action.

There are various polymers used to produce in situ gels, such as poloxamer 407 and carbomer 934P, which are important for obtaining thermosetting properties. In situ gel allows for continuous release of clotrimazole, improving treatment and patient compliance by reducing the frequency of use compared to conventional methods. In their study, Harish et al. mentioned several key parameters, including gel temperature, viscosity, drug release profile, antifungal activity, and stability. The prepared gel showed a suitable gelation temperature for oral administration and

sufficient viscosity to adhere to mucosal surfaces. A sustained release profile indicates prolonged release of the drug, providing consistent antifungal activity against Candida species. Stability studies confirmed that the gel maintained its physical and chemical properties over time. This review concludes that in situ gels containing clotrimazole offer a promising approach for the effective treatment of oral candidiasis. This novel delivery system increases the therapeutic value of clotrimazole by providing sustained drug delivery, increased bioavailability, and improved patient compliance, thereby improving clinical outcomes in the treatment of fungal infections.[64]

2.10 Nagarwal et al. (2008) provided a comprehensive analysis of a type of novel oral drug delivery approach aimed at improving drug efficacy and patient compliance. Phase transition system (PTS) uses a sol-gel transfer mechanism that occurs in response to physiological conditions to enhance drug delivery in the oral cavity. Nagarwal et al. explained the basic principles of in situ gels, in which a solutions transforms into gel when exposed to specific stimuli such as temperature, pH, or ionic strength. For oral delivery, coagulation and pH-sensitive systems are ideal. The authors emphasize the importance of choosing the right polymers, such as poloxamer, which exhibits thermoreversible solubility, and carbopol, which has a date that responds to changes in pH. Nagarwal et al. also described the advantages of oral gels at the site: ease of administration, long-term retention in the workplace, and drug delivery capability. These benefits lead to improved bioavailability, reduced dosage, and better patient compliance. This review also discusses the ability of in-situ gels to protect drugs from the harmful environment of the stomach, thus maintaining their effectiveness. Several case studies are presented that demonstrate the use of gels in the field to deliver a variety of therapeutic agents, including antifungal, antibacterial, and anti-inflammatory drugs. These studies demonstrate the successful *in-situ* fabrication of gels with desired physical properties such as appropriate incubation temperature, viscosity, and drug release profile. For example, the antifungal power of clotrimazole in topical skin form is considered important for the treatment of oral candidiasis. The authors also addressed the challenges associated with developing gels in the PTS setting, such as ensuring behavioral consistency, improving drug loading, and maintaining long-term stability. This highlights the need for in vitro and in vivo evaluations to ensure the safety and efficacy of these systems. In conclusion, the review demonstratd the potential of phase transfer systems in oral tissues as a drug delivery system. This technology greatly improves local and stable drug delivery, improving treatment outcomes and patient compliance.[65]

Chapter 3

Aims and objectives

AIM:

- To formulate and evaluate Aceclofenac loaded in-situ gel for the treatment of Periodontitis.

OBJECTIVES:

- Selection of drug and polymer
- Preparation of drug (aceclofenac) loaded in-situ gel
- Characterization with the help of SEM, TEM, TG/DTA, XRD, FTIR
- Evaluation of the Syringibility, pH, melting point, spreadibility, swelling, gelation time and temperature
- In-vitro drug realease study
- Conduction of anti-oxidation study

PLAN OF WORK:

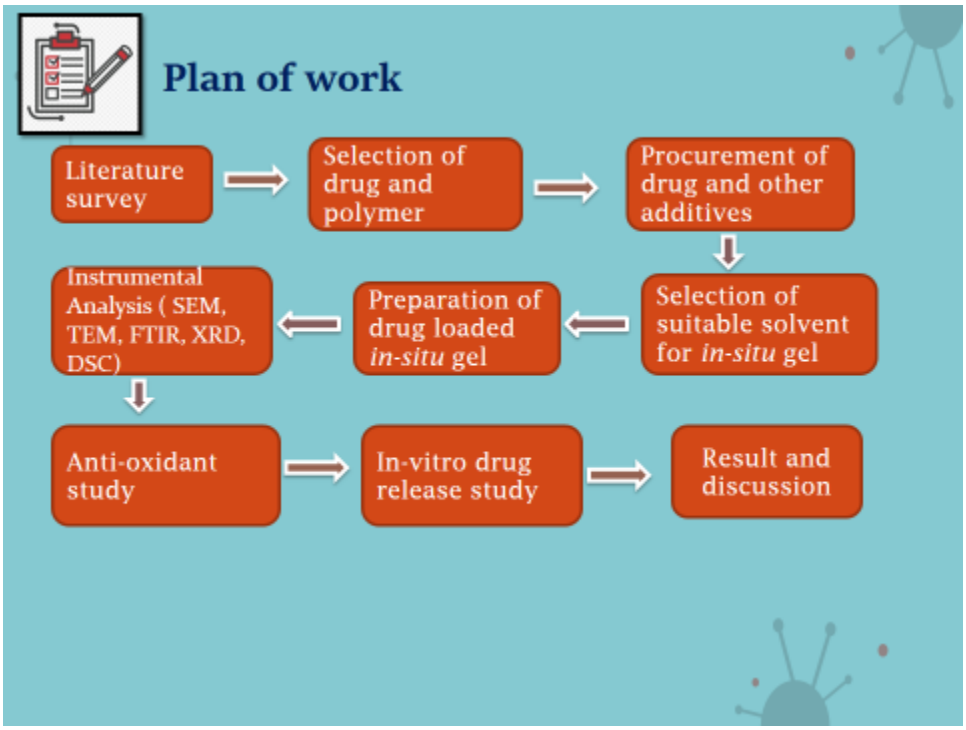


Figure 5- Flow chart showing plan of work

Chapter 4:

Materials and Reagents

4.1 Aceclofenac [66,67,68,69]

- **Drug class-** Nonsteroidal anti-inflammatory drugs (NSAIDs)
- **Description-** Aceclofenac is a kind of oral non-steroidal anti-inflammatory medicine (NSAID) with strong anti-inflammatory and analgesic effects; it is the proper treatment for rheumatoid arthritis, ankylosing spondylitis, and osteoarthritis. The placebo-controlled trials have shown that it has a higher anti-inflammatory effect or at least it gave the same effect as the standard NSAIDs. Aceclofenac really suppresses cyclo-oxygenase (COX) enzyme which makes prostaglandins, inflammation mediators, arising the synthesis of heat, pain, edema, and inflammation. Aceclofenac is around one of the members of the BCS Class II which are poorly soluble in water. It has good permeability which can move across the articular cartilage. For the joints, where the cartilage is lost, causing joint pain, joint stiffness, crepitus, and local inflammation of the joint it has a high permeability to the joints.

- **Molecular Formula-** C16H13Cl2NO4

- **Chemical Structure-**

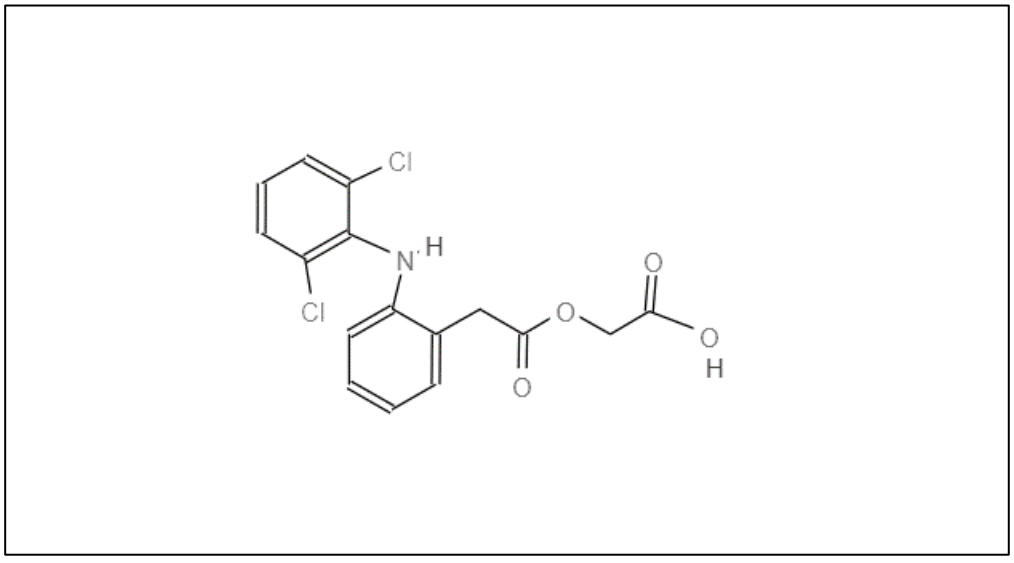


Figure 6- Chemical structure of aceclofenac

- **IUPAC Name-** 2-[2-[2-(2,6-dichloroanilino)phenyl]acetyl]oxyacetic acid

- **Molecular Weight-** 354.2 g/mol

- **Melting Point-**

- **Solubility-** Aceclofenac is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF). The solubility of aceclofenac in ethanol is approximately 10 mg/ml and approximately 30 mg/ml in DMSO and DMF. Aceclofenac is sparingly soluble in aqueous buffers.
- **Mechanism of action-** Aceclofenac inhibits COX-2, which in turn reduces the generation of different inflammatory mediators from the arachidonic acid (AA) pathway, such as prostaglandin E2 (PGE2), IL-1β, and TNF. It is believed that diclofenac, which is derived from aceclofenac, mediates the inhibition of IL-6. Reactive oxygen species generation is reduced by the suppressed activity of inflammatory cytokines. Human articular chondrocytes have been demonstrated to produce less nitrous oxide when exposed to aceclofenac. Furthermore, aceclofenac inhibits the expression of L-selectin (CD62L), a lymphocyte-expressed cell adhesion protein, which prevents neutrophils from adhering to endothelium. The synthesis of glycosaminoglycan in human osteoarthritic cartilage is thought to be stimulated by aceclofenac, possibly via the inhibition of IL-1 production and activity. 4'-hydroxyaceclofenac produces the chrondroprotective effects by inhibiting the release of proteoglycan from chrondrocytes and suppressing IL-1-mediated promatrix metalloproteinase-1 and metalloproteinase-3 synthesis.
- **Adverse effect-**

  -Common side effects of Aceclofenac include: Dizziness, indigestion, abdominal pain, nausea, diarrhea, increase liver enzymes.

-Less common side effects of Aceclofenac include: Flatulence, gastritis, constipation, vomiting, mouth ulcers; Itching, rash, dermatitis, urticarial, increased blood urea, increased blood creatinine.

-Rare side effects of Aceclofenac include: Anemia, anaphylactic reactions (including shock), hypersensitivity, visual disturbances, shortness of breath, black stools, facial swelling.

- **Route of elimination-** The majority of the drug's clearance occurs in the urine, where elimination processes account for 70–80% of the total. About two thirds of the dosage is eliminated in the urine, primarily as aceclofenac in its hydroxylated and glucuronidated forms. Feces contain around 20% of the dosage that is eliminated.

- **Clearance-**The mean clearance rate is approximately 5 L/h

4.2 Chitosan [70,71, 72]

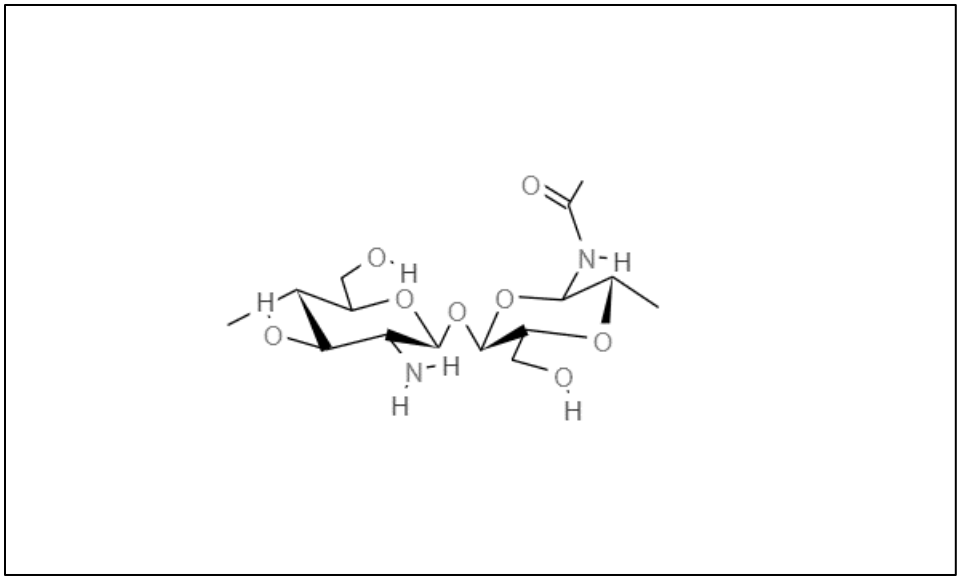
- **Category-** Linear polysaccharide
- **Description-** Chitosan is a linear polysaccharide composed of acetylated N-acetyl-D-glucosamine and randomly distributed deacetylated D-glucosamine linked together with β-(1→4) bonds. The chitin exoskeletons of shrimps and other crustaceans are converted to chitosan by soaking them in sodium hydroxide, an alkaline chemical.
- **Synonyms-**

  Poliglusam

  Chicol

  Flonac C

- **Chemical Structure-**



Figures 7: Chemical structure of chitosan

- **IUPAC Name-**
  Methyl N-[(2S,3R,4R,5S,6R)-5-[(2S,3R,4R,5S,6R)-3-amino-5-[(2S,3R,4R,5S,6R)-3-amino-5-[(2S,3R,4R,5S,6R)-3-amino-5-[(2S,3R,4R,5S,6R)-3-amino-5-[(2S,3R,4R,5S,6R)-3-amino-5-[(2S,3R,4R,5S,6R)-3-amino-4,5-dihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-4-hydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-4-hydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-4-hydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-4-hydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-4-hydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-2-[(2R,3S,4R,5R,6S)-5-amino-6-[(2R,3S,4R,5R,6R)-5-amino-4,6-dihydroxy-2-(hydroxymethyl)oxan-3-yl]oxy-4-hydroxy-2-(hydroxymethyl)oxan-3-yl]oxy-4-hydroxy-6-(hydroxymethyl)oxan-3-yl]carbamate
- **Molecular Formula-** C56H103N9O39
- **Molecular Weight-** 1526.5 g/mol
- **Physical Description-** Yellow powder, solid
- **Chemical Classes-** Biological Agents -> Polysaccharides
- **Hazards Summary-** No adverse effects except minor skin irritation observed in toxicity studies
- **Melting Point-**102.5°C
- **Odor-** Odorless

- **Solubility-** dilute aqueous acid (pH <6.5).: soluble

4.3 Sodium-β-Glycerophosphate [73,74, 75]

- **Description-** Sodium β-glycerophosphate pentahydrate is a phosphatase inhibitor. It delivers phosphate ions to osteoblasts and encourages the mineralization of the bone matrix. It is also used in the production of scaffolds and hydrogels that are used in tissue engineering as well as for cell proliferation. Additionally, it serves as an addition in isolation media by providing phosphate ions for its isolation. In vitro, it can be employed for altering metabolic activity of bone cells towards favoring mineralization.
- **Synonyms-**

  Sodium-β-Glycerol Phosphate Hydrate,

  β-GP,

  β-Glycerolphosphate,

  Disodium β-glycerol phosphate

- **Chemical Structure-**

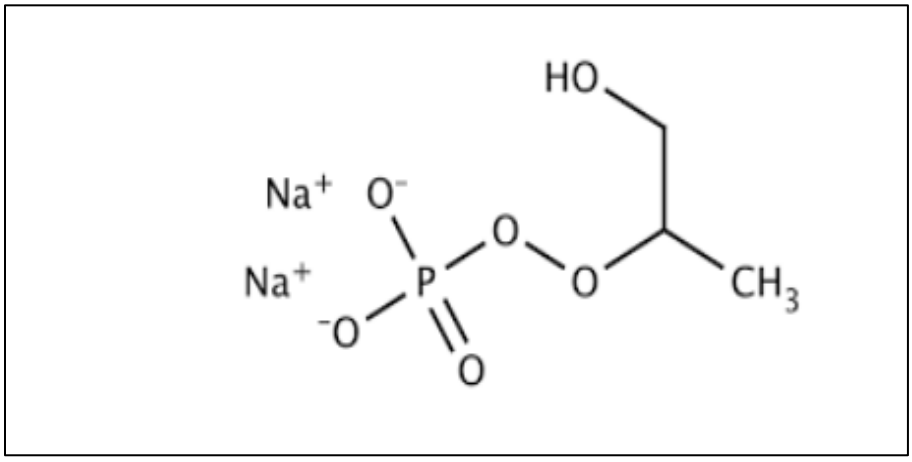


Figure 8: Chemical structure of Sodium-β-glycerophosphate

- **IUPAC Name-** 1,3-dihydroxypropan-2-yl dihydrogen phosphate;sodium;pentahydrate

- **Molecular Formula-** $C_3H_7Na_2O_6P.5H_2O$
- **Molecular Weight-** 306.11 g/mol
- **Physical Description-** White crystalline powder
- **Chemical Classes-** Organic acid, metal salts.
- **Melting Point-** >300°C
- **Odor:** Odorless
- **Solubility:** Freely soluble in water

4.4 Sodium bicarbonate [76,77,78]

- **Description-** Sodium bicarbonate (IUPAC name: sodium hydrogencarbonate), commonly known as baking soda or bicarbonate of soda, is a chemical compound with the formula NaHCO3. It is a salt composed of a sodium cation (Na+) and a bicarbonate anion (HCO3−). Sodium bicarbonate is used for the symptomatic treatment of acid indigestion, upset stomach and heartburn, as well as the treatment of metabolic acidosis in cases like severe renal disease or circulatory inadequacy due to shock.
- **Class-** Organic carbonic acids and derivatives
- **Synonyms-**

  Sodium bicarbonate

  Sodium hydrogen carbonate

  Baking soda

  Sodium hydrogencarbonate

- **Chemical structure-**

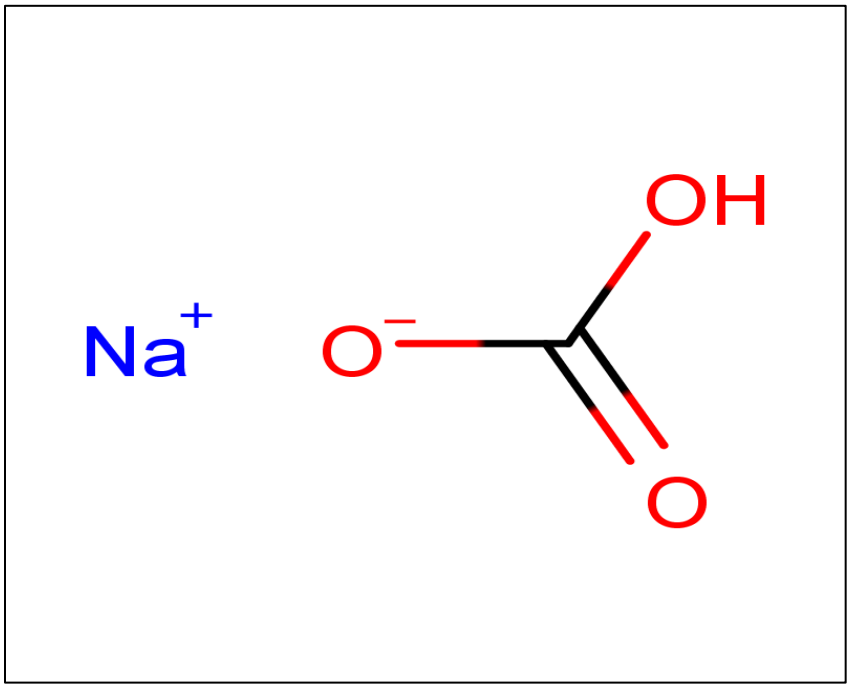


Figure 9: Chemical structure of Sodium bicarbonate

- **Molecular Formula-**

  NaHCO3

  CHNaO3

- **Molecular Weight-** 84.007 g/mol
- **Physical Description-** White crystalline powder
- **Class-** Organic carbonic acids and derivatives
- **Adverse effect-**

  -Eyes: Causes severe eye irritation.

  -Skin: Causes skin irritation.

  -Ingestion: Harmful if swallowed.

  -Inhalation: Irritation to the respiratory system.

- **Odor:** Odorless
- **Melting point:** 50°C
- **Density:** 2.1 g/cm³
- **Taste:** Cooling, slightly alkaline taste
- **Solubility:** Soluble in water. Insoluble in ethanol

4.5 Halloysite Nanotube: [79,80,81]

- **Description:** An aluminosilicate clay mineral having the structural formula $Al_2(OH)_4Si_2O_5 \cdot nH_2O$, halloysite is mostly found in the form of nanotubes (HNTs). With an exterior diameter ranging from 30 to 190 nm and a length of 0.5 to 2 μm, these nanotubes possess an unusual tubular structure because of the difference in tetrahedral SiO4 and octahedral Al(OH)3 layers. Because HNTs are easily functionalized, they can be used in the disciplines of biology, environmental science, and catalysis, improving features like stability. By lowering the use of pesticides and promoting plant development, they can serve as nano containers for the regulated release of substances and enhance agricultural operations. HNTs are also employed in tissue engineering, medication delivery, and packaging.
- **Synonym:** Kaolin Clay
- **Molecular Formula:** $Al_2Si_2O_5(OH)_4 \cdot 2H_2O$
- **Molecular Structure:**

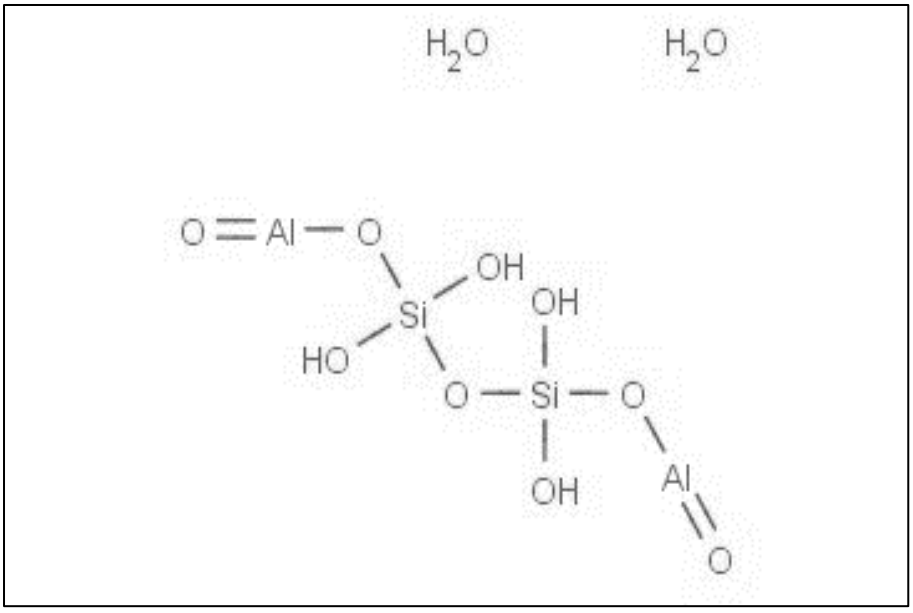


Figure 10: Chemical structure of Halloysite nanotube

- **IUPAC:** [dihydroxy(oxoalumanyloxy)silyl] oxy-dihydroxy-oxoalumanyloxysilane
- **Form:** Crystalline, elongated mineral that can be found both hydrated and dehydrated. When it is hydrated, it is made up of curving sheets made of unit layers of kaolin.
- **Colour:** White to gray or brown solid
- **Molecular weight:** 246.19 g/mol
- **Density:** 2.53(True specific gravity)
- **Pore Size:** 1.26-1.34 ml/g pore volume
- **Synonyms:** Kaolin clay
- **Appearance:** White powder
- **Diameter:** 50-300 nm
- **Length:** 1-10μm

Chapter 5

Method and Characterization

5.0 Pre-formulation studies

5.1 Physical characterization

The physical characters like colour, texture of the drug was identified by visual examination

5.2 Melting point

5.2.1 Evaluation of melting point:

One end of the capillary tube was sealed by applying heat with the help of Bunsen burner. Aceclofenac was then put inside the capillary tube through the open end of the tube. After a certain amount of aceclofenac was put inside the capillary tube, the tube gently tapped on the closed end to fill the tube with 1-2 mm height. In the tube holder of the device (), the capillary tube was placed to evaluate the melting point of the drug. The knob that controls the temperature was properly adjusted. Both the initial temperature when the drug just started to melt and the final temperature when the drug completely melted was determined and noted. By taking the average of both the temperatures, the melting point of drug was determined. To obtain an optimum result, the procedure was repeated thrice.

5.3 Standard curve of aceclofenac

5.3.1 Preparation of standard curve for aceclofenac

5.3.1.1 Preparation of 0.01N phosphate buffer, pH 6.8

1.387 gm of Potassium dihydrogen orthophosphate and 3.508gm of Potassium dihydrogen orthophosphate was taken and dissolved in water to obtain 100ml of 0.01N phosphate buffer pH (6.8).

5.3.1.2 Determination of absorption maxima

The UV absorption maximum of aceclofenac (10 µg /ml) dissolved in phosphate buffer pH (6.8) was determined by scanning the solution in the region of 200-400 nm with a UV Spectrophotometer (UV-1800, Shimadzu, Japan).

5.3.1.3 Determination of standard curve of aceclofenac

100ppm stock solution was prepared by dissolving 10 mg of aceclofenac accurately weighed in 100 ml phosphate buffer 6.8 pH. From the stock solution, aliquots of 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20µg/m1 were scanned at 273.60nm (max) using a UV Spectrophotometer (UV-1800, Shimadzu, Japan) with spectral bandwidth of 1 nm ± 0.3 nm wavelength precision. All samples were analyzed three times to reduce error.

5.4 Method of preparation

5.4.1 Preparation of HNT for loading of drugs

20 g of HNT was taken in a petridish without any cover. The petridish was then placed inside a vacuum dryer for 2 hours, under full vacuum at 60°C. After 2 hours, the petridish was removed from vacuum dryer and the dried HNT was transferred in an eppendorf and was kept inside a dessicator.

5.4.2 Loading of aceclofenac inside HNT

Table 1 : Different formulations containg HNT and aceclofenac.

Formulation	Amount of HNT (mg)	Amount of aceclofenac (mg)
F1	100	50
F2	100	100
F3	50	100

Three formulations F1, F2 and F3 were prepared in ratios given in table_. First the given amount of drug was dissolved in 5 ml ethanol, then given amount of HNT and 18 ml of water was added. After few minutes of stirring of the dispersion with glass rod, it was sonicated for 30 minutes. Then the solution was kept in a petridish and the petridish was placed inside a vacuum dryer for 2 hours under full vacuum at 60°C. After the petridish was taken out from the vacuum dryer, it was kept inside dessicator overnight.

5.4.3 Freeze drying of HNT

The solution was transferred in a round bottom flask (RBF) and the RBF was placed inside a lyophilizer for 6 hours, whose temperature was set at -40°C. After freeze drying was completed

the dried sample was scrapped and placed inside a eppendorf and the eppendorf was kept inside a dessicator.

5.4.4 Preparation of in-situ gel

Two solutions, i.e, solution A and B were prepared. Solution A was prepared by dissolving 100 mg of MMW chitosan in 4.5 ml of 35% HCl. Solution B was prepared by dissolving 25 mg of GP and 21 mg of $NaHCO_3$ in 0.5 ml of distilled water. After preparation of solution A and solution B, it was kept inside a refrigerator at 4°C. After the solution attained 4 C, Solution B was added dropwise to solution A with continuous stirring. In that solution C (containing drug loaded HNT) was added.

After adequate stirring of the solution, the solution was transferred in a test tube. The test tube was then placed in a water bath whose temperature was controlled at 37°C. The time taken by the solution to transform into gel was noted.

5.5 Characterization of prepared formulation

5.5.1 Spreadability Test

After the gel was weighed to a maximum of 0.5 g, it was deposited on glass-coated graph paper. Next, we placed a second glass on top of the mass of gel. The diameter length of many sides was measured in order to calculate the average gel diameter. After that a load of 100 g was placed on the second glass and allowed to stand for 30 seconds and again the diameter length of many sides was measured to determine the average increased diameter.

5.5.2 Stability Testing:

The effects of ambient temperature and low temperature on *in-situ* gel formulations were investigated by preparing two sets of samples labeled F1, F2 and F3. One set of the sample was stored in a refrigerator at 4°C, while the other set was stored inside a room at 25±2°C and 60±5% humidity. These storage conditions were maintained for one month to evaluate the stability and performance of the gels under ambient temperature and low temperature.

5.5.3 Fourier Transform Infrared Spectroscopy

A sampling technique called attenuated total reflectance (ATR) allows samples, whether they are liquid or solid, to be examined directly without additional preparation. It creates an evanescent wave that enters the sample and provides important molecular information by using total internal reflection. Since ATR makes it easier to measure almost any substance by allowing for the neat analysis of both solid and liquid samples, it is frequently used in conjunction with Fourier transform infrared (FTIR) spectroscopy together known as ATR-FTIR spectroscopy. It is an efficient analytical technique used for the investigation of the chemical property and composition of various materials. FTIR was employed to determine whether a drug and a polymer have any relationship. To learn more about the correlations, individual infrared spectra of the drug, F2 and F2G were acquired. Perkinelmer spectrum software was used for analysis on a Spectrum Two FT-IR Spectrometer. Scanning was done across a range of 4000-400cm^{-1} for three minutes. Using the attenuated total reflectance (ATR) method, the samples were put against a high refractive index prism and internally reflected in the prism. The interaction of the infrared light with the sample causes the chemical bonds to vibrate at different frequencies. In order to determine which functional groups are present in the sample and to learn more about the interactions between the different sample components, the resulting absorption spectra is recorded and examined.

5.5.4 TEM

The morphology of the samples were analysed using Cryo TEM L120C ThermoFisher operating at 120 kV and 36000× magnification. A 300 mesh carbon coated grid has been taken with film side up in a pair of self clamping forcep. Suitable concentration of sample has been prepared with water. Samples were sonicated with a bath sonicator for 8-10 min. 1 drop of sample placed on the grid. It was stained with 1% phosphotungstic acid for 30 seconds, then incubated it for 30-90 seconds. Excess liquid was removed with a piece of filter paper. It was then examined in the TEM.

TEM involves the process of transmitting a high-energy electron beam through an ultrathin sample specimen. Images are created on the basis of transmission or attenuation of electrons, further being magnified for in-person micro- and nano-scale observation and study. It is one of the methods for mapping size, shape, and elemental structure in the nano-scale size range with extremely high resolution.

As such, the wavelength of an electron is much shorter compared with a photon; hence, it can focus much finer than light. Therefore, the TEM is capable of viewing particles at a much higher magnification and resolution than possible with a light microscope. In addition to this, it creates higher-resolution images compared to a scanning electron microscope that is capable of scanning and viewing only the surface of a sample. In the case of the TEM, scientists can view samples down to the atomic level, less than 1 nm.

5.5.5 SEM

The samples were sputter-coated with a thin layer of electrically conductive gold under a vacuum for charge neutralization. The SEM analysis was carried out using a Field Emission Scanning Electron Microscope (FESEM), FEI QUANTA FEG650S. The scanning acceleration voltage was 5 kV. The powdered samples were dubbed onto the mounting stubs, and the wafers were cut into specimens and were pasted onto the mounting stubs. The morphology of the powdered sample and wafers were viewed after gold sputter coating. A focusing stream of electrons are directed and scanned on the surface of a sample by a scanning electron microscope (SEM) that makes use of special detectors to deploy a spectrum of the signals generated. The electrons in the beam collide with the atoms present in the sample. These collisions give rise to a set of signals that can be utilized to detect the surface. Both real-time images can be presented on an external monitor using software that correlates the position of the beam with the electrons that are measured by the detectors.

5.5.6 Powder X-Ray Diffraction analysis (PXRD):

XRD analysis verifies the crystallographic structure of a substance. The X-Ray Diffraction analysis of In- situ gel was performed by Bruker Eco D8 advance. The samples were crushed into fine powder to obtain homogeneity and to increase the surface area for diffraction. Small amount of aceclofenac, powdered blank and powdered F2G was pressed on the sample and was placed in the instrument's sample stage one by one. When X-rays strike the atoms in the sample, they diffract, forming a diffraction pattern that is detected by a detector based on X'pert Highscore XRD software to analyses the diffraction. The samples were scanned at range of 20 from 5° to 90°.

5.5.7 Swelling Study

The capability of in-situ gel to absorb water and swell was determined by weighing the lyophilized gel (w_0) and placing lyophilized gel in perforated cap and then putting the cap in Phosphate buffer 6.8 at 37^o C. Then the weight of the wet sample (w_t) was recorded on day 1,2,3,4 and 5. The degree of swelling was calculated by using the following equation:

Swelling degree = $(W_t - W_0)/ W_0 \times 100$

Where W_0 stands for the freeze-dried hydrogel and W_t stands for the weights of the swollen hydrogel.

5.5.8 Syringeability Test

An efficient method of administering the in-situ gel to treat severe tooth cavities and periodontitis is by injecting the medication directly into the periodontal pocket using an injection needle. Using a 21 G needle, the syringeability of the in situ gels under non-physiological conditions was assessed.

Using syringe attached with 21 G needle, the solution (containing solution A, solution B and drug loaded HNT) was loaded inside the syringe to test the syringeability

5.5.9 pH Determination of *in-situ* gel:

To determine pH of in-situ gel, pH paper was used. At first some saliva was collected and spreaded uniformly over clean palm using a sterile swab. Ensure that the saliva is adequately spreaded, i.e has covered adequate area. A pH paper was taken and put in contact with the saliva for few second to get a colour on the pH paper. Compare the colour developed on the pH paper with the reference chart to determine the pH of the saliva initially.

Similarly dip the pH paper in different formulations of in-situ gels, i.e blank, F1G, F2G, F3G and wait for few seconds to develop colour on it and eventually determine the pH of the formulations. Similarly compare the colour on the pH paper with the reference chart to determine their respective pH.

5.5.10 Gelation Temperature

To determine gelation temperature of the formulation, 'tube inversion method' was employed. In a 20 ml glass tube, about 5 ml sample was filled and the tube was placed in a temperature adjustable water bath. The temperature was increased from 30^{o}C to 40^{o}C and the sample was observed for change in appearance of gel solution. At each temperature, the tube was inverted and the temperature at which no fluidity of the solution was observed was recorded as the gelation temperature. Every measurement was done three times.

5.5.11 Gelation Time:

Gelation time of the formulation was found out by visual observation. Here 5 ml of the sample was taken in a glass tube and was sealed with parafilm. The tube was then placed in a temperature controlled water bath, whose temperature was set at 37◦C. The time taken to form stable gel, with stiff consistency was noted. Every measurement was done three times.

5.5.12 Optical Microscopy:

The physical appearances of halloysite nanotube (HNT), F2 formulation and F2G (lyophilized *in-situ* gel) were examined with the help of compound light microscope. The purpose of using optical microscopy is to magnify and visualize small particles or details that are not visible to the naked eyes, allowing for detailed observation. A tiny portion of the HNT, F2, and F2G samples were placed on a glass slide and with the help of light, additional structural analysis was done under a microscope.

5.5.13 Antioxidant study

Four formulations, F2, $F1_g$, $F2_g$, $F3_g$ were soaked overnight in phosphate buffer solution of 6.8 pH. After that the soaked solutions were centrifuged at 6000 rpm for 15 minutes. With the help of micro pipette, 100µl of each stock solutions of the samples were pipetted out in microplate.

Each stock solution of the samples was diluted twice, resulting in four serial dilutions (560 µg/ml, 280 µg/ml, 140 µg/ml, 70 µg/ml). The positive control was ascorbic acid, and the negative control was DPPH solution. As a blank, distilled water was utilized. 150µl of a 0.1 mM DPPH solution was made and added to the sample well, negative, and positive control wells. A 96-well microplate was placed in the dark for half an hour. To reduce inaccuracy, the absorbance reading was obtained three times.

5.5.14 TG/DTA

Using a Perkin Elmer TG/DTA Diamond Thermal Analyzer and an aluminum crucible, the thermal behavior was examined. The samples were examined in a dynamic air setting (flow rate of 100 mL·min−1 and synthetic air of 5.0 Linde Gas). Samples were preheated to 300 °C at a rate of 10 °C/min after being equilibrated at 30 °C, all the while being watched for weight loss. A flow of nitrogen of 30 mL per minute was established.

5.5.15 *In-vitro* drug release

In this investigation, the release of aceclofenac from the *in-situ* gel was determined using three magnetic stirrers. Phosphate buffer 6.8 was used as the dissolution medium. Initially, three dialysis membranes were activated and three formulations having 10 mg, 15mg and 20mg of drug content was put inside the membranes. The membranes were partially immersed in beakers containing 100 ml dissolution media each. The beaker is then placed in the magnetic stirrer at 30 rpm. At predefined time intervals, samples were collected, and the quantity of aceclofenac released from the formulated gels was determined using a UV-VIS spectrophotometer with a λmax of 273.60 nm.

5.5.16 Kinetics of release

The pharmacokinetics of a drug delivery vector can be understood with the use of a kinetic model. Solubility, particle size, crystallinity, and amount of drug, as well as the nature of the vector that encapsulates the drug, all play a crucial role in the release pattern of the drug from the vector. Different kinetic models such as zero-order ($Q_t = K_0t + Q_0$), first-order ($Q_t = Q_0e^{-Kft}$), Higuchi ($Q_t = K_H\sqrt{t} + Q_0$), Korsmeyer-Peppas ($Q_t/Q_\infty = K_kt^n$) and Hixon-Crowell ($Q_0^{1/3}/Q_t^{1/3} = K_st$) are used to describe the kinetics of drug release. For these equations, Q_t is the amount of drug released at time t, Q_0 is the initial amount of drug, K_0, K_f, K_H, K_s, K_k are release rate constants for Zero-order kinetics, First-order kinetics, Higuchi model, Hixon-Crowell model and Korsmeyer-Peppas model, respectively.

Chapter 6

Results and discussions

6.1 Physical Characterization of drug

The organoleptic characters like appearance, colour and odour of the received sample of aceclofenac was studied. It shows all properties which comply with standards as per IP. The details have been summarised in table _.

Table 2: Physical properties of aceclofenac

Sl no.	Property	Observation
1.	Visual appearance	powder
2.	Colour	Pale white
3.	Odour	Odourless

6.2 Melting point of drug

According to IP, "melting point of a substance is defined as those points of temperature at which the substance begins to melt and is completely melted except as defined otherwise for certain substances". Standard melting point range of pure aceclofenac is found to be 149-153 ^{o}C.[66] The recorded melting point of aceclofenac is 151^{o}C. The recorded melting point of drug was within the range indicating its purity.

6.3 Standard curve of aceclofenac

6.3.1 Absorption maxima

The UV absorption maximum was recorded at λ_{max} 273.60 nm of aceclofenac (10 µg/ ml) dissolved in phosphate buffer pH (6.8).[82]

6.3.2 Calibration curve

The aliquots of aceclofenac in phosphate buffer pH 6.8 ranging from 2 µg/ ml to 20 µg/ ml showed linearity at λmax 273.60 nm. The absorbance data and its standard curve is shown in table_ and figure_ respectively.

Table 3 : Absorbance of aliquoits of aceclofenac at λmax 273.60nm.

Concentration (µg/ ml)	Absorbance
2	0.0495
4	0.0825
6	0.1125
8	0.164
10	0.220
12	0.2516
14	0.287
16	0.3226
18	0.364
20	0.398

77

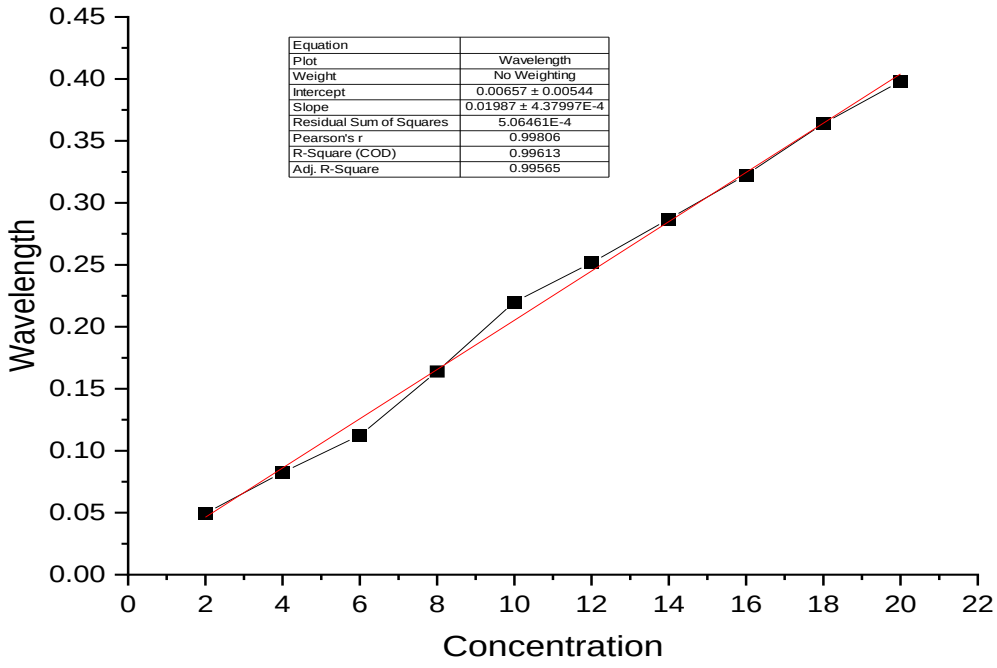


Figure 11: Standard curve of aceclofenac

6.4 Spreadability Test

The spreading of gel controls its therapeutic efficacy. The spreading of gel aids in even distribution of gel to the affected area, that's why the gel should spread adequately and satisfy optimal quality for application in gum. Moreover, this is thought to be a significant element in patient compliance to therapy.[83,84]

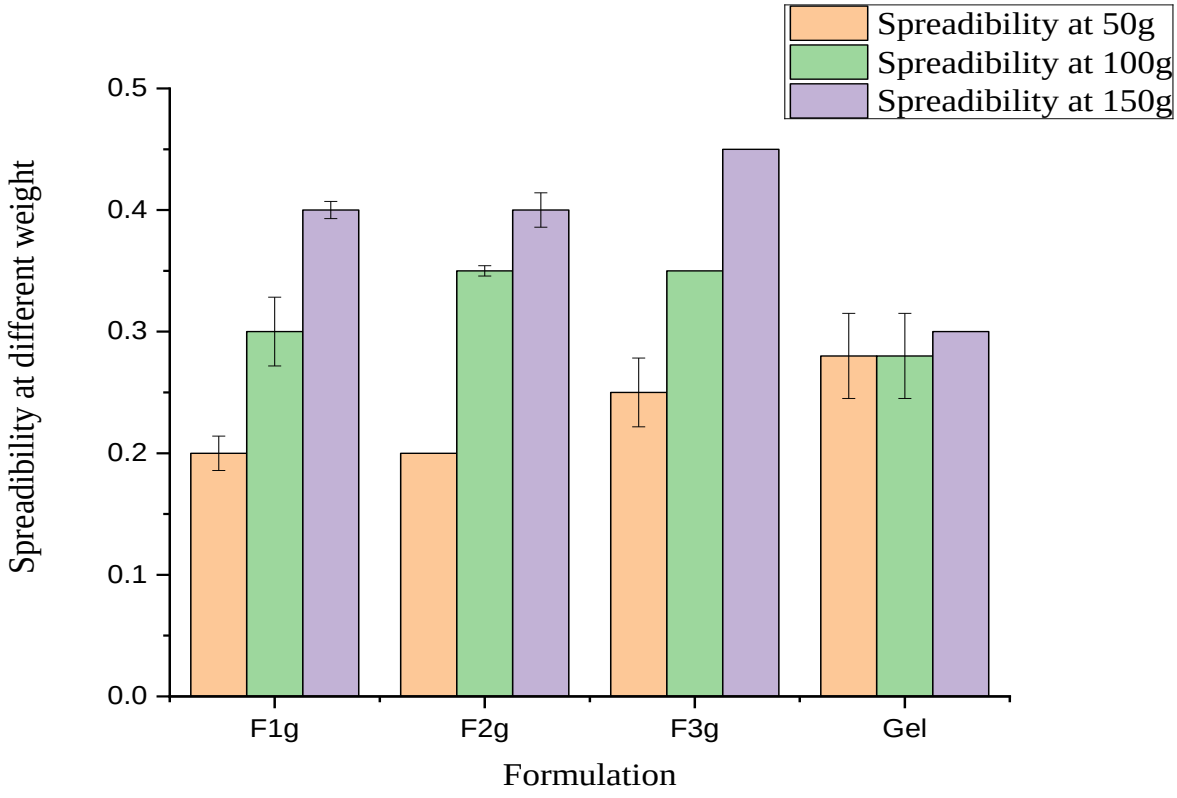


Figure 12: Chemical structure of Sodium-β-glycerophosphate

6.5 Stability testing

Continuous testing of gels for the treatment of dental caries evaluates the performance and reliability of the gel over time under different conditions. Stability testing is an important part of the development of *in-situ* gels for the treatment of dental caries. Testing ensures the gel's long-term effectiveness, safety and durability in a variety of environmental conditions.[85] The results of the stability test of the *in-situ* gel for the dental caries treatment showed stability of the gel stored under refrigerated conditions (4°C) and no discoloration or contamination during the test. However, gels stored at room temperature (25 ± 2 °C) and humidity (60% ± 5) showed significant color change (brown) and fungal development. These findings suggest that closed gels are sensitive to high temperature and humidity, leading to reduced stability and risk of contamination. Therefore, it is recommended to store the gel in a refrigerator (4°C) to maintain its quality, integrity and safety throughout its use.

6.6 Fourier Transform Infrared Spectroscopy

The presence of benzene derivative is indicated by the characteristic bands in the aceclofenac spectrum, which are located at 3316.83 cm $^{-1}$ (N-H stretching), 1769.50 cm^{-1} and 1713.90 cm^{-1} (C=O stretching, carboxylic acid), 1581.53 cm^{-1} (Skeleton vibration of aromatic C-C stretching), 1504.59 cm^{-1} (N-O stretching, nitro compound), 1444.20 cm^{-1} (O-H bending, carboxylic acid), 1344.28 cm^{-1} (O-H in plane bending), 1251.23 cm^{-1} (C-N stretching, aromatic amine), 1142.37 cm-1 (C-O stretching, aliphatic ether), 1054.39 cm^{-1} (C-O stretching, primary alcohol), 964.61 cm-1 (C=C bending), and 663.73 cm^{-1} (C=C bending). [1, 2, 3]. The C-X group's (X= Cl, Br, I) vibration was detected in the frequency range of 1129-480 cm^{-1}, which is indicative of halo compounds. C-Cl stretching is indicated by the FTIR absorption band at 850.53 cm^{-1}, 612.57 cm^{-1}, and.513.43 cm^{-1} .[86,87]

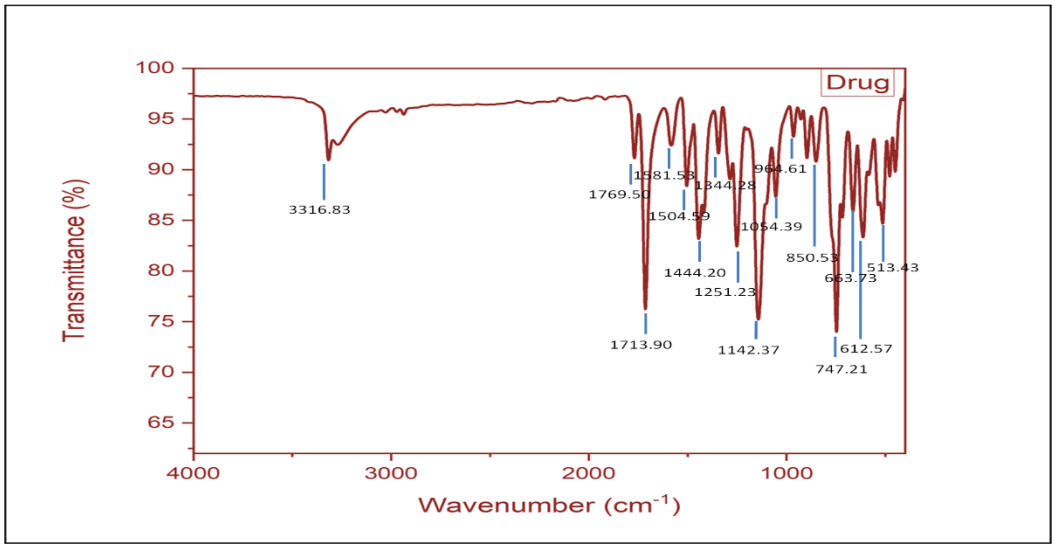
In case of HNT peaks at 3624.25 cm^{-1} is due to Al-OH stretching vibration and OH stretching of inner surface hydroxyl group. The peak at 1114.86 cm-1 is due to Si-O-Si degradation. The bending vibration of NH2 is responsible for the peak at 1629.85 cm^{-1}. The peaks at 1033.65 cm^{-1}, 912.33 cm^{-1} and 752.24 cm^{-1} are due to in-plane stretching of Si-O and O-H deformation vibration of internal hydroxyl groups.[88]

For sample F2, characteristic peaks were found at 3691.75 cm^{-1}, 3622.32 cm^{-1} corresponding to Al-OH stretching vibrations of halloysite nanotubes. There was a peak at 3319.49 cm $^{-1}$ and

3275.13 cm^{-1} corresponding to the N-H stretching, which confirmed aceclofenac was loaded inside the lumen. Characteristic peak of 1713.90 cm^{-1} was slighty shifted to 1716.65 cm^{-1} and this represent C=O stretching of carboxylic acid present in aceclofenac. The peaks at 1602.85 cm^{-1} of aceclofenac were slightly shifted. The peak at 1344.38 cm^{-1} represented the characteristic peaks of aceclofenac. The peaks at 1087.85 cm^{-1} and 1037.70 cm^{-1} suggest the presence of Si-O stretching vibrations, characteristic of halloysite nanotubes. Peaks at 912.33 cm^{-1} and 750.31cm^{-1} are indicative of Al-O stretching vibrations, suggesting the presence of HNT.[89]

For sample F2G, peaks at 3693.68 cm^{-1} may corresponds to Al-OH stretching vibrations of halloysite nanotubes or O-H stretching vibration of hydroxyl groups, possibly from chitosan. The peak at 2936.01 cm^{-1} and 2856.58 cm^{-1} are of C-H stretching vibrations, which are commonly found in organic compounds like aceclofenac and chitosan.[5] 1367 cm^{-1} is the characteristic peaks of aceclofenac. 1321.24 cm^{-1} is the peak that corresponds to C-N stretching vibrations, possibly indicating the presence of amine groups in chitosan. 1031.92 cm^{-1} and 1012.63 cm^{-1} are the peaks representing C-O stretching vibrations, which are commonly assigned for carbohydrates like chitosan and glycerophosphate. The stretching vibrations of C-Cl, which are indicative of chlorinated substances such as aceclofenac, may be the cause of the peak at 651.94 cm^{-1}. The peak at 516.92 cm^{-1} may be due to Si-O-Si bending vibrations of halloysite nanotubes.[90]

(a)



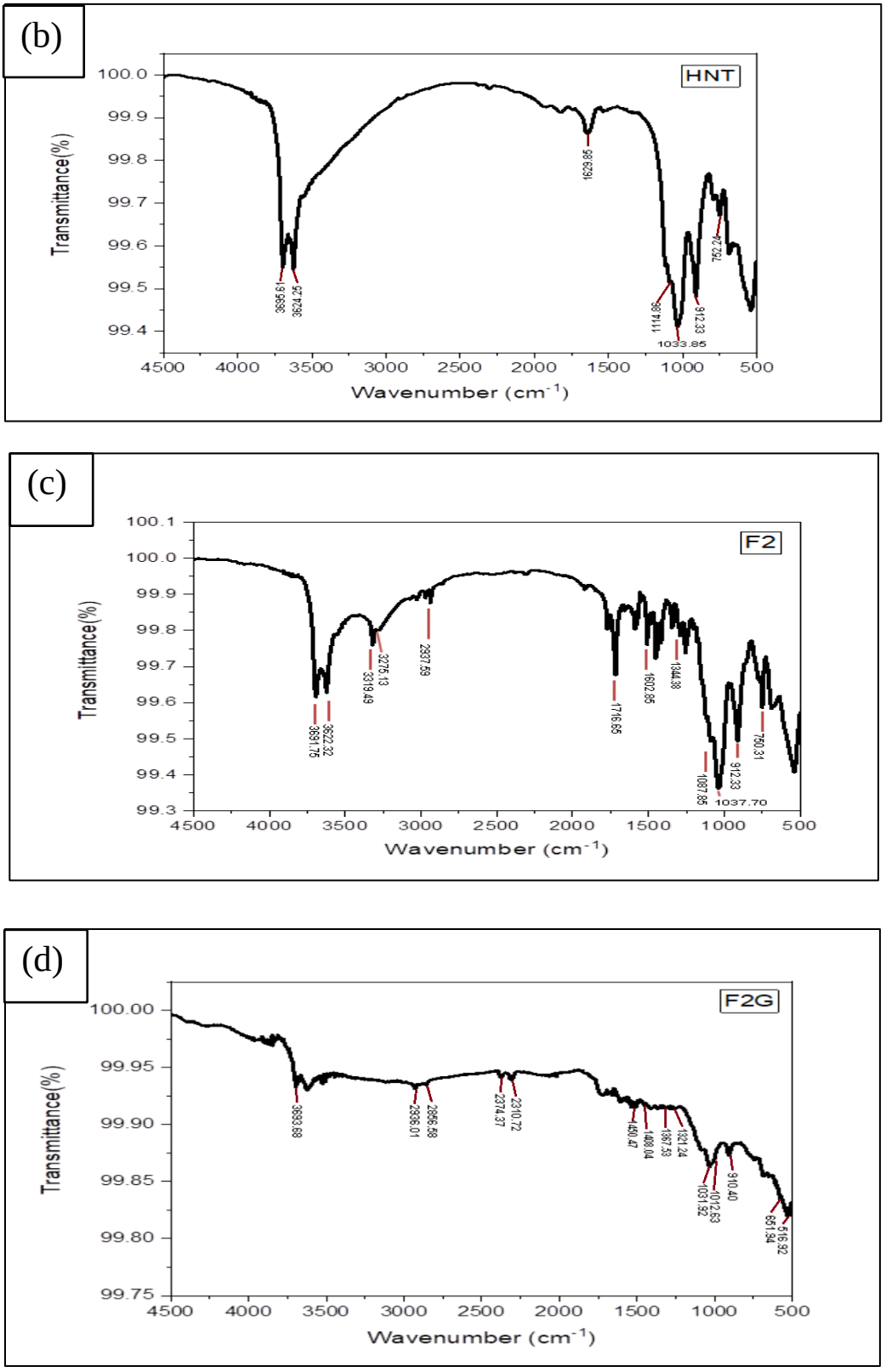


Fig 13: FTIR images of different samples, (a) Drug, (b) HNT, (c) F2, (d) F2G

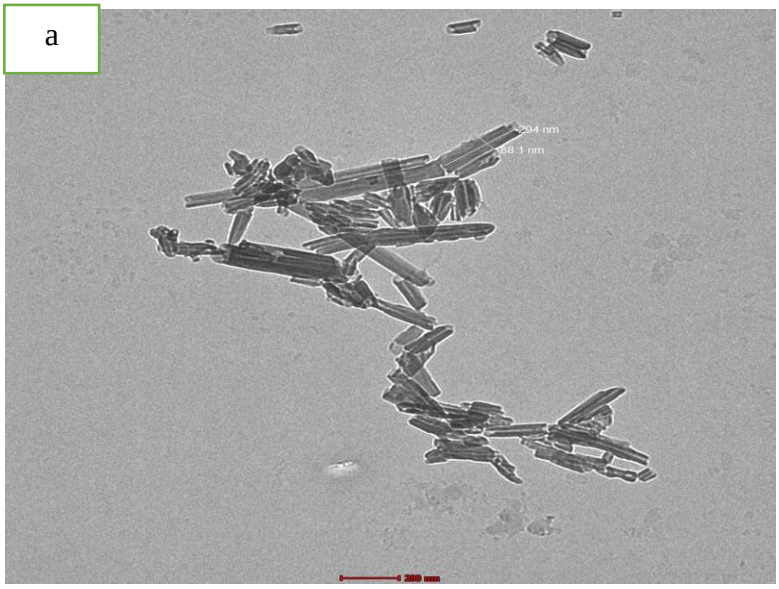
6.7 TEM analysis

TEM analysis was done to get more structural information on halloysite nanotubes, the drug loaded into it, and the formulations. Hollow tubular structure of HNT was clearly observed from the TEM images with a length of 294 nm and diameter of 88.1 nm. In F_2 Formulation too, the tubular structure was observed.[91] The presence of black globular structure in the inner lumen of HNT indicated that the drug, aceclofenac was successfully loaded inside the HNT. The fig.__ shows blurred tubular structure, which indicates that the drug loaded HNT was well incorporated inside the gel. It was seen that the drug loaded HNTs were well dispersed in the in-situ gel, as no huge aggregations could be observed in the TEM images. This homogeneous dispersion is an important factor in achieving the enhancement of the mechanical and thermal properties of the gel.

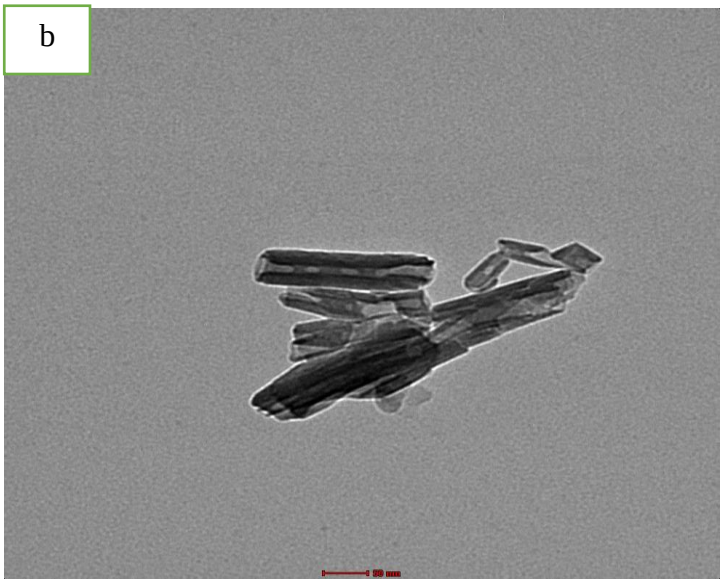
The structural characteristics and morphology of the halloysite nanotubes, drug incorporated HNT and their uniform distribution within the gel matrix have been examined insidiously in the TEM analysis. The TEM observations include high-resolution images that allow closer looks at the dimension of nanotubes and interactions within the gel matrix.[92]

Like a light microscope, the TEM also works on same basic principle but uses electrons instead of light. Since electrons have a wavelength that is significantly smaller than light's, in theory, the ideal resolution possible with TEM images is orders of magnitude better than what can be obtained using a light microscope.[2] Hence, TEMs are capable of exhibiting even the finest details of the internal structure, often down to the level of individual atoms.[93]

a



b



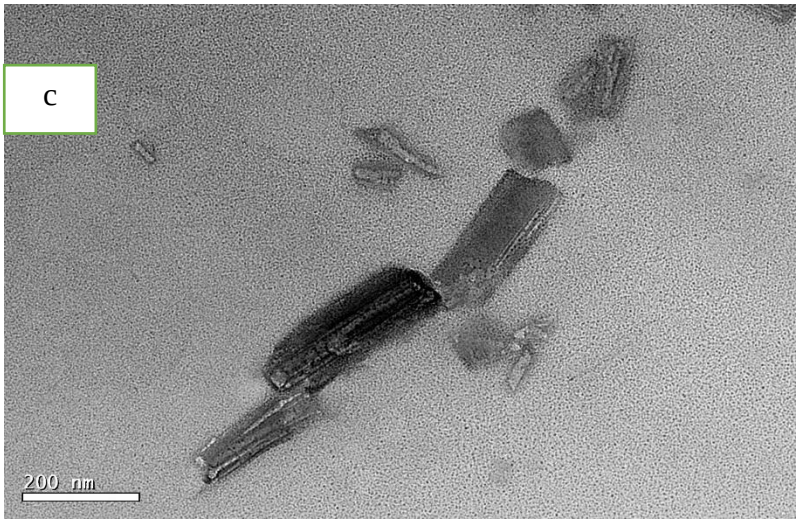


Figure 14: TEM images of (a) HNT, (b) F_2 and (c) F_2G

6.8 SEM analysis

SEM analysis was used to study the morphological structure of the materials-

Halloysite nanotubes (Fig. a), Aceclofenac loaded HNT, ($F_{2)}$ (Fig. b), and freeze dried samples, (F_2G and blank) (Fig. c, d).

As seen from the SEM images, the nanotubes were not in clear tubular shapes and they were in agglomerated form. The probable reason for not showing clear tubular shape can be that the SEM images were generated in sub-micron range and not in nanometer range.[94]

Sheet like structure were seen from the SEM image of aceclofenac loaded HNTs, F2. The reason of Sheet like appearance was due to the fact that the aceclofenac loaded HNTs were freeze dried and were scrapped to collect the sample. Sheet like structure had smooth surface for freeze dried sample.

In case of SEM image of F_2G, scattered HNTs were seen inside filament like structure. Scattered HNTs indicate that the drug loaded nanotubes are evenly spread inside gel and is well incorporated inside the gel which in turn indicate the stability of the gel.

For SEM image of blank sample, a filament like network was seen, which indicate the formation of gel.

Scanning electron microscopy (SEM) is an extremely flexible technique that allows us to gather high-resolution pictures and complete surface information on the specimens. In comparison to optical microscopy, the scanning electron microscope (SEM) method is more effective since the images are created by scanning the surface with a concentrated electron beam which in turn gives better resolution of the images. These SEM devices can also have resolutions of a few nanometers to less than one nanometer.[95]

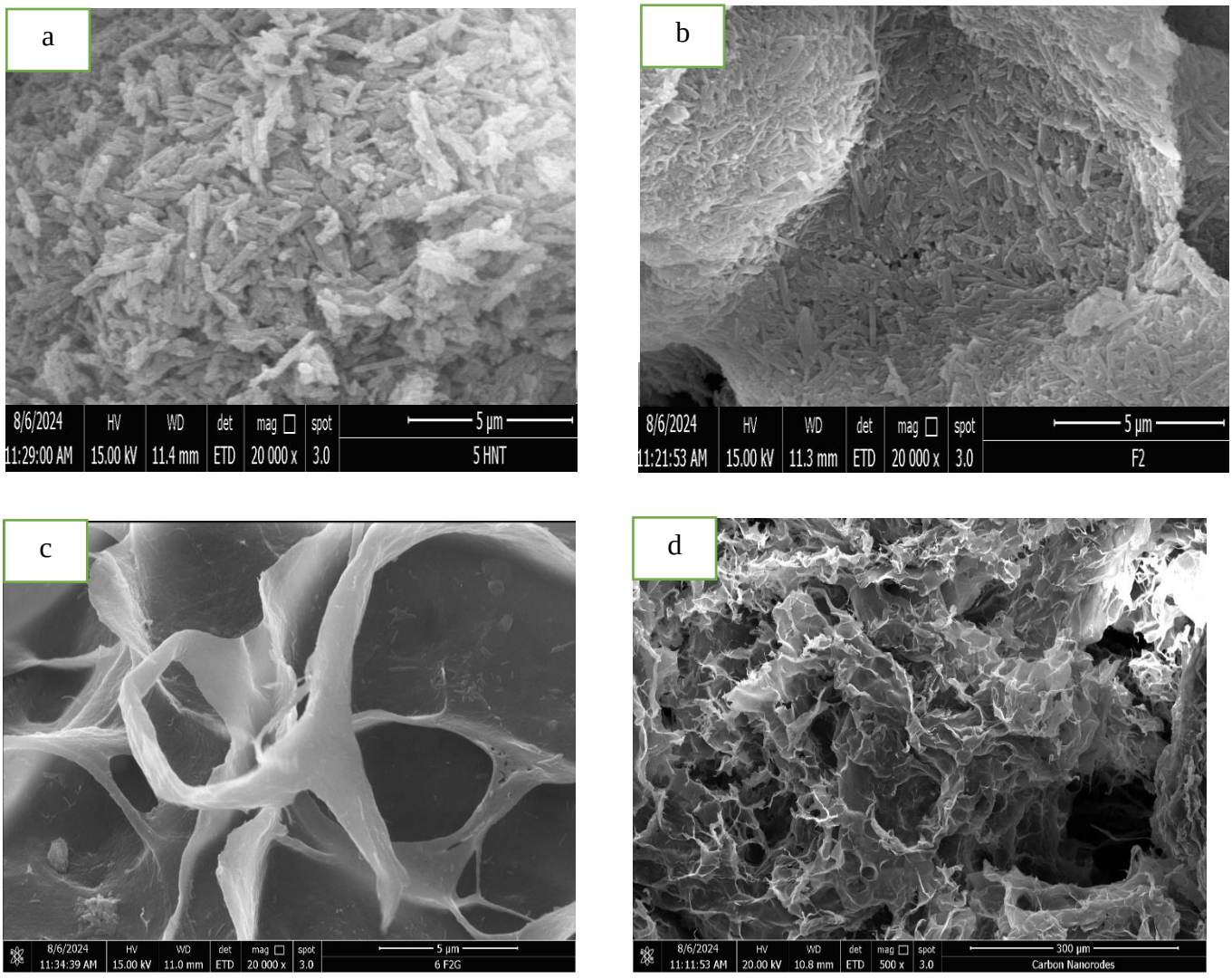


Figure 15: SEM images of (a) HNT (b) F_2 (c) F2G and (d) Blank

6.9 Powder X-Ray Diffraction analysis (PXRD):

The outcome of a PXRD experiment is usually a diffraction pattern or a plot showing the intensity of diffracted X-rays as a function of the scattering angle. In X-ray diffraction (XRD) studies, diffraction patterns provide crucial information about the structural properties of a sample like crystallite Size, crystal structure identification, crystal lattice, arrangement of atoms and texture.[96] Figure15 shows PXRD graph of (a) aceclofenac (b) blank (c) F2G.

The characteristic peaks of drug (aceclofenac) at 2θ (8.65°, 11.4°, 14.4°, 16.75°, 17.40°, 18.40°, 19.3°, 22.150°, 24.40°, 25.850°, 31.150°, 32.05°, 33.050°, 36.450°, 37.0°, 39.20°, 40.450) were noted, amongst which 18.40°,22.150°, 24.40°, 25.850°, and 32.05° were the most sharp and distinct.

For blank sample, peaks at 9.65° and 19.75° were found indicating the presence of Chitosan. Peak at 9.65° was due to hydrated crystalline form and peak at 19.75° was due to semi-crystalline nature of chitosan.

For F2G, small, low intensity peaks at 9.3°, 12.1°, 12.6°, 16.25°,17.8°, 18.5°, 20.3°, 25.15° were found. It denotes that few of the sharp and distinct peaks of aceclofenac were missing, and few were of low intensity. Among these peaks at 9.3° and at 20.3° may be due to chitosan and at 12.1° and 25.15° may be due to HNT.[56] These all fact indicates that the drug was entrapped inside the tube and the tube was surrounded by chitosan. The number and intensity of peaks reduced after the lyophilization of an in-situ gel containing a drug inside HNT, thus indicating a drug and excipients loss of crystalline nature, hence increasingly amorphous in the formulation.

The XRD analysis explains that the formulation contaning aceclofenac, chitosan, and HNT reduces crystallinity after lyophilization. The intensity of the F2G formulation was also reduced that may indicate the successful loading of the drug inside the nanotube. Therefore, XRD analysis confirms the amorphous nature of the F2G formulation due to a decrease in diffraction pattern intensity. We can draw a conclusion from the fact that the formulation is amorphous in nature and that the drug is uniformly dispersed in the matrix.

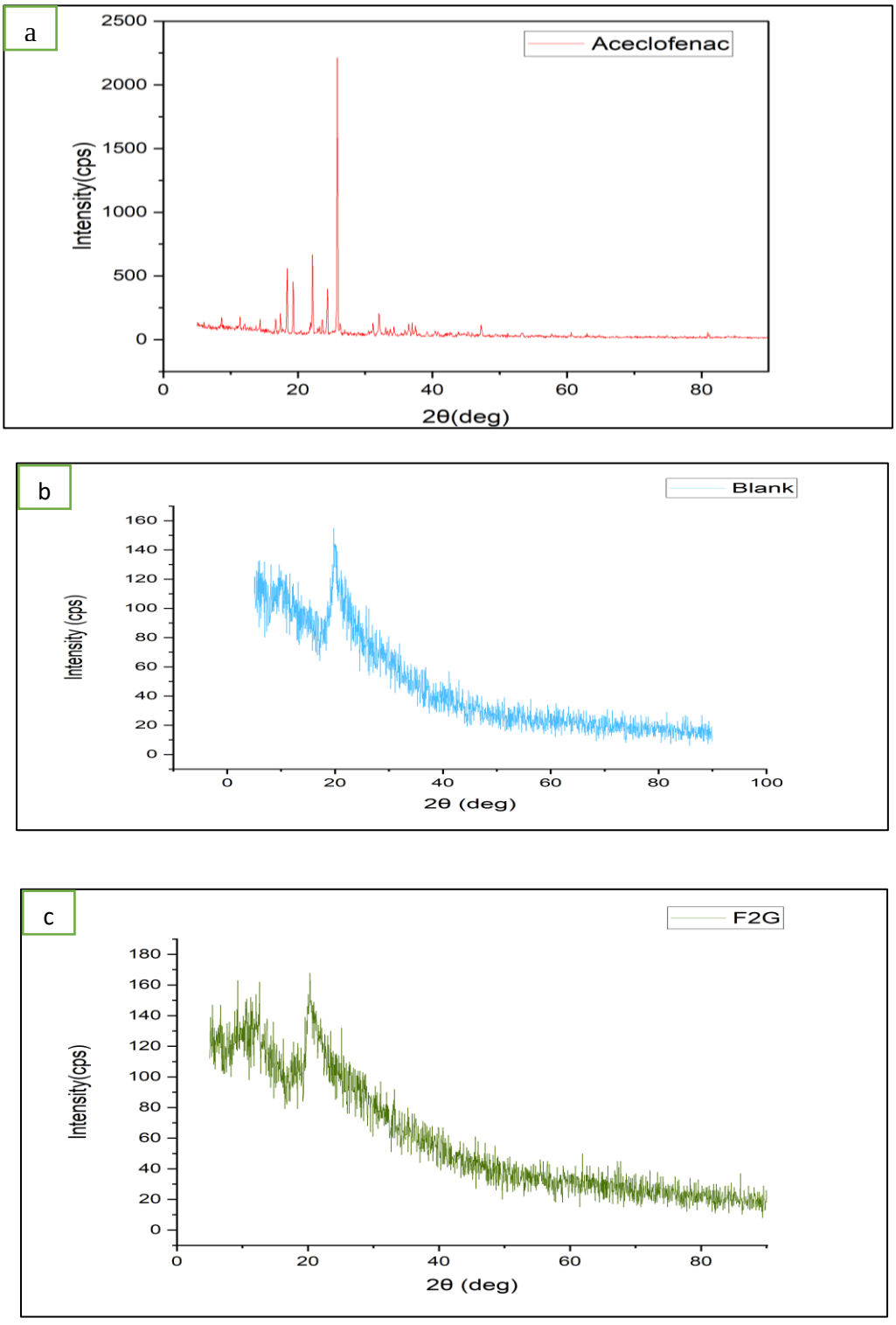


Figure 16: PXRD graph of a.Aceclofenac, b. Blank and c. F_2G

6.10 Swelling Study

Table 4: Data of swelling study

Formulation	Weight (mg) Day 1	Weight (mg) Day 2	Weight (mg) Day 3	Weight (mg) Day 4	Weight (mg) Day 5
Blank	295	340	324	261	260
F_1G	166	172	192	161	160
F_2G	193	244	225	212	212
F_3G	224	222	189	209	208

Formulation	Swelling degree (%) Day 1	Swelling degree (%) Day 2	Swelling degree (%) Day 3	Swelling degree (%) Day 4	Swelling degree (%) Day5
Blank	1436.5	1670.83	1587.5	1259.37	1254.16
F_1G	764.58	795.83	900	738.54	733.33
F_2G	1014.58	1170.83	1004.16	1071.875	1071.875
F_3G	1066.66	1056.25	884.375	988.54	983.33

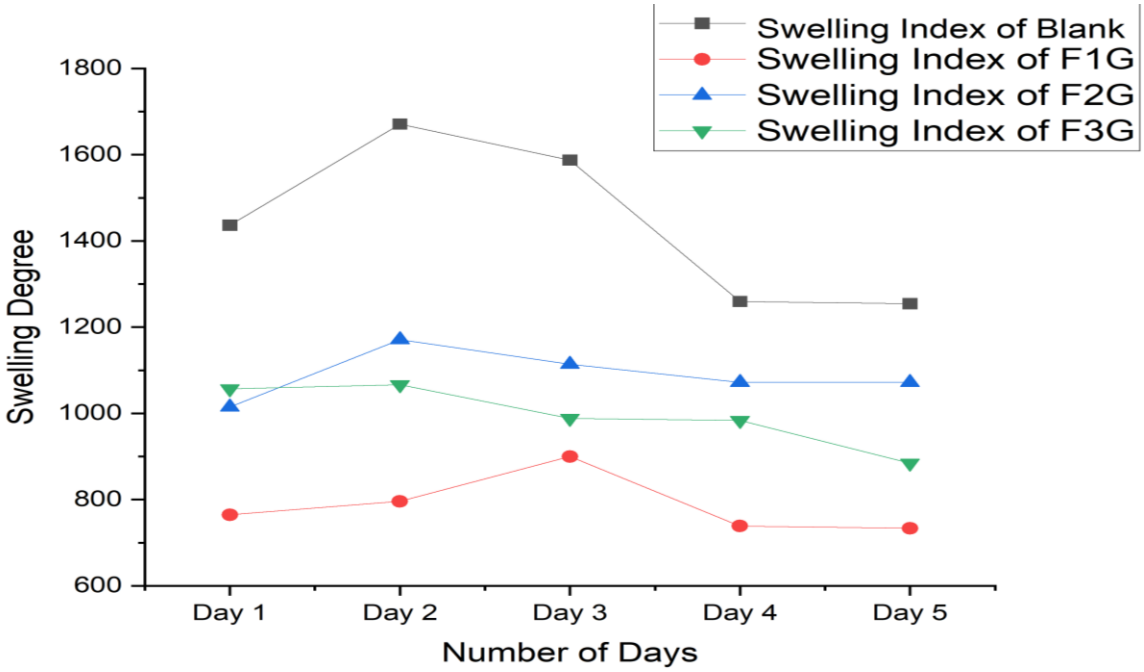


Figure 17: Chemical structure of Sodium-β-glycerophosphate

The results presented information that the in-situ gel depicted a gradual upsurge in the swelling index throughout time, which afterward soon got decreased and got constant. The swelling index at 1, 2, 3 and 4, and 5 days were measured and the conclusion was that the gel started swelling on day 1 and showed sharp increase in water uptake capacity of gel, then decreased and finally became constant.

The in-situ gel's ability to absorb liquids and inflate and expand with time is shown by the increasing swelling index. This characteristic enables the gel to stay in contact with the tissues of the oral cavity, which allows the active pharmaceutical ingredient to release gradually and is advantageous for the intended use. It is clear from the steady rise in the swelling index that the gel's composition is appropriate for usage in the oral cavity. This is important because, in order to transfer the therapeutic substance directly to the location of infection or inflammation, the gel needs to stick to the targeted area when treating periodontitis.[97]

6.11 Syringeability Test

All the three formulations (i.e F1G, F2G and F3G) were found to have good syringeabilty indicating that there will be less wastage of the formulation solution and sufficient amount of solution can be easily administered to the periodontal pocket increasing patient compliance and comfort.

Due to physiological stimuli, such as temperature, pH, or ionic strength of body fluids, in-situ gels, which are solutions at room temperature, transit into semi-solid gels. The delivery of medications to the target site using injectable in situ gel forming sol to gel systems has received a lot of attention recently. The advantages of easy syringeability (administration) include high local drug concentration, prolonged release/action, and decreased dosing frequency, all of which increase patient compliance and comfort.[98]

Figure 18: Chemical structure of Sodium-β-glycerophosphate

6.12 pH Determination of *in-situ* gel:

For the oral *in-situ* gel to be stable and efficient, its pH is very crucial. This approach is a straightforward qualitative analysis. In order to verify the correctness and reproducibility of the data, the experiment was conducted thrice.[99] The results showed that the blank and F2G formulation exhibited pH 7. The pH of the F1 and F2 formulations, however, was found to be less than 7. All of the formulations are appropriate for use in treating tooth cavities because their pH values fall between 6.2 and 7.6 in saliva. Additionally, this guarantees the in-situ gel compositions' tissue compatibility, safety, and efficacy. It was made sure that the pH paper was handled with sterile instruments and clean hands to prevent contamination.

6.13 Gelation Temperature

The gelation temperature of the prepared formulation was noted and few notable findings were observed. At 37^{o} C, a stable gel was formed, characterized by its stiff consistency and its ability to withstand deformation. This denotes the creation of a strong gel network at 37^{o}C and also proves its suitability for various biomedical applications.

Gel formation was also seen at temperatures slightly less than 37℃, specifically at 35℃ and 36℃ but the stability was low. In comparison to the gel created at 37℃, the gels generated at these temperatures showed some degree of liquefaction or decreased stiffness.

This implies a temperature-dependent gelation process, in which variations from the ideal temperature may affect the gel network's stability and structural integrity.[100]

At low temperatures, chitosan/β-GP solutions behave like typical semi-dilute solutions. However, as heating from 5°C to 70 °C, the elastic modulus (G′) rises exponentially, showing that incipient gelation is near 37 °C. The β-GP concentration at which gelation occurs corresponds to 1-2 molar times the concentration of amine groups of chitosan. This suggests that upon addition, chitosan transfers its charge to β-GP, resulting in a reduction of charge density over the polymer chain.

6.14 Gelation Time:

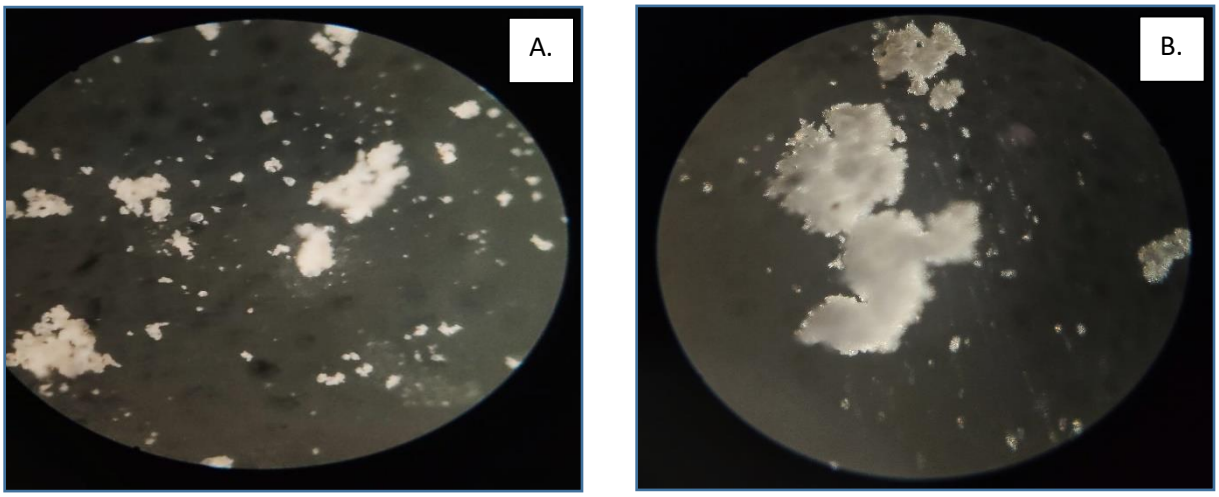
The gelation time of the sample was found to be approximately 1 minute to 1.5 minutes at 37◦C. The gel was remarkably resistant to deformation and had a firm consistency. Furthermore, no signs of liquefaction were seen throughout the time period.

The fast gelation within 1 minute to 1.5 minutes in this study validates the rapid formation of a stable gel network in a short period of time and suggests that it is a well-optimized formulation. Rapid gelation is necessary to accommodate the formed gel within the dental cavity quickly. This rapid gelation kinetics can be explained by the composition of the gel precursor solutions used, the interaction of chitosan and β-GP and also the mechanism of gelation.

The firm consistency of the in-situ gel denotes the high degree of mechanical strength and its resilience to external stress. The gel's capability to endure deformation without disintegrating its structure or endure liquefaction at 37◦C makes it fit for such biomedical applications.[101]

6.15 Optical Microscopy:

Optical microscopy analysis was performed at a magnification of 100x to investigate the structures of HNT, F2 and F2G. However, it was difficult to observe the structures in depth because of the trinocular microscope's limited magnification power. For both the F2 formulation and the HNT, the pictures showed a lump-like development and for the F2G formulation, a filamentous structure was seen. More thorough and practical insights were obtained through additional characterization utilizing Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM).



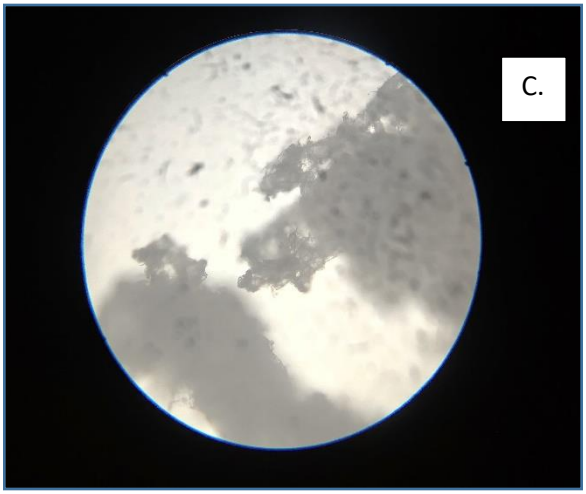


Figure 19: Optical microscopic photo A. F2, B. HNT, C. F2G

6.16 Antioxidant Study

Table 5- Results of antioxidant study

Formulation=F2

Concentration(ppm)	**Absorbance (nm)**	**Antioxidant %**
560	0.136	62.75%
280	0.120	55.37%
140	0.109	50.48%
70	0.104	47.99%
35	0.005	2.307%

Formulation=F1G

Concentration(ppm)	**Absorbance (nm)**	**Antioxidant %**
560	0.8634	392.99%
280	0.1225	55.75%
140	0.065	29.58%
70	0.048	21.84%
35	0.008	3.64%

Formulation=F2G

Concentration(ppm)	Absorbance(nm)	Antioxidant %
560	0.506	244.44%
280	0.103	49.75%
140	0.061	29.45%
70	0.033	15.94%
35	0.012	5.79%

Formulation=F3G

Concentration(ppm)	Absorbance (nm)	Antioxidant %
560	0.1767	80.83%
280	0.108	49.40%
140	0.095	43.45%
70	0.052	23.78%
35	0.0183	8.37%

6.17 TG/DTA

For drug, mass loss (93%) occurred between 175℃ to 300℃. This may be due to degradation of drug due to increase of heat.

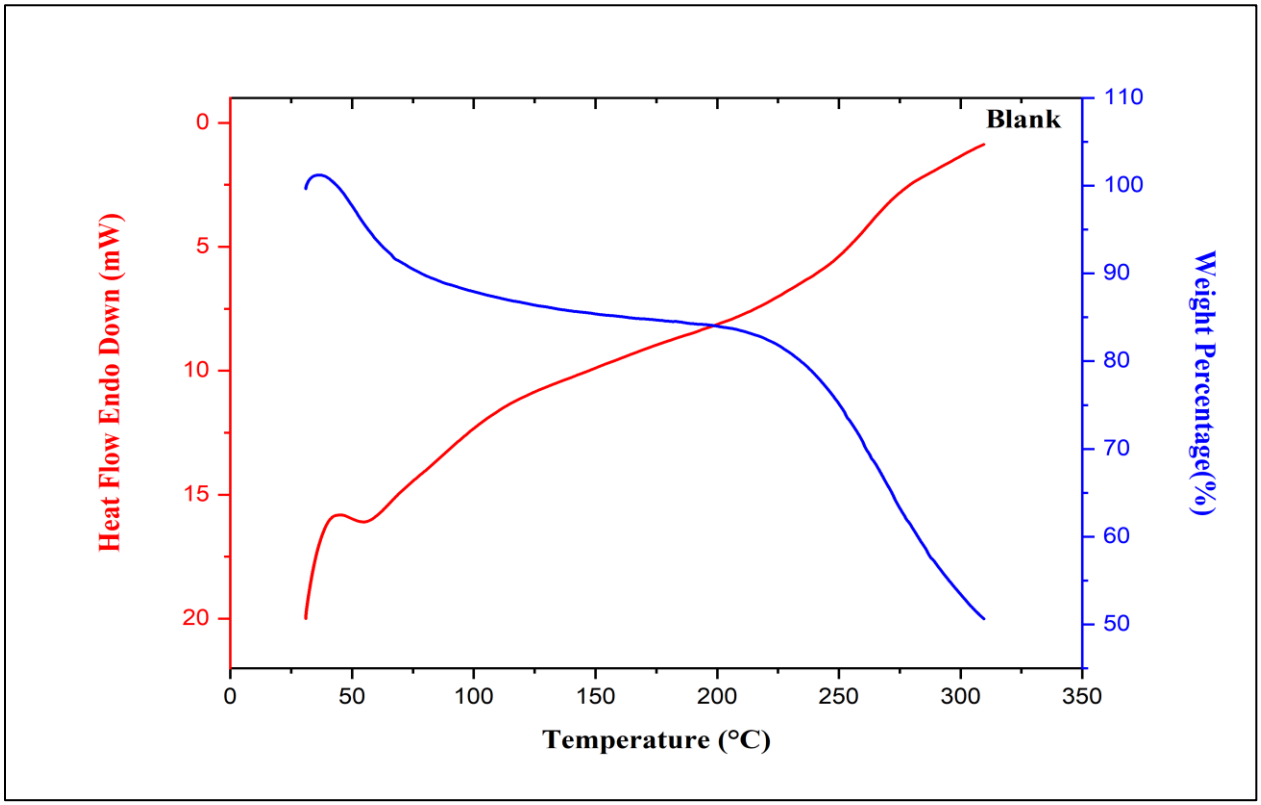
For blank, the first mass loss (9%) between 37 ℃ and 67 ℃ corresponding to the exothermic peak below 75 ℃ is due to dehydration. The second and third mass losses occurred from 85 ℃ to 182 ℃ (5%) and from 225℃ to 309 ℃ (31%), which may be due to degradation of lyophilized gel.

For F2G, the first mass loss (6%) between 37 ℃ and 69 ℃ corresponding to the endothermic peak may be due to the release of water molecule. The second mass loss (27%) from 222℃ to 308℃ corresponding to the exothermic peak, may be due to the degradation aceclofenac, chitosan and sodium-β glycerol phosphate hydrate.

The thermal decomposition of the drug gives two endothermic peaks. The first endothermic peak is due to melting at 167.6 °C and the second endothermic peak at 315.8°C is due to boiling point.

For blank sample, an exothermic peak was obtained at 43.24°C, which may indicate breakage of cross-linking between sodium-β glycerol phosphate hydrate and chitosan also the breakage of hydrogen bond and release of water molecule.

For F2G, the TGA graph obtained had two exothermic peak at 42.98°C and 278.79°C. First peak at 42.39°C might be due to breakage of crosslinking of *in-situ* gel and release of water molecule. The second peak at 190.08°C might be due to further decomposition of aceclofenac, chitosan and sodium-β glycerol phosphate hydrate.



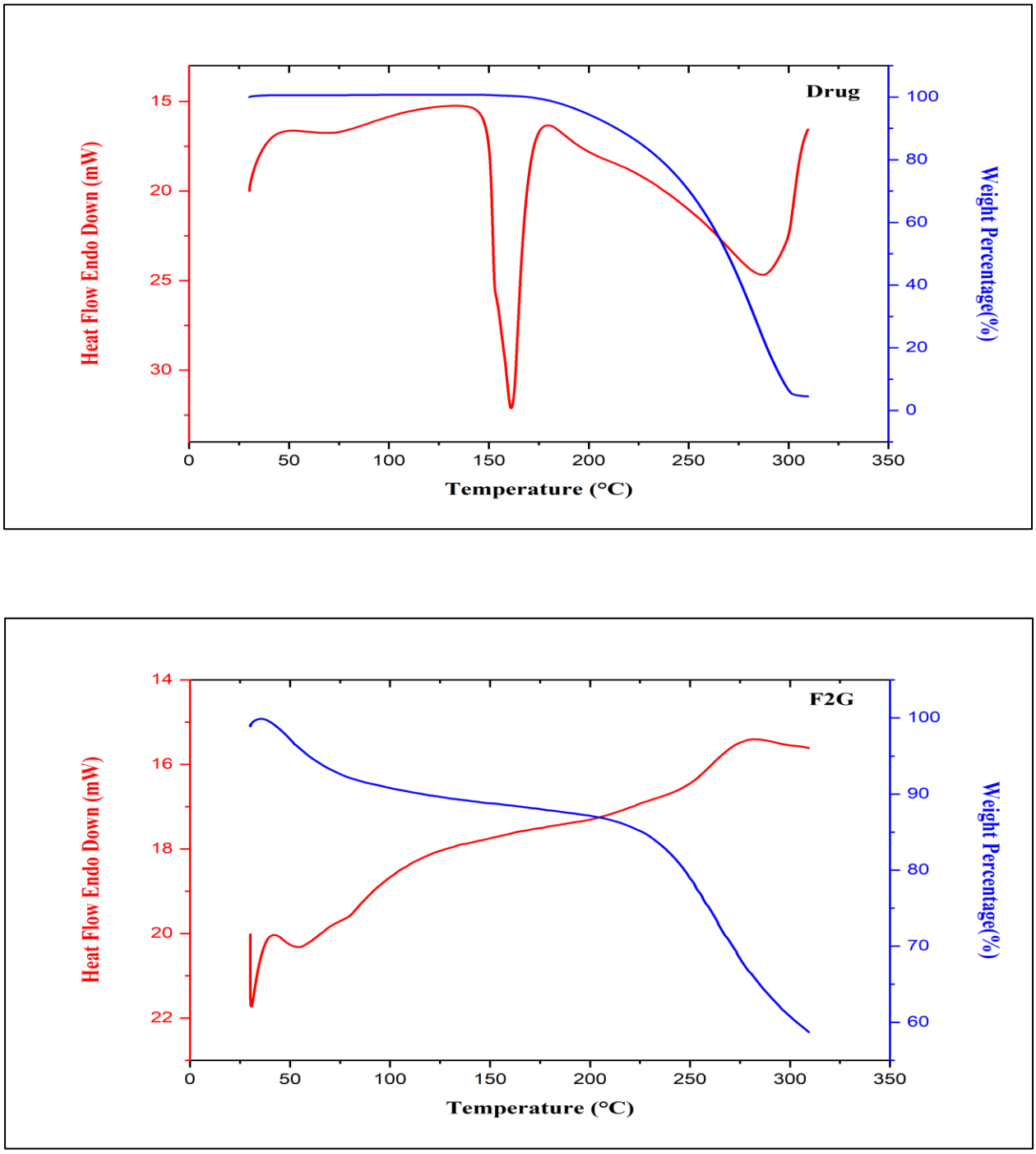


Figure 20: TG/DTA curves of blank, drug and F2G

6.18 In-vitro drug release study

The in vitro release study of formulated gels namely F1G, F2G and F3G was carried out to assess their drug release profiles (figure 21). After an initial 4-hour observation period, it was observed that F1G exhibited a drug release of 31.5725%, F2G released 35.42333% of the drug and F3G released 30.73625%.

Subsequently, the study extended for another 4 hours, totalling 8 hours of examination. At the end of this extended period, the drug release percentages were as follows: F1, 79.135%; F2, 86.165%; F3, 75.99875%.

An interesting observation emerged regarding the influence of Halloysite nanotubes on drug release. The formulations containing same amount of HNT and aceclofenac (F2) exhibited comparatively higher drug release than those with lower or higher amount of HNT than drug (F1, F3). This observation suggests that the HNT-to-drug ratio plays a significant role in drug release kinetics.

The results of this study suggest that the amount of HNT used in the formulation of *in-situ* gel can have a significant impact on the release of drug from the *in-situ* gel. Individual dissolution calculations of all formulations have been summarized in table 6, 7 and 8

Table 6: %CDR Calculation of F1G formulation

Time (hr)	Absorbance	Concentration(μg)	Cumulative amount in 100ml	Cumulative concentration in mg	Cumulative % drug release
1	0.217	4.535	453.5	0.4535	4.535
2	0.5205	12.1225	1212.25	1.21225	12.1225
3	0.8865	21.2725	2127.25	2.12725	21.2725
4	1.2985	31.5725	3157.25	3.15725	31.5725
5	1.748	42.81	4281	4.281	42.81
6	2.223	54.685	5468.5	5.4685	54.685
7	2.71	66.86	6686	6.686	66.86
8	3.201	79.135	7913.5	7.9135	79.135

Table 7: % CDR Calculation of F2G formulation

Time (hr)	Absorbance	Concentration(µg)	Cumulative amount in 100ml	Cumulative concentration in mg	Cumulative % drug release
1	0.354	7.96	796	0.796	5.306666667
2	0.859	20.585	2058.5	2.0585	13.72333333
3	1.4705	35.8725	3587.25	3.58725	23.915
4	2.161	53.135	5313.5	5.3135	35.42333333
5	2.895	71.485	7148.5	7.1485	47.65666667
6	3.6565	90.5225	9052.25	9.05225	60.34833333
7	4.4295	109.8475	10984.75	10.98475	73.23166667
8	5.2055	129.2475	12924.75	12.92475	86.165

Table 8: %CDR Calculation of F3G formulation

Time (hr)	Absorbance	Concentration(µg)	Cumulative amount in 100ml	Cumulative concentration in mg	Cumulative % drug release
1	0.411	9.385	938.5	0.9385	4.6925
2	1.0005	24.1225	2412.25	2.41225	12.06125
3	1.7015	41.6475	4164.75	4.16475	20.82375
4	2.4945	61.4725	6147.25	6.14725	30.73625
5	3.355	82.985	8298.5	8.2985	41.4925
6	4.2535	105.4475	10544.75	10.54475	52.72375
7	5.182	128.66	12866	12.866	64.33
8	6.1155	151.9975	15199.75	15.19975	75.99875

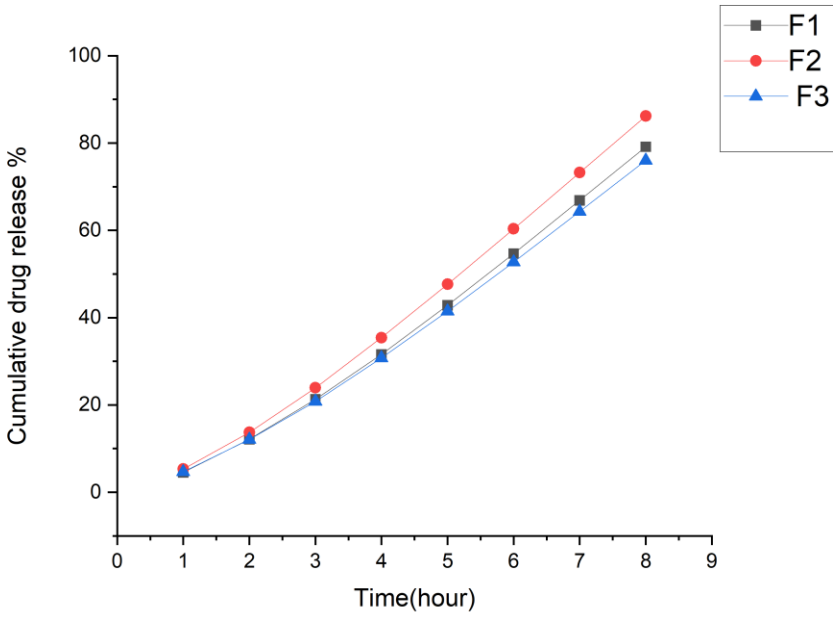


Figure 21: % Cumulative drug release vs. Time of F1G, F2G and F3G

6.19 Release Kinetics

The release of a drug from the *in-situ* gel formulation depends on many factors including, pH, temperature, drug solubility, polymer amount, HNT amount, desorption of the surface-bound drug, drug diffusion through the HNT, gel matrix swelling and erosion, and the combination of erosion and diffusion. *A in-situ* gel comprise of drug, HNT, and polymer, and the drug is released following some mechanism. Korsmeyer-Peppas model describes drug release from a polymeric system neither Fickian nor non-Fickian mechanisms transport (n>1).

After analyzing data obtained from Korsmeyer Peppas it was observed that n values in all formulations are more than 1 indicating Super Case-II transport, meaning the release mechanism is dominated by polymer swelling or relaxation, and this is faster than simple diffusion or erosion processes. Data obtained by using Zero order equation (table 18, figure 21) we observed R2 values of release profiles of all formulations as 0.9946 to 0.9961 confirming the linearity of profiles. The mechanism of drug release conforms to that of zero order too. The profiles obtained from the Hixon- Crowell model are linear as supported by the R2 values (0.9946- 0.9961). The release mechanism conforms to that of the Hixon- Crowell model if it is assumed that the structure of particles gradually decreases keeping the shape of initial particles. The profiles obtained by using the Higuchi model and First-order model show less linearity compared to that of other models Korsmeyer Peppas, Zero order, and Hixon- Crowell model. Table 17 presents the summary of r^2 value of various models. Table 19, 20, 21, 22 represent summary of 1^{st} order kinetic, Higuchi

model, Hixon-Crowell model, Korsmeyer Peppas respectively and figure 22,23,24, and 25 presents

Table 9: Summary of r^2 value of various models

Formulation	Zero order	First order	Higuchi	Hixson-Crowell	Korsmeyer-	Peppas
	r^2	r^2	r^2	r^2	r^2	n
F1G	0.9946	0.9268	0.956	0.9946	0.9998	1.377
F2G	0.9961	0.9043	0.9602	0.9961	0.9997	1.345
F3G	0.9951	0.9372	0.9572	0.9951	0.9999	1.342

Table 10: Calculation of zero-order kinetics.

	Zero order: cumulative % drug release vs. time		
Time (hr)	**(F1G)**	**(F2G)**	**(F3G)**
1	4.535	5.30667	4.6925
2	12.1225	13.72333	12.06125
3	21.2725	23.915	20.82375
4	31.5725	35.42333	30.73625
5	42.81	47.65667	41.4925
6	54.685	60.34833	52.72375
7	66.86	73.23167	64.33
8	79.135	86.165	75.99875

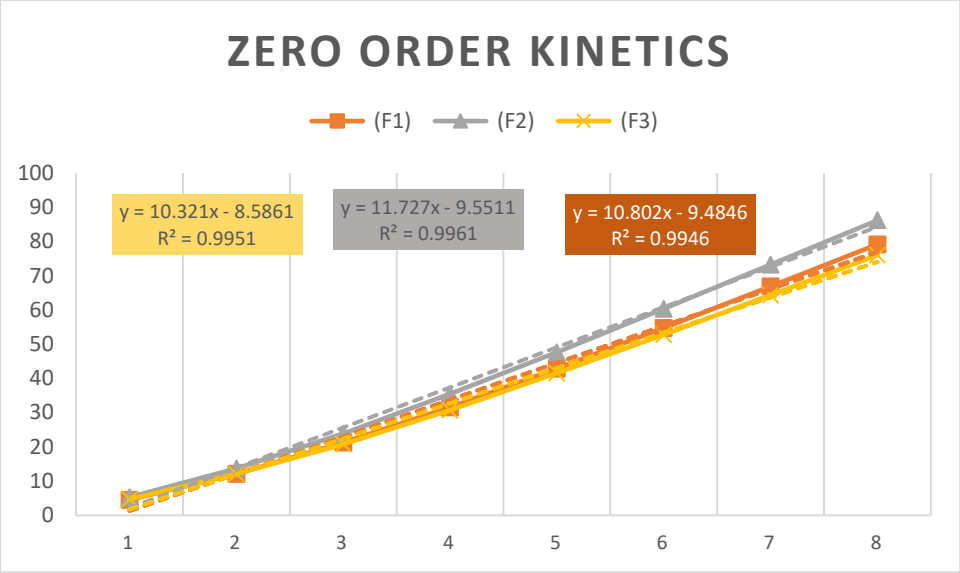


Figure 22: Zero-order release kinetics.

Table 11: Calculation of first-order kinetics

	First Order		
Time (hr)	F1G	F2G	F3G
1	1.979844	1.976319	1.979127
2	1.943878	1.935893	1.94418
3	1.896126	1.881299	1.898595
4	1.835231	1.810076	1.840506
5	1.75732	1.718861	1.767212
6	1.656242	1.598261	1.674643
7	1.520353	1.427621	1.552303
8	1.319418	1.140979	1.380234

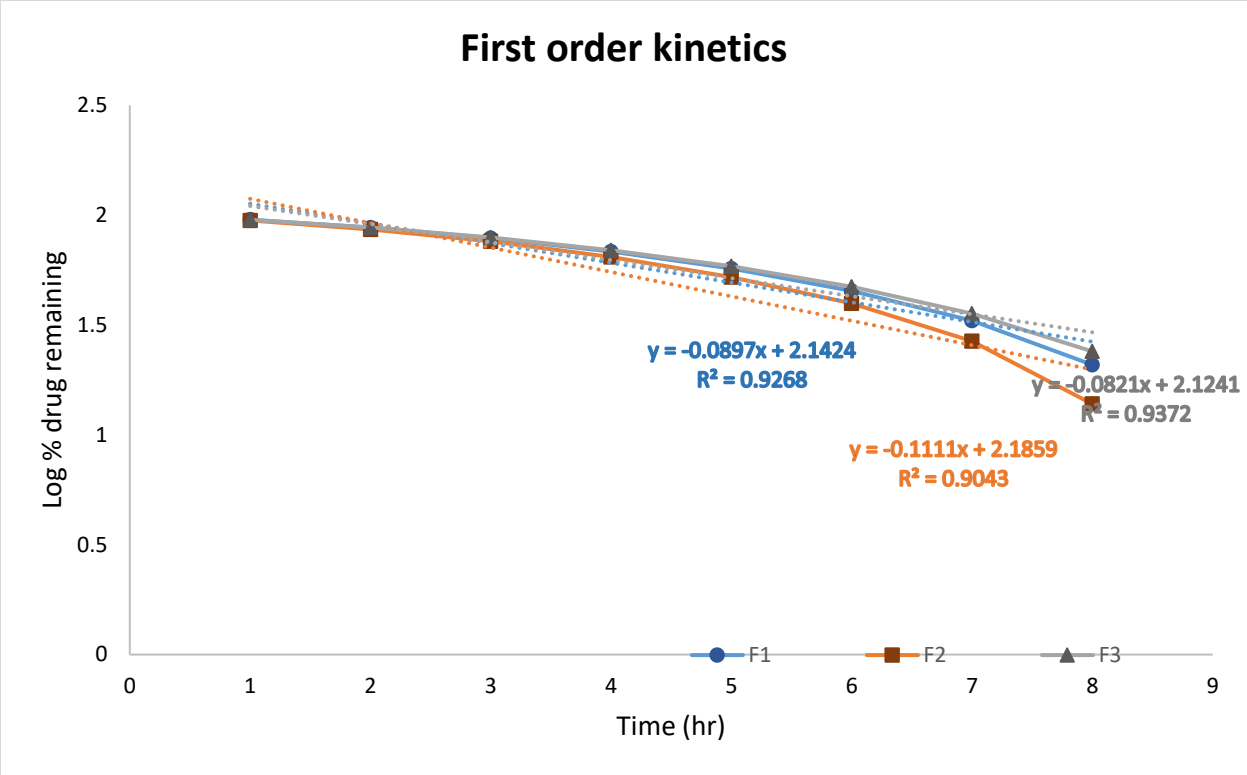


Figure 23 : First-order release kinetics.

Table 12: Calculation of Higuchi model

Higuchi: %CDR vs. sq. root of time				
Time(hr)	Sq root of time	(F1)	(F2)	(F3)
1	1	4.535	5.30667	4.6925
2	1.414213562	12.1225	13.72333	12.06125
3	1.732050808	21.2725	23.915	20.82375
4	2	31.5725	35.42333	30.73625
5	2.236067977	42.81	47.65667	41.4925
6	2.449489743	54.685	60.34833	52.72375
7	2.645751311	66.86	73.23167	64.33
8	2.828427125	79.135	86.165	75.99875

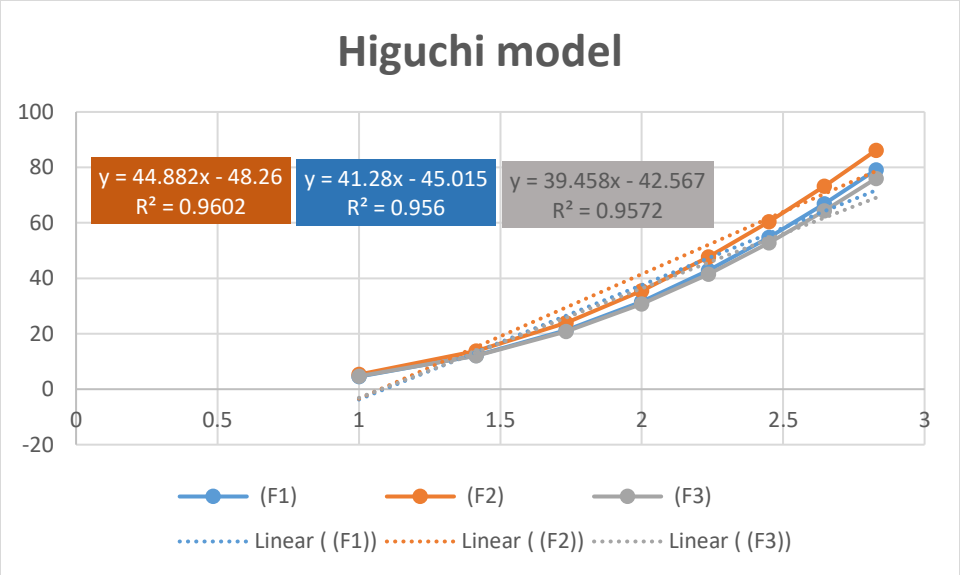


Figure 24: Higuchi release kinetics.

Table 13: Calculation of Hixon-Crowell model

Hixon-Crowell model			
Time (hr)	F1G	F2G	F3G
1	31.82167	31.56444	31.76917
2	29.2925	28.75889	29.31292
3	26.2425	25.36167	26.39208
4	22.80917	21.52556	23.08792
5	19.06333	17.44778	19.5025
6	15.105	13.21722	15.75875
7	11.04667	8.922777	11.89
8	6.955	4.611667	8.000417

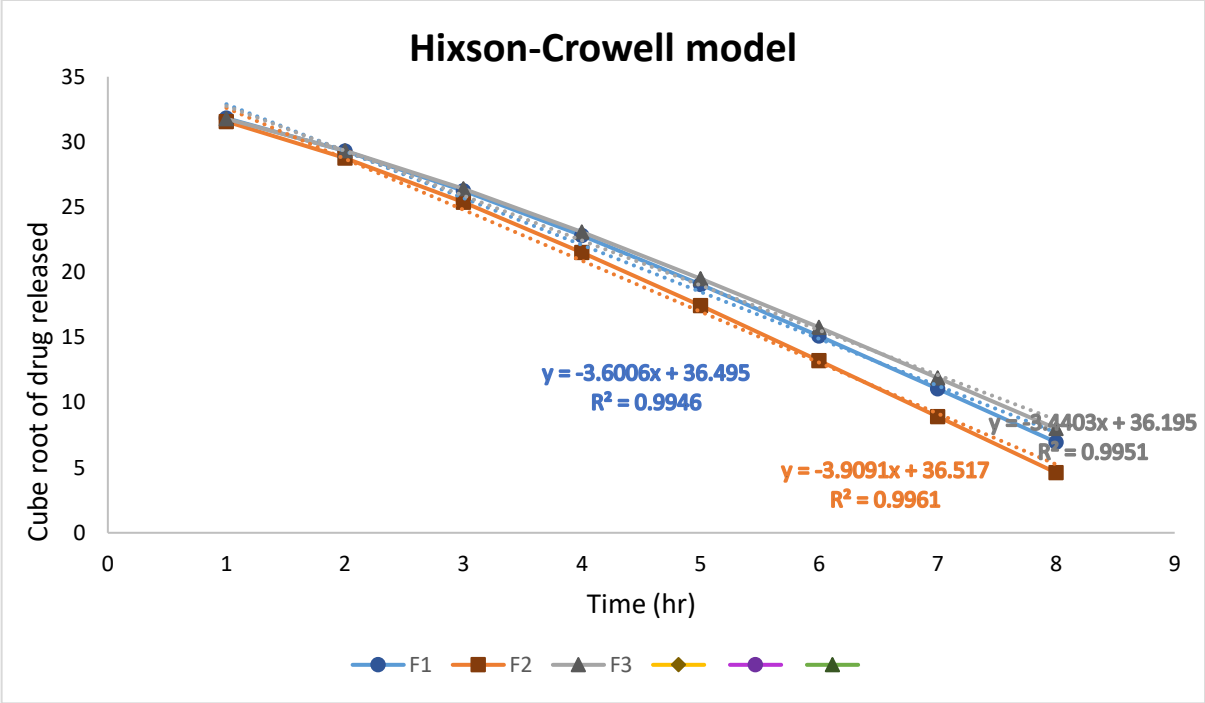


Figure 25: Hixon-Crowell Kinetics.

Table 14: Calculation of Korsmeyar-Peppas model

Korsmeyar-Peppas Model			
Log time	F1	F2	F3
0	0.656577	0.724822	0.671404
0.30103	1.083592	1.13746	1.081392
0.477121	1.327819	1.37867	1.318559
0.60206	1.499309	1.549289	1.487651
0.69897	1.631545	1.678124	1.61797
0.778151	1.737868	1.780665	1.722006
0.845098	1.825166	1.864699	1.808414
0.90309	1.898369	1.935331	1.880806

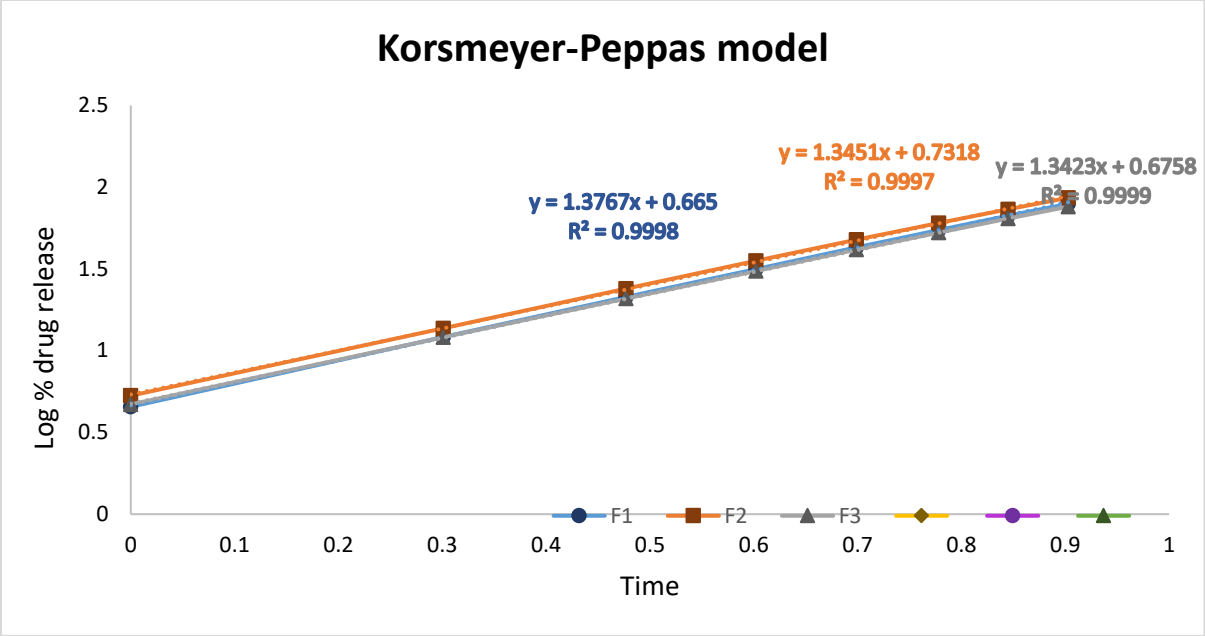


Figure 26: Korsmeyar-Peppas kinetics.

Chapter 7

Summary and Conclusion

7.0 Summary and conclusion

In-situ gels have grown as one of the most useful tools in treating periodontitis because they realize the possibility of localized and sustained drug delivery right into the periodontal pocket. The efficacy of these gels for drug delivery to the infected site is related to some key factors, which involve the type of polymer, release rate of drugs from them, and their adhesion to mucosal surface. In the family of polymers, the *in-situ* gel of gellan gum, pectin, and chitosan has gained attention because they are either biocompatible, biodegradable, non-irritant, and/or are able to form gels at physiological conditions. The gelation and viscosity features of the in-situ gel may further be altered according to the requirement by modifying the polymer concentration and further by changes in environmental triggers like temperature, pH, or ion presence.

This indicates that *in-situ* gel formulations give major improvements above conventional drug delivery systems in periodontitis treatment. They provide controlled and sustained release of drug contents, which increases bioavailability and decreases dosing frequency. Localized release avoids systemic side effects and keeps the drug in contact with the infected area for a longer time, hence increasing the therapeutic effect. Use of natural polymers pectin and chitosan also guarantees the biocompatibility and minor promise of allergic response.

However, there are challenges to overcome for *in-situ* gels: instabilities and low drug encapsulation potential for some hydrophobic drugs. The gel formulation needs careful optimization, so as not to compromise early disintegration or drug release. Furthermore, restrictions, such as avoidance of eating or drinking for a certain period of time after application, could result in a lack of patient compliance.

In situ gels are a versatile and effectivene approach for the treatment of periodontitis through sustained drug release, localized delivery, and minimal systemic exposure. Appropriate selection of polymer and optimization of the properties of gels can lead to a sustained increase in therapeutic effectiveness in mainstream treatment.

Before formulating an aceclofenac-loaded in situ gel for the treatment of periodontitis, pre-formulation studies were conducted to evaluate the drug and polymers used. Key physical characteristics of aceclofenac, including its melting point, were examined, and solubility assessments were performed. The drug's λmax was determined, and a standard curve was plotted. Instrumental analyses such as FTIR and PXRD were performed for the drug and the formulated gel to assess potential interactions between ingredients.

- FTIR spectra were used to determine any chemical interactions between aceclofenac and the polymers. The characteristic peaks of aceclofenac in the formulation showed some reduction in intensity, suggesting the formation of weak interactions, like Van der Waals forces, between the drug and the polymers. The absence of significant peaks in the blank gel confirmed that no drug was present in this sample.
- The PXRD data revealed the crystalline nature of aceclofenac, indicated by sharp diffraction peaks. However, a notable decrease in the diffraction pattern was observed in the drug-loaded gel, suggesting that the crystalline form of aceclofenac may convert to an amorphous form when incorporated into the gel.
- SEM and TEM analysis provided insights into the surface morphology and dimensions of HNT and *in-situ* gel. The smooth surface of the HNT and *in-situ* gel suggests effective polymer coating, with no notable fractures observed.
- The thermal stability of the aceclofenac-loaded in situ gel was assessed using TG/DTA. The drug exhibited a significant mass loss due to thermal degradation between 175°C and 300°C. The F2G formulation showed two primary stages of mass loss, linked to water release and the degradation of aceclofenac, chitosan, and β-GP. This analysis revealed the presence of two exothermic peaks, corresponding to the breakdown of cross-links and further decomposition of the gel components.
- The antioxidant potential of the aceclofenac-loaded gel was measured at various concentrations. The F2G formulation displayed significant antioxidant activity at higher concentrations, which is crucial for mitigating oxidative stress in periodontal disease.

Chapter 8
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