Kinetic modelling and antibacterial study of drug released from clay layers and lab synthesized transdermal patch.

Thesis submitted towards partial fulfilment of the requirements for the award of the Degree of Master of Technology in Nano Science and Technology

By

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CERTIFICATE OF RECOMMENDATION

This is in order to certify that the thesis entitled " **Kinetic modelling and antibacterial study of drug released from clay layers and lab synthesized transdermal patch.**" is a legitimate work done by Aditya Debapriya Gayen under our supervision and guidance for the purposes of fulfilling the requirement for the degree of Master of Technology in Nano Science and Nanotechnology in the School of Materials Science and Nanotechnology from the academic session 2021-2023.

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I hereby declare that this thesis contains a literature survey and original research work by the undersigned candidate for the Master of Technology (Nano Science and Technology) program during the academic year 2021-2023. The information in this document has been gathered and presented according to academic standards and ethical conduct. I also declare that I have cited and referred to all materials and results that are not original to this work as required by these rules and conduct.

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“I do not know what I may appear to the world, but to myself I seem to have been only like a boy playing on the sea-shore, and diverting myself in now and then finding a smoother pebble or a prettier shell than ordinary, whilst the great ocean of truth lay all undiscovered before me.”

— Isaac Newton

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Abstract

In the world of medicine, a class of drug called antibiotics is used to treat bacterial infections. They function by either eradicating the bacteria or slowing their growth, so preventing the spread of the infection. Clay has become a material that shows promise for drug loading. Large surface area, ability to produce nanoscale particles or layered structures, and high drug-loading capacity are all characteristics of clay. Drug molecules can be stored in the interlayer gaps of clay minerals, where they are shielded from deterioration and given greater stability. The Clay minerals like montmorillonite, kaolinite, and halloysite have these properties. Here Gatifloxacin an antibiotic drug is loaded into montmorillonite clay layers. Characterization methods like FTIR, XRD and SEM are conducted, for further confirmation and efficacy of the loaded drug, different culture of the bacteria (Staphylococcus aureus) which is a commonly found bacteria was made and the zone of inhibition of the bacteria upon application of drug was observed. The release behaviour of medications from different drug delivery devices may be predicted using release kinetics. To characterise the release kinetics and forecast the release behaviour under various situations, mathematical models such the zero-order, first-order, Higuchi, and Korsmeyer-Peppas models are frequently utilised. Burst and sustained releases were conducted to observe which model fits the best. The drug loaded clay was incorporated into patches and their release kinetics as well as the antibacterial efficacy test were performed. It was observed that the patches were successful to inhibit the bacterial growth.

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1 Introduction

1.1 Nanoscience and Technology

Nanoscience is an interdisciplinary field of scientific study that focuses on understanding, manipulating, and exploring phenomena at the nanoscale. The nanoscale typically refers to dimensions at or below 100 nanometres (one billionth of a meter). Nanoscience seeks to comprehend the unique properties and behaviours of materials and structures at this tiny scale, often revealing unexpected and novel characteristics.

Nanotechnology, on the other hand, is the application of knowledge from nanoscience to create, design, and use functional structures, devices, and systems. It involves engineering and manipulating materials and components at the atomic and molecular levels to develop innovative products and solutions.

Multidisciplinary Nature: Nanoscience and nanotechnology are highly interdisciplinary, drawing expertise from fields like physics, chemistry, biology, materials science, and engineering.

Historical Origins: The term "nanotechnology" was popularized by physicist Richard Feynman in his 1959 lecture, but the concepts and phenomena it encompasses have existed in nature and science for centuries.

Quantum Effects: At the nanoscale, quantum effects become prominent, leading to unique properties such as size-dependent optical and electronic behaviours in nanomaterials.

Applications in Medicine: Nanotechnology has revolutionized medicine, enabling the development of nanoparticles for targeted drug delivery, nanoscale imaging tools, and even the creation of artificial organs and tissues.

Materials Advancements: Nanomaterials, like carbon nanotubes and graphene, possess exceptional mechanical, electrical, and thermal properties, with applications in industries ranging from electronics to aerospace.

Environmental Impact: Nanotechnology also addresses environmental challenges, with nanomaterials used for water purification, pollution monitoring, and renewable energy solutions.

Consumer Products: Nanotechnology has permeated everyday consumer products, from stain-resistant clothing to sunscreen with nanoscale zinc oxide particles.

Nanoelectronics: Miniaturization of electronic components to the nanoscale has led to the development of faster and more efficient electronic devices, such as smaller transistors in computer chips.

Energy Efficiency: Nanotechnology contributes to improved energy storage and conversion technologies, including more efficient batteries, solar cells, and fuel cells.

Ethical and Safety Concerns: Alongside its potential, nanotechnology raises ethical and safety concerns regarding the environmental and health impacts of nanomaterials, necessitating responsible research and regulatory oversight.

1.2 Bio-nanotechnology

Bio-nanotechnology is an interdisciplinary field that combines the principles and tools of nanoscience and nanotechnology with biology and biotechnology to create innovative solutions for healthcare, agriculture, and environmental applications. It involves manipulating and engineering biological molecules, cells, and organisms at the nanoscale to develop novel materials, devices, and processes.

Molecular Precision: Bio-nanotechnology allows scientists to work with biological molecules, such as DNA, proteins, and enzymes, at the molecular level. This precision enables the design of custom-made biomaterials and nanoscale devices.

Medical Applications: One of the primary focus of bio-nanotechnology is in medicine. It encompasses the development of targeted drug delivery systems, biosensors for disease detection, and nanoscale imaging techniques. These advancements have the potential to revolutionize diagnostics and treatments, offering personalized and more effective healthcare solutions.

Biological Machines: Researchers are exploring the creation of biological machines and nanobots that can perform specific tasks within the human body, such as drug delivery, tissue repair, or even augmenting natural physiological functions.

Regenerative Medicine: Bio-nanotechnology plays a significant role in regenerative medicine by providing nanoscale scaffolds and materials that support tissue regeneration and organ transplantation.

Agriculture and Food: Bio-nanotechnology has applications in agriculture, where nanoscale materials and sensors can be used for targeted delivery of nutrients and pesticides. Additionally, it aids in food safety by detecting contaminants and pathogens in real-time.

Environmental Remediation: Nanomaterials engineered through bio-nanotechnology can remove pollutants and toxins from water and soil, offering sustainable solutions for environmental remediation.

DNA Nanotechnology: Researchers have developed techniques to use DNA as a programmable building material, creating intricate nanoscale structures and devices, including DNA origami and DNA-based nanomachines.

Nanoparticles in Drug Delivery: Nanoparticles made from biocompatible materials are used for targeted drug delivery, reducing side effects and improving the effectiveness of treatments.

Nanoscale Imaging: Advances in bio-nanotechnology have led to the development of super-resolution microscopy techniques, allowing scientists to visualize biological structures at the nanoscale, including individual molecules and cellular processes.

Biosensors: Bio-nanotechnology has led to the creation of highly sensitive biosensors that can detect specific biomolecules, pathogens, and disease markers, revolutionizing diagnostics.

Ethical Considerations: As with any emerging technology, bio-nanotechnology raises ethical questions, particularly regarding the manipulation of living organisms at the nanoscale and issues related to safety and biosecurity.

Interdisciplinary Collaboration: Bio-nanotechnology thrives on collaboration between biologists, chemists, physicists, engineers, and medical researchers, fostering a rich environment for innovation.

1.3 Montmorillonite clay

In honour of the French province of Montmorillon, where it was initially discovered, smectite clay has been given the name "montmorillonite clay." It is a naturally occurring clay mineral that is a member of the phyllosilicate class of minerals known for its layered structure [1]. Because of its special qualities, montmorillonite clay is frequently utilised in a wide range of industries, including geology, agriculture, construction, and health and beauty.

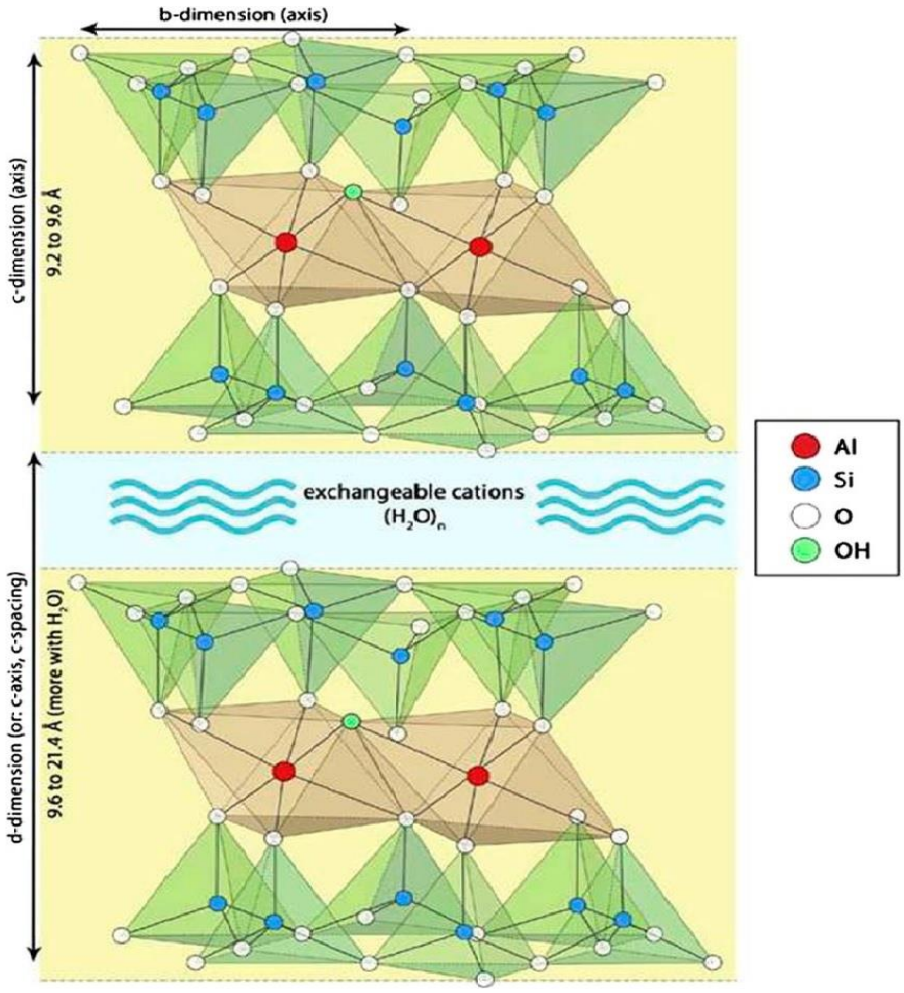


Figure 1-1 Montmorillonite structure

1.3.1 Chemical Composition of montmorillonite clay

The chemical formula for montmorillonite clay is $(Na, Ca)_{0.33}(Al, Mg)_2(Si_4O_{10})(OH)_2\,nH2O$. It is a hydrous aluminium phyllosilicate mineral. Its silicate sheet layers are layered and filled with exchangeable cations like sodium (Na^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), and aluminium (Al^{3+}). The high cation exchange capacity (CEC) and swelling behaviour of montmorillonite clay are special characteristics that are due to these exchangeable cations [2].

1.3.2 Structure

The silicate sheets are piled in layers to form the structure of montmorillonite clay. Tetrahedral and octahedral sheets are stacked in an alternating arrangement to make up each silicate sheet. The octahedral sheet is made up of aluminium (Al) or magnesium (Mg) atoms coupled to oxygen (O) atoms in an octahedral arrangement, whereas the tetrahedral sheet is made up of silicon (Si) atoms bonded to oxygen (O) atoms in a hexagonal configuration [3]. The weak van der Waals forces that hold the layers together are what allow montmorillonite clay to swell and contract in response to changes in moisture content.

1.3.3 Properties

1.3.3.1 Cation Exchange Capacity (CEC)

The ability of Montmorillonite clay to draw in, hold onto, and exchange cations is known as its high cation exchange capacity (CEC). This characteristic makes montmorillonite clay helpful in agriculture for improving soil fertility and as a detoxifying agent in water purification operations. It enables it to adsorb and cling onto minerals, ions, and other molecules.

1.3.3.2 Swelling Behaviour

Due to its layered structure and high moisture absorption capacity, montmorillonite clay exhibits a distinctive swelling behaviour. The silicate sheets' interlayer gaps may widen when exposed to water, causing the clay to inflate and gain volume. Due to this characteristic, montmorillonite clay can be used to construct foundry moulds, fill cracks in buildings, and seal drilling fluids.

1.3.3.3 Thermal Stability

Due of its strong thermal stability, montmorillonite clay can withstand high temperatures. This characteristic makes it excellent for usage as thermal insulators in building materials as well as fire-resistant materials.

1.3.3.4 Adsorption and Absorption

Since montmorillonite clay has a high capacity for both adsorption and absorption, it may both adsorb and absorb a wide range of compounds, including poisons, heavy metals, and organic molecules. Due to this characteristic, montmorillonite clay is valuable in both environmental clean-up procedures as well as uses involving detoxification, such as cosmetic products.

1.3.3.5 Plasticity

The ability to be moulded and shaped without losing its structural integrity is known as plasticity, and it is a property of montmorillonite clay [4].

1.3.4 Applications

1.3.4.1 Geology

Geology relies heavily on montmorillonite clay, especially when it comes to the development of clay minerals in sedimentary rocks. The clay-rich rock type bentonite, which is utilised in drilling fluids for oil and gas exploration, is where it is typically found. Due to its special characteristics, including its high swelling behaviour and capacity to build impermeable barriers, montmorillonite clay is utilised in drilling muds to manage pressure, stabilise boreholes, and stop fluid migration [5].

1.3.4.2 Agriculture

In order to increase the fertility and structure of soil, montmorillonite clay is frequently employed in agriculture. Its high cation exchange capacity (CEC) enables it to retain and release minerals like potassium (K), calcium (Ca), and magnesium (Mg) that are crucial for plant growth while also enhancing soil water retention and aeration. As a soil conditioner, fertiliser carrier, and part of livestock feed, montmorillonite clay helps improve animal digestion and nutrient absorption.

1.3.4.3 Construction

Montmorillonite clay is used to enhance the qualities of building materials like cement, concrete, and plaster. As a plasticizer, it improves the materials' workability and lessens material cracking. Due to its flexibility and capacity to bind things together, montmorillonite clay is also utilised in the creation of pottery, bricks, and tiles.

1.3.4.4 Environmental Remediation

The removal of pollutants from soil and water has been a common use for montmorillonite clay in environmental remediation. As a result of its strong adsorption and absorption capacities, it is effective at adsorbing heavy metals, pesticides, and other pollutants, assisting in the clean-up of contaminated areas and the improvement of water quality.

1.3.4.5 Bio-Medical Uses

1.3.4.5.1 Drug Delivery

The use of montmorillonite clay as a medication delivery system carrier has been investigated. It is useful for encapsulating pharmaceuticals and releasing them gradually due to its high surface area, porosity, and capacity to expand and intercalate molecules [6]. A possible contender for numerous therapeutic uses, montmorillonite clay-based drug delivery systems have showed promise in enhancing drug stability, bioavailability, and targeted administration to certain areas in the body.

1.3.4.5.2 Wound Healing

The ability of montmorillonite clay to heal wounds has been studied. Montmorillonite clay has the ability to absorb excess moisture from wounds, produce a moist environment that aids in wound healing, and act as a physical barrier against outside contaminants. Additionally, it demonstrates antibacterial and anti-inflammatory qualities, which can aid in reducing inflammation and preventing infection, facilitating the healing process [7].

1.3.4.5.3 Bone Tissue Engineering

Due to its biocompatibility and capacity to imitate the mineral makeup of bone, montmorillonite clay has been investigated as a viable material for bone tissue engineering. Incorporating it into scaffolds or composites can improve their mechanical qualities, encourage

cell adhesion, proliferation, and differentiation, and aid in bone regeneration [8]. The capacity of montmorillonite clay to release ions, such as calcium and silicon, which are crucial for bone formation, has also been investigated.

Montmorillonite, a natural clay mineral with a high surface area and biocompatibility, is increasingly employed in bone tissue engineering. Incorporated into scaffold materials, it enhances mechanical strength, bioactivity, and cell adhesion. Its ion exchange capacity enables controlled drug release, particularly of essential ions like calcium and magnesium, facilitating localized growth factor delivery for bone regeneration. Furthermore, montmorillonite's osteoinductive properties encourage osteogenic differentiation of mesenchymal stem cells, making it a valuable component in the quest to develop innovative therapies for bone injuries and diseases, thus representing a promising avenue for future research and application in the field of bone tissue engineering.

1.3.4.5.4 Dental Applications

The dental uses of montmorillonite clay, such as toothpaste, mouthwash, and dental composites, have been researched. It has been researched for its capacity to remineralize teeth, limit bacterial growth, and absorb poisons and pathogens. Dental products made from montmorillonite clay have demonstrated promise in terms of avoiding dental cavities, lowering plaque production, and enhancing general oral health.

1.3.4.5.5 Hemostasis

To stop bleeding during surgical procedures or after traumatic injuries, montmorillonite clay has been employed as a hemostatic agent. Montmorillonite clay can rapidly absorb blood and form a clot when applied to a bleeding location, aiding in the cessation of the bleeding and fostering hemostasis. In circumstances where quick blood clotting is essential, including in emergency medical treatment or surgical procedures, this feature makes it valuable [9].

1.4 Antibiotics

Antibiotics are a group of medications that are used to treat bacterial infections. They function by preventing the growth of or eradicating the infection-causing microorganisms. Modern

medicine is dependent on antibiotics because they are successful at treating bacterial infections and have saved many lives.

Antibiotics come in a wide variety of forms, and they can be divided into several groups based on how they work, how broadly they can be used, and how they are administered. The different types of antibiotics are:

Penicillin

One of the oldest and most often used classes of antibiotics is the penicillin family. They function by preventing the development of bacterial cell walls, which results in the bacterial death.

Macrolides

Macrolides cause bacteria to stop producing proteins, which kills the organism. They are frequently used to treat skin infections and infections of the respiratory tract.

Cephalosporins

In terms of how they work, cephalosporins are comparable to penicillin, but they have a wider range of activity and are effective against more types of bacteria.

Tetracyclines

Tetracyclines cause bacteria to die by preventing their ability to synthesise proteins. Acne and specific kinds of respiratory tract infections are frequently treated with them.

Fluoroquinolones

Fluoroquinolones kill bacteria by blocking the enzymes needed for DNA replication, which is how they function [10].

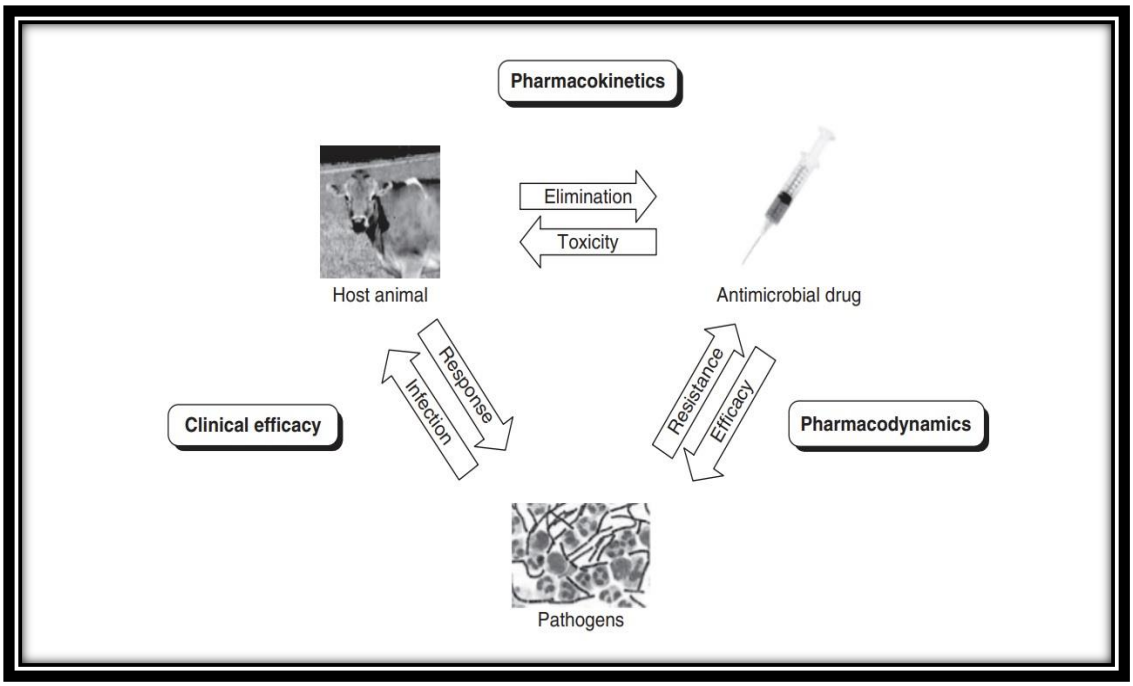


Figure 1-2 Schematic of the chemotherapeutic triangle depicting the relationships between the host animal, antimicrobial drug, and target pathogens.

1.5 Gatifloxacin

1.5.1 Chemical Structure

One-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-3-quinolinecarboxylic acid is the chemical name for the antibiotic gatifloxacin. Its molecular weight is 375.4 g/mol and its chemical formula is $C_{19}H_{22}FN_3O_4$ [11]

1.5.2 Mechanism of Action

Bacterial DNA gyrase and topoisomerase IV, which are involved in DNA replication and repair, are inhibited by gatifloxacin, which then has an antibacterial effect. The bacterial DNA synthesis process is interfered with by gatifloxacin by blocking these enzymes, which prevents the bacteria from replicating properly and finally results in their death.

1.5.3 Spectrum of Activity

A large variety of Gram-positive and Gram-negative bacteria, as well as unusual microorganisms, can be successfully treated with the broad-spectrum antibiotic gatifloxacin. Along with several Gram-negative bacteria like Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa, it has minimal efficacy against some common respiratory tract

pathogens such Haemophilus influenzae, Moraxella catarrhalis, and Streptococcus pneumoniae. Mycoplasma pneumoniae and Chlamydophila pneumoniae are two examples of the atypical bacteria that gatifloxacin is effective against.

1.5.4 Indications

Gatifloxacin is used to treat a number of bacterial diseases, including respiratory tract infections such community-acquired pneumonia, acute exacerbations of chronic bronchitis, and sinusitis. conditions of the skin, soft tissues, the urinary tract, and other bacterial illnesses brought on by susceptible organisms are also treated with it.

1.6 Bacteria and wound microbiology

Wound microbiology is a fascinating and critical field of study within the realm of microbiology and medicine. It involves the investigation of the diverse communities of microorganisms that can colonize and potentially infect wounds in the human body. Bacteria play a central role in wound microbiology, as they are often the primary culprits in wound infections. Understanding the dynamics of these microscopic organisms in wounds is crucial for effective wound management and treatment.

Wounds create a unique ecological niche that invites the colonization of various microorganisms. These microorganisms can be broadly classified into two categories: commensals and pathogens. Commensal bacteria are normally present on the skin and mucous membranes without causing harm. However, when the skin's protective barrier is breached due to injury, surgery, or chronic conditions, these commensals can become opportunistic pathogens.

Pathogens, on the other hand, are microorganisms that have the potential to cause harm and infections. Common wound pathogens include Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, and Escherichia coli. The severity of wound infections can range from mild to life-threatening, depending on the type of bacteria involved, the host's immune response, and the wound's overall condition.

Aerobic and facultative microorganisms	Type of wound	Anaerobic bacteria	Type of wound
Coagulase-negative staphylococci	A, C	*Peptostreptococcus asaccharolyticus*	A, C
Micrococcus sp.	C	*Peptostreptococcus anaerobius*	A, C
Staphylococcus aureus	A, C	*Peptostreptococcus magnus*	A, C
Beta-hemolytic streptococcus (group C)	A	*Peptostreptococcus micros*	A, C
Beta-hemolytic streptococcus (group G)	C	*Peptostreptococcus prevotii*	A, C
Streptococcus spp. (fecal)	A, C	*Peptostreptococcus indolicus*	C
Streptococcus spp. (viridans)	A, C	*Peptostreptococcus* sp.	A, C
Corynebacterium xerosis	C	*Streptococcus intermedius*	C
Corynebacterium sp.	A, C	*Clostridium perfringens*	A, C
Bacillus sp.	A	*Clostridium clostridioforme*	A, C
		Clostridium cadaveris	A, C
Escherichia coli	A, C	*Clostridium baratii*	C
Escherichia hermanii	A	*Clostridium septicum*	A
Serratia liquefaciens	C	*Clostridium histolyticum*	A, C
Klebsiella pneumoniae	A, C	*Clostridium tertium*	A
Klebsiella oxytoca	A, C	*Clostridium ramosum*	C
Enterobacter cloacae	A, C	*Clostridium sporogenes*	A, C
Enterobacter aerogenes	C	*Clostridium difficile*	C
Citrobacter freundii	C	*Clostridium bifermentans*	A
Proteus mirabilis	A, C	*Clostridium limosum*	A
Proteus vulgaris	C	*Eubacterium limosum*	C
Providencia stuartii	A	*Propionibacterium acnes*	A, C
Morganella morganii	C		
Acinetobacter calcoaceticus	A, C	*Bacteroides fragilis*	A, C
Pseudomonas aeruginosa	A, C	*Bacteroides ureolyticus*	A, C
Stenotrophomonas maltophilia	A	*Bacteroides ovatus*	A
Sphingobacterium multivorum	C	*Bacteroides uniformis*	A, C
		Bacteroides stercoris	C
Candida parapsilosis	A	*Bacteroides capillosus*	C
Candida krusei	A	*Bacteroides thetaiotaomicron*	C
		Bacteroides caccae	C
		Prevotella oralis	A, C
		Prevotella oris	A, C
		Prevotella disiens	A
		Prevotella bivia	C
		Prevotella buccae	C
		Prevotella sp.	A
		Prevotella corporis	A, C
		Prevotella intermedia	A
		Prevotella melaninogenica	C
		Porphyromonas asaccharolytica	A, C
		Gram-negative pigmented bacillus	A, C
		Fusobacterium necrophorum	C
		Veillonella spp.	A

[a] Adapted from reference 27 with permission of the publisher.
[b] Acute wounds (A) included primarily cutaneous abscesses and postsurgical wounds; chronic wounds (C) included primarily leg ulcers, foot ulcers and pressure sores. A total of 367 isolates were cultured from the 106 wounds (61 acute wounds and 45 chronic wounds).

Figure 1-3 Different aerobic and anaerobic bacteria present in wound microenvironment

Wound Type: The microbiota in a wound can vary based on its type. For example, surgical wounds may have different bacterial profiles than chronic ulcers or traumatic injuries.

Host Factors: The health and immune status of the individual can impact the susceptibility to wound infections. Immunocompromised individuals are more vulnerable to severe infections.

Wound Environment: The oxygen levels, pH, moisture content, and nutrient availability in the wound environment can influence the types and numbers of bacteria that thrive there.

Foreign Bodies: The presence of foreign bodies, such as sutures, implants, or debris, can serve as breeding grounds for bacteria and complicate wound healing.

Chronic wounds, such as diabetic foot ulcers and pressure sores, are particularly challenging in wound microbiology. In these cases, the microbiota can become more complex and polymicrobial, with a diverse array of bacteria, fungi, and even antibiotic-resistant strains. The persistence of these infections can lead to delayed wound healing and serious complications.

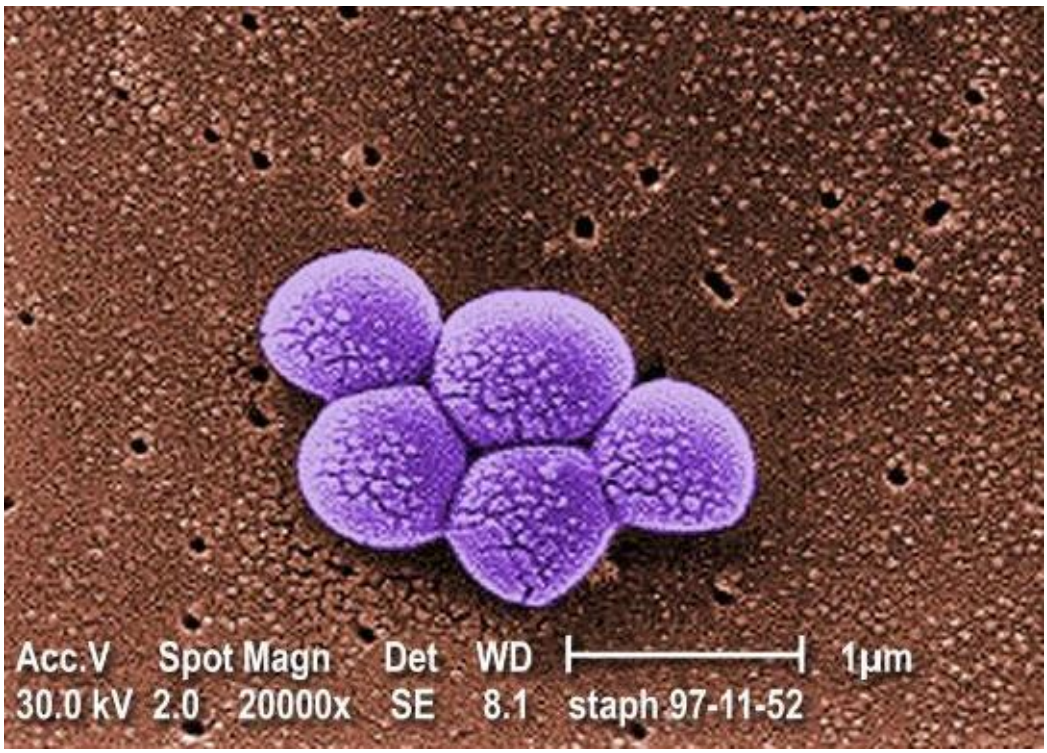


Figure 1-4 SEM image of digitally colourized Staphyllococcus Aureus

The study of wound microbiology has evolved with advancements in diagnostic techniques. Traditional culture-based methods have limitations in capturing the full diversity of wound microorganisms, and modern molecular methods like polymerase chain reaction (PCR) and next-generation sequencing (NGS) are increasingly used for more accurate profiling.

Treatment strategies for wound infections involve both local and systemic approaches. These may include wound debridement (removal of infected tissue), topical antimicrobial agents, dressings that promote a moist wound environment, and systemic antibiotics when necessary. In severe cases, surgical interventions may be required to address deep-seated infections or remove foreign bodies.

1.7 Loading of Drug into clay layers

1.7.1 Adsorption

The mechanism by which drug molecules are drawn to and cling to the surface of montmorillonite clay particles is known as adsorption. By merely combining the medication with a suspension of clay particles in a liquid media, the drug molecules can be directly loaded onto the clay particles. The interactions between the drug molecules and the clay atoms include van der Waals forces, hydrogen bonds, and electrostatic interactions. The adsorption procedure may be influenced by the drug's physicochemical qualities, the surface area and charge of the clay particles, and other factors.

1.7.2 Intercalation

The mechanism by which drug molecules are drawn to and cling to the surface of montmorillonite clay particles is known as adsorption. By merely combining the medication with a suspension of clay particles in a liquid media, the drug molecules can be directly loaded onto the clay particles. The interactions between the drug molecules and the clay atoms include van der Waals forces, hydrogen bonds, and electrostatic interactions. The adsorption procedure may be influenced by the drug's physicochemical qualities, the surface area and charge of the clay particles, and other factors.

1.7.3 Encapsulation

The technique of encapsulation, which usually involves the creation of nanoparticles or nanocomposites, is how drug molecules are contained within the clay particles. This can be done by either covering the clay particles with materials that contain drug molecules or by introducing drug molecules into the clay during the synthesis or creation of the clay particles. The drug molecules may be protected by an encapsulating barrier, improving their stability and possibly regulating their release [12].

1.7.4 Benefits of Drug Loading into Montmorillonite Clay

1.7.4.1 Controlled Drug Release

Drugs can be slowly released in a regulated manner by montmorillonite clay. To achieve a sustained and regulated drug release profile, the interlayer spacing of the clay particles can act as a reservoir, progressively releasing the loaded drug molecules.

1.7.4.2 Protection of Drugs

In order to protect loaded pharmaceuticals from environmental elements including light, moisture, and oxidation, which could cause the drugs to deteriorate and lose some of their effectiveness, montmorillonite clay can operate as a protective habitat [13].

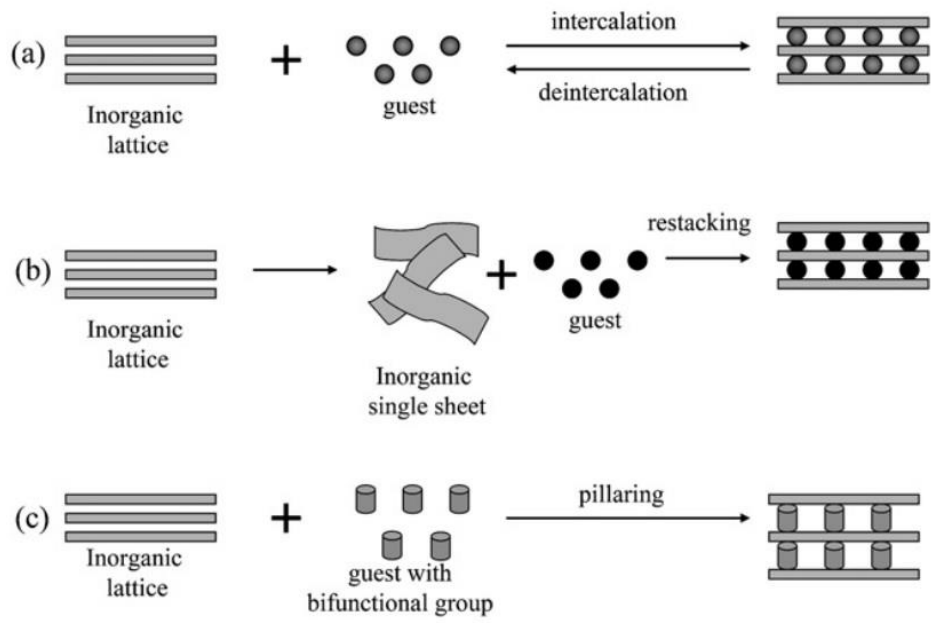


Figure 1-5 Reaction routes to incorporate biomolecules into layered nano-materials for biomolecule loading and protection

1.7.4.3 Increased Drug Stability

Some medications, especially those that are susceptible to deterioration or have low solubility, have been demonstrated to be more stable when dissolved in montmorillonite clay. The loaded medications may have a longer shelf life and have improved bioavailability due to the clay's propensity to adsorb substances, which can shield the drugs from deterioration or precipitation [14].

1.7.4.4 Enhanced Drug Delivery

The size, shape, and surface characteristics of drug-loaded montmorillonite clay particles can be designed to have particular effects on how they interact with biological tissues and cells [15]. This might lead to better medication delivery to the intended tissues or cells, increasing the therapeutic potency of the loaded medicines.

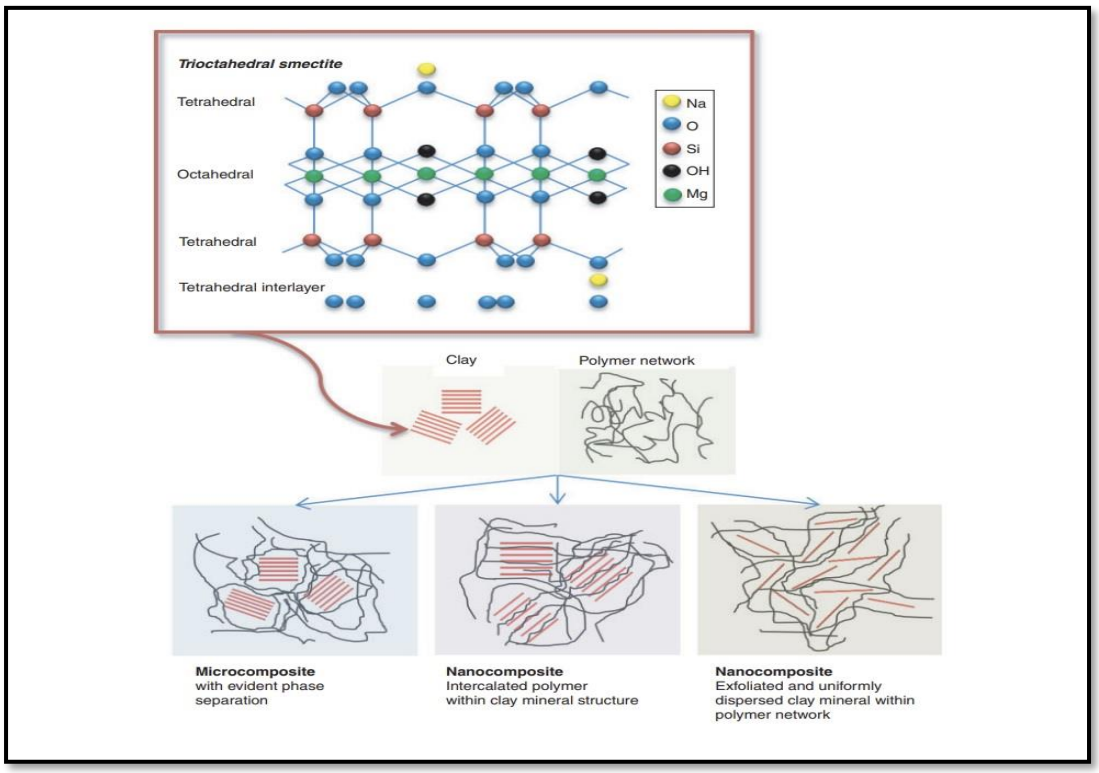


Figure 1-6 Schematic representation of clay mineral (trioctahedral smectite) and clay mineral-polymer composites.

1.7.4.5 Biocompatibility

Due to the fact that montmorillonite clay is a naturally occurring mineral and has been employed in a variety of biomedical applications, including drug delivery, without posing any significant toxicity risks, it is usually regarded as being biocompatible. For each unique clay-drug system, comprehensive biocompatibility testing is still required to guarantee safety and efficacy [16].

In biomedical applications, drug loading into montmorillonite clay has showed promise as a possible drug delivery method. The current work is focused on loading gatifloxacin into

montmorillonite clay layers, characterize the drug loading, analyse the release kinetics, efficacy of the released drug on a bacterium cultured in lab.

1.8 Reference

[1] F. Uddin, "Monmorillonite: An Introduction to Properties and Utilization," *Curr. Top. Util. Clay Ind. Med. Appl.*, pp. 3–24, 2018, doi: 10.5772/intechopen.77987.

[2] S. Cadars *et al.*, "New insights into the molecular structures, compositions, and cation distributions in synthetic and natural montmorillonite clays," *Chem. Mater.*, vol. 24, no. 22, pp. 4376–4389, 2012, doi: 10.1021/cm302549k.

[3] M. Matusewicz *et al.*, "Microstructural investigation of calcium montmorillonite," *Clay Miner.*, vol. 48, no. 2, pp. 267–276, 2013, doi: 10.1180/claymin.2013.048.2.08.

[4] M. Segad, B. Jönsson, T. Åkesson, and B. Cabane, "Ca/Na montmorillonite: Structure, forces and swelling properties," *Langmuir*, vol. 26, no. 8, pp. 5782–5790, 2010, doi: 10.1021/la9036293.

[5] Q. Rao and Y. Leng, "Molecular Understanding of CO2 and H2O in a Montmorillonite Clay Interlayer under CO2 Geological Sequestration Conditions," *J. Phys. Chem. C*, vol. 120, no. 5, pp. 2642–2654, 2016, doi: 10.1021/acs.jpcc.5b09683.

[6] S. Jayrajsinh, G. Shankar, Y. K. Agrawal, and L. Bakre, "Montmorillonite nanoclay as a multifaceted drug-delivery carrier: A review," *J. Drug Deliv. Sci. Technol.*, vol. 39, pp. 200–209, 2017, doi: 10.1016/j.jddst.2017.03.023.

[7] M. Horue, M. L. Cacicedo, M. A. Fernandez, B. Rodenak-Kladniew, R. M. Torres Sánchez, and G. R. Castro, "Antimicrobial activities of bacterial cellulose – Silver montmorillonite nanocomposites for wound healing," *Mater. Sci. Eng. C*, vol. 116, p. 111152, 2020, doi: 10.1016/j.msec.2020.111152.

[8] K. S. Katti, D. R. Katti, and R. Dash, "Synthesis and characterization of a novel chitosan/montmorillonite/ hydroxyapatite nanocomposite for bone tissue engineering," *Biomed. Mater.*, vol. 3, no. 3, 2008, doi: 10.1088/1748-6041/3/3/034122.

[9] C. Liu *et al.*, "Efficient antibacterial dextran-montmorillonite composite sponge for

rapid hemostasis with wound healing," *Int. J. Biol. Macromol.*, vol. 160, pp. 1130–1143, 2020, doi: 10.1016/j.ijbiomac.2020.05.140.

[10] P. T. Reeves, "Antibiotics: Groups and Properties," *Chem. Anal. Antibiot. Residues Food*, pp. 1–60, 2011, doi: 10.1002/9781118067208.ch1.

[11] C. M. Perry, J. A. B. Balfour, and H. M. Lamb, "Gatifloxacin," vol. 58, no. 4, pp. 683–696, 1999.

[12] C. Viseras, C. Aguzzi, P. Cerezo, and M. C. Bedmar, "Biopolymer-clay nanocomposites for controlled drug delivery," *Mater. Sci. Technol.*, vol. 24, no. 9, pp. 1020–1026, 2008, doi: 10.1179/174328408X341708.

[13] J. M. Oh, T. T. Biswick, and J. H. Choy, "Layered nanomaterials for green materials," *J. Mater. Chem.*, vol. 19, no. 17, pp. 2553–2563, 2009, doi: 10.1039/b819094a.

[14] C. Aguzzi, P. Cerezo, C. Viseras, and C. Caramella, "Use of clays as drug delivery systems: Possibilities and limitations," *Appl. Clay Sci.*, vol. 36, no. 1–3, pp. 22–36, 2007, doi: 10.1016/j.clay.2006.06.015.

[15] W. Chrzanowski, S. Y. Kim, and E. A. Abou Neel, "Biomedical applications of clay," *Aust. J. Chem.*, vol. 66, no. 11, pp. 1315–1322, 2013, doi: 10.1071/CH13361.

[16] J. H. Yang, J. H. Lee, H. J. Ryu, A. A. Elzatahry, Z. A. Alothman, and J. H. Choy, "Drug–clay nanohybrids as sustained delivery systems," *Appl. Clay Sci.*, vol. 130, pp. 20–32, 2016, doi: 10.1016/j.clay.2016.01.021.

2 Scope of work

2.1 Importance of loading of antibiotic in clay materials

For a various of reasons, drugs are infused into clay materials. Clay materials provide biocompatibility, increased drug solubility, drug protection and stabilisation, targeted medication delivery, and controlled drug release. They offer a special and beneficial medication delivery mechanism that enables accurate dosage, greater stability, increased bioavailability, and targeted distribution to particular body locations. Clay materials are adaptable to multiple drug delivery methods and formulations due to their simplicity of modification and incorporation.

2.2 Oral versus topical application of antibiotics

Antibiotics can be administered to the body through two different methods, either orally or topically, to cure infections.

Antibiotics are administered orally in the form of pills, capsules, or syrups, which are subsequently ingested and circulated throughout the body by the bloodstream [1]. When a higher concentration of the antibiotic is required to reach the infection site or when the illness has spread throughout the body, oral antibiotics are frequently used to treat systemic infections. Internal infections include bloodstream infections, urinary tract infections, and respiratory infections can all be successfully treated with oral antibiotics.

On the other hand, topical administration entails putting antibiotics in the form of creams, ointments, gels, or lotions directly onto the skin or mucous membranes. Topical antibiotics are used to treat localised bacterial skin infections, wounds, burns, and other skin disorders that affect the skin or mucous membranes. [2][3] Topical antibiotics function by eradicating or preventing bacterial development on the skin's surface.

2.3 Bacterial Culture

By offering a regulated environment for their multiplication and research, bacterial culture plays a critical role in the scientific study of bacteria. The main goal was to learn more about the metabolism, growth patterns, and responses to diverse stimuli of bacteria. This technique enabled researchers to understand the complex systems that control bacterial behaviour.

A crucial element of bacterial culture is the culture medium, which was carefully designed. To boost bacterial growth, it involved combining vital nutrients, along with salts and growth agents. Agar was used to produce a suitable matrix in the case of solid media. The culture media underwent a sterilisation procedure to remove any impurities that could hinder bacterial development in order to guarantee a sterile environment [4].

The selected bacteria were then added to the pre-made culture medium using specified techniques. The start of the growth process was aided by adding a precise amount of the bacterial sample to the medium. In order to prevent contamination and preserve the culture's purity, this phase required meticulous monitoring [5].

The cultivated media was put inside an incubator after being introduced with the bacteria. This carefully regulated setting offered the right temperature, humidity, and other elements for bacterial development [6]. Depending on the bacterial strain and the goals of the experiment, the incubation period changed. In this way the cultured bacteria were produced.

2.4 Advantages of patches over oral medication

1. Patches offer controlled and sustained drug administration, releasing medication gradually and consistently over a long period of time. Avoiding the fluctuating levels that can happen with other medicine forms, including pills taken orally, helps maintain a steady blood concentration of the drug [7].

2. Patches can lessen the need for frequent dosage. Patches may be created to deliver medication over a longer period of time, allowing for less frequent administration than several daily pill doses. This may increase patient comfort and drug compliance.

3. Patches may be practical and simple to use. Patches can frequently be worn covertly under clothing and are typically easy to apply to the skin. Patients who struggle to swallow pills or who may have other oral drug administration restrictions may find this to be especially helpful.

4. The dose of patches may be flexible. Some transdermal patches offer flexibility in drug management by allowing easy dose modulation or medication discontinuation when necessary.

2.5 Release from patch

The organised and controlled release of antibacterial chemicals from specialised adhesive patches, which facilitates their absorption into the skin or a targeted area, is known as antibacterial release from a patch. Due to its targeted and continuous distribution of antimicrobial agents, which considerably aids in infection management and control, this technology has become more popular in medical applications [8].

The patch's actual design is essential to the release of antibiotic compounds. These patches often have various layers, each of which has a different function. The antibacterial agent is contained within the patch, frequently in a reservoir or matrix layer, allowing for controlled release over a predetermined time period and progressive diffusion.

Applications

The uses of antibacterial patches cover a range of medical situations:

Wound Management: To prevent and cure infections in wounds, cuts, and abrasions, antibacterial patches are used in wound care.

Postoperative Infections: By helping to lower the risk of infections at surgical sites, these patches facilitate postoperative healing.

Infections Caused by Catheters: Antibacterial release patches are helpful in preventing infections caused by catheter use.

Burn Treatment: Antibacterial patches are used in the treatment of burns to aid in wound healing and the prevention of infections.

Implant Surfaces: Antibacterial patches are used on implant surfaces to reduce infections brought on by medical equipment.

2.6 Work done in the thesis

Drug Loading:

This section involved the process of incorporating a specific drug into a carrier material, such as clay or a polymer. The method used for drug loading, the drug-to-carrier ratio, and the efficiency of loading were explored.

Characterization:

Detailed characterization of the drug-loaded carrier material was conducted. Techniques like spectroscopy, microscopy, and X-ray diffraction may have been employed to determine the physical and chemical properties of the loaded material.

Kirby Bauer Test:

The Kirby Bauer method, a standard technique in microbiology, was likely used to assess the antimicrobial activity of the drug-loaded material. It involves testing the material against various bacteria to determine its effectiveness as an antimicrobial agent.

Drug Calibration:

Calibration of the drug assay method was performed to accurately quantify the amount of drug released during the in vitro release experiments.

In vitro Release in SSF Medium:

In this section, the drug-loaded material's release behaviour in simulated stomach fluid (SSF) was studied. The kinetics of drug release, release profiles, and factors influencing release were likely investigated.

In vitro Release in SBF Medium:

Similar to the SSF study, the drug-loaded material's release in simulated intestinal fluid (SBF) was examined. Differences in release behaviour between SSF and SBF could be discussed.

MIC calculation of Drug-Loaded Clay:

The mic (minimum inhibitory concentration) of the drug-loaded clay, likely against specific bacteria, was determined. This is crucial for assessing the material's antimicrobial potency.

Patch Formulation:

Development of drug-loaded patches for topical or transdermal delivery. This would involve selecting appropriate materials, formulating the patches, and ensuring drug uniformity.

Release from Patch:

Evaluation of drug release kinetics from the formulated patches. Factors affecting drug release, such as patch composition and design, were investigated.

Efficacy of Patch:

Kirby Bauer test was performed and the patches were successful in showing anti-bacterial effect by producing a zone of inhibition.

2.7 Reference

[1] P. O'Toole *et al.*, "Oral Antibiotic Therapy," *J. Arthroplasty*, vol. 29, no. 2 SUPPL., pp. 115–118, 2014, doi: 10.1016/j.arth.2013.09.050.

[2] H. Todo, T. Oshizaka, W. R. Kadhum, and K. Sugibayashi, "Mathematical model to predict skin concentration after topical application of drugs," *Pharmaceutics*, vol. 5, no. 4, pp. 634–651, 2013, doi: 10.3390/pharmaceutics5040634.

[3] M. Schäfer-Korting, W. Mehnert, and H. C. Korting, "Lipid nanoparticles for improved topical application of drugs for skin diseases," *Adv. Drug Deliv. Rev.*, vol. 59, no. 6, pp. 427–443, 2007, doi: 10.1016/j.addr.2007.04.006.

[4] J. Monod, "a Certain Number," *Annu. Rev. M*, vol. 3, no. Xl, pp. 371–394, 1949.

[5] J. C. Lagier, S. Edouard, I. Pagnier, O. Mediannikov, M. Drancourt, and D. Raoult,

"Current and past strategies for bacterial culture in clinical microbiology," *Clin. Microbiol. Rev.*, vol. 28, no. 1, pp. 208–236, 2015, doi: 10.1128/CMR.00110-14.

[6] R. J. Arnold, J. A. Karty, A. D. Ellington, and J. P. Reilly, "Monitoring the growth of a bacteria culture by MALDI-MS of whole cells," *Anal. Chem.*, vol. 71, no. 10, pp. 1990–1996, 1999, doi: 10.1021/ac981196c.

[7] S. Dhiman, T. G. Singh, and A. K. Rehni, "Transdermal patches: A recent approch to new drug delivery system," *Int. J. Pharm. Pharm. Sci.*, vol. 3, no. SUPPL. 5, pp. 26–34, 2011.

[8] J. E. Riviere and M. G. Papich, "Potential and problems of developing transdermal patches for veterinary applications," *Adv. Drug Deliv. Rev.*, vol. 50, no. 3, pp. 175–203, 2001, doi: 10.1016/S0169-409X(01)00157-0.

3 Literature Review

The purpose of this literature review is to present a thorough summary of the state of research on drug loading into clay-based materials at this time. The pattern at which a chemical is released from a modulated source over time is known to as release kinetics. It is used to describe how medications, chemicals, or other things release from various delivery systems in the domains of pharmacology, materials science, and chemical engineering.

In a study by [1] method has been devised that uses the neurotransmitter release time course to determine how medicines affect the release process. In the research, it is demonstrated that the time course of release is insensitive to the copper ion concentration inside cells is distributed over time.

In another research by [2] it is shown that how to use a mathematical model to characterise paracetamol release curves from gradient matrix systems (GMS) with various geometries (slabs and spheres). Diffusion is considered as the rate-regulating phase of the release process. The variations in matrix structure caused by the various initial loading concentrations and by changes during the release process are accounted for by position- and time-dependent diffusion coefficients. It is possible to fully explain the release of paracetamol from spherical and slab model systems.

In the research by [3] it is concluded that materials like clays are frequently employed in the pharmaceutical industry. It was observed that they interact with medications, limiting their absorption, when supplied concurrently. Therefore, by controlling the release, such interactions can be used at advantages to yield technological and pharmacological benefits. In this field, formulations such as natural clay, commercial clay, synthetic clay, composite clay-polymers, nanocomposites clay-polymers, films, and hydrogel composite clay-polymers are used to affect the release of medications and thereby increase their bioavailability. Montmorillonite clay is used in this project for loading the drug.

In a research by [4] showed a study on drug dissolution and concluded that some cationic drugs and certain non-ionic drugs have attraction for montmorillonite clay. Weakly bound anionic drugs would be expected to have less bioavailability. The two-step process of a cation-exchange reaction followed by strong surface chemisorption the mechanism for how cationic medicines attach to montmorillonite. Montmorillonite clay is used as the matrix and the drug

Gatifloxacin is loaded inside the montmorillonite clay. Gatifloxacin is antibiotic, also known as1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-[3-methyl-1-piperazinyl].Various bacterial infections are treated with this drug. It is a synthetic fluoroquinolone with antibacterial activity against both Gram-negative and Gram-positive pathogens [5].

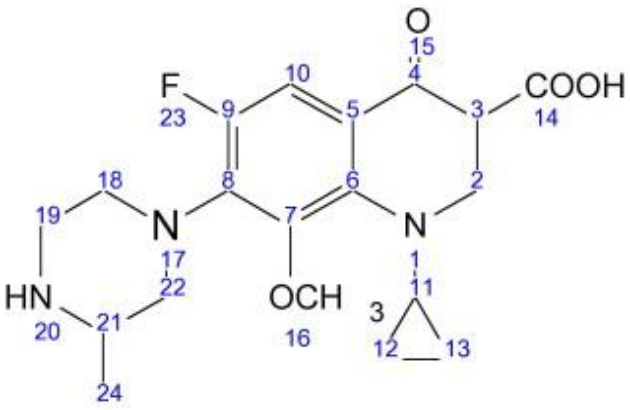


Figure 3-1 Chemical structure of Gatifloxacin

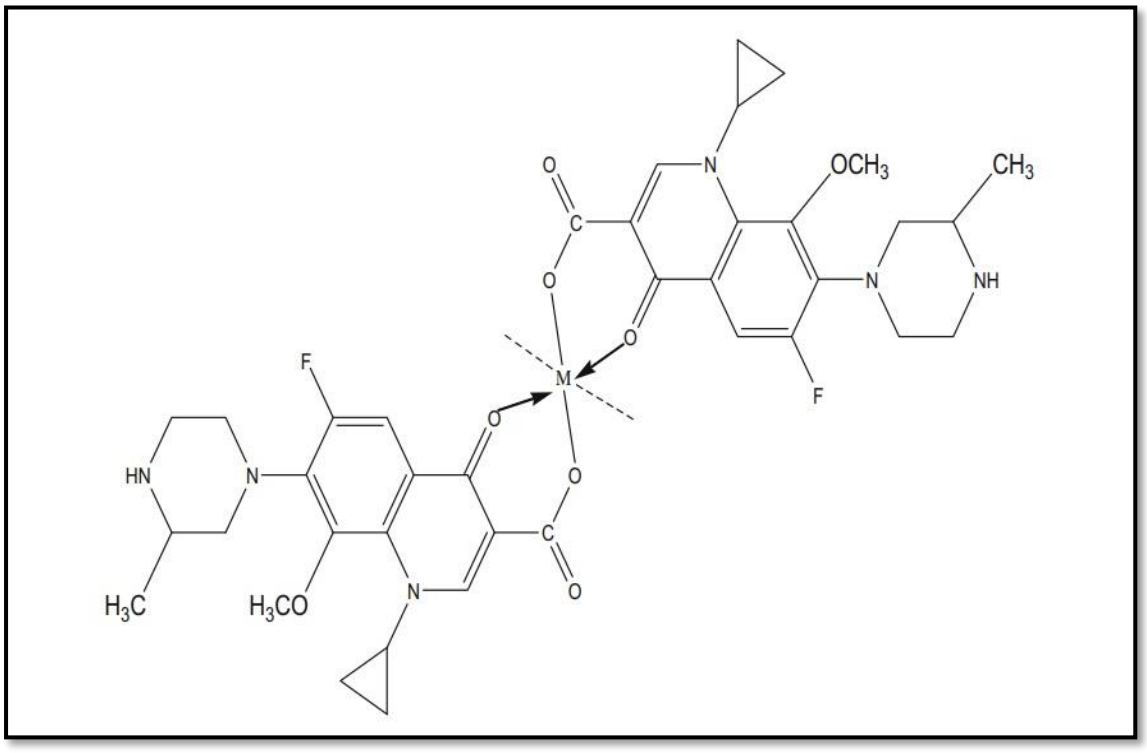


Figure 3-2 The proposed structures of the gatifloxacin–metal complexes

In a research, 873 isolates suffering from infection were tested for gatifloxacin's in vitro activity in Japan between 1997 and 1998. Gatifloxacin showed activity against streptococci, Escherichia coli, Klebsiella pneumoniae, Moraxella catarrhalis, Haemophilus influenzae, and Neisseria gonorrhoeae. The activity was equivalent to that against Gram-negative bacteria and two to 32 times greater than that of ciprofloxacin and levofloxacin against Gram-positive bacteria. When it came to quinolone-resistant staphylococci, Enterococcus faecalis, E. coli, and Enterobacter cloacae, gatifloxacin was more effective than the other quinolones. Gatifloxacin was also effective against H. influenzae and Streptococcus pneumoniae that were resistant to ampicillin and either penicillin or macrolide antibiotics. From this research work [6] we can also conclude that the MIC for gatifloxacin is (0.10-0.39) mg/L and 50% and 90% MIC are both 0.39 mg/L.

From a review by [7] it is concluded that a transdermal patch is a medicated adhesive patch applied to the skin in order to transdermally administer a particular dosage of medication into the circulation. A transdermal patch has certain advantage over other types of drug administrative methods like oral medicine, intravenous administration, topical administration etc. is that does controlled release of the medication into the patient, typically through either a porous membrane covering a reservoir of medication or through body heat melting thin layers of medication embedded in the adhesive. The only drawback of transdermal patch is that epidermis is difficult barrier.

The conclusion of the research by [8] states that the goal of the thorough and in-depth clinical pharmacology research for gatifloxacin was to evaluate the pharmacokinetics and safety of this novel fluoroquinolone antibiotic. Male and female volunteers from diverse age ranges, races, and patient demographics were included in the research.

The individuals received either single-dose or multiple-dose regimens of gatifloxacin throughout the research, either orally or intravenously. Additionally, the programme comprised more than 6,200 individuals who were engaged in randomised clinical studies.

Furthermore, the pharmacokinetic profile of gatifloxacin was predicted. This indicates that it is possible to predict its behaviour throughout the body, including absorption, distribution, metabolism, and excretion. Gatifloxacin showed comparable pharmacokinetic properties in both oral and intravenous forms. It had a long enough half-life to support once-daily dosage,

low serum binding (meaning it doesn't attach to blood proteins significantly), and only moderate build-up with several doses. It was also promptly absorbed after administration.

In addition to having a good pharmacokinetic profile, the antibiotic gatifloxacin also showed strong antibacterial action notably against gram-positive cocci. Common pathogens like Streptococcus pneumoniae and Staphylococcus aureus, which cause a variety of infectious disorders, are examples of gram-positive cocci.

A study by [9] states that the aim is to assess the types of bacteria found in individuals with skin infections and their patterns of antibiotic susceptibility. Swab sticks were employed by the researchers to collect skin samples, which were then streaked onto culture plates containing blood agar, MacConkey agar, and nutrient agar. After that incubation is done, the collected bacteria were evaluated and classified using accepted microbiological standards.

Staphylococcus aureus, Escherichia coli, Klebsiella species, Proteus species, and Pseudomonas aeruginosa were among the five bacterial species identified by the study's findings. Among these, Proteus species had the lowest percentage incidence (9.17%) and Staphylococcus aureus had the greatest proportion (33.03%).

A greater proportion of isolates were found in male patients (61.47%), according to the distribution of isolates by gender. The age groups with the fewest isolates were those between the ages of 31 and 40 (1.83%), while those between the ages of 11 and 20 (40.37%) and 1 to 10 years (25.69%) had the most.

The results of the antibiotic sensitivity test revealed that all of the drugs tested were effective against the Klebsiella species, Proteus species, and Staphylococcus aureus.

Table 3-1 Organisms found on skin infection

Organism	Number of isolates	Percentage occurrence
Staphylococcus aureus	36	33.03%
Klebsiella sp	18	16.51%
Escherichia coli	30	27.52%
Pseudomonas aeruginosa	15	13.76%
Proteus sp	10	9.17%
Total	109	100%

In the research by [10] showed that the release of a drug from its formulation is a critical element that considerably affects its efficacy. The physicochemical qualities of the medication, the excipients utilised, dosage form design, manufacturing process variables, and formulation design are just a few of the variables that affect how the drug releases from the dosage form. Therefore, to ensure the optimal therapeutic effect, understanding the medication release pattern is crucial.

To understand and assess the drug release profile from dosage forms, researchers have created a number of models. These models support the development of powerful formulas. Some of the popular models include the following:

Higuchi Model: This model explains how a matrix system releases drug. It asserts that drug release happens by diffusion across the matrix since the rate of release is related to the square root of time.

Hixson-Crowell: This model is relevant when the formulation's particle surface area and diameter alter. On the basis of the cube root of the remaining drug mass, it describes the drug release.

Korsmeyer-Peppas Model: This model is used to examine drug release from polymeric delivery devices that are both swelling and non-swelling. Both non-Fickian or anomalous transport mechanisms and Fickian diffusion are taken into consideration.

Baker-Lonsdale Mode: This model, which describes drug release from spherical matrices, is derived from the Higuchi model. It offers information on the kinetics of drug release from spherical dosage forms.

Weibull Equation: The release of medications from dosage forms is described by this equation. It expresses the total amount of drug 'm' in solution at time 't'. It is usual practise to fit dissolution data using the Weibull model.

Hopfenberg Model: Drug release from surface-eroding polymers is predicted by this model. It accounts for the polymer matrix's erosion as the drug release mechanism.

Gompertz Model: This exponential model explains the drug's in-vitro dissolving profile. The drug release is thought to begin with a lag phase and then grow exponentially over time.

Gallagher and Corrigan Model: The percentage of medication released from biodegradable polymeric systems is described by this model. It considers things like drug diffusion and polymer degradation as release mechanisms.

Cooney Model: The drug release from spheres and cylinders undergoing surface erosion is explained by this model. It regards surface layer degradation as the main mechanism of drug release.

In a research study by [11] a transdermal patch is a sort of medicated bio adhesive patch that is used to apply medication to the skin in order to penetrate the epidermis and enter the bloodstream. In comparison to other modes of administration, including oral, topical, intravenous, and intramuscular delivery, transdermal drug delivery has a number of advantages.

The transdermal patch's ability to deliver a regulated dose of medication to the patient's system is a significant benefit. The heat from the body melting the tiny layers of medication implanted in the adhesive patch or a porous membrane covering a reservoir of medication can both cause this controlled release. Transdermal medication delivery ensures a constant blood level profile by offering regulated release, which lowers systemic side effects and may even increase efficacy when compared to conventional dose forms.

A transdermal drug delivery system's main goal is to minimise variations within and between individuals while delivering medications into the bloodstream through the skin at a predetermined rate. The therapeutic drug levels are maintained and the best possible treatment results are guaranteed by this controlled and predictable distribution.

Transdermal delivery does have some restrictions, though. The skin acts as a very strong barrier, enabling only little molecules to get through it without difficulty. As a result, this technique can only be used to deliver tiny molecule drugs. Larger-molecule drugs might have trouble penetrating the skin barrier and hence be unsuitable for transdermal administration.

Transdermal delivery of medication has a number of benefits. Injections or oral intake are not necessary because it offers a non-invasive mode of administration. Additionally, it may lead to

a more stable drug concentration in the blood, reducing the negative effects brought on by high and low drug levels. For medications with low oral bioavailability, high first-pass metabolism, or regulated release patterns, transdermal administration can be especially helpful.

Drugs intended for transdermal distribution are developed to have characteristics that make skin absorption easier. These medications typically consist of tiny molecules with specific physical and chemical characteristics that enable them to successfully permeate the epidermal barrier.

3.1 Release Kinetics

The quantitative analysis of a substance's time-dependent release from a dosage form or delivery mechanism is known as release kinetics. Analysis of the substance's release mechanism and rate over time is required. In the realm of pharmaceutical sciences, where it is essential to comprehend and regulate the release of medications from varied formulations, release kinetics is particularly pertinent.

The release kinetics of a drug delivery system describes how the medication is released from the dosage form and made accessible for interaction or absorption at the target site in the body. The medicine, the formulation, and the environment all interact intricately during this process.

The physicochemical characteristics of the medicine, the formulation's design, and the environment all affect how quickly a substance is released into the environment. These variables affect how and how quickly drugs are released, which can be essential for getting the desired therapeutic result.

Different mathematical models and equations are used to comprehend release kinetics. These models explain the empirically observed release profiles and shed light on the underlying mechanisms. Models including zero-order kinetics, first-order kinetics, the Higuchi model, the Korsmeyer-Peppas model, and the Weibull model are frequently employed [12].

Understanding release kinetics enables researchers to improve drugs delivery methods for better therapeutic results. Based on the desired therapeutic goals, it aids in devising dosage forms that offer targeted distribution, controlled and sustained release, or particular release profiles.

Researchers may modify the release kinetics to produce specific drug release profiles and maximise therapeutic efficacy by adjusting variables such formulation composition, drug particle size, matrix properties, and environmental conditions. This information is essential for creating new medication delivery systems and optimising current formulations for effective and precise drug delivery.

3.2 Fick’s law of Diffusion

A fundamental principle that governs the process of diffusion is known as Fick's law of diffusion, after the German physicist Adolf Fick. The term "diffusion" describes how molecules or other small objects move randomly through space from one region of higher concentration to another region of lower concentration.

According to Fick's law of diffusion, the surface area accessible for diffusion, the concentration gradient, and the substance's diffusion coefficient are all directly related to the rate of diffusion (flux) of a substance over a concentration gradient. It has the following mathematical expression:

$J = - D \times (dC/dx)$

Where:

J = Diffusion flux, which is the amount of substance (mass, moles, or particles) passing through a unit area per unit time.

D = Diffusion coefficient or diffusivity of the substance, which is a measure of how easily the substance can diffuse through a particular medium.

(dC/dx) = Concentration gradient of the substance, which is the change in concentration per unit distance.

Fick's law states that the material moves net down the concentration gradient as a result of the diffusion flux (J), which is directed from higher concentration (C1) to lower concentration (C2) regions.

According to this equation, the concentration gradient (dC/dx) and diffusion coefficient (D) are exactly proportional to each other. The "dx" word, which denotes the diffusion path thickness,

also suggests that diffusion is inversely proportional to it. A higher rate of diffusion will be produced by a larger surface area or a steeper concentration gradient [13].

A key idea that guides numerous processes in disciplines including chemistry, biology, and materials science is Fick's law of diffusion. It is frequently employed to comprehend and foresee how compounds will behave in various systems and is essential in fields including drug delivery, membrane transport, and applications in material science.

3.3 Zero Order Release Kinetics

Zero-order release kinetics refers to a type of drug release pattern where the rate of release remains constant over time, regardless of the concentration of the drug. This means that a fixed amount of drug is released per unit of time.

Zero order release kinetics equation:

$dQ/dt = K_0$

dQ/dt = Rate of drug release per unit time

K_0 = Zero order release constant

This equation is derived on the assumption that the rate of drug release is independent of drug concentration and is controlled by a zero-order kinetic process.

Consider a matrix or reservoir system that is stocked with drugs. Q(t) represents the quantity of medication that is still present in the matrix at any given moment. Through the matrix's surface area, the medication is released from the matrix. We assume that the matrix's accessible surface area for drug release is constant [14].

Now, the drug release rate is defined as the variation in the amount of drug still present in the matrix with respect to time:

$dQ/dt = - dQ/dt$

The rate of drug release is constant and independent of drug concentration since zero-order release kinetics are being considered. As a result, we can write the release rate as follows:

$dQ/dt = K_0$

K_0 = Zero-Order release rate constant.

3.4 First order release kinetics

When a drug release pattern uses first-order kinetics, the rate of release is inversely correlated with the amount of drug still in the body. To put it another way, when the medication concentration falls, the rate at which it is released also does.

The following equation can be used to characterise the first-order release kinetics:

$dQ/dt = - k * Q$

Where:

dQ/dt = Rate of drug release per unit of time.

K = First-order release rate constant.

Q = Amount of drug remaining in the system at a given time.

The equation's negative sign denotes the drug concentration's gradual decline as it is released from the system.

Let's consider a drug-loaded matrix or reservoir system to gain a better understanding of first-order release kinetics. Diffusion is used to release the drug, and as it does so, the concentration of the drug in the body gradually drops [15].

The drug concentration within the system acts as the driving force for diffusion in first-order release kinetics. The greater the concentration gradient and, consequently, the faster the release rate, the higher the drug concentration.

The drug's diffusion coefficient, the surface area available for release, and the properties of the matrix or reservoir system are all included in the release rate constant (k), which also takes other variables into account that have an impact on release kinetics.

The drug's diffusion coefficient, the surface area available for release, and the properties of the matrix or reservoir system are just a few of the variables that are considered by the release rate constant (k), which also affects the release kinetics.

According to first-order release kinetics, a drug's release from a dosage form or delivery system should, in theory, gradually slow down over time. With a faster release rate initially when the drug concentration is high and a gradually slower release rate as the drug concentration decreases, the release follows an exponential decay pattern.

3.5 Higuchi Release Kinetics

The Higuchi model, put forth by Yasuo Higuchi, is a mathematical representation of the diffusion-based release of a substance from a matrix system. The amount released is specifically stated to be proportional to the square root of the release time, demonstrating the relationship between the amount of substance released and the release time.

According to the Higuchi model, drug release happens as a result of drug molecules diffusing from a matrix system. The matrix can be a substance that is solid and contains the drug, like a tablet or a polymer matrix. Drug molecules move through the matrix from areas of high concentration to areas of lower concentration during the diffusion process, which results in their release [16].

$$Q = K \times t^{0.5}$$

Q = amount of substance released at time t.

K = Higuchi dissolution constant.

t = release time.

The cumulative amount of substance released (Q), in accordance with the model, is inversely proportional to the square root of the release time (t). This suggests that the release rate is time-dependent and decreases with passing time.

The diffusion process is described by Fick's second law of diffusion, on which the Higuchi model is based. It is predicated on the idea that the substance will diffuse out of the matrix over time in a linear release pattern. This suggests that rather than other factors like erosion or

surface effects, the release is primarily controlled by the substance's diffusion through the matrix.

The release behaviour of drug delivery systems is frequently analysed and predicted using the Higuchi model, especially in cases where diffusion is the predominant mechanism of release. It contributes to understanding the variables affecting drug release, aids in the design and optimisation of controlled-release formulations, and offers insights into release kinetics.

The Higuchi model, though, makes idealised assumptions and simplifies things, so the applicability of the model will depend on the particular drug and matrix system under study. For some systems, additional factors like swelling, erosion, or complicated release mechanisms may need to be considered.

3.6 Korsmeyer-Peppas Model

The power law model, also referred to as the Korsmeyer-Peppas model, is a frequently employed mathematical model to describe drug release from various pharmaceutical dosage forms. Considering the involved release mechanism, it offers a quantitative relationship between the quantity of drug released and the time of release.

The Korsmeyer-Peppas

$M_t/M_\infty = K * t^n$

M_t = Amount of drug released at time t.

M_∞ = Total amount of drug that can be released (usually at infinite time or complete dissolution).

K = Release rate constant.

t = Release time.

n = Release exponent, which characterizes the release mechanism.

According to the Korsmeyer-Peppas model, the drug release time (t) and amount (M_t/M_∞) follow a power law relationship. While the release rate constant (K) incorporates various

factors affecting the release kinetics, the release exponent (n) reflects the release mechanism involved.

The transport mechanism regulating the drug release is revealed by the release exponent (n). Different release mechanisms are associated with various values of n:

Transport Case I (Fickian Diffusion):

n = 0.5: Indicates Fickian diffusion-controlled release, in which the diffusion process is influenced by the concentration gradient.

Non-Fickian or Anomalous Transport

1. $0.5 < n < 1$ (Suggests a combination of diffusion and additional transport processes like polymer relaxation, swelling, or erosion. Represents non-Fickian or anomalous transport.)

2. $n = 1$ (A zero-order release kinetics indicator means that the release rate is unaffected by the drug's concentration.)

3. $n > 1$ (Super case II transport is reflected, pointing to a release mechanism that is influenced by matrix erosion or swelling.)

Regression analysis is used to fit experimental data on drug release at various time points to the Korsmeyer-Peppas model in order to determine the release rate constant (K) and the release exponent (n). The resulting K and n values aid in describing the release behaviour and comprehending the primary release mechanism.

The Korsmeyer-Peppas model is frequently used in the development of pharmaceutical formulations. The drug release profiles from various dosage forms, including tablets, capsules, and transdermal patches, can be predicted and optimised. Researchers can customise drug release to achieve desired therapeutic outcomes, including sustained or targeted release, by modifying K and n values in formulation and design [17].

3.7 Reference

[1] C. Lustigt, H. Parnas, and L. Segelt, “Release Kinetics as a Tool to Describe Drug Effects on Neurotransmitter Release,” 1990.

[2] E. M. G. Van Bommel, R. F. R. Dezentj6, D. J. A. Crommelin, and J. G. Fokkens, “Drug release kinetics from the gradient matrix system" Mathematical modelling,” 1991.

[3] L. A. de S. Rodrigues *et al.*, “The systems containing clays and clay minerals from modified drug release: A review,” *Colloids and Surfaces B: Biointerfaces*, vol. 103. pp. 642–651, Mar. 01, 2013. doi: 10.1016/j.colsurfb.2012.10.068.

[4] J. W. Mcginityx and J. L. Lach, “I n Vitro Adsorption of Various Pharmaceuticals to Montmorillonite.”

[5] N. Sultana, A. Naz, M. S. Arayne, and M. A. Mesaik, “Synthesis, characterization, antibacterial, antifungal and immunomodulating activities of gatifloxacin-metal complexes,” *J. Mol. Struct.*, vol. 969, no. 1–3, pp. 17–24, Apr. 2010, doi: 10.1016/j.molstruc.2010.01.036.

[6] Tsurumaki Youko, Manda Hinako, Takei Masaya, and Hosaka Masaki, “In vitro antimicrobial activity of gatifloxacin against 873 clinical isolates from respiratory tract, urinary tract and surgical infections during 1997-1998 in Japan,” 1997.

[7] D. Patel, S. A. Chaudhary, B. Parmar, and N. Bhura, “THE PHARMA INNOVATION Transdermal Drug Delivery System: A Review,” 2012. [Online]. Available: www.thepharmajournal.com

[8] D. M. Grasela, “Clinical Pharmacology of Gatifloxacin, a New Fluoroquinolone.” [Online]. Available: https://academic.oup.com/cid/article/31/Supplement_2/S51/484496

[9] I. U. Nwankwo, K. C. Edward, C. N. Nwoba, and C. V. Okwudiri, “Evaluation of Bacterial Species in Patients with Skin Infection and their Antibiogram,” *South Asian J. Res. Microbiol.*, pp. 10–16, May 2021, doi: 10.9734/sajrm/2021/v9i430214.

[10] M. Padmaa Paarakh, P. Ani Jose, C. M. Setty, and G. V. P. Christoper, “RELEASE KINETICS-CONCEPTS AND APPLICATIONS.”

[11] A. Sultana and A. Mannan, “Transdermal patch: An overview,” 2020. [Online]. Available: www.ijrti.org

[12] S. Dash, P. N. Murthy, L. Nath, and P. Chowdhury, “Kinetic modeling on drug release from controlled drug delivery systems,” *Acta Pol. Pharm. - Drug Res.*, vol. 67, no. 3, pp. 217–223, 2010.

[13] H. J. V. Tyrrell, “The origin and present status of fick’s diffusion law,” *J. Chem. Educ.*, vol. 41, no. 7, pp. 397–400, 1964, doi: 10.1021/ed041p397.

[14] K. Nagai, T. Shibanuma, and M. Hashimoto, “Zero-order desorption kinetics based on phase equilibrium,” *Surf. Sci.*, vol. 145, no. 1, pp. 4–9, 1984, doi: 10.1016/0039-6028(84)90756-8.

[15] J. B. Schwartz, A. P. Simonelli, and W. I. Higuchi, “Drug Release from Wax Matrices II,” *J. Pharm. Sci.*, vol. 57, no. 2, pp. 278–282, 1968, doi: 10.1002/jps.2600570207.

[16] J. Siepmann and N. A. Peppas, “Higuchi equation: Derivation, applications, use and misuse,” *Int. J. Pharm.*, vol. 418, no. 1, pp. 6–12, 2011, doi: 10.1016/j.ijpharm.2011.03.051.

[17] I. Y. Wu, S. Bala, N. Škalko-Basnet, and M. P. di Cagno, “Interpreting non-linear drug diffusion data: Utilizing Korsmeyer-Peppas model to study drug release from liposomes,” *Eur. J. Pharm. Sci.*, vol. 138, no. June, p. 105026, 2019, doi: 10.1016/j.ejps.2019.105026.

4 Instrumentations

4.1 UV-Vis Spectrophotometer

A UV-Vis spectrophotometer, often known as a spectrophotometer, is a scientific tool used to measure a sample's ability to transmit or absorb UV and visible light. It is a flexible instrument that is frequently used in a variety of disciplines, including chemistry, biochemistry, molecular biology, physics, and environmental research [1].

The Beer-Lambert Law, which states that the absorbance of light by a sample is directly proportional to the concentration of the absorbing substance and the path length of light through the sample, forms the foundation for UV-Vis spectrophotometers.

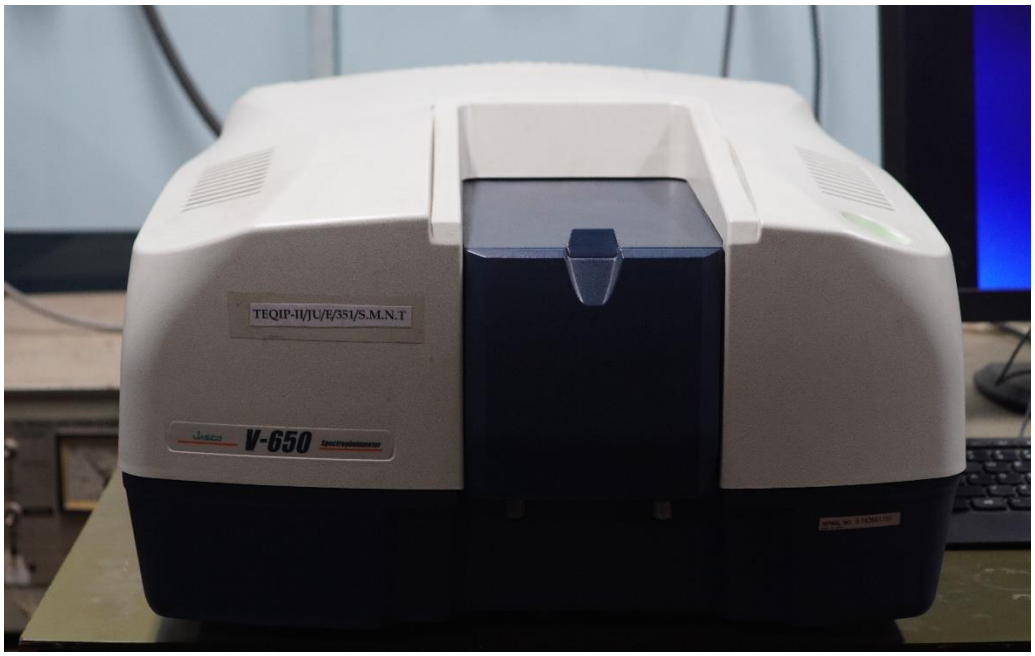


Figure 4-1 UV-Vis Spectrophotometer

Components:

Light Source: It offers a source of ultraviolet and visible light, often a tungsten lamp for visible light and a deuterium lamp for ultraviolet light.

Monochromator: It divides the entering light into distinct wavelengths so that the appropriate wavelength can be chosen for measurement.

The sample holder: which might be a liquid, solid, or gas, is held in the sample holder. Typically, the sample is put in a cuvette, which is a clear vessel.

Photodetector: It gauges how much light is reflected off of or absorbed by the sample. Photodiodes or photomultiplier tubes are common examples of detector types.

Electronics and Display: These parts regulate the operation of the instrument, handle the data processing, and show the results.

Different wavelengths of absorbance or transmittance information are provided by UV-Vis spectrophotometers. To extract useful information or ascertain the characteristics of the sample, the collected data can be further analysed using a variety of mathematical models, such as calibration curves, the standard addition method, or data fitting methods.

Due to their adaptability, simplicity of use, and wide range of applications in studying the interaction of light with matter, UV-Vis spectrophotometers are frequently employed in research, quality control, and academic laboratories.

Ultraviolet-Visible (UV-Vis) spectrometry boasts a wide array of applications across diverse fields. It is employed for quantitative analysis, enabling the determination of compound concentrations in chemistry, pharmaceuticals, and environmental monitoring. Qualitative analysis relies on its ability to identify functional groups and chromophores. In pharmaceuticals, UV-Vis spectrometry ensures the quality and purity of active ingredients. Environmental analysis employs it to monitor pollutants in air, water, and soil. It is instrumental in biochemistry and life sciences for studying biomolecules like DNA and proteins. In the material sciences, UV-Vis spectroscopy characterizes nanomaterials, and in the food industry, it assesses the quality and nutritional content of products. Art restoration relies on its ability to identify pigments and dyes, while water treatment uses it to gauge UV disinfection efficiency. These applications underscore UV-Vis spectrometry's versatile and essential role in scientific research and industrial processes.

4.2 Fourier Transform Infrared (FTIR) Spectrometer

In a wide range of scientific disciplines and industries, Fourier Transform Infrared Spectroscopy (FTIR) has become a common analytical technique. It works by interacting with a sample's molecular vibrations via infrared light, giving important information about the sample's molecular structure and makeup.

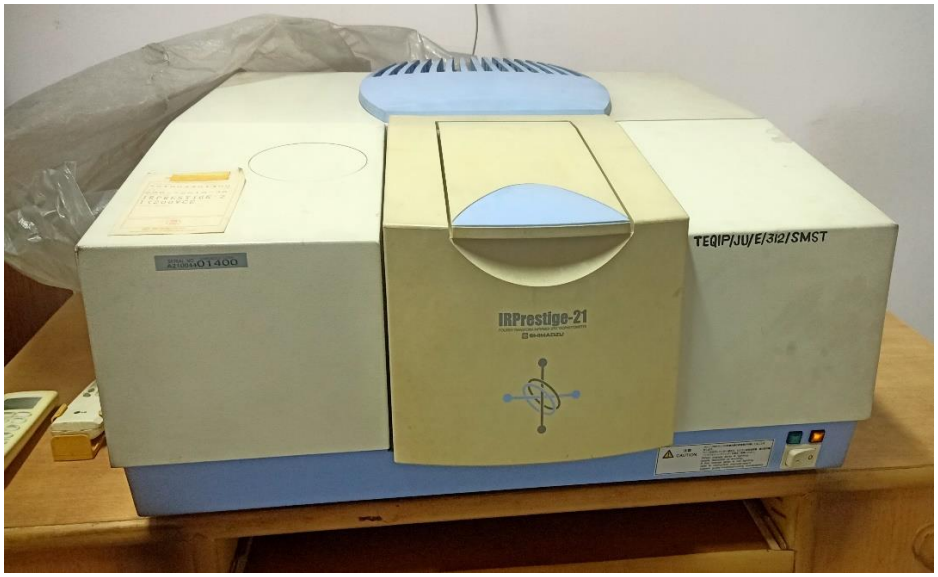


Figure 4-2 FTIR Spectrometer

In FTIR [2], a sample is exposed to an infrared light beam, which passes through it and absorbs certain frequencies that correspond to the molecular vibrations. The obtained spectrum is then obtained after detecting the remaining transmitted light. The intensity of the light that is absorbed or transmitted is shown as a function of frequency or wavenumber in this spectrum.

One important component of FTIR is the analysis of the obtained spectrum. Every peak or band in the spectrum is associated with a particular molecular vibration or set of vibrations. Unknown substances can be found by comparing these peaks to known reference spectra or databases. In disciplines like pharmaceuticals, forensics, environmental science, and food analysis, this qualitative analysis is especially helpful.

Additionally, FTIR makes it possible for quantitative analysis by gauging the strength of the spectrum's peaks. The concentration of a specific substance in an unknown sample can be ascertained by calibrating the instrument with known concentrations of that substance.

Numerous applications, such as quality control, environmental monitoring, and pharmaceutical analysis, benefit from this quantitative ability.

FTIR has numerous uses in a variety of industries and fields of study. FTIR is used in chemical analysis to identify and characterise both organic and inorganic compounds. It facilitates the separation and identification of individual components, assisting in the analysis of complex mixtures.

For a number of purposes, including drug formulation, stability testing, and counterfeit detection, the pharmaceutical industry uses FTIR. It aids in determining functional groups present in pharmaceutical ingredients and confirming the purity of drugs. Additionally, FTIR is essential to forensic science because it helps investigators analyse trace evidence like fibres, paint, and drugs. It assists in identifying the substances that are present at crime scenes and offers crucial evidence for forensic analyses.

4.3 X-Ray Diffractometers

In order to study the crystallographic structure of materials, X-ray diffractometers (XRD) are highly sophisticated devices used in the fields of materials science, chemistry, geology, and other scientific disciplines. In order to understand more about the arrangement of atoms or ions within a material, XRD [3] examines the diffraction patterns that are produced when X-rays interact with a crystalline sample.

4.3.1 Bragg's Law

Bragg's Law states that the crystal planes will diffract X-rays when they hit a crystal lattice at a particular angle. The constructive interference between the diffracted X-rays produces a diffraction pattern with discrete peaks.

$n \lambda = 2 d \sin(\theta)$

n = The order of the diffraction peak. It can take on integer values such as 1, 2, 3, and so on, corresponding to the first, second, third, etc., order of diffraction.

λ = The wavelength of the incident X-rays.

d = The spacing between adjacent crystal planes.

θ = The angle between the incident X-ray beam and the crystal plane.

According to the equation, the X-ray wavelength is an integer multiple of the path difference between waves diffracted from two adjacent crystal surfaces. At particular angles of diffraction, the intensity is increased as a result of this constructive interference. One may determine the distance between crystal surfaces by measuring the diffraction angles and knowing the X-ray wavelength.

4.3.2 Data Analysis in XRD

Peak Position: Information regarding crystal symmetry and lattice spacing may be obtained from the placements of the diffraction peaks. The identification of crystallographic phases contained in the sample is made possible by comparison with reference data.

Peak Intensity: The crystallographic phases' atomic configuration and concentration are related to the peak's intensity. It may be applied to quantitative analysis and crystallinity assessment.

Peak Shape and Breadth: Information about crystallite size, strain, and lattice defects may be obtained from the shape and breadth of the diffraction peaks.

4.3.3 Applications of X-ray Diffraction

Phase identification: XRD is frequently used to pinpoint the crystalline phases that are present in a substance, assisting in substance characterisation and standardisation.

Crystal Structure Determination: XRD enables the determination of the crystal structure, including unit cell characteristics and atomic locations, by analysing the diffraction patterns.

Quantitative Analysis: XRD may be used to quantify the proportions of various phases in a sample, revealing details on the phases' makeup and transitions between them.

Texture Analysis: Understanding the preferred orientation or texture of crystalline materials, such as metals and ceramics, is crucial for interpreting their mechanical and physical characteristics. XRD can do this.

Thin Film Analysis: For research in semiconductor, optoelectronic, and nanotechnology, XRD is used to examine the crystallographic structure and thickness of thin films.

4.4 Scanning Electron Microscopy (SEM)

The discipline of materials characterization and analysis has been completely changed by scanning electron microscopy (SEM) [4], a sophisticated imaging technology. SEM, in contrast to conventional optical microscopes, uses a concentrated electron beam to examine the surface topography and morphology of a material with extraordinary resolution and depth. This non-destructive method offers important insights into the microstructure, elemental makeup, and surface characteristics of a variety of materials.

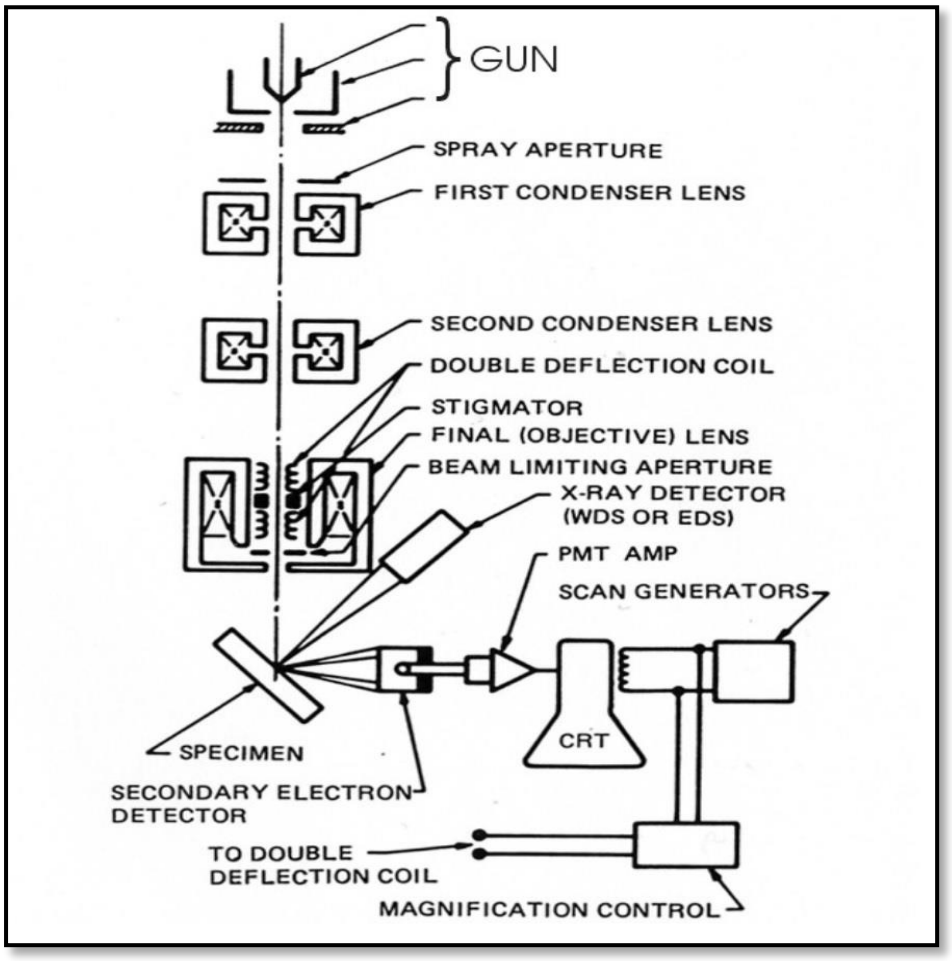


Figure 4-3 SEM Structure

4.4.1 Working Principle

The interaction of a focussed stream of electrons with the sample surface is the basis of scanning electron microscopy's (SEM) working theory. SEM has excellent imaging capabilities that enable close analysis of surface topography and morphology at high resolution. The advanced review will cover electron beam creation, sample interaction, signal detection, and picture formation in addition to a thorough explanation of the SEM's operational principles.

4.4.1.1 Electron Beam Generation

An electron source, usually a tungsten or LaB6 filament, is used to produce an electron beam in the SEM. Field emission guns (FEG) or cold field emission guns (CFEG) may be used in more sophisticated SEM systems to increase brightness and beam stability. A narrow, concentrated beam of electrons is produced by accelerating the electron beam to high energy using an anode voltage.

4.4.1.2 Sample Interaction

The sample surface is the target of the focussed electron beam. Different signals are released as the electrons contact with the sample, giving important details about the surface properties. The main interactions in SEM are as follows:

Elastic Scattering: Elastic scattering is the process through which incident electrons shift their orientation without losing energy. Backscattered electrons (BSE), which are produced as a result of this contact, are identified and employed to create contrast in the SEM image. BSE are sensitive to the sample's atomic number and chemical make-up, giving information about its elemental make-up.

Inelastic Scattering: When the incident electrons provide the sample energy, the sample's atoms are excited or ionised, which causes inelastic scattering. Secondary electrons (SE), which are produced by this interaction and released from the sample surface to create the SEM image, are collected. The surface morphology and topography of the sample are thoroughly described by SE.

X-ray Emission

Characteristic X-rays are released when the high-energy electrons interact with the material because the inner shell electrons relax. Energy-Dispersive X-ray Spectroscopy (EDS) can be used to analyse these X-rays, which include information on the sample's elemental composition.

4.4.1.3 Signal Detection and Image Formation

With the help of the proper detectors, the secondary electrons and backscattered electrons can be collected. While backscattered electron detectors are positioned below the sample to pick up BSE, secondary electron detectors are positioned above the sample surface to pick up SE emissions. Using advanced electronics and imaging technologies, these signals are enhanced, processed, and turned into a digital image. With the use of changes in electron emissions, the resulting image depicts the sample's surface morphology and topography while adding contrast.

4.4.2 Application

Scanning Electron Microscopy (SEM) serves as a multifaceted tool with diverse applications spanning numerous scientific and industrial domains. By utilizing a focused electron beam to scan a sample's surface, SEM delivers high-resolution, three-dimensional images, unveiling crucial details about a specimen's topography, composition, and morphology. In material science, SEM facilitates the study of material microstructures and properties, while in nanotechnology, it empowers researchers to explore and manipulate nanoscale structures. Biologists and life scientists employ SEM for visualizing cell structures and biological specimens. Geologists utilize it for geological sample analysis, and the semiconductor industry relies on SEM for quality control and failure analysis. Additionally, SEM assists in materials and coatings analysis, forensic investigations, cultural heritage preservation, environmental research, and the study of material failures, demonstrating its vital role in advancing scientific knowledge and solving real-world challenges across a wide spectrum of disciplines.

4.5 Biosafety Cabinet (BSC)

A specialised containment device called a biosafety cabinet (BSC) [5], often referred to as a biological safety cabinet or a microbiological safety cabinet, is created to provide a secure working environment for handling dangerous biological agents. To prevent exposure to potentially hazardous bacteria, biosafety cabinets are frequently used in laboratories, healthcare facilities, and research institutes.

Figure 4-4 Biosafety Cabinet

A biosafety cabinet functions by combining HEPA filtration, airflow control, and containment characteristics to stop the discharge of dangerous contaminants and safeguard the user and the surrounding area. Based on their airflow patterns, biosafety cabinets can be divided into Class I, Class II, and Class III, which are the three basic categories.

Class I biosafety cabinets pull air from the surrounding space through a front opening, protecting both the operator and the environment. The air is vented back into the space or through a different exhaust system after being cleaned using a HEPA filter. Class I cabinets offer staff security but do not safeguard the samples being handled within.

Class II biosafety cabinets are the most typical ones found in labs. By combining airflow and HEPA filtration, they offer safety for both the staff and the samples. Class II cabinets contain an operator front access opening and a work surface that directs airflow downward to create a hygienic environment for handling samples. A portion of the air is vented outside the cabinet through a specialised exhaust system while the majority is recirculated inside the cabinet through HEPA filters.

Glove boxes and class III cabinets, commonly referred to as containment and protection levels, offer the highest level of both. They are made to deal with extremely dangerous and contagious substances. Airtight gloves are connected for operator manipulation in Class III cabinets, which are totally sealed and gas-tight. In order to stop the escape of dangerous compounds, the cabinet maintains negative pressure and filters all exhaust air through HEPA filters.

4.5.1 Applications

Microbiological Research

Cell culture, handling of infectious agents, and handling of microbiological cultures all need the use of biosafety cabinets. They offer a controlled environment to stop cross-contamination and safeguard lab workers.

Clinical Diagnostics

Clinical laboratories use biosafety cabinets to handle patient samples, run diagnostic procedures, and handle potentially infectious items.

Pharmaceutical and Biotechnology

The pharmaceutical and biotechnology industries depend on biosafety cabinets to create new drugs, produce sterile goods, and perform research. In addition to safeguarding against cross-contamination, they assist in maintaining the purity and sterility of materials.

Animal Research

In animal research facilities, biosafety cabinets are used for processes involving animals and the containment of potential allergens.

4.6 Centrifuge

A centrifuge is a laboratory tool that uses centrifugal force to separate liquid mixture components according to their densities. It is made up of a rotor that rotates quickly, creating centrifugal forces that induce heavier particles or substances to settle out or separate from the lighter ones.

A centrifuge's operation is based on the idea of centrifugal force [6], which is the outward pressure felt by an item moving in a circular motion. When a centrifuge is in use, the rotor quickly spins and produces a centrifugal field that produces forces that are several times stronger than the force of gravity.

Figure 4-5 Centrifuge

Figure 4-6 Centrifuge loaded with tubes

The liquid combination that has to be separated is loaded into the rotor in tubes or containers. The denser materials move to the bottom of the tube or container as the rotor spins due to the centrifugal force acting on the combination.

Types of Centrifuges

Fixed Angle Centrifuge: In a fixed-angle centrifugal system, the sample tubes are arranged at a fixed angle to the axis of rotation. Between 25 and 45 degrees is the usual range for this angle. Fixed-angle centrifuges are excellent for pelleting cells or precipitates as well as for separating solid particles from liquids.

Swinging-Bucket Centrifuge: Centrifuges with swinging buckets feature tube holders that move outward while the rotor spins. As a result, the separated components can create clear layers at the bottom of the sample tubes, which remain upright throughout the centrifugation process. Centrifuges with a swinging bucket are frequently used for separating samples with various densities and for separating samples along a density gradient.

Ultracentrifuge

Ultracentrifuges are extremely fast centrifuges with a maximum speed that frequently exceeds 100,000 rotations per minute (RPM). For separating particles or large molecules like proteins,

DNA, and RNA, they are employed. Due to their rapid rotation, ultracentrifuges can produce outstanding separation resolution.

4.7 Magnetic Stirrer

An adaptable laboratory tool known as a magnetic stirrer uses a rotating magnetic field to stir or mix liquid samples. It comprises of a motor-driven magnet that is typically enclosed in a heat-resistant plate and a stir bar that is submerged in the liquid to be stirred. The stir bar is commonly composed of Teflon-coated magnetic material.

A magnetic stirrer is a multipurpose laboratory tool that uses a rotating magnetic field to stir or mix liquid samples. It comprises of a stir bar, normally made of Teflon-coated magnetic material, that is inserted in the liquid to be stirred and a motor-driven magnet, usually enclosed in a heat-resistant plate [7].

Figure 4-7 Magnetic Stirrer

When compared to alternative mixing techniques, magnetic stirrers provide a number of benefits. They are simple to set up and use, and because they are contactless, there is no chance of cross-contamination. Additionally, highly adjustable, magnetic stirrers give users precise control over stirring direction and speed. They can also be utilised with a wide range of sample

sizes and viscosities, making them appropriate for a variety of applications in chemistry, biology, and research labs.

4.8 Hot Air Oven and Micro-Oven

4.8.1 Hot Air Oven

A hot air oven, also called a convection oven, is a heating appliance that achieves homogeneous heating through hot air circulation. It comprises of a sealed chamber with heat-producing heating components. The heated air is distributed evenly and kept at a constant temperature across the chamber by a fan operating inside the oven.

A hot air oven works by raising the temperature of the air inside the chamber using its heating elements [8]. The fan then circulates the heated air, creating an area that is evenly heated. Hot air ovens are frequently employed in sterilisation, drying, and heating processes that need for exact temperature control. They are frequently used in labs, hospitals, and other industrial environments to sterilise instruments, dry glassware.

Figure 4-8 Hot Air Oven

4.8.2 Micro-Oven

On the other hand, a microwave oven heats material using electromagnetic waves at a microwave frequency. It works by producing microwaves, which excite the water molecules, causing them to vibrate and produce heat. The material nearby is thus heated quickly and effectively as a result of the heat transfer.

Figure 4-9 Micro-Oven

4.9 Sonicator

A sonicator, commonly referred to as an ultrasonic homogenizer or ultrasonic cleaner, is a multipurpose laboratory tool that applies mechanical energy to samples using high-frequency sound waves. It is principally employed in a number of processes, including cell lysis, homogenization, emulsification, dispersion, and degassing.

A sonicator works by creating ultrasonic waves with the help of a transducer. The transducer generates high-frequency mechanical vibrations, typically at a frequency of 20 kHz to several megahertz. These vibrations cause high-intensity sound waves to be produced, and they move through liquid media [9].

Figure 4-10 Ultrasonic Cleaner

High-pressure and low-pressure cycles are produced alternately as the sound waves go through a sample. Cavitation is the name given to this phenomenon. In the liquid, tiny gas bubbles or voids emerge during the low-pressure cycles. The bubbles quickly implode or collapse when the high-pressure cycles take place, producing powerful localised forces. The sample is broken up, mixed, and dispersed as a result of the mechanical agitation caused by cavitation.

4.10 Autoclave

An autoclave is a pressure chamber used to sterilise tools, supplies, and media by exposing them to hot, high-pressure saturated steam [10]. To guarantee the eradication of bacteria, viruses, fungus, and other microbes, autoclaves are frequently employed in laboratories, healthcare facilities, and industrial settings.

The combination of high-pressure steam and heat is the basis for how an autoclave functions in order to produce efficient sterilisation. To form a closed system, the autoclave chamber is

sealed, and steam is produced by boiling water. A high-pressure atmosphere is created as the steam moves the air out of the chamber as it fills it.

Steam can reach temperatures exceeding 100 degrees Celsius (212 degrees Fahrenheit) because the autoclave's increased pressure causes water to boil at a higher temperature. Sterilisation is ensured by the high temperature and pressurised steam, which kills bacteria and their spores.

Autoclave Cycle Stages

Heating and Pressurisation: By heating the autoclave and creating steam, the temperature and pressure inside the chamber are raised. To keep the proper sterilisation conditions, the pressure is carefully managed.

Sterilisation Phase: The sterilisation phase starts when the necessary temperature and pressure are reached. The sterilising objects are penetrated by the high-pressure steam, which successfully kills spores and germs.

Depressurization and Cooling: Following the sterilisation process, the chamber's pressure is gradually let out. The temperature drops as the steam is evacuated or condensed, allowing the sterilised objects to cool before being removed from the autoclave safely.

4.11 Micro Pipettes and Tip Boxes

Micro pipettes, sometimes referred to as pipettors or micropipettes, are precise tools used for precisely measuring and transferring small amounts of liquid. They are widely utilised in fields including biotechnology, clinical diagnostics, and scientific research where accurate liquid handling is essential [11].

Micro pipettes come in single-channel and multi-channel variants, among other configurations. While multi-channel pipettes allow simultaneous pipetting of numerous samples, enhancing efficiency and throughput, single-channel pipettes are used to handle one sample at a time.

Figure 4-11 Micro pipette and Tip Boxes

4.12 Incubator

An incubator for biological samples, such as cells, tissues, microbes, and embryos, is a specially constructed tool used in labs and other scientific research facilities to establish and maintain the best circumstances for their growth and development. It gives the particular organisms or materials being examined a controlled habitat that closely resembles the natural circumstances needed for their survival.

A biological incubator's major function is to precisely control and maintain temperature, humidity, and occasionally other environmental parameters like CO2 levels or lighting conditions. The biological samples are guaranteed to be viable and survive during all stages of growth, replication, and experimentation thanks to this regulated environment.

A chamber with a heating source, temperature sensors, and a control system are the basic components of an incubator. The temperature sensors continuously monitor and modify the internal temperature as necessary, while the heating element aids in preserving a steady and uniform temperature inside the incubator. For investigations that need lower temperatures, some incubators also offer cooling features.

Controlling humidity is yet another essential component of biological incubators. Many organisms, especially those raised in tissue culture, need a certain amount of humidity to survive. To maintain the proper air moisture level, incubators may use water reservoirs or humidification systems.

Additionally, biological incubators frequently have movable racks or shelving to accommodate different sample sizes and arrangements. To safeguard the integrity of the samples, they may also have safety features like alarms and automated shutdown procedures in the event of temperature variations or power outages.

Research in many disciplines, including cell biology, microbiology, genetics, and developmental biology, makes extensive use of biological incubators. They are essential for the growth and research of microbes like as bacteria, viruses, and other cells. Incubators are also essential in the culture of embryos in fertility clinics and animal breeding facilities, as well as in assisted reproductive technologies.

4.13 Reference

[1] M. L.C. Passos and M. L. M.F.S. Saraiva, "Detection in UV-visible spectrophotometry: Detectors, detection systems, and detection strategies," *Meas. J. Int. Meas. Confed.*, vol. 135, pp. 896–904, 2019, doi: 10.1016/j.measurement.2018.12.045.

[2] C. Berthomieu and R. Hienerwadel, "Fourier transform infrared (FTIR) spectroscopy," *Photosynth. Res.*, vol. 101, no. 2–3, pp. 157–170, 2009, doi: 10.1007/s11120-009-9439-x.

[3] D. Bonvin, R. Yellepeddi, and A. Buman, "Application and perspectives of a new innovative XRF-XRD spectrometer in industrial process control," *JCPDS-International Cent. Diffr. Data 2000, Adv. X-ray Anal.*, vol. 42, no. C, pp. 126–136, 2000.

[4] A. Abdullah and A. Mohammed, "Scanning Electron Microscopy (SEM): A Review," *Proc. 2018 Int. Conf. Hydraul. Pneum. - HERVEX*, pp. 77–85, 2019.

[5] R. H. Kruse, W. H. Puckett, and J. H. Richardson, "Biological safety cabinetry," *Clin. Microbiol. Rev.*, vol. 4, no. 2, pp. 207–241, 1991, doi: 10.1128/CMR.4.2.207.

[6] H. Anlauf, “Recent developments in centrifuge technology,” *Sep. Purif. Technol.*, vol. 58, no. 2, pp. 242–246, 2007, doi: 10.1016/j.seppur.2007.05.012.

[7] W. G. Pfann and D. Dorsi, “Magnetic stirring technique,” *Rev. Sci. Instrum.*, vol. 28, no. 9, p. 720, 1957, doi: 10.1063/1.1715985.

[8] S. A. S. Alkadhim, “Hot Air Oven for Sterilization: Definition & Working Principle,” *SSRN Electron. J.*, pp. 1–7, 2019, doi: 10.2139/ssrn.3340325.

[9] R. Baqai and S. Hafiz, “Microwave oven in microbiology laboratory.,” *J. Pak. Med. Assoc.*, vol. 42, no. 1, pp. 2–3, 1992.

[10] R. Kumar Patel, “Design of an Autoclave,” *Int. J. Res. Appl. Sci. Eng. Technol.*, vol. V, no. IX, pp. 1378–1380, 2017, doi: 10.22214/ijraset.2017.9197.

[11] A. Yeung, T. Dabros, J. Masliyah, and J. Czarnecki, “Micropipette: A new technique in emulsion research,” *Colloids Surfaces A Physicochem. Eng. Asp.*, vol. 174, no. 1–2, pp. 169–181, 2000, doi: 10.1016/S0927-7757(00)00509-4.

5 Experiment/Methodologies

5.1 Exchange of Sodium ion with Potassium ions in mesoporous clay

The difference in ion size and affinity causes the exchange of potassium (K+) and sodium (Na+) ions in MMT (montmorillonite) clay during drug loading. A type of layered clay mineral called MMT clay has a negatively charged surface that is balanced by exchangeable cations found in the interlayer spaces, such as sodium, potassium, calcium, etc.

Figure 5-1 K-MMT and synthesized Na-MMT

5.1.1 Procedure

The objective of the study was to perform an ion exchange process to convert K-MMT (Montmorillonite clay containing potassium ions) into Na-MMT (Montmorillonite clay containing sodium ions). The process involved several systematic steps to achieve the desired exchange efficiently.

To make a homogeneous clay solution, first weigh 5 grams of K-MMT and combine it with 100 ml of distilled water. Next, carefully incorporate a 0.1N NaCl solution into the clay solution.

Shake the mixture vigorously or stir with magnetic stirrer and allow the potassium and sodium ions to interact within the clay lattice for a predetermined amount of time.

5 minutes of centrifugation at 4000 RPM should be used to separate the liquid phase from the clay that has been exchanged. Decant the excess NaCl and other soluble substances from the liquid phase, leaving the converted Na-MMT in the centrifuge tube.

The clay exchange should then be subjected to X-ray diffraction (XRD) analysis to confirm the structural change from K-MMT to Na-MMT. The exchanged clay sample's XRD pattern ought to show distinctive peaks connected to Na-MMT, offering verifiable proof of an effective ion exchange.

Rinse the exchanged clay several times in deionized water to get rid of any persistent NaCl or other impurities to make sure it is pure. To achieve the highest level of purity, take care not to disturb the clay's structure when rinsing [1].

Finally, gather the cleaned Na-MMT clay and let it dry naturally for 24 hours to get rid of any moisture. The finished clay ought to have a fine texture and be prepared for further investigation and testing.

5.2 Drug loading into the clay

The process involves loading drugs into clay layers to create a drug-clay nanocomposite. Clay minerals, such as Na-MMT (sodium montmorillonite), are known for their layered structure and high surface area, making them ideal candidates for drug delivery systems. The loading of drugs into clay layers aims to improve drug stability, enhance drug release control, and increase the efficiency of drug delivery to specific targets.

The first step in the drug loading procedure is to combine 100 ml of water with 1 gm of clay (Na-MMT) in a container. The pH of the mixture is carefully monitored to make sure it stays between 4.8 and 5.7.

The drug of interest, gatifloxacin, is then added to the clay-water mixture after the pH has been adjusted. After that, the drug molecules are absorbed or intercalated into the clay's interlayer spaces. Drug concentration, clay type, and pH are a few variables that have an impact on the absorption process. The drug will be distributed uniformly throughout the clay structure with proper absorption.

After the drug has been incorporated into the clay mixture, the mixture is magnetically stirred for 24 hours. The prolonged mixing time promotes thorough mixing and the trapping of drug molecules in the clay's interlayer spaces. The goal of this drug-clay nanocomposite formation is to improve drug stability and regulate drug release over time.

Following the stirring process, the drug-loaded clay nanocomposite is separated from the liquid phase using centrifugation at 4000 rpm for 20 minutes. Centrifugation helps isolate the drug-loaded clay from any unbound drug molecules and excess water, resulting in a concentrated and well-defined drug-clay nanocomposite.

The dried solid drug-loaded clay is then gathered and uniformly distributed over a petri dish to begin the drying process. After about 48 hours of drying, the water and solvent that were still present in the nanocomposite evaporate, leaving a dry and stable drug-clay nanocomposite [2].

The dried nanocomposite is ground into a powder in order to produce fine particles and uniformity. Large aggregates are broken down during the grinding process, creating smaller particles with more surface area, which is advantageous for applications involving drug delivery.

5.3 Kirby Bauer Test

The Kirby-Bauer test, also known as the disc diffusion method or antibiotic sensitivity test, is a standard technique used to determine the antibiotic susceptibility of bacteria. This test plays a crucial role in guiding healthcare professionals in choosing the most effective antibiotic treatment for bacterial infections.

During the Kirby-Bauer test, bacterial isolates are cultured on a solid agar medium, spread evenly across the surface. Small, circular discs infused with different antibiotics are then placed on the agar. As the antibiotics diffuse outward from the discs, they create zones of inhibition, areas where bacterial growth is prevented due to the antibiotic's efficacy.

The 24-hour turnaround time of the test results makes it a realistic option for directing first antibiotic therapy. Because infections can spread quickly, early, focused treatment is essential for improving patient outcomes. The Kirby-Bauer test's quickness enables medical professionals to make timely, educated decisions that improve patient care.

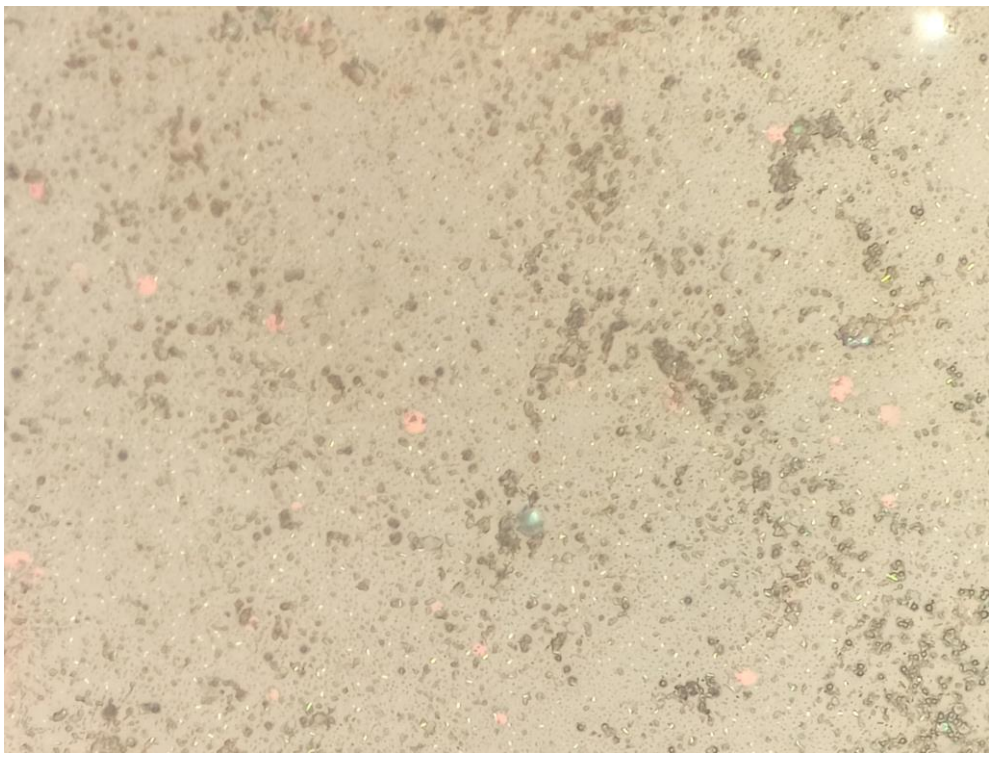


Figure 5-2 Staphylococcus Aureus culture under microscope.

The Kirby-Bauer test can be used in a variety of healthcare settings because it is affordable and does not require for special equipment. This is especially helpful in environments with limited resources or places where access to advanced laboratory technologies is limited. The test is widely used in a variety of healthcare settings around the world due to its price and simplicity [3].

The test helps health officials discover emerging resistance trends and modify treatment recommendations by tracking patterns of antibiotic resistance in various bacterial populations.

Antibiotic resistance monitoring is essential for creating strategies that effectively combat resistance and safeguard public health.

It is crucial to recognise the restrictions of the Kirby-Bauer test. The minimum inhibitory concentration (MIC) of antibiotics necessary to completely stop bacterial growth is not provided; instead, it offers a qualitative estimate of antibiotic susceptibility. MIC values are essential for adjusting dosages for severe infections and spotting long-term changes in bacterial susceptibility.

5.3.1 Procedure

Preparation of Bacterial Culture: A pure bacterial culture of Staphylococcus aureus obtained first. In order to do this, bacteria from a stock culture or a clinical sample are streaked onto the proper agar medium, such as nutrition agar or blood agar. To avoid contamination and get a pure culture with distinct bacterial colonies, make sure to follow aseptic techniques throughout.

Inoculating the Agar Plate: The agar plates should be at room temperature before beginning to avoid condensation on the surface, which can impede bacterial development. Few well-isolated colonies from the pure culture with a sterile swab or inoculating loop was taken. Then it was spread evenly across the agar plate's surface using a streaking technique. This ensures the bacterial inoculum is distributed uniformly and encourages steady development.

Applying Antibiotic Discs: The antibiotic discs should be delicately placed on the agar plate's surface using sterile forceps or a disc dispenser. To ensure appropriate adherence, make sure the discs are evenly spaced apart and gently placed onto the agar surface. Immediately after application, the antibiotics will start to diffuse into the surrounding agar medium, four discs were placed one containing drug loaded clay (GMD-5), one containing pure drug (Gatifloxacin+H_2O), one containing pure clay (Na-MMT+H_2O) and one for control containing water

Incubation: The agar plate should be put into an incubator as soon as the discs have been placed; this temperature is normally 37°C (98.6°F). Incubate the plates for a predetermined amount of time, often 16 to 18 hours. A bacterial layer may be seen growing on the surface of the agar during the incubation time.

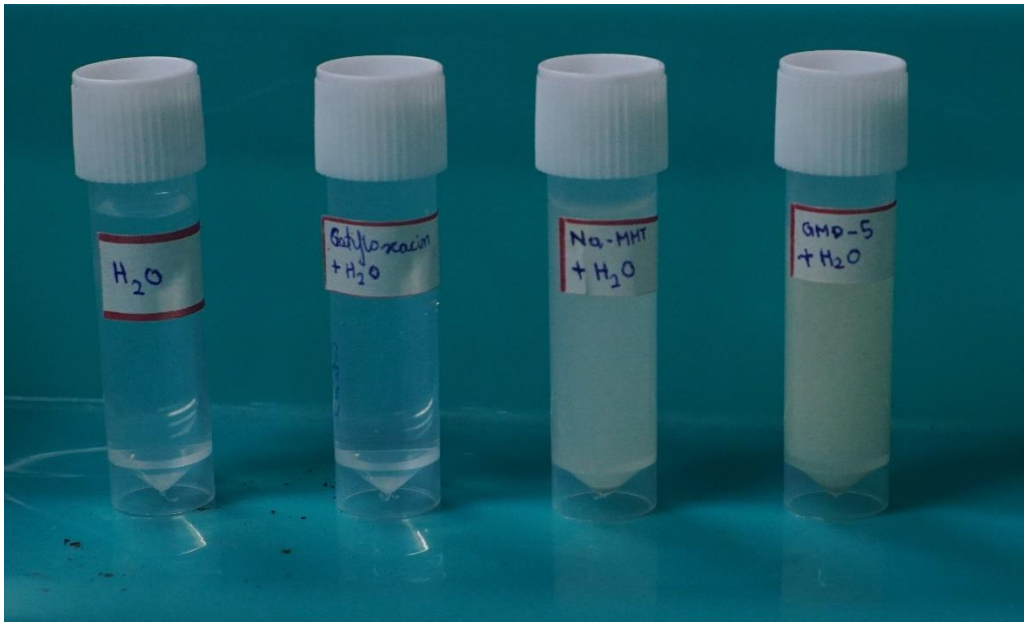


Figure 5-3 Four solutions made for the disc absorption

After incubation it was observed that that the pure drug disc and disc containing drug loaded clay made a somewhat circular zone where the growth of the bacteria (Staphylococcus Aureus) was restricted.

5.4 Drug Calibration Curve

An essential tool for establishing the relationship between a substance's concentration and the related analytical signal, such as absorbance or peak area, is a calibration curve. To measure sample concentrations that are unknown, analytical chemistry and pharmaceutical analysis frequently use this method.

To produce the calibration curve, a set of standard solutions with known concentrations of the target material are first made. These reference materials span a range that corresponds to the expected concentration ranges in the samples [4].

Using the selected analytical tool, such as a spectrophotometer or chromatograph, the standard solutions are examined. For each standard, the associated analytical signal is measured, such as absorbance for UV-Vis spectroscopy or peak area for chromatography.

The known concentrations of the standard solutions are then displayed on the x-axis (horizontal) and their corresponding analytical signals on the y-axis (vertical), creating the

calibration curve. To depict the relationship between concentration and signal, the data points are joined by a curved or rounded line.

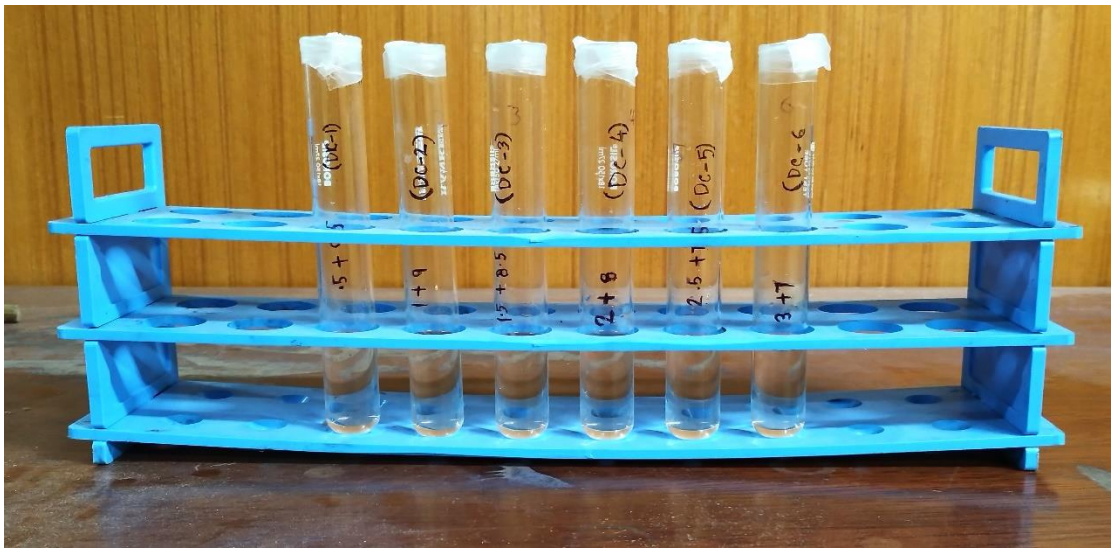


Figure 5-4 Drug calibration solutions

Table 5-1 Standard solution preparation chart

Set No	Stock Solution (Gatifloxacin) [ML]	Base Solution (DI Water) [ML]	Total Solution [ML]	Concentration [µg/ML]	Absorbance
1	0.5	9.5	3	5	0.069531
2	1	9	10	10	0.235283
3	1.5	8.5	10	15	0.320546
4	2	8	10	20	0.513607
5	2.5	7.5	10	25	0.716207
6	3	7	10	30	0.781645

The graph starts from [0,0] coordinate and the plot will be discussed in the results.

5.5 In Vitro Release of Drug in SSF (Simulated Skin Fluid)

Simulated Skin Fluid (SSF) is a laboratory-prepared solution designed to mimic the pH and ion concentration of human skin. It serves as a valuable tool in various research fields, especially in dermatology and pharmaceutical sciences, where understanding the behaviour of substances on the skin surface is crucial.

A certain volume of aqueous solution was taken for preparation of SSF. Human skin surface pH typically ranges from 4.5 to 6.5, with an average around pH 5. The pH of the aqueous solution was modulated using hydrochloric acid (HCl) or sodium hydroxide (NaOH).

In this study, the drug-loaded clay was immersed in 10ml of simulated sweat fluid (SSF) at a pH of 5, and the system was continuously stirred using a magnetic stirrer. SSF was selected as the appropriate solution due to its ion concentration, closely resembling that of human sweat.

At various time intervals during the 168-hour observation period, 3ml aliquots of the sample were systematically collected. The collected samples were promptly replaced with an equivalent volume of fresh SSF to maintain a constant total volume in the system. This approach ensured that the drug release behaviour was continuously monitored without disturbing the overall experimental setup [5].

The study aimed to comprehend how the drug interacted with the clay matrix and how the properties of SSF influenced its release. By analysing the dissolution kinetics and diffusion characteristics of the drug-loaded clay in a sweat-mimicking environment, the research sought to gain insights into potential applications in sweat-targeted drug delivery systems.

Throughout the experiment, the drug's release patterns were documented and analysed, providing valuable data for the development of novel drug delivery systems targeted at sweat-related applications.

5.6 In Vitro Release of Drug in SBF (Simulated Body Fluid)

The Simulated Body Fluid (SBF) [6] was prepared using various chemical components and their respective quantities. It included 8.035 g of Sodium Chloride (NaCl), 0.355 g of Sodium Bicarbonate ($NaHCO_3$), 0.225 g of Potassium Chloride (KCl), 0.231 g of Dipotassium Hydrogen Phosphate Trihydrate ($K_2HPO_4.3H_2O$), 0.311 g of Magnesium Chloride

Hexahydrate ($MgCl_2.6H_2O$), and 0.292 g of Calcium Chloride ($CaCl_2$). Additionally, 0.072 g of Sodium Sulphate (Na_2SO_4) and 6.118 g of Tris(hydroxymethyl)aminomethane [$(CH_2OH)_3CNH_2$] (Tris Base) were also included in the formulation. The pH of the solution was adjusted using 39 ml of Hydrochloric Acid 1(N) HCl. During the preparation process, the components were added in a specific order, and the mixture was thoroughly stirred. The resulting SBF was formulated to resemble the ion concentrations found in human blood plasma, providing an accurate model for research purposes. This SBF was then used to immerse the drug-loaded clay and observe the drug release behaviour over a period of 168 hours.

In this study, the drug-loaded clay was immersed in 10ml of simulated body fluid (SBF) and subjected to stirring using a BOD incubator. SBF is a solution with ion concentration akin to human blood plasma. The pH of the SBF solution was meticulously maintained at 7.4 by employing a 1(N) HCl solution

Throughout the experimental duration of 168 hours, 3ml aliquots of the sample were systematically collected at different time intervals. Each time a sample was taken, it was promptly replaced with an equal volume of fresh SBF, ensuring a constant total volume in the system. This approach allowed for continuous monitoring of the drug's release behaviour without disruption to the overall experimental setup.

Over the course of 168 hours, the drug's release behaviour in the SBF was carefully monitored. The use of the passive voice emphasises that the drug-loaded clay is the main object of interest, not the experimenter, leading to an objective and rigorously scientific description of the experimental design and results.

The detailed documentation and analysis of the medication's release patterns produced useful information for the creation of innovative drug delivery systems with potential applications in the biomedical and pharmaceutical industries.

5.7 MIC calculation of Drug Loaded Clay Complex

The 96-well plate method with absorbance measurements from ELISA Reader is used to determine the Minimum Inhibitory Concentration (MIC) [7].

The microorganism (Staphylococcus Aureus) culture (100 microlitres) were prepared.

The Drug loaded clay solutions (100 microlitres) were prepared and sterile water (100 microlitres) were added to the wells. A two-fold serial dilution method were used (e.g., 2x, 4x, 8x, 16x, and so on) to add the drug loaded clay solutions in the wells with required dilution and constant volume.

The plates are set up by labelling the wells according to the drug loaded clay concentrations, and drug loaded clay is added to the corresponding wells.

The bacterial solutions were added to wells uniformly (10 microlitres) each.

Control wells were also made, with positive control wells containing only the microorganism without the drug loaded clay

The initial absorbance reading was taken at start.

The plate was incubated by sealing it with a lid or cover and then incubating it at the optimal temperature for 24 hours [8].

Absorbance measurements were taken after the appropriate incubation period using an ELISA Reader and the ELISA Reader determines the absorbance of each well, which is directly related to microbial growth.

The data was analysed by plotting the difference in absorbance readings against the drug loaded clay concentrations. The MIC is determined as the lowest concentration of the antimicrobial agent that shows no visible growth, as represented by a lack of significant increase in absorbance compared to the negative control.

Table 5-2 Table for wells containing drug loaded clay and the bacterial solution

Cell	Abs (Initial)	Abs (Final)	Change in Absorbance(F-I)	Concentration(mg/ml)
A	0.0632	0.5270	0.4638	0.0078125
B	0.059	0.3288	0.2698	0.015625
C	0.0552	0.1091	0.0539	0.03125
D	0.0696	0.1583	0.0887	0.0625
E	0.0734	0.1166	0.0432	0.125
F	0.1087	0.1487	0.0400	0.25
G	0.2137	0.1970	-0.0167	0.5
H	0.2811	0.2411	-0.0400	1

The cells represent the different wells where the drug loaded clay was added with the bacterial liquid culture, initially an absorbance value was noted and another absorbance reading was taken at the 24^{th} hour, simultaneously a control was included in the experiment. In the table a difference in Absorbance column was created to plot against concentration in order to compare with the control and get the MIC of the drug loaded clay.

Table 5-3 Control table

Cell	Abs (Initial)	Abs (Final)	Concentration(mg/ml)	Change in Absorbance(F-I)
A	0.0629	0.4379	0.0078125	0.375
B	0.0515	0.1927	0.015625	0.1412
C	0.0550	0.2611	0.03125	0.2061
B	0.0528	0.2791	0.0625	0.2263
E	0.0582	0.2417	0.125	0.1835
F	0.0569	0.2094	0.25	0.1525
G	0.0567	0.2221	0.5	0.1654
H	0.0555	0.2553	1	0.1998

5.8 Formulation of Patch

The transdermal antibiotic patches [9] were meticulously prepared, involving nuanced adjustments to the methodology, with a focus on utilizing the polymer ethyl cellulose (EC) and polyvinylpyrrolidone (PVP). The procedure entailed a series of deliberate steps. Firstly, a cylindrical glass mould, open on both ends, underwent partial wrapping with aluminium foil. Subsequently, within this enclosed space, a precisely measured 5 ml of a 4% (w/v) aqueous polyvinyl alcohol (PVA) solution was introduced. This amalgamation was then exposed to a controlled drying process lasting 6 hours at a temperature of 50°C, resulting in the formation of a vital backing membrane essential for the subsequent transdermal patch assembly [10].

Continuing the process, the matrix was meticulously fashioned. A composite was prepared by combining ethyl cellulose and polyvinylpyrrolidone in a 2:1 ratio, along with 100 mg of drug-loaded clay, all of which were homogeneously dispersed within ethanol. To enhance the properties of the matrix, a plasticizer, Di-n-butyl phthalate, was introduced at a concentration of 10% (v/v). The resulting blend, characterized by its uniform dispersion, was methodically cast onto the previously prepared PVA backing membrane. This casting was undertaken with precision to ensure uniformity in the application.

The casted composition within the mould was then subjected to a drying phase at a consistent temperature of 50°C for a period of 6 hours. This carefully controlled desiccation process enabled the matrix to solidify, thereby laying the foundation for the final transdermal patch.

Upon the completion of the drying process, the resultant dried patch was delicately extracted from the mould. It was then placed within a desiccator to maintain its structural integrity and preserve its inherent properties. This step was instrumental in facilitating subsequent comprehensive analysis and in-depth studies, thereby providing the groundwork for further research and potential applications within the transdermal drug delivery systems.

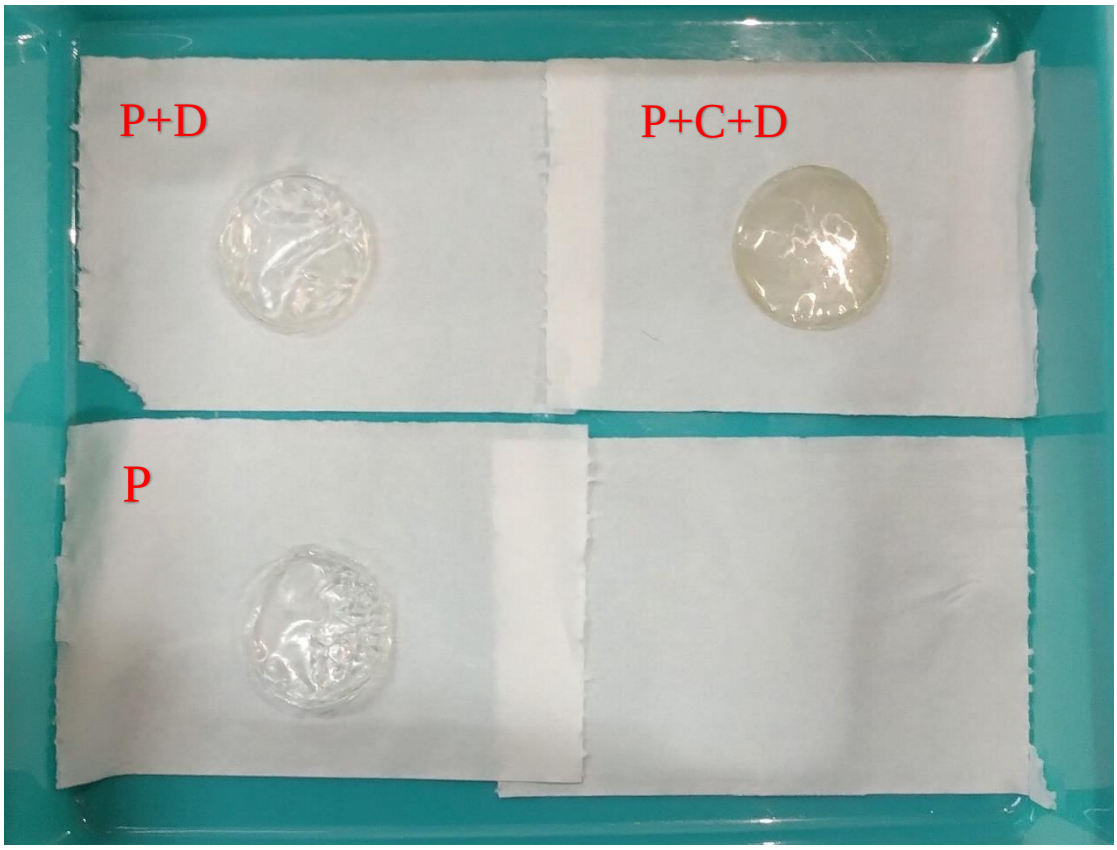


Figure 5-5 (P-Control patch, it contains patch polymers only), (P+D- It is the patch incorporated with drug) & (P+C+D-It is the patch incorporated with drug loaded clay)

5.9 Ex Vivo Release from Patch

The formulated patches were individually placed onto the stratum corneum side of human skin, which had been previously soaked overnight in a simulated body fluid solution (SBF) with a pH of 7.4. The skin, along with the patch, was carefully positioned within a Franz diffusion cell in such a manner that the dermis side of the skin faced the receptor part of the cell, which contained 100ml of SBF at room temperature. To ensure thorough mixing, a magnetic bead was employed to continuously stir the solution in the receptor compartment at a rate of 40 RPM.

During the experimental process, 1 mL of the solution was collected from the receptor part at various time intervals, and an equivalent volume of SBF was added to maintain the total volume. Subsequently, the collected samples were subjected to analysis employing a UV-Vis spectrophotometer, which facilitated the determination of antibiotic concentration that had permeated through the skin.

Throughout this study, several distinct release kinetics models were established for data analysis, enabling a comprehensive examination of the permeation behaviour of the antibiotic through the skin. These models served as valuable tools for gaining insights into the drug's diffusion and release kinetics in the context of the formulated patches and their interaction with the stratum corneum layer of human skin [11].

Ex-vivo release studies from patch applications are crucial experiments conducted in controlled laboratory settings to assess how effectively transdermal or topical patches deliver drugs or active substances. During these studies, patches are applied to an external membrane or tissue that mimics the physiological conditions of the skin. Samples are collected at specific time intervals, and advanced analytical techniques are employed to quantify the concentration of the released drug or substance. This information yields insights into the patch's release kinetics, helping researchers understand factors like burst release, sustained release, and overall release profiles. Ex-vivo release studies hold significant importance in optimizing patch formulations for consistent drug release, ensuring quality control in manufacturing, facilitating comparative evaluations, and satisfying regulatory requirements for the approval of transdermal or topical drug products.

5.10 Reference

[1] M. L. Whittaker, L. N. Lammers, S. Carrero, B. Gilbert, and J. F. Banfield, "Ion exchange selectivity in clay is controlled by nanoscale chemical-mechanical coupling," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 116, no. 44, pp. 22052–22057, 2019, doi: 10.1073/pnas.1908086116.

[2] W. C. G. Butler and S. R. Bateman, "A flow-through dissolution method for a two component drug formulation where the actives have markedly differing solubility properties," *Int. J. Pharm.*, vol. 173, no. 1–2, pp. 211–219, 1998, doi: 10.1016/S0378-5173(98)00238-5.

[3] J. Hudzicki, "Kirby-Bauer Disk Diffusion Susceptibility Test Protocol Author Information," *Am. Soc. Microbiol.*, no. December 2009, pp. 1–13, 2012, [Online]. Available: https://www.asm.org/Protocols/Kirby-Bauer-Disk-Diffusion-Susceptibility-

[4] H. Furukawa *et al.*, “The comparative verification of calibration curve and background fundamental parameter methods for impurity analysis in drug materials,” *X-Ray Spectrom.*, vol. 46, no. 5, pp. 382–387, 2017, doi: 10.1002/xrs.2788.

[5] V. P. Shah and R. L. Williams, “Topical Drug Bioavailability, Bioequivalence, and Penetration,” *Top. Drug Bioavailability, Bioequivalence, Penetration*, pp. 61–67, 2014, doi: 10.1007/978-1-4939-1289-6.

[6] T. Kokubo and S. Yamaguchi, “Simulated body fluid and the novel bioactive materials derived from it,” *J. Biomed. Mater. Res. - Part A*, vol. 107, no. 5, pp. 968–977, 2019, doi: 10.1002/jbm.a.36620.

[7] A. Murugappan, J. S. Sudarsan, and A. Manoharan, “Effects of using Lignite mine drainage for irrigation on soils - A case study of perumal tank command area in Tamilnadu State,” *J. Ind. Pollut. Control*, vol. 22, no. 1, pp. 149–160, 2006.

[8] I. Wiegand, K. Hilpert, and R. E. W. Hancock, “Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances,” *Nat. Protoc.*, vol. 3, no. 2, pp. 163–175, 2008, doi: 10.1038/nprot.2007.521.

[9] S. T. Prajapati, C. G. Patel, and C. N. Patel, “Formulation and Evaluation of Transdermal Patch of Repaglinide,” *ISRN Pharm.*, vol. 2011, pp. 1–9, 2011, doi: 10.5402/2011/651909.

[10] P. Maji, A. Gandhi, S. Jana, and N. Maji, “Preparation and Characterization of Maleic Anhydride Cross-Linked Chitosan-Polyvinyl Alcohol Hydrogel Matrix Transdermal Patch,” *J. PharmaSciTech*, vol. 2, no. 2, pp. 62–67, 2013.

[11] R. Gannu, Y. Vamshi Vishnu, V. Kishan, and Y. Madhusudan Rao, “Development of Nitrendipine Transdermal Patches: In vitro and Ex vivo Characterization,” *Curr. Drug Deliv.*, vol. 4, no. 1, pp. 69–76, 2006, doi: 10.2174/156720107779314767.

6 Results and Discussion

6.1 XRD Analysis

After exchange of ions and loading of drug into the clay nano-layers it is important to characterize the composite material, for the first characterization technique X-Ray Diffraction Crystallography is used.

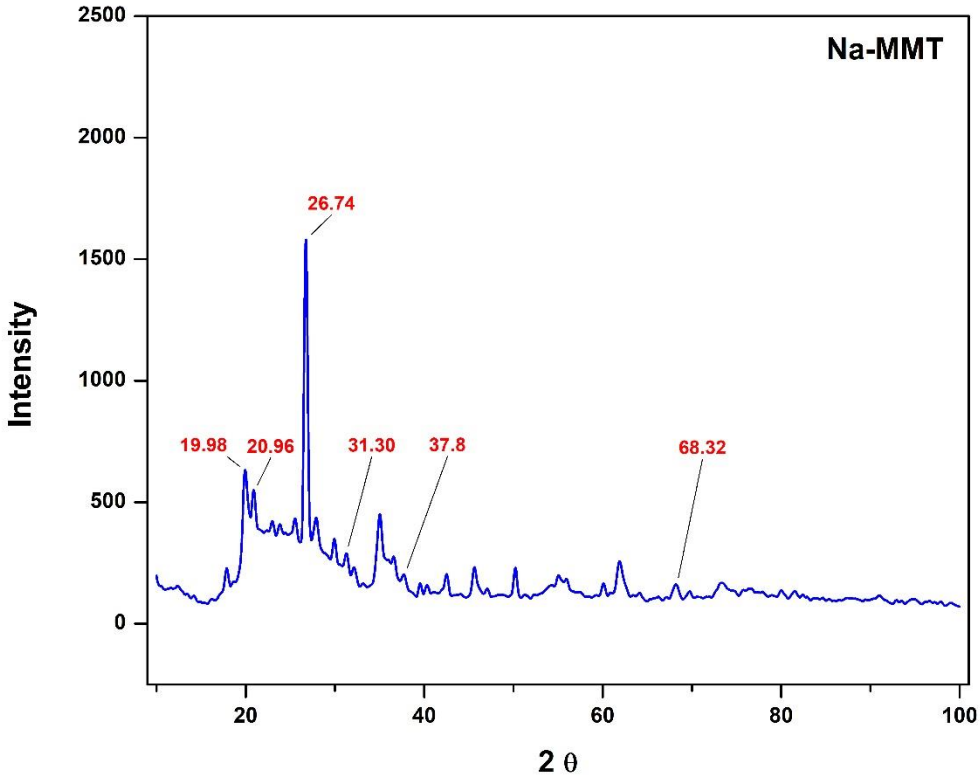


Figure 6-1 X-Ray Diffraction spectrum of Sodium Montmorillonite Clay

In the XRD spectrum of Na-MMT it is observed that the first hump-based peak is located at 19.98°, the other major peaks according to the research by [1] and [2] are also marked in the spectrum confirms the characterization of Sodium ion exchanged montmorillonite clay (Na-MMT). The other major peaks are 20.96°,26.74°,31.30°,37.80°and 68.32°. There are many unmarked small peaks present in the spectrum which relates to the crystallinity of quartz. Into this Na-MMT the drug gatifloxacin is added and the changes in the XRD spectrum is given below.

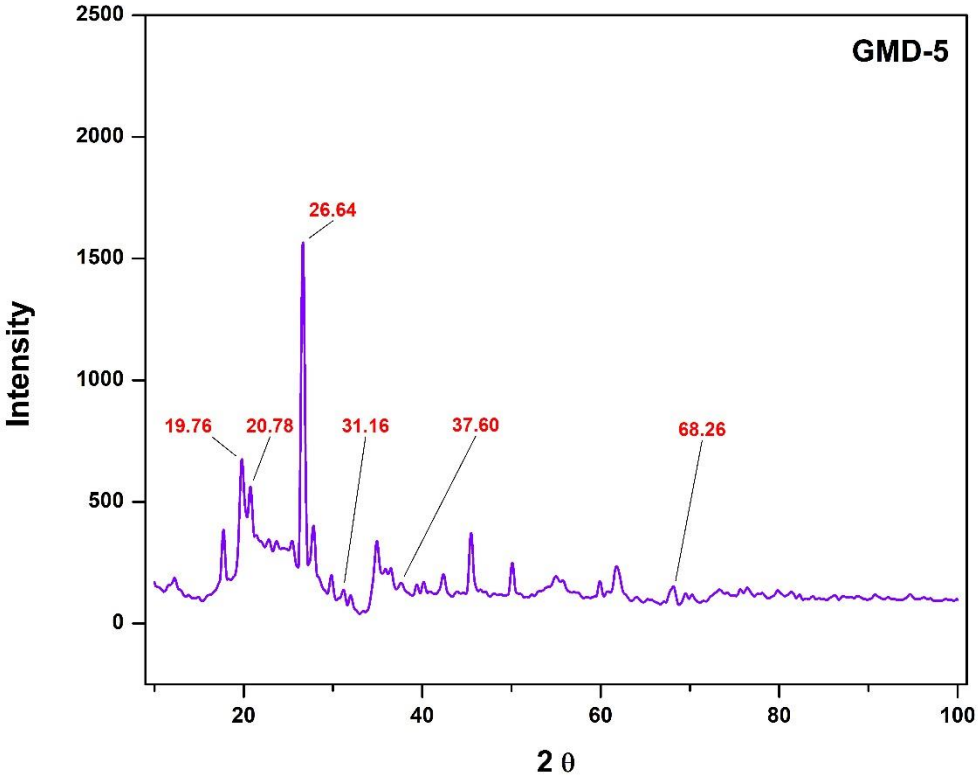


Figure 6-2 Ray Diffraction spectrum of Gatifloxacin loaded montmorillonite clay (GMD-5)

In this GMD-5 we can observe that peaks are present at 19.67°,20.78°,26.64°,31.16°,37.60° and 68.26°. This observation major peaks in Na-MMT after drug is loaded has shifted toward left by a significant magnitude. 19.98° has shifted by 0.31°,20.96° has shifted by 0.18°,26.74° has shifted by 0.10°,31.30° has shifted by 0.14°,37.80° has shifted by 0.20° and 68.32° has shifted by 0.06°. The average shift is 0.165° towards left.

From Bragg's Law: $2d \sin \theta = n \lambda$

d = The distance between the atomic layers, λ = Wavelength of the incident X-Ray

Thus, θ is inversely proportional d, and shift of peaks towards left indicates lower value of θ which in turn means increase in “d” value, increase in “d” value indicates the increase in distance between atomic layers which signifies loading of drug in the clay layer.

6.2 FTIR Analysis

After the sample underwent FTIR Spectroscopy the following spectrum was observed for Na-MMT

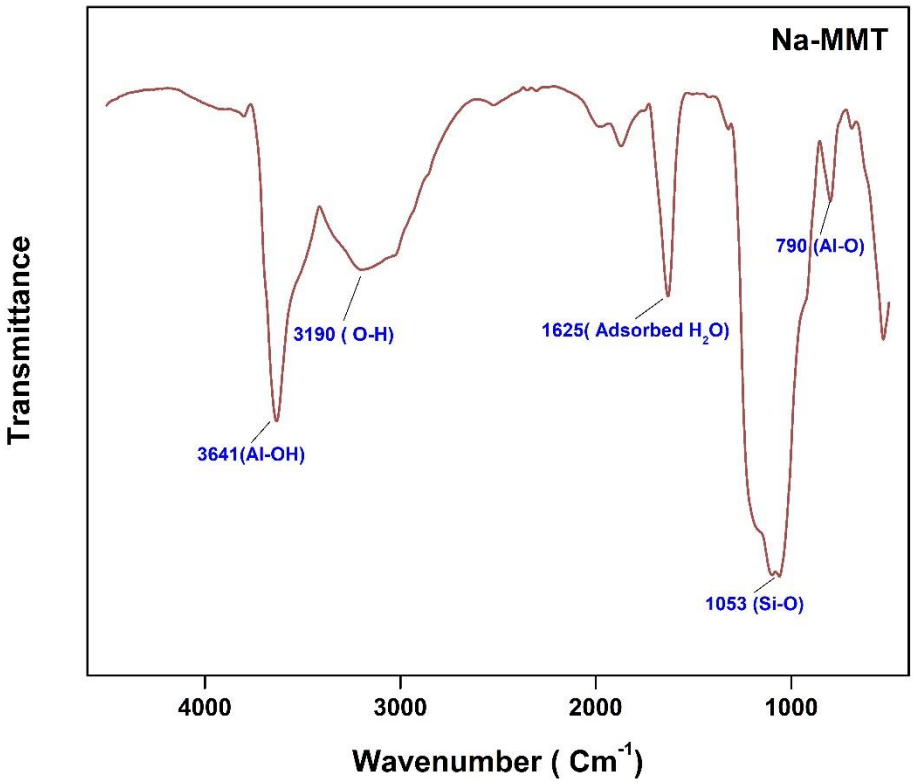


Figure 6-3 FTIR Spectra of Na-MMT

Si-O-Si Bending: Bending vibrations of silicon-oxygen-silicon (Si-O-Si) linkages in the clay's structure can be seen in the range of 996-1071Cm^{-1} and in this plot it is observed at 1053 Cm^{-1}

Al-O Stretching: The stretching vibrations of Al-O bonds correspond to the connections between aluminium atoms and oxygen atoms in the clay mineral, the range is 950-850 Cm^{-1}, in this plot it is observed at 790 Cm^{-1}

Adsorbed Water: Peaks related to water molecules adsorbed on the clay's surface might show up around 3700-3000 Cm^{-1} (stretching vibrations of O-H bonds in adsorbed water) observed at

3190 Cm^{-1} also around 1650-1600 Cm^{-1} (bending vibration of adsorbed water) absorbed near 1625 Cm^{-1}.

Hydroxyl Groups (Al-OH and Si-OH Stretching):Generally found around 3600-3200 Cm^{-1}. The hydroxyl groups attached to aluminium (Al-OH) and silicon (Si-OH) atoms in the clay structure contribute to stretching vibrations in this region. Here it is observed at 3641 Cm^{-1}.

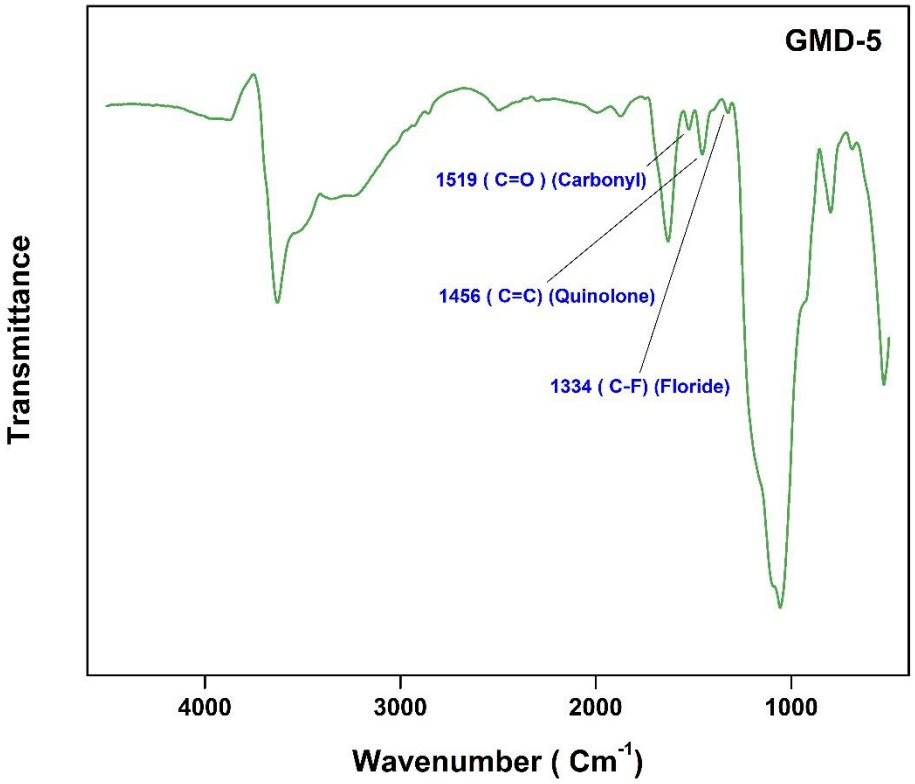


Figure 6-4 FTIR of GMD-5

After loading of the drug Gatifloxacin into Na-MMT it forms the composite GMD-5, conclusively apart from the bonds present in the pure Na-MMT, GMD-5 has few unique bonds present and are marked in the spectrum. The different bonds are discussed below.

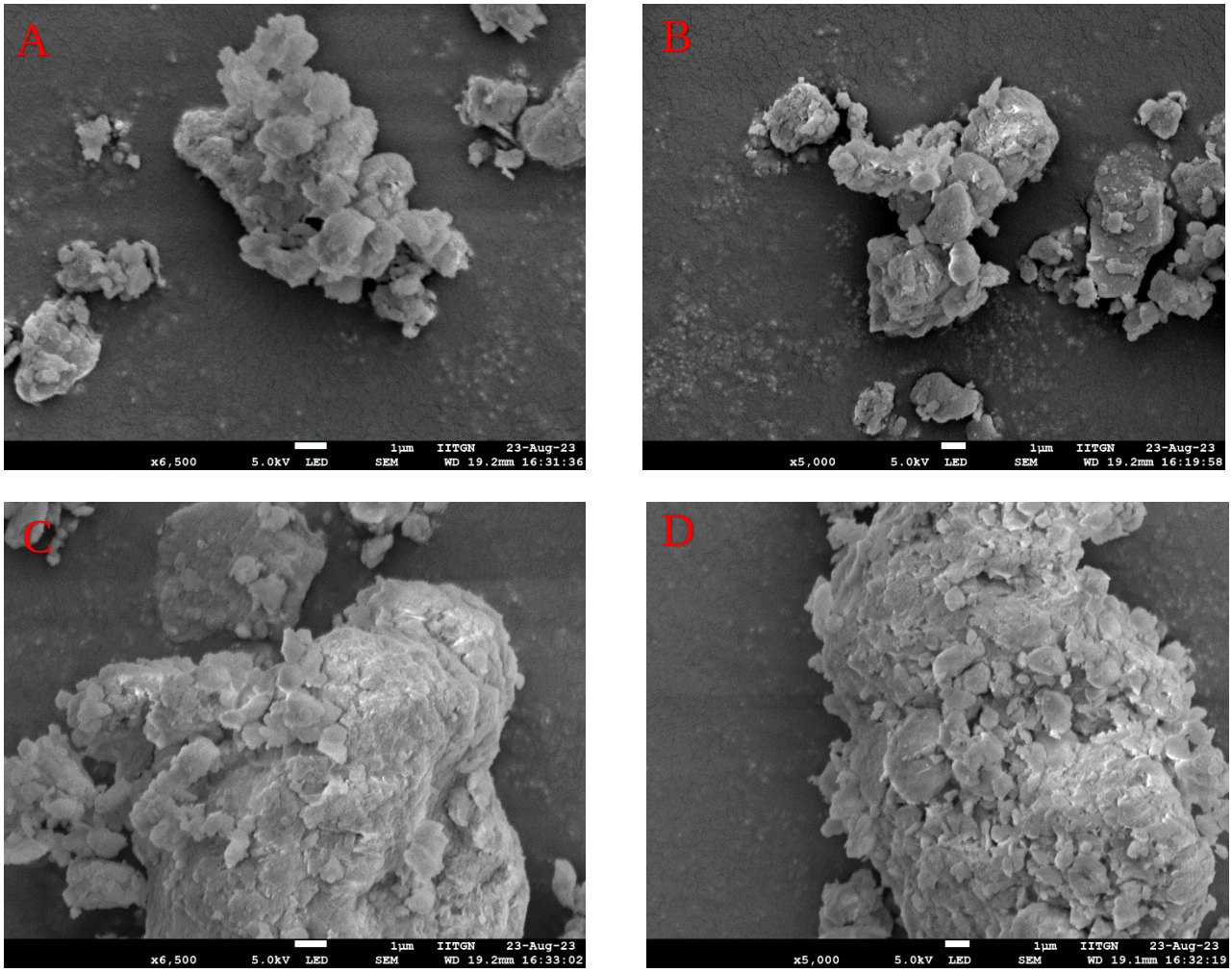
C=O Stretching (Carbonyl): This peak can be found around 1710-1750 Cm^{-1}, corresponding to the carbonyl group (C=O) in the molecule the peak found in the spectrum is located at 1519 Cm^{-1}.

C-F Stretching (Fluoride): Gatifloxacin falls under the category of Fluoroquinolone group which has a unique carbon-fluoride bond. Peaks related to the C-F bond stretching vibrations are typically seen in the range of 1200-1350 Cm^{-1}. Here it is located at 1334 Cm^{-1}.

Aromatic C=C Stretching (Quinolone Ring): Peaks due to aromatic C=C stretching vibrations in the quinolone ring might be found in the range of 1450-1600 Cm^{-1}. Here it is located at 1456 Cm^{-1}.

All these bonds correspond to the fact that the drug loading process was successfully completed and the required elements and their corresponding bonds are present in the Drug loaded clay composite [3].

6.3 SEM Images



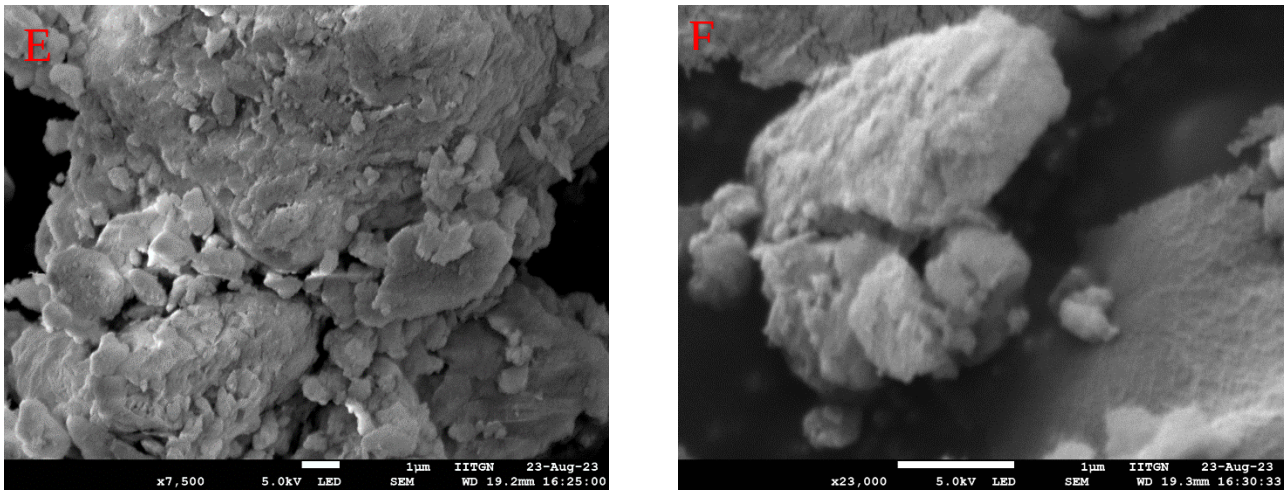


Figure 6-5 SEM Images of GMD5 at different resolution ((B, D)5000x, (A, C)6500x, (E)7500x and (F)23000x)

6.4 Zone of Inhibition Analysis

The zone of inhibition is a critical technique in Kirby Bauer Test used to evaluate the antibacterial activity of substances, such as drugs or drug-loaded materials, against bacteria. The presence of a clear zone surrounding the substance-soaked discs on an agar medium indicates the inhibition of microbial growth due to the antimicrobial properties of the substance. The results from these provide valuable insights into the effectiveness of the tested substances against specific microorganisms.

The results obtained for the drug-loaded clay samples exhibited significant zones of inhibition. This observation suggests that the drug, when loaded onto the clay matrix, retains its antibacterial activity and is capable of inhibiting the growth of the target microorganisms effectively.

In this test few discs were soaked in water, pure MMT clay, pure drug and drug loaded clay. In the water and Pure MMT clay there were no zone present which showed bacterial growth, in the pure drug and drug loaded clay we observed zone where no bacteria were present.

The most important conclusion is that the drug is properly loaded into the layers of the clay,

Figure 6-6 Zone of inhibition

In this above-mentioned picture, we can observe pure drug (Gatifloxacin) loaded disc created a zone of inhibition its displayed in circular red structure and the drug loaded clay also created a zone of inhibition which is marked in red oval in shape from which we can conclude that the drug was properly loaded into the clay matrix because in the water and pure MMT soaked discs showed no such zone.

6.5 Calibration Curve Analysis

A series of standard solutions with known drug concentrations was prepared in order to build a drug calibration curve. Concentrations of drugs were made and their respective absorbance is measured and was plotted, the resulting graph produced is called the calibration curve of Gatifloxacin.

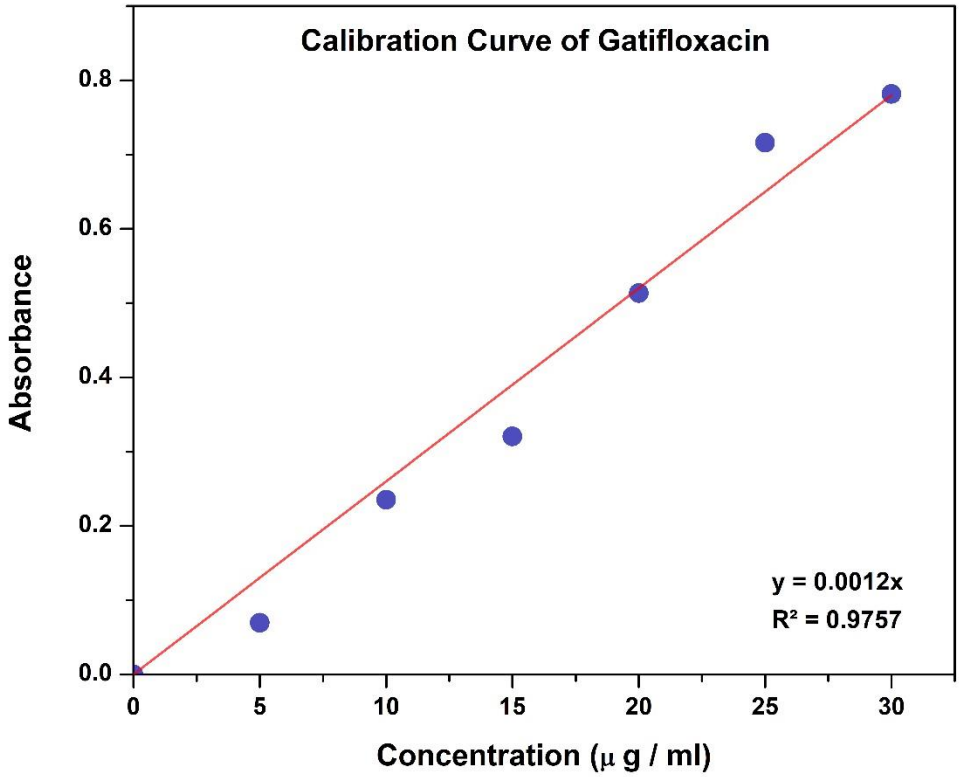


Figure 6-7 Calibration curve of Gatifloxacin

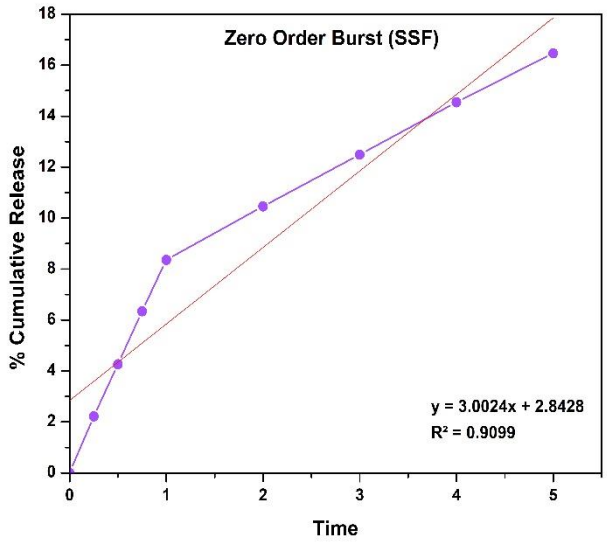
Applying linear regression analysis to the calibration curve helps determine the equation of the best-fit line, allowing for the conversion of instrument responses into drug concentrations. This equation enables the quantification of unknown samples by comparing their measured responses to the curve, facilitating accurate determination of drug concentrations.

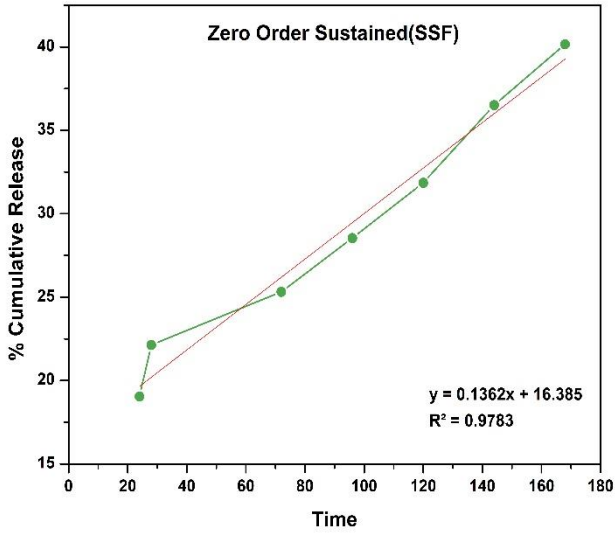
The calibration curve when plotted gives an equation which can be further used find out absorbance of higher concentration or vice versa, here we get the slope 0.0012 and regression coefficient as 0.9757 these values are exclusive for gatifloxacin drug.

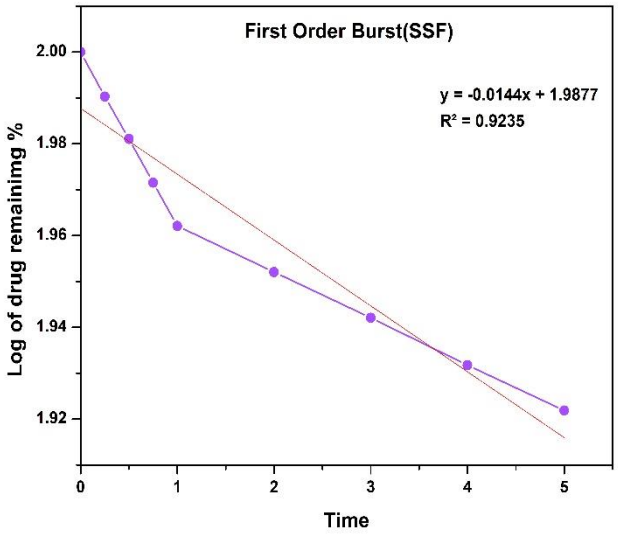
In the pharmaceutical industry, calibration curves for drugs are essential for quality control, drug formulation, and stability testing. They ensure that the manufactured drugs meet specified potency levels and remain consistent over time. By periodically recalibrating instruments using fresh standard solutions and recalculating the calibration curve, accurate drug concentration measurements can be maintained, supporting the safety and efficacy of pharmaceutical products.

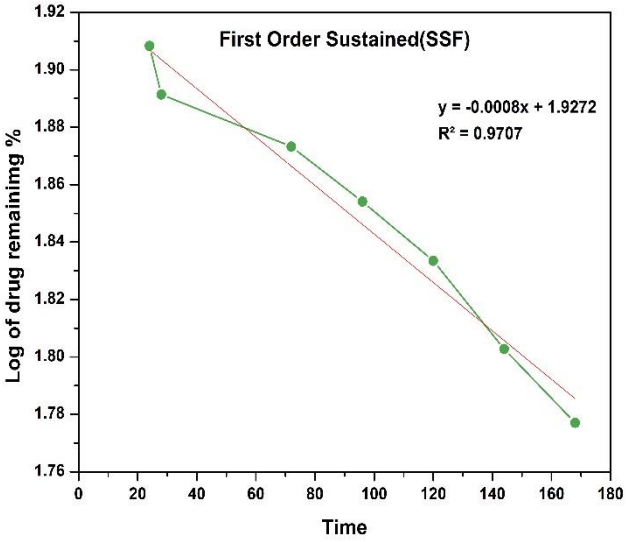
6.6 In Vitro Release in SSF

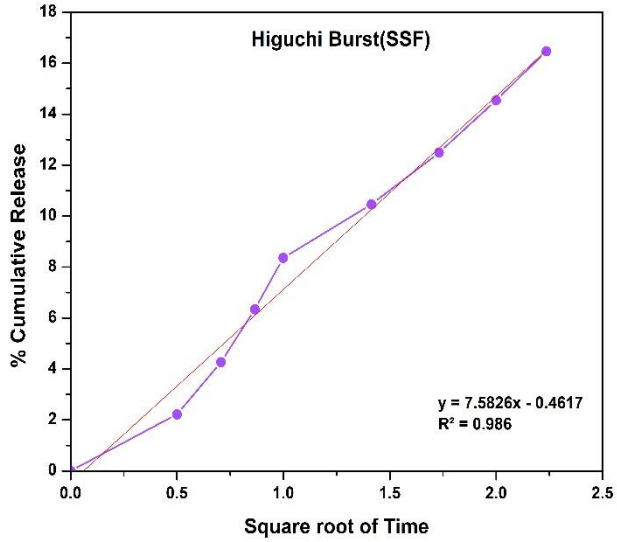
In the release from SSF (Simulated Skin Fluid) different release kinetic models are evaluated. Separate release for burst and sustained condition were performed and the respective regression coefficient were noted down, the SSF solution has pH of 5.5 which resembles the skin, this was done in order to study the release at skin condition.

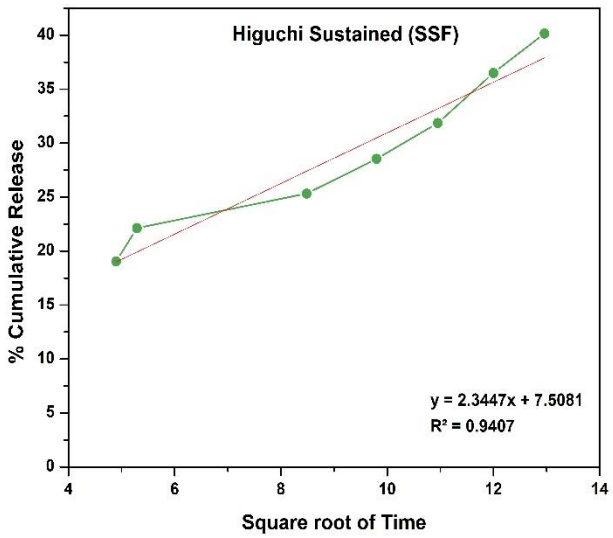


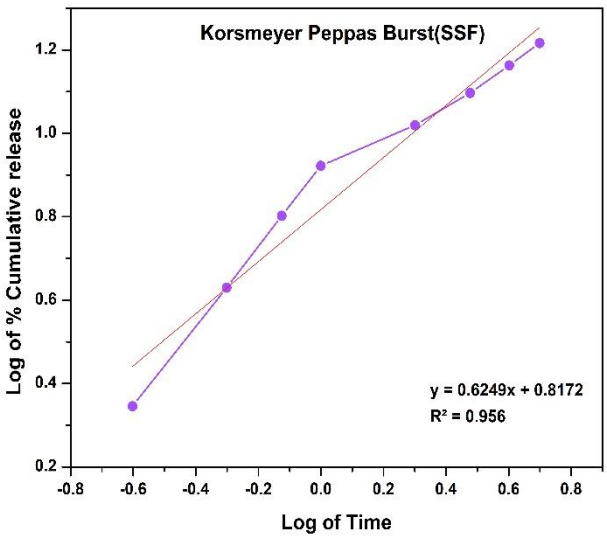












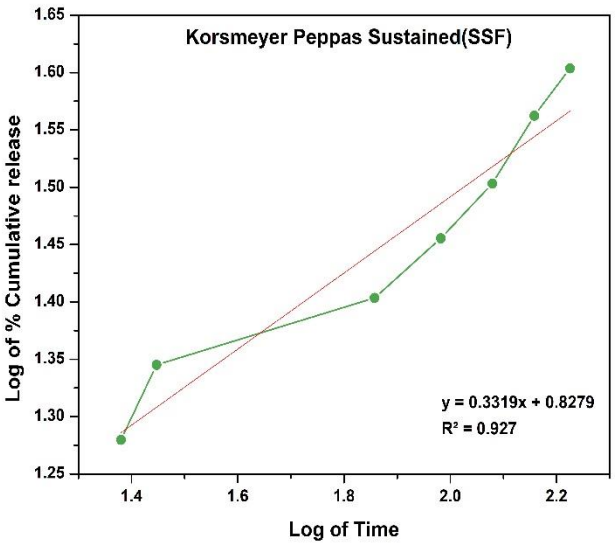


Figure 6-8 Different In-Vitro release kinetic graph in SSF Medium

In the plotted graphs burst and sustained release for Zero Order, First Order, Higuchi and Korsmeyer Peppas model are done, the following data are tubulised.

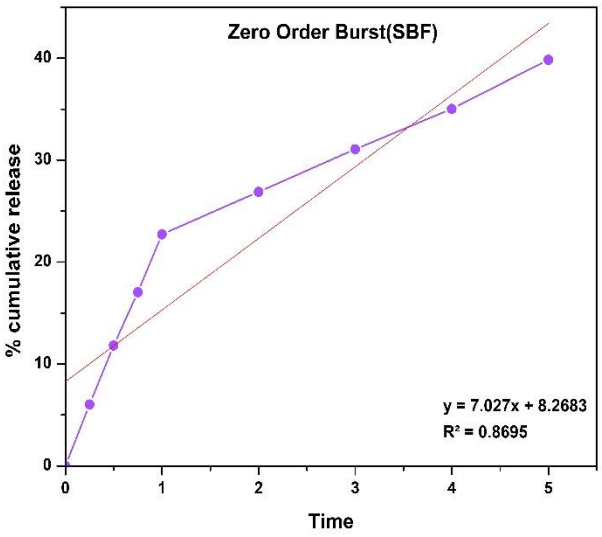
Table 6-1 In Vitro Release in SSF

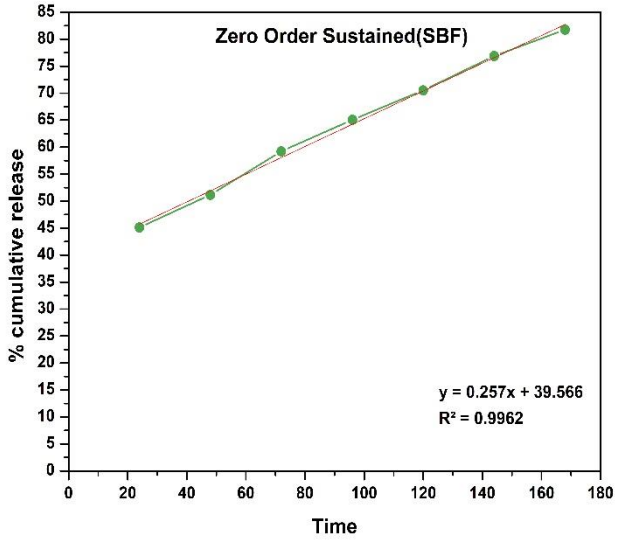
Release Type	Zero Order (R^2)	First Order (R^2)	Higuchi (R^2)	Korsmeyer Peppas (R^2)	Korsmeyer Peppas (n)
Burst	0.9099	0.9235	0.9191	0.956	0.6249
Sustained	0.9783	0.9707	0.9737	0.927	0.3319

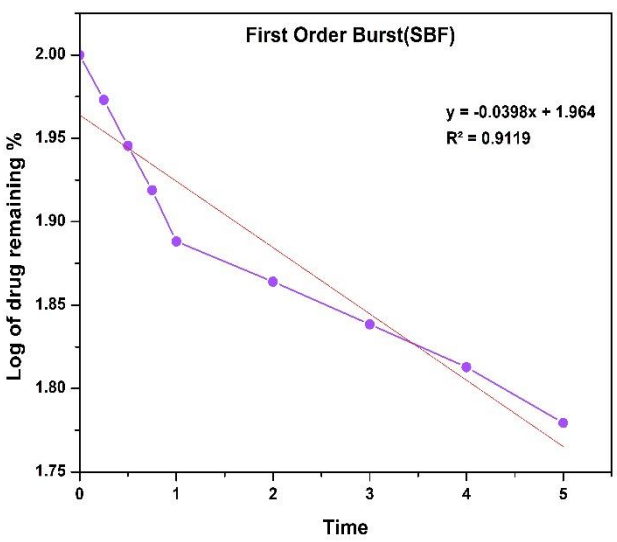
For burst release of drug in SSF medium it is concluded that the best fit regression coefficient is coming for Korsmeyer Peppas and for sustained release the best fit regression coefficient is coming for Zero Order Model, the n value for Korsmeyer Peppas for burst and sustained release are 0.6249 and 0.3319 which corresponds with non Fickian diffusion and Fickian Diffusion respectively.

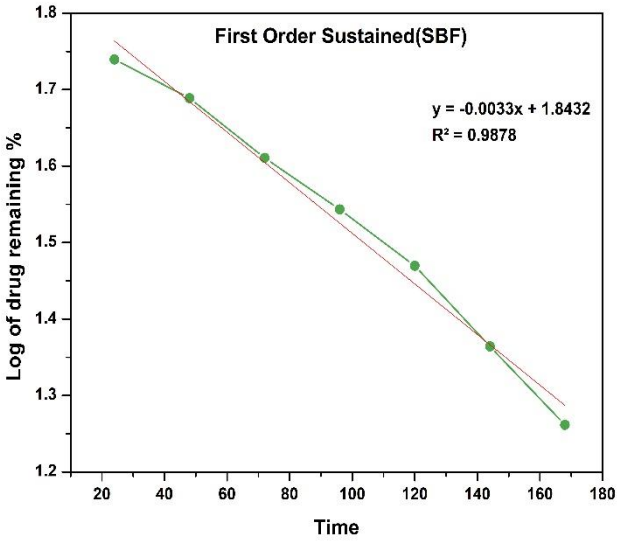
6.7 In Vitro Release in SBF

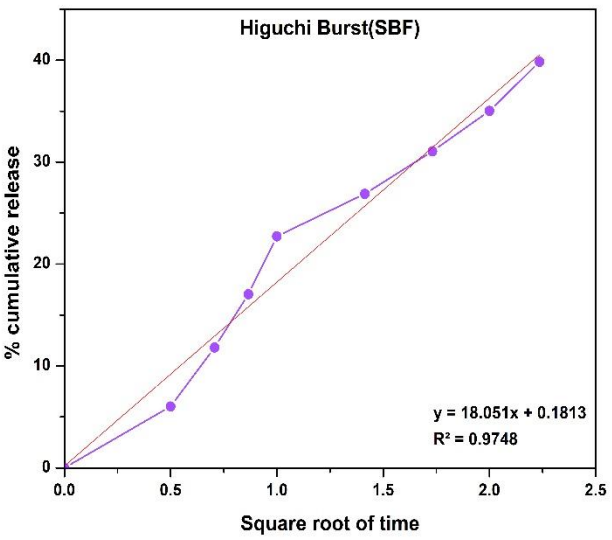
In the release from SBF (Simulated Body Fluid) different release kinetic models are evaluated. Separate release for burst and sustained condition were performed and the respective regression coefficient were noted down, the SBF solution has pH of 7.5 which resembles the body fluid, the different release models are given below.











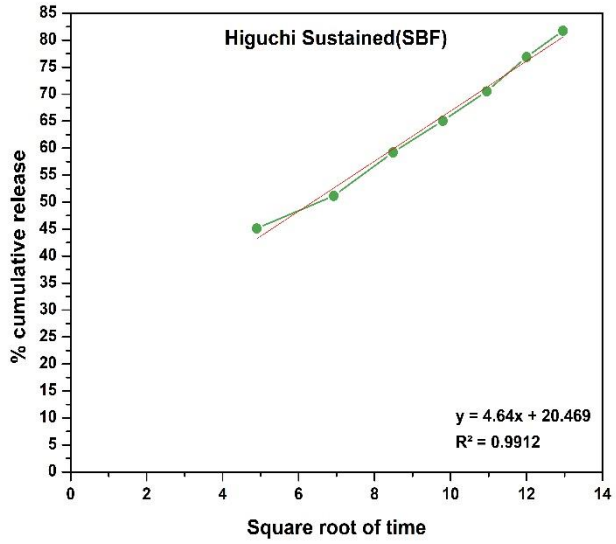
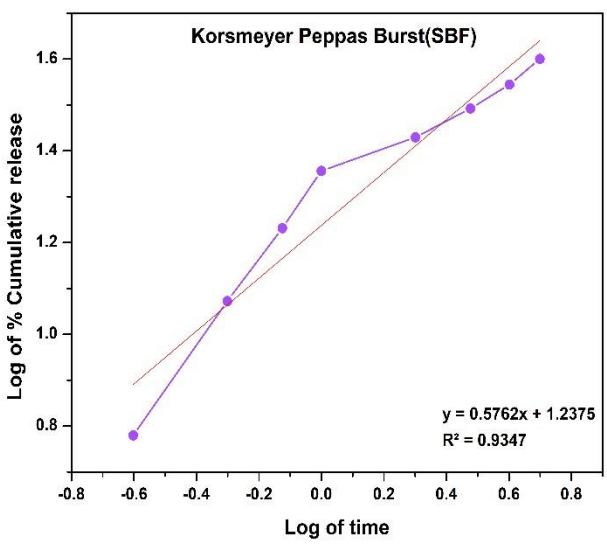
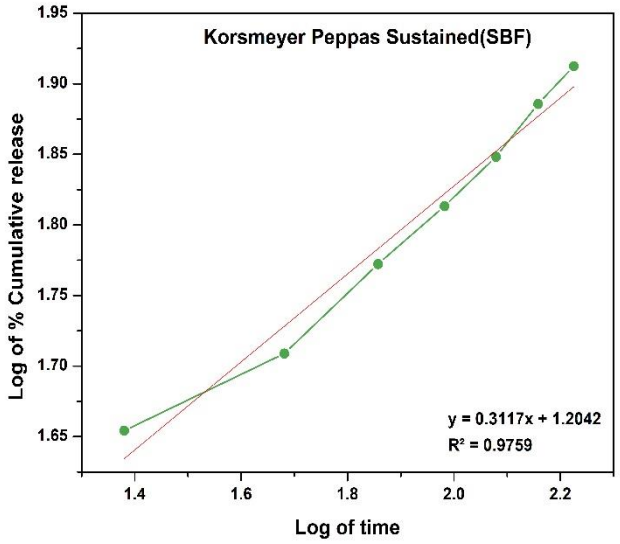


Figure 6-9 Different In-Vitro release kinetic graph in SSF Medium





The in vitro release from GMD-5 in SBF medium, the plotted graph's burst and sustained release for Zero Order, First Order, Higuchi and Korsmeyer Peppas model are done, the following data are tubulised.

Table 6-2 In-Vitro Release in SBF

Release Type	**Zero Order (R^2)**	**First Order (R^2)**	**Higuchi (R^2)**	**Korsmeyer Peppas (R^2)**	**Korsmeyer Peppas (n)**
Burst	0.8695	0.9119	0.9748	0.9347	0.5762
Sustained	0.9962	0.9878	0.9912	0.9759	0.3117

For burst release of drug in SBF medium it is concluded that the best fit regression coefficient is coming for Higuchi Model and for sustained release the best fit regression coefficient is coming for Zero Order Model, the n value for Korsmeyer Peppas for burst and sustained release are 0.5762 and 0.3117 which corresponds to non Fickian and Fickian diffusion respectively.

A substance's pattern of release from a solid dosage form, such as a tablet, capsule, ointment or any other form of medication into a liquid medium is referred to as a dissolution profile. How quickly and how much a drug or molecule releases from its dosage form is an important characteristic to consider in the pharmaceutical and chemical fields because it affects both the drug's effectiveness and bioavailability. The combined dissolution profile for SBF and SSF is constructed and given below.

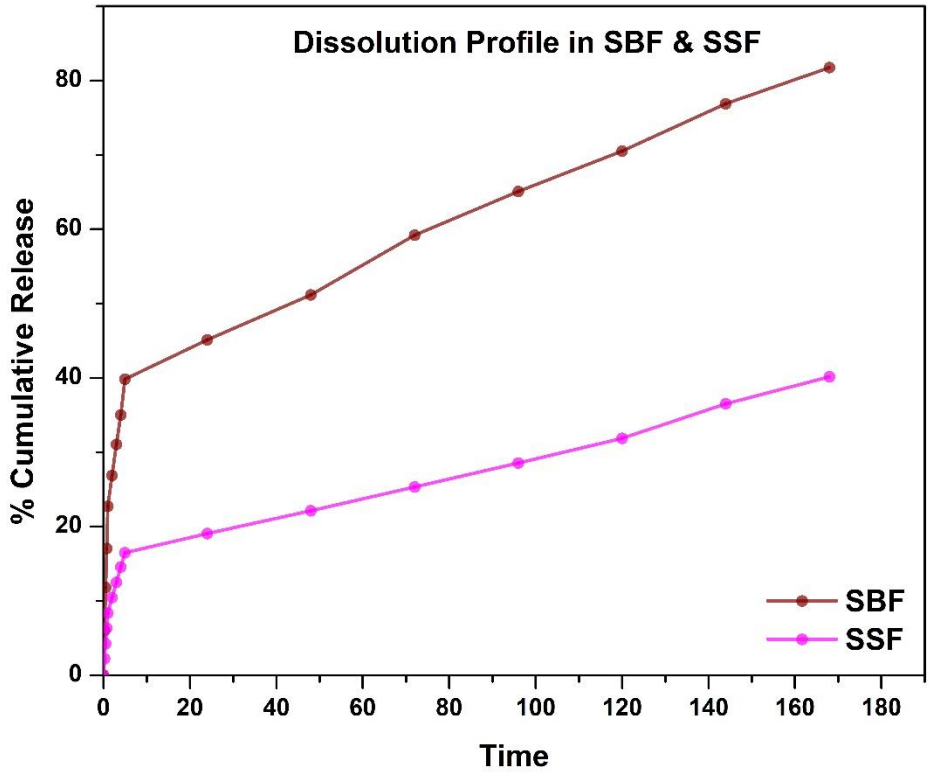


Figure 6-10 Dissolution Profile of GMD-5 in SBF and SSF medium

From the Dissolution profile of GMD-5 in SBF and SSF medium it is concluded that the percentage cumulative release in SBF medium is higher compared to the percentage cumulative release in SSF at 168 hours. This typically gives the release idea of release over and inside a skin wound.

6.8 MIC (Minimal Inhibitory Concentration) calculation of drug loaded clay (GMD-5)

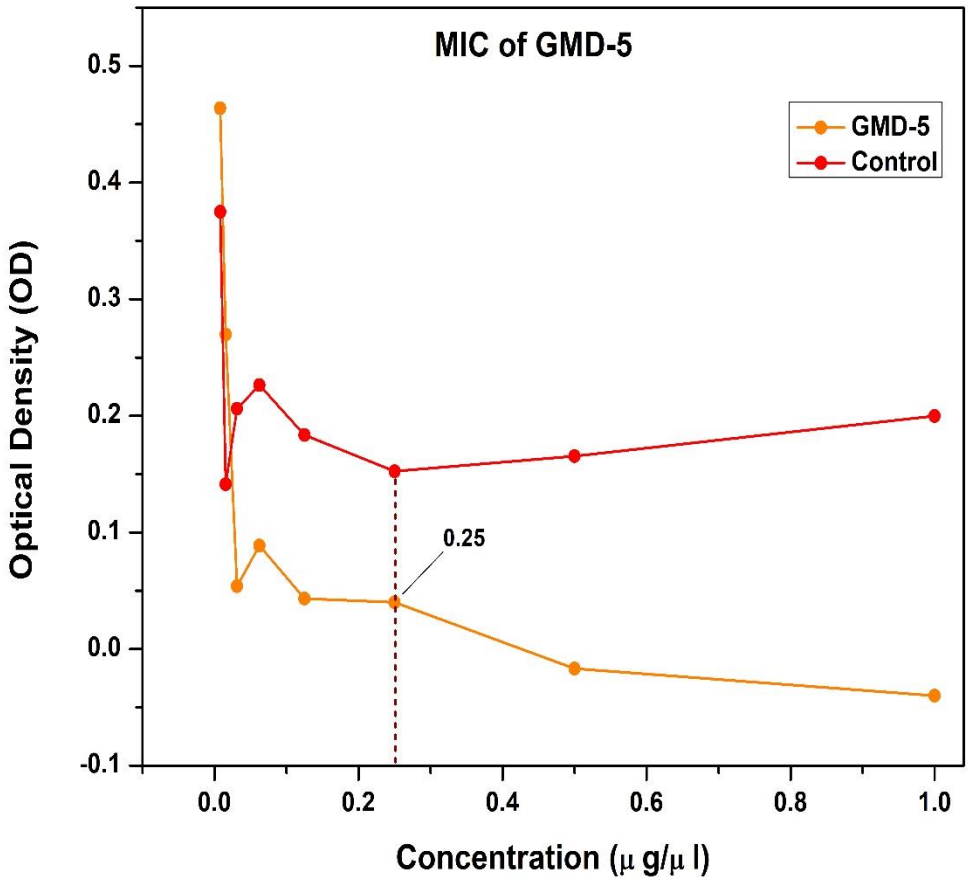


Figure 6-11MIC graph of GMD-5

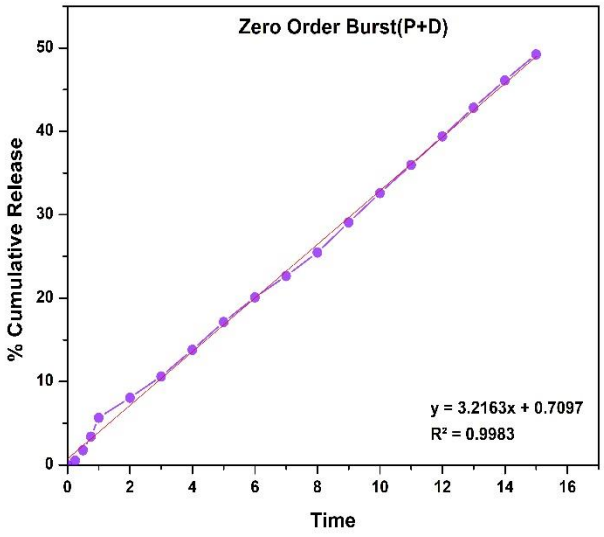
The MIC is the minimum inhibitory concentration, this is the lowest concentration at which the specified material shows antibacterial effect, going to a lower concentration will show no antibacterial effect even in the presence of drug/antibacterial material.

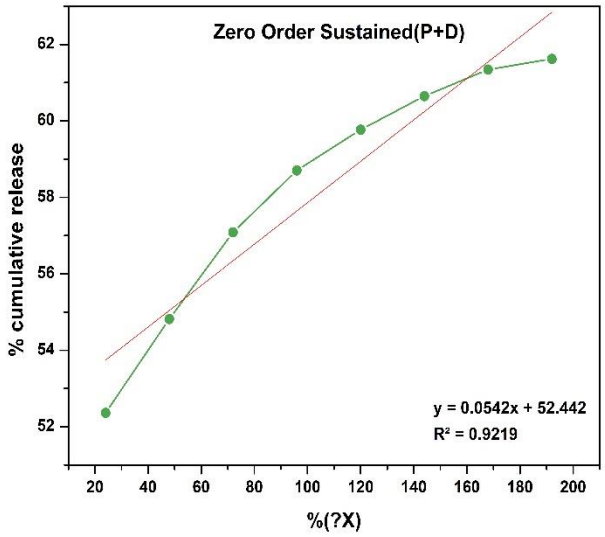
In the aforementioned graph is optical density versus concentration over the span of 24 hours, the first concentration point at which the control is growing but the curve for GMD-5 is going downward is the MIC, the X-axis is hybrid of time and concentration the time set is not marked because in order to calculate the MIC it is needed to interpolate with consideration of concentration and the interpolated MIC is coming at 0.25 µg/µl. Hence the MIC for GMD-5 is 0.25 µg/µl

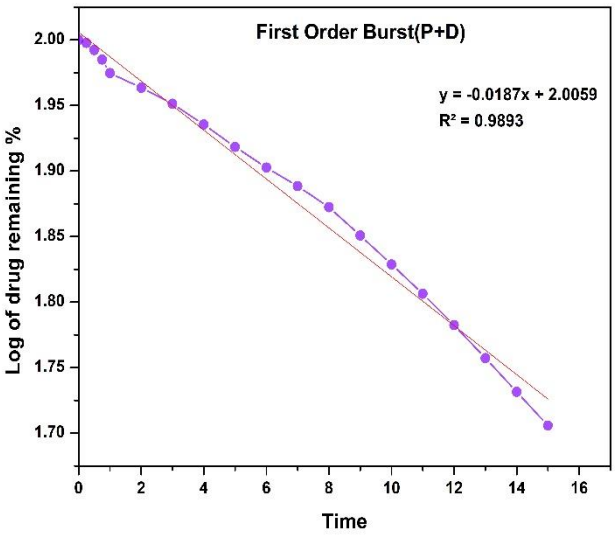
6.9 Ex-Vivo Release from Patch in SBF Medium

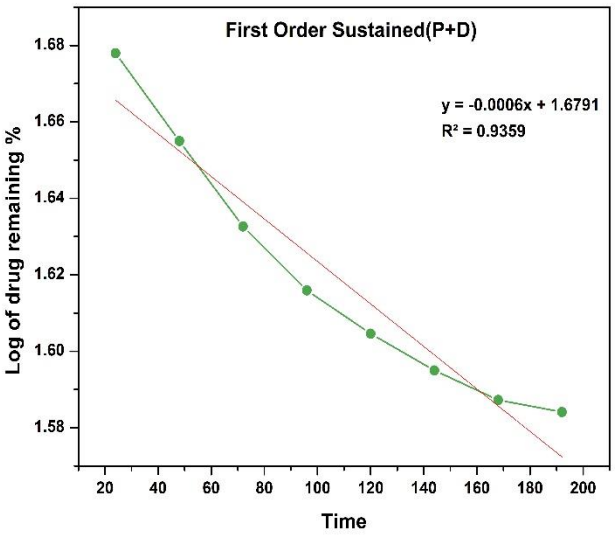
Two different ex-vivo release from patch in SBF medium are performed and are evaluated, two unique conditions are made, in one condition the rerelease is studied from patch loaded with pure drug its denoted as (P+D) and in another condition the patch is loaded with drug loaded clay (GMD-5) and its denoted as (P+C+D).

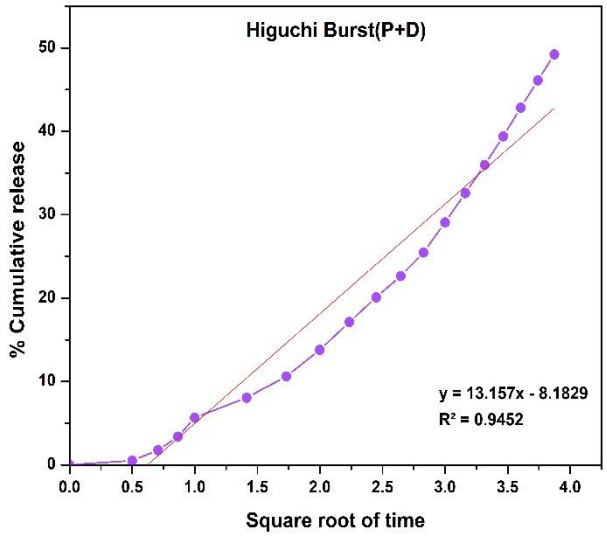
1. The (P+D) release kinetics:

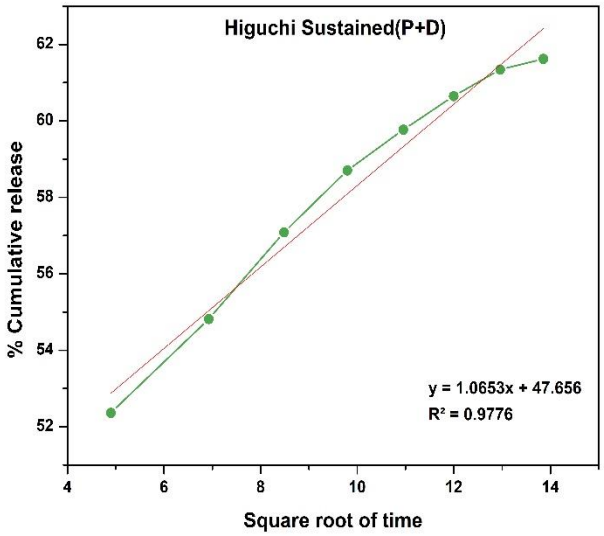


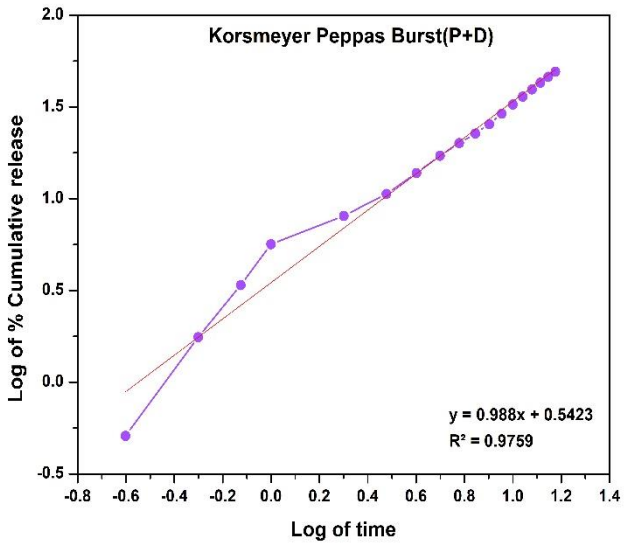












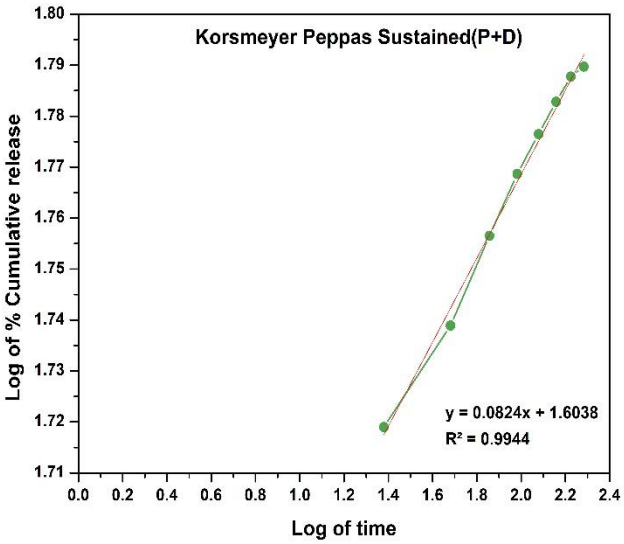


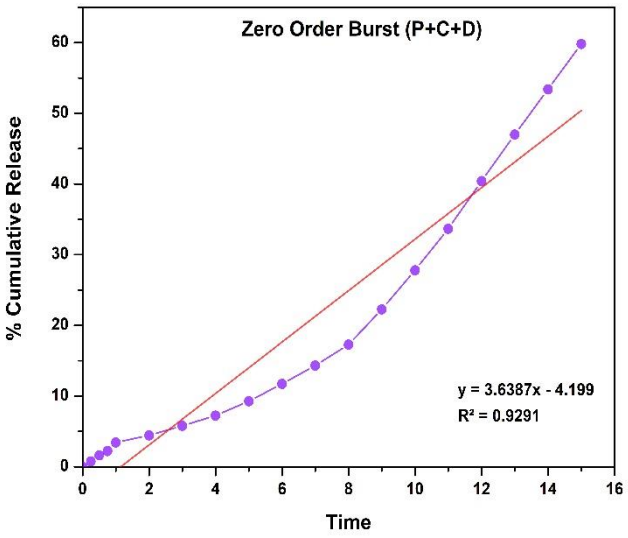
Figure 6-12 Different ex-vivo release kinetics graph in SBF medium released from (P+D)

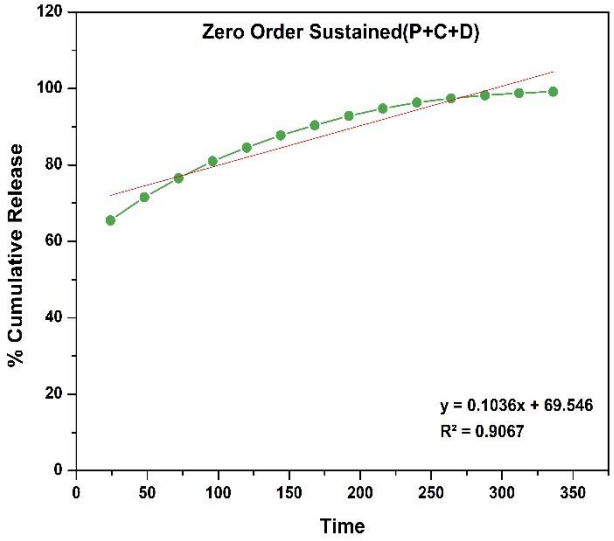
Table 6-3 Ex-Vivo release from (P+D in SBF)

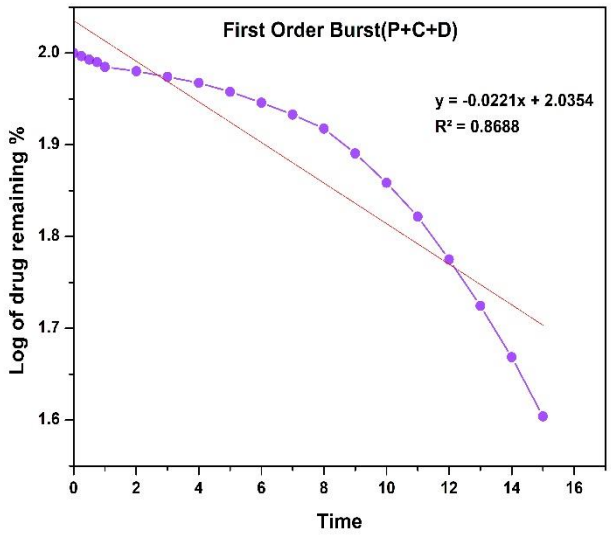
Release Type	**Zero Order (R^2)**	**First Order (R^2)**	**Higuchi (R^2)**	**Korsmeyer Peppas (R^2)**	**Korsmeyer Peppas (n)**
Burst	0.9983	0.9893	0.9452	0.9759	0.988
Sustained	0.9219	0.9359	0.9776	0.9944	0.0824

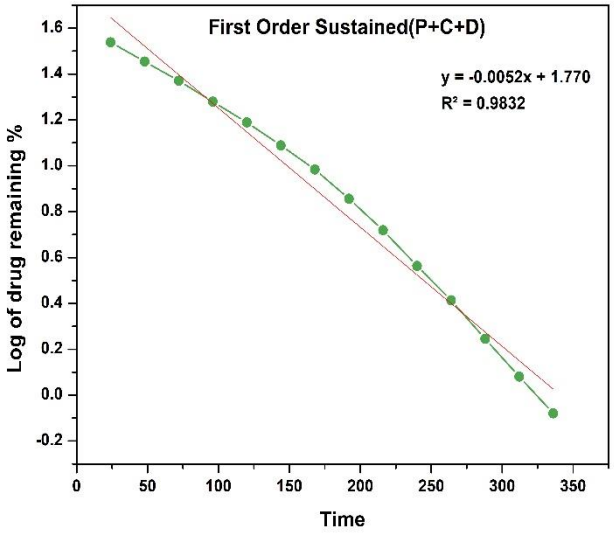
From the tabulated data the best fit regression coefficient for ex vivo release kinetics of (P+D) in SBF medium is Zero order for burst release and Korsmeyer Peppas for Sustained release. The Korsmeyer Peppas coefficient for burst and sustained release is 0.9880 and 0.0824 respectively which corresponds to non Fickian (Super Case II Transport) and Fickian Diffusion.

2 The (P+C+D) Release Kinetics:









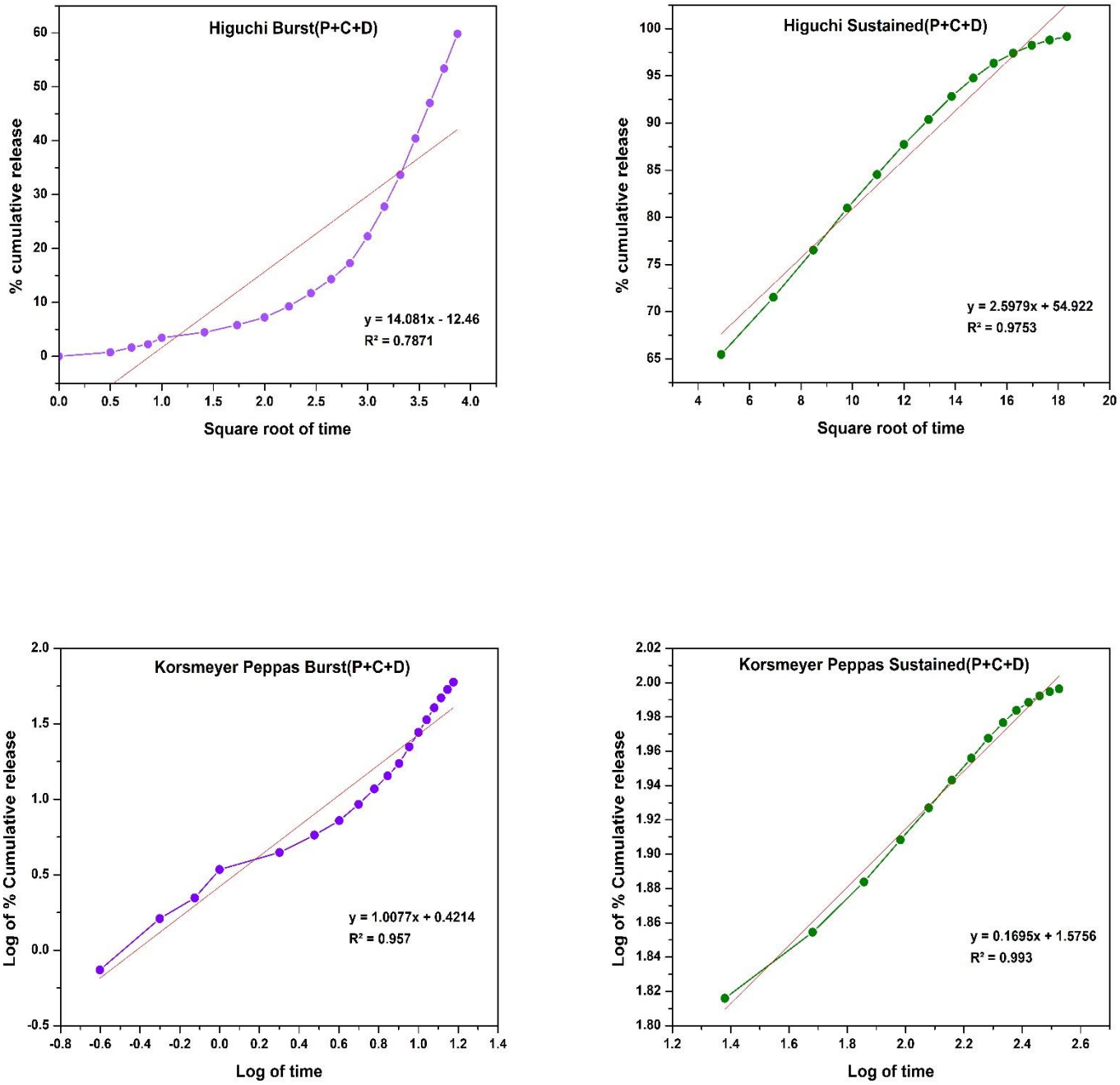


Figure 6-13Different ex-vivo release kinetics graph in SBF medium released from (P+C+D)

Table 6-4 Ex-Vivo release form (P+C+D) in SBF.

Release Type	Zero Order (R^2)	First Order (R^2)	Higuchi (R^2)	Korsmeyer Peppas (R^2)	Korsmeyer Peppas (n)
Burst	0.9291	0.8688	0.7871	0.9570	1.0077
Sustained	0.9067	0.9832	0.9753	0.9930	0.1695

From the tabulated data the best fit regression coefficient for ex vivo release kinetics of (P+C+D) in SBF medium is Korsmeyer Peppas for both burst and sustained release. The Korsmeyer Peppas coefficient for burst and sustained release is 1.0077 and 0.1695 respectively which corresponds to non Fickian (Super Case II Transport) Diffusion.

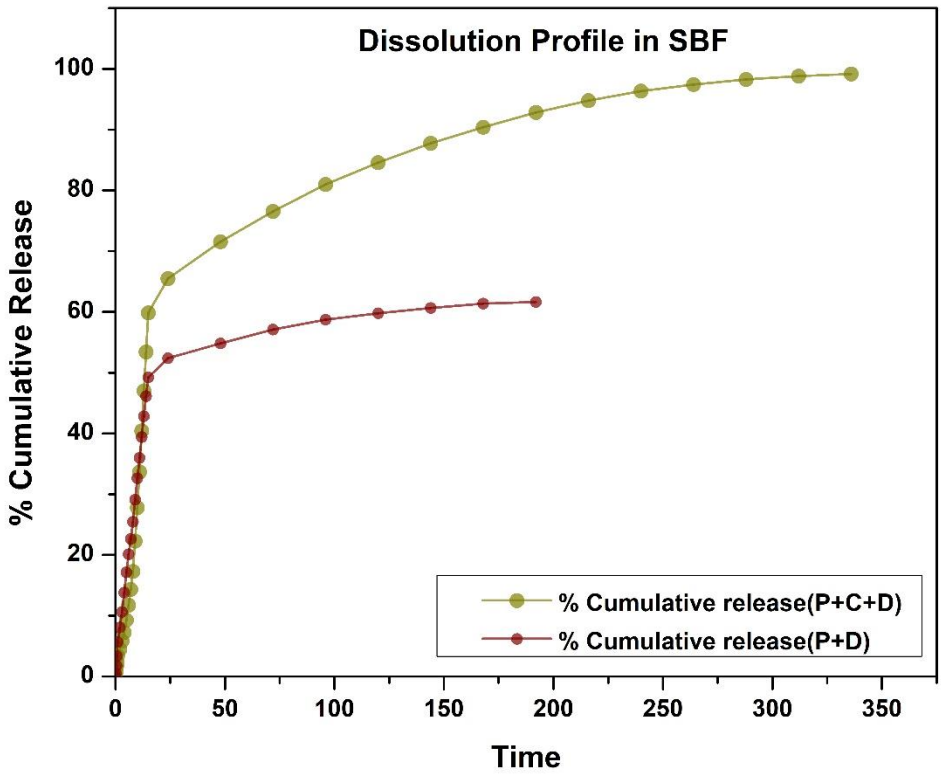


Figure 6-14 Dissolution profile of (P+D) and (P+C+D) in SBF medium

From the dissolution profile of (P+D) and (P+C+D) a major conclusion which can be drawn is that then the drug is loaded into clay and incorporated into the patch the release is more sustained and is well distributed over a longer period of time compared to the drug when loaded in nascent patch.

From the dissolution profile on analysis of data, the percentage cumulative release at which the MIC of drug released is 7.22% for (P+C+D) and 3.37% for (P+D) and their corresponding time of release are 4 hours and 45 minutes respectively.

6.10 Antibacterial efficacy of the formulated patch

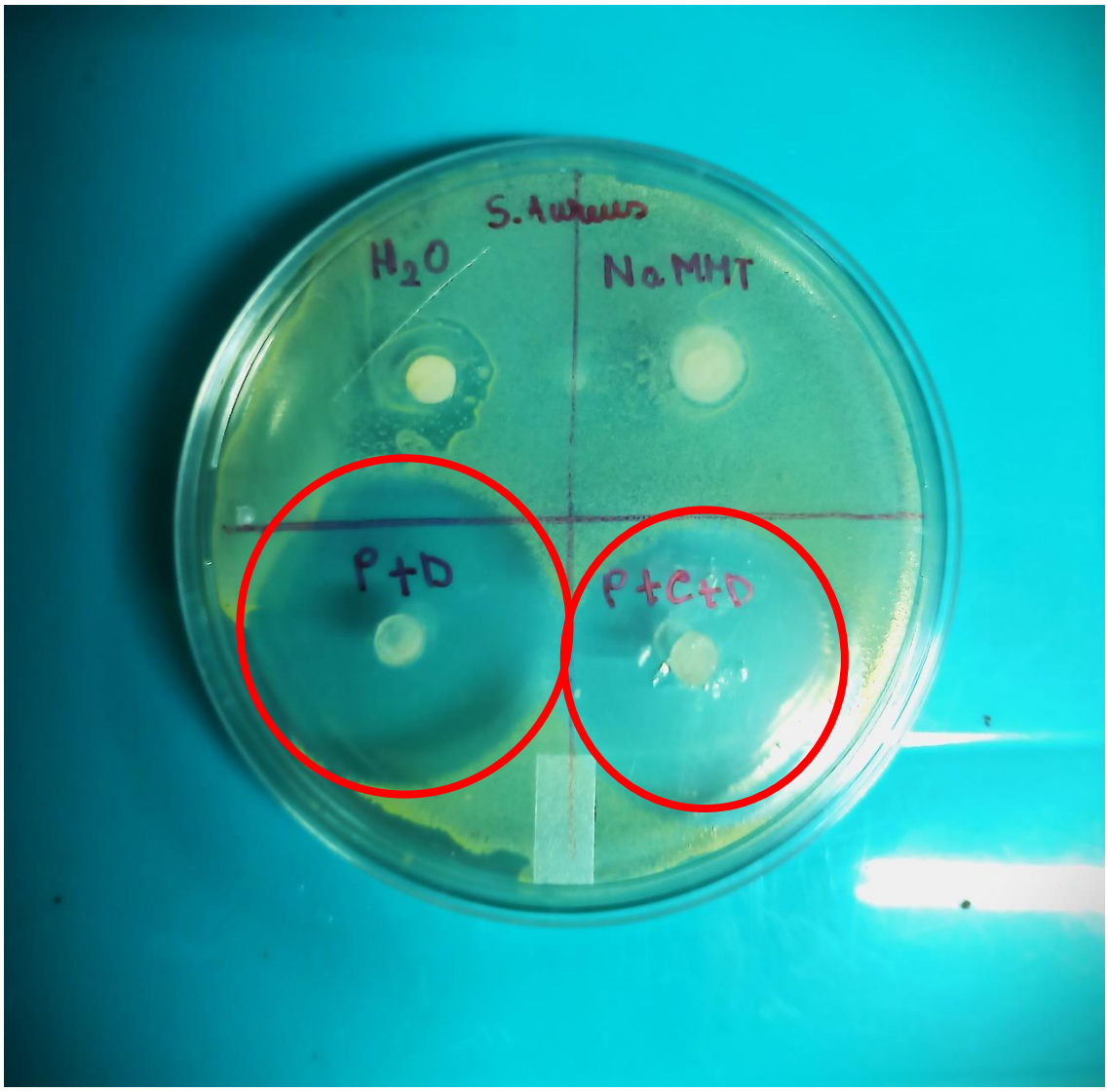


Figure 6-15 Zone of inhibition shown by antibacterial patch circular discs

The most crucial conclusion which can be drawn is that the patch discs are showing antibacterial effect against the most commonly found bacteria (Staphylococcus Aureus) on skin

infection. The drug loaded clay patch (P+C+D) produced a comparatively smaller circle because the drug is loaded in clay layers and are being continuously released, this give a more sustained release characteristic.

6.11 Reference

[1] I. A. P. U. T. P. C. T. (PCT), "NOVEL CRYSTALLINE FORMS OF GATIFLOXACIN"

[2] B. A. Fil, C. Özmetin, and M. Korkmaz, "Characterization and electrokinetic properties of montmorillonite," *Bulg. Chem. Commun.*, vol. 46, no. 2, pp. 258–263, 2014.

[3] S. Arthanari *et al.*, "Preparation and characterization of gatifloxacin-loaded alginate/poly (vinyl alcohol) electrospun nanofibers," *Artif. Cells, Nanomedicine Biotechnol.*, vol. 44, no. 3, pp. 847–852, 2016, doi: 10.3109/21691401.2014.986676.

7 Conclusion

In order to confirm that the drug is loaded into the matrix, in the XRD plot, the characteristics peaks are present at 19.67°,20.78°,26.64°,31.16°,37.60° and 68.26° it is observed that the characteristic peaks have shifted left by a small value. 19.98° has shifted by 0.31°,20.96° has shifted by 0.18°,26.74° has shifted by 0.10°,31.30° has shifted by 0.14°,37.80° has shifted by 0.20° and 68.32° has shifted by 0.06°. The average shift is 0.165° towards left.

From the FTIR plot it is confirmed that the desired drug (Gatifloxacin) is loaded and present in the matrix, before loading the plot shows the bonds of the matrix and after loading the plot shows the bonds of montmorillonite matrix as well as other characterizing bonds of gatifloxacin like C=O stretching (Carbonyl), C-F stretching (Fluoride) and aromatic C=C Stretching (Quinolone Ring).

For antibacterial test, it was tested for Kirby Bauer Test and was concluded that the drug loaded clay and drug both produced a zone of inhibition which was circular in shape and no bacterial colony were present in the zone whereas the control contained bare Na-MMT and showed no such zone. This was tested for the most common bacteria Staphylococcus Aureus held responsible for skin infection. The conclusion which can be drawn is that the drug is loaded into the Na-MMT matrix, show good antibacterial property and shows a release pattern.

For burst release of drug in SSF medium it is concluded that the best fit regression coefficient is coming for Korsmeyer Peppas and for sustained release the best fit regression coefficient is coming for Zero Order Model, the n value for Korsmeyer Peppas for burst and sustained release are 0.6249 and 0.3319 which corresponds with non Fickian diffusion and Fickian Diffusion respectively.

For burst release of drug in SBF medium it is concluded that the best fit regression coefficient is coming for Higuchi Model and for sustained release the best fit regression coefficient is coming for Zero Order Model, the n value for Korsmeyer Peppas for burst and sustained release are 0.5762 and 0.3117 which corresponds to non Fickian and Fickian diffusion respectively.

From the plot of optical density versus concentration the MIC (Minimum Inhibitory Concentration) for the drug loaded clay is observed at 0.25 µg/µl.

After Patch formulation the best fit regression coefficient for ex vivo release kinetics of (P+D) in SBF medium is coming for Zero Order for burst release and Korsmeyer Peppas for Sustained release. The Korsmeyer Peppas coefficient for burst and sustained release is 0.9880 and 0.0824 respectively which corresponds to non Fickian (Super Case II Transport) and Fickian Diffusion.

For ex vivo release kinetics of (P+C+D) in SBF medium the best fit regression coefficient is coming for Korsmeyer Peppas for both burst and sustained release. The Korsmeyer Peppas coefficient for burst and sustained release is 1.0077 and 0.1695 respectively which corresponds to non Fickian (Super Case II Transport) Diffusion. Also, the dissolution profile shows that's the release from patch containing drug loaded clay (GMD-5) has longer release time compared to pure drug patches.

From the dissolution profile on analysis of data, the percentage cumulative release at which the MIC of drug is released is 7.22% for (P+C+D) and 3.37% for (P+D) and their corresponding time of release are 4 hours and 45 minutes respectively.

Kirby Bauer test is performed for synthesized patch material cut into circular discs and both the (P+D) and (P+C+D) discs shows a circular zone of inhibition. It is concluded that the patches are properly synthesized and retain the antibiotic property as well as the sustained release property of the GMD-5.

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[2] W. Chrzanowski, S. Y. Kim, and E. A. Abou Neel, "Biomedical applications of clay," *Aust. J. Chem.*, vol. 66, no. 11, pp. 1315–1322, 2013, doi: 10.1071/CH13361.

[3] J. M. Oh, T. T. Biswick, and J. H. Choy, "Layered nanomaterials for green materials," *J. Mater. Chem.*, vol. 19, no. 17, pp. 2553–2563, 2009, doi: 10.1039/b819094a.

[4] Ю. И. Ярец, В. Н. Мартинков, and Н. И. Шевченко, "Особенности проявления биохимических идентификационных признаков у клинических штаммов S. aureus, образующих биопленку," *Физико-Химическая Биология Как Основа Современной Медицины : Тезисы Докладов Участников Международной Научной Конференции, Посвященной 75-Летию Со Дня Рождения Профессора Е. В. Барковского (Минск, 21 Мая 2021 Г) / Под Ред. В. В. Хрусталёва, А. Д. Таганови*. pp. 367–368, 2021.

[5] C. Viseras, C. Aguzzi, P. Cerezo, and M. C. Bedmar, "Biopolymer-clay nanocomposites for controlled drug delivery," *Mater. Sci. Technol.*, vol. 24, no. 9, pp. 1020–1026, 2008, doi: 10.1179/174328408X341708.

[6] K. Sachs-Barrable, J. W. Darlington, and K. M. Wasan, "The effect of two novel cholesterol-lowering agents, disodium ascorbyl phytostanol phosphate (DAPP) and nanostructured aluminosilicate (NSAS) on the expression and activity of P-glycoprotein within Caco-2 cells," Lipids *Health Dis.*, vol. 13, no. 1, pp. 1–10, 2014, doi: 10.1186/1476-511X-13-153.