NILOTINIB - LOADED POLY LACTIDE - CO - GLYCOLIDE NANOPARTICLES FOR CHRONIC MYELOID LEUKEMIA TREATMENT: DEVELOPMENT AND THEIR PHYSICOCHEMICAL CHARACTERIZATIONS

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CERTIFICATE OF APPROVAL

This is to certify that **SRIJITA GOPE** bearing **Registration No: 163663 of 2022-23** has carried out the research work entitled "**NILOTINIB - LOADED POLY LACTIDE – CO - GLYCOLIDE NANOPARTICLES FOR CHRONIC MYELOID LEUKEMIA TREATMENT: DEVELOPMENT AND THEIR PHYSICOCHEMICAL CHARACTERIZATIONS "** independently with proper care and attention under my supervision and guidance in the Pharmaceutics Research Laboratory in the Department of Pharmaceutical Technology, Jadavpur University. She has incorporated her findings into this thesis of the same title, being submitted by her. In partial fulfilment of the requirements for the degree of **MASTERS OF PHARMACY** from Jadavpur University. I appreciate her endeavour to do the project and her work has reached my gratification.

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DECLARATION OF ORIGINALITY AND COMPLIANCE OF ACADEMIC ETHICS

I hereby declare that this thesis contains a literature survey and original research work by the undersigned candidate as part of her Master of Pharmaceutical Technology studies. All information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all materials, and the results are original to this work.

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Date –

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Srijita Gope

Dedicated to my guide
and my family

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Introduction

Blood cancers alter the formation and function of blood cells. Most of these malignancies begin in the bone marrow, where blood originates. Stem cells in bone marrow mature and differentiate into red blood cells, white blood cells, and platelets. Most blood cancers occur when an abnormal form of blood cell grows uncontrollably, disrupting the normal blood cell development process. These abnormal blood cells, also known as cancerous cells, inhibit your blood from performing several important activities, such as fighting infections and preventing serious bleeding [1].

Types of Blood Cancer :

- **Leukemia -** Leukemia is a blood cancer characterized by altered hematopoietic progenitors and widespread bone marrow invasion. Cancers of the hematopoietic tissues, such as bone marrow and the lymphatic system, develop from white blood cells and lymph nodes. White blood cells typically divide and increase according to the body's needs. Leukemia disrupts the normal growth pattern of blood cells, leading to uncontrolled expansion. Acute leukemia causes bone marrow to overproduce premature white blood cells and stop normal production, resulting in a loss of the body's ability to fight the disease [2]. Tobacco use, past treatment with chemotherapeutics, radiation exposure, rare congenital diseases, certain blood abnormalities, family history, age, and gender are all risk factors for leukemia. The survival rate of leukemia patients has improved dramatically due to recent advances in innovative therapy techniques and targeted medications [3].

Leukaemia is classified into four types: Acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), and chronic myeloid leukemia (CML) [4]. In 2020, leukemia was responsible for around 2.5% and 3.1% of all new cancer incidence and death, respectively. The risk of leukemia varies by age, sex, and geographical region. These inequalities could be attributed to differences in the incidence of various environmental and genetic risk factors for leukemia. Acute Myelogenous Leukemia (AML) is the most prevalent kind of leukemia, affecting both adults and children. This kind of leukemia is also known as Acute Non-Lymphocytic Leukemia (ALL). Besides this, leukemia also includes Acute Lymphocytic Leukemia (ALL) and Chronic Myelogenous Leukemia (CML), which typically develops in adulthood. CML is caused by a Philadelphia chromosomal defect, which results in an abnormal gene termed BCR-ABL. Chronic lymphocytic leukemia

(CLL) is the most frequent type of adult leukemia that can last for years without therapy. Global statistics show that this type of leukemia never develops in children [4]. Other bone marrow chronic disorders, such as Chronic Myelogenous Leukemia, cause chronic leukemia by creating either very few or very many bone marrow cells. Acute bone marrow leukemia is caused by chronic bone marrow abnormalities, which include dysplasia and bone marrow tissue proliferation. Unlike other cancers, leukemia is not a solid tumor that can be surgically removed by a doctor. Leukemia treatment is complex due to the involvement of bone marrow. Today's leukemia treatments include chemotherapy, which is the most prevalent form. Treatment options for leukemia include a single drug or a combination of chemical agents, biological therapy (which uses the immune system to prevent cancer progression), and kinase inhibitors. Inhibitors used to treat persistent bone marrow leukemia reduce the risk of several complications. To treat cancer, there are various ways, such as controlling known oncogene proteins or undergoing bone marrow transplantation with a healthy donor [4].

- **Lymphoma -** Lymphoma is a cancer that affects the lymphatic system, namely the nodes. This is a type of white blood cell that targets lymphocytes. The most prevalent type of lymphoma is Hodgkin lymphoma. Non-Hodgkin lymphoma includes all kinds of lymphoma. There are over 70 different types of lymphoma. These includes both slow-growing and aggressive organisms. Both adults and children can develop Hodgkin and non-Hodgkin lymphoma. Lymphocytes, also known as B and T lymphocytes, are responsible for most lymphomas (T cells). Malignant cells may spread throughout the body via lymphatic vessels as the disease progresses. This can lead to the formation of tumors [5]. The type and stage of lymphoma indicate treatment options and prospects of cure. Lymphoma is classified into two types;
  a. **Non-Hodgkin lymphoma** : It is a lymphatic system malignancy. Non-Hodgkins lymphoma is the type of lymphoma that occurs most frequently (NHL). It is more prevalent in older people. Treatment options for non-Hodgkin lymphoma include, among other things, immunotherapy, chemotherapy, radiation, targeted therapy, and stem cell transplantation [6].
  b. **Hodgkin lymphoma** : This disease affects the immune system. If it is identified and treated quickly, Hodgkin's disease is one of the most curable types of cancer. Immunotherapy, chemotherapy, and stem cell transplantation are possible treatments for Hodgkin lymphoma [7].

- **Myeloma :** The malignancy known as myeloma affects the plasma cells, which are lymphocytes that produce antibodies to prevent infections. Myeloma impacts the immune system, which makes the body more susceptible to infection. A mutation in the genome of the plasma cell causes myeloma. DNA acts as the blueprint, or instructions, for the cell. As new plasma cells are produced by the bone marrow, the DNA is altered. The aberrant plasma cell proliferates. It's myeloma. An antibody is produced abnormally by myeloma cells. Both lymphoma and myeloma do not expand. The majority of the problems brought on by this illness are produced by para-protein in the body and abnormal plasma cells in the bone marrow. Active bone marrow is damaged by idiopathic myeloma. It is comprised of the rib cage, spine, skull, arms, legs, shoulders, and pelvis [8].

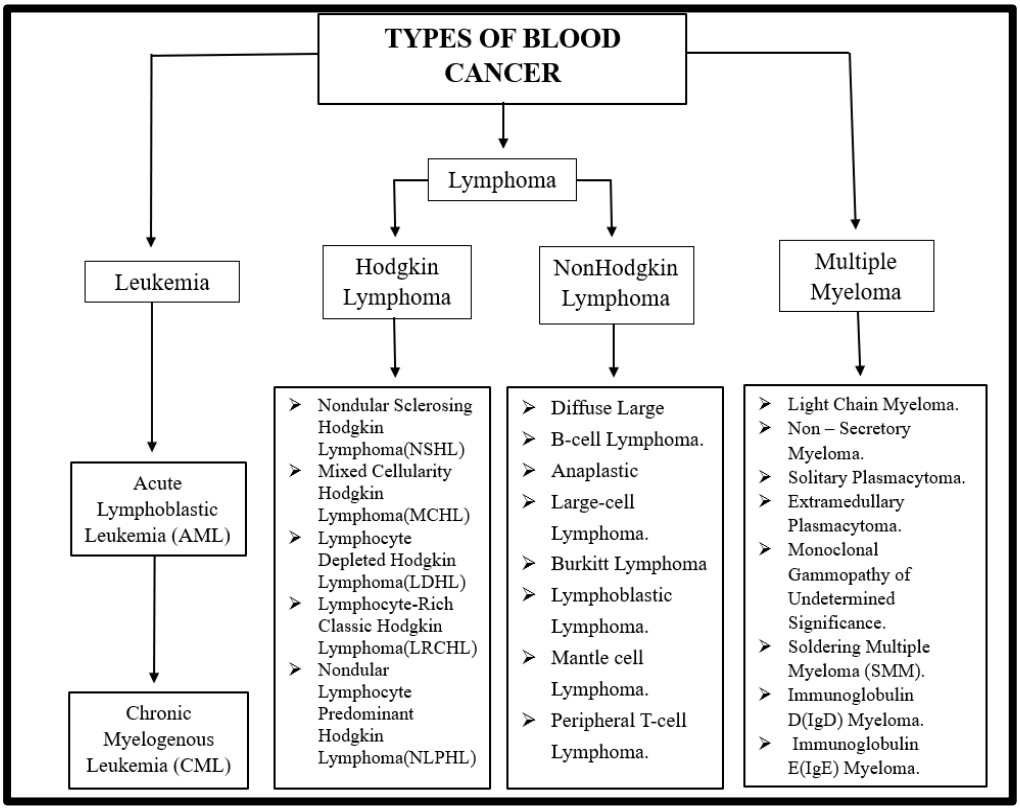


Fig 1 : Types of Blood Cancer

Strategies for treatment of Leukemia

- **Chemotherapy :** The most popular method of treating leukemia is chemotherapy. Among the medications frequently used to treat AML are methotrexate, cytarabine, thioguanine, mitoxantrone, and daunorubicin. Among the medications used to treat ALL include vincristine, prednisone, asparaginase, doxorubicin, and daunorubicin [9].

For CLL, popular treatments include chlorambucil, cyclosporin, and fludarabine; for CML, common treatments include hydroxyurea and the alkylating agent busulfan. Clinicians are increasingly using an adaptable combination of multiple chemical medications to increase the therapeutic effect [10].

Chemotherapy, as a systemic treatment, can only manage leukemia; it cannot eradicate it. The medications cause negative side effects and harm normal cells in addition to killing leukemia cells. Antioxidant consumption is one of the most frequent side effects. A sequence of oxidative stress responses are brought on by the chemotherapy medications' attempts to eliminate reactive oxygen species (ROS) during the course of the treatment, which also cause them to create ROS and deplete the body's stored antioxidants. Malnutrition, obesity, endocrine issues, hypothyroidism, cardiovascular illnesses, lung conditions, neurological conditions, osteonecrosis, secondary malignancy and persistent pain are additional therapeutic adverse effects [11].

- **Radiation Therapy :** Radiotherapy uses ionizing radiation, which is released when radionuclides decay, to kill and suppress sick tissues. Iodine-131, yttrium-90, lutetium-177, copper-67, bismuth-213, astatine-211, actinium-225, and other radionuclides are among the most often utilized ones. Radiation sensitivity is quite high in hematological malignancies. The objective of radioimmunotherapy can be accomplished by loading the carrier with the proper radionuclides to target blood tumor cell markers. Among the key benefits of radiation immunotherapy are the following: (1) The radiation dose within the tumor is high, but the harm to healthy organs is less. (2) The tagged molecules are useful even if cells do not internalize them. (3) Both the curative effect and the lethal range are significant [12].

  The primary side effect of radiation therapy is bone marrow suppression, which can be severe and cause leukopenia, anemia, and thrombocytopenia. Radiation therapy can be used alone or in conjunction with chemotherapy as a pretreatment method for stem cell transplantation [2].

- **Stem Cell Transplantation :** A variety of basic hematopoietic cells possessing an infinite or extended capacity for self-renewal and the ability to differentiate in multiple directions are referred to as hematopoietic stem cells. The only clinically effective treatment for leukemia at this time is hematopoietic stem cell transplantation. Transplantations can be classified into three categories based on the source of hematopoietic stem cells: bone marrow transplantation, peripheral blood stem cell

transplantation and umbilical cord blood stem cell transplantation. High-intensity chemotherapy or radiation therapy is necessary to eradicate the sick leukemia cells prior to hematopoietic stem cell transplantation. Healthy hematopoietic stem cells are then injected into the body to await the cells' recovery [13].

- **Targeted Therapy :** One therapeutic method called targeted therapy works by interfering with the essential molecules of leukemia. Three categories of small molecule inhibitors that target gene mutations, inhibitors that target important signaling pathways and antibodies or antibody-coupled medications that target cell surface molecules make up their principal therapeutic methods [14].
- **Bone Marrow Microenvironment :** There are two types of compartments in the bone marrow microenvironment (BMM): cellular and noncellular. The two primary cell types found in the intercellular compartment are hematopoietic and non-hematopoietic. Extracellular matrix (ECM), oxygen and liquid environment (which contains chemokines, growth factors, and cytokines) are examples of noncellular compartments. In the bone marrow microenvironment, the intercellular compartments are created or influenced by all of these. Unusual interstitial cell compositions in the bone marrow microenvironment are closely associated with the onset and progression of leukemia, as well as with the differentiation, migration, proliferation, survival, and medication resistance of malignant tumor cells. Therefore, it is crucial to treat leukemia by focusing on the bone marrow microenvironment or developing customized response drug release mechanisms based on the microenvironment's features. Immature bone marrow cells cannot differentiate under the pathogenic circumstances of leukemia. Myeloid-derived suppressor cells (MDSCs), which overexpress arginase, reactive oxygen species (ROS), and nitric oxide (NO), aggregate as a result of these cells. These can be taken into account while creating a nanodelivery system in order to account for the sensitive drug release. Targeting hypoxia regions as a potential leukemia therapy strategy should be considered, as the bone marrow microenvironment under pathological conditions associated with leukemia is hypoxic [15].
- **Nanoparticles for Treating Leukemia :** Nowadays, drug therapy (chemical or gene drugs) is the most commonly used treatment option for leukemia, regardless of whether it is used in conjunction with radiation therapy, hematopoietic stem cell transplantation, or both. However, conventional chemotherapy typically has a lower treatment window due to the poor pharmacokinetic properties of chemical drugs; additionally, traditional chemotherapeutic drugs cannot act specifically on lesion cells; and they may negatively

affect normal cells and cause serious side effects. For protein and gene drugs, whose features degrade easily and have difficulty in entering cells, appropriate nanoparticle carriers are crucial for improving drug targeting, minimizing side effects, and shielding pharmaceuticals from nuclease degradation [16].

a. **Nanoparticles for small molecules**: Drug solubility can be changed, and nanoparticles can increase a drug's stability in vivo. Nanoparticles can also be made to carry medications, as opposed to free drugs, to particular cells or tissues, which might effectively reduce the adverse effects of the drugs and avoid various drug resistance processes [17].
b. **Nanoparticle for Antibody drug Therapy**: Antibody medications have a safe and specific binding affinity for the target antigen. Compared to conventional antibody medications, the specificity of the nanoparticles loaded with antibody therapeutics is better. Higher efficacy, selective leukemia cell death, and particular binding to specific antigens are the primary components of the specific performance [18].
c. **Nanoparticles for Gene Therapy** : Currently, leukemia gene therapy is in the preclinical and experimental stages. There are four main types of RNA gene drugs: nucleic acid aptamers, antisense oligonucleotide (ASO), small interfering RNA (siRNA), and microRNA (miRNA). At the RNA level, they are able to treat illnesses. However, due to their negative charge, RNA gene medicines have difficulty penetrating hydrophobic cell membranes. If left unprotected or unaltered, they would quickly break down in serum. Nonviral vectors have lesser toxicity and a lower immune response than viral vectors, making them superior options for RNA drug delivery [19].
d. **Nanoparticles for Combined Therapy** : The etiology of leukemia is complex. Getting the best potential therapy outcomes for a single pathologic mechanism is frequently unattainable. Better synergy can therefore be produced when various medications are employed for diverse mechanisms of treatment, regardless of clinical or basic research. It has been suggested that future research concentrate on the combined therapy. In terms of multidrug delivery, nanoparticles have also advanced significantly [20].
e. **Nanoparticles for Photodynamic Therapy** : Photodynamic therapy (PDT) is a newer and more advanced therapeutic option for leukemia, in addition to conventional chemical and gene therapies. PDT shows significant promise in treating tumors because it has fewer side effects and lower drug resistance than

traditional chemotherapy and radiation treatments. The PDT mechanism uses photosensitizers with varying half-lives in each tissue. The concentration of photosensitizer in the tumor tissue surpasses that of the surrounding normal tissues after a specific duration. The leftover photosensitizer in the tumor tissue would then be excited by the particular wavelength, which would cause the photosensitizer to release reactive oxygen species (ROS) that would kill the tumor cells. The most widely used photosensitizers are merocyanine 540, tetrahydroxybenzene chloride and aluminum phthalocyanine chloride. PDT has the ability to efficiently filter the residual leukemia cells and reduce leukemia relapse, particularly following autologous bone marrow transplantation [21].

Types of Nanoparticles : Two of the most common kinds of nanoparticles are organic and inorganic. The first category includes compact, hybrid, liposome, dendrimer, and micellar polymeric nanoparticles. The second group includes metal nanoparticles, silica, fullerenes, and quantum dots. In addition, nanoparticles can also be categorized based on their size, shape, and chemical composition.

- **Nanoparticles from Organic materials** : Extracellular vehicles (EVs), liposomes, and polymeric nanoparticles (PNPs) are the three types of organic nanoparticles that researchers report using the most. PNPs are among the most well-liked natural solutions to a number of nanoparticle-based problems since they are biocompatible and biodegradable and may be produced from biological or synthetic polymers. They can be obtained using a variety of techniques, including two-step emulsification, filtering, supercritical technologies, and nanoprecipitation. They could change in size and stability while they are being produced [22].

  Liposomes are spherical vesicles made of a bilayer of fatty lipids. Proteins, phospholipids, cholesterol, and surfactants can all be combined to create nanoparticles. Liposomes can be produced in a variety of methods, including sonication, extrusion etc. They can be viewed as delivery systems that can also hold particular biomolecules and other nanomaterials because they can hold both water and water-based medications.

  One of the most widely used extracellular vehicles (EVs) in research are exosomes, which range in size from 70 to 150 nm. All cell types release them, and they contribute in controlling pathophysiology and cell metabolism. Currently, the most potent and biocompatible medicines on the market are exosome-like nanoparticles. They can be produced artificially or spontaneously.

- **Nanoparticles from Inorganic materials** : Inorganic nanoparticles can be classified into three categories: nanoparticles made up with metal oxides, or MNPs; quantum dots; and metallic nanoparticles. Peptides, proteins, polysaccharides, and other macromolecules specifically can be utilized to raise overall NP stability and decrease dangerous heavy metal leakage. The nanoparticles GQDs, InP, CdSe, InAs, and CdTe are frequently employed in biosensing and bioimaging. Precious and magnetic metals such as copper (CuNPs), silver (AgNPs), gold (AuNPs), and palladium (PdNPs) make up MNPs. These MNPs have potential use as theranostics agents, while magnetic ones may be employed as biosensors and contrast agents. Biocompatible MNPs include iron oxide (Fe3O4), zirconia (ZrO2), zinc oxide, mesoporous silica nanoparticles (MSNs), cerium (CeO2), and titania (TiO2). They can be utilized for implants and cell imaging and have excellent chemical stability, antioxidant properties, and catalytic capabilities [22].

PLGA Nanoparticle : One of the most widely utilized biodegradable polymers is poly(lactic-co-glycolic acid) (PLGA), which hydrolyzes to produce the metabolite monomers lactic acid and glycolic acid (Fig 2). The utilization of PLGA for medication administration or biomaterial applications is associated with a reduced systemic toxicity due to the endogenous nature of these two monomers, which the body can quickly metabolize through the Krebs cycle by forming Carbon dioxide and water. The European Medicines Agency (EMA) and the US FDA have approved PLGA for use in a variety of human medication delivery systems. Depending on the molecular weight and copolymer ratio, the degradation time might range from a few months to a few years.Monomer ratios are typically used to identify different types of polylactic acid. For instance, the copolymer known as PLGA 50:50 is made up of 50% glycolic acid and 50% lactic acid. Because poly (lactic acid) (PLA) degrades more slowly than PLGA, it has also been employed less frequently [23].

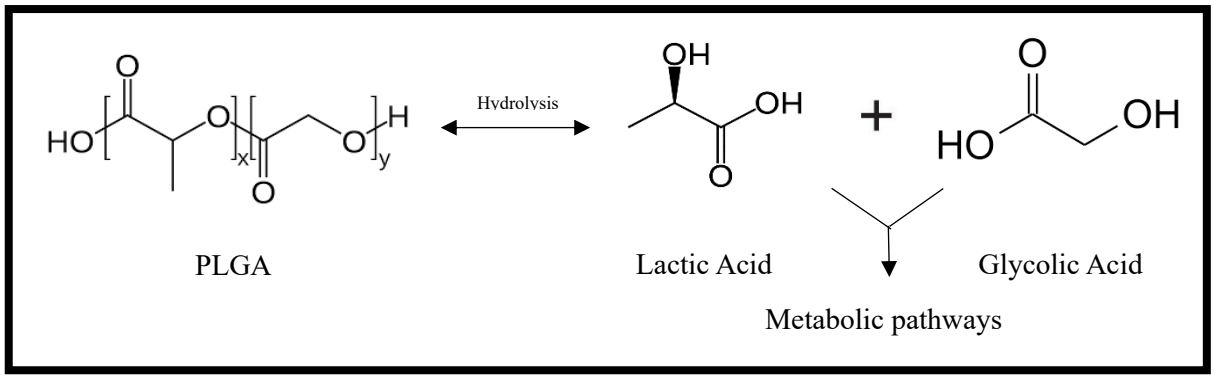


Fig 2 : Hydrolysis of PLGA

Both clathrin-mediated endocytosis and fluid phase pinocytosis play a role in the internalization of PLGA nanoparticles in cells. After 10 minutes of incubation, PLGA-nanoparticles quickly exit endo-lysosomes and reach the cytoplasm. Because of this, interactions between nanoparticles and vesicular membranes are facilitated, which causes temporary, localized membrane instability and allows nanoparticles to escape into the cytosol (Fig 3). Hydrophobic particles are recognized by the body as foreign particles. The reticuloendothelial system (RES) removes them from the bloodstream and stores them in the liver or spleen. This mechanism is a major biological obstacle to nanoparticle-based medication delivery. Opsonin proteins present in blood serum connect to injected nanoparticles, causing them to adhere to macrophages and get internalized through phagocytosis [24].

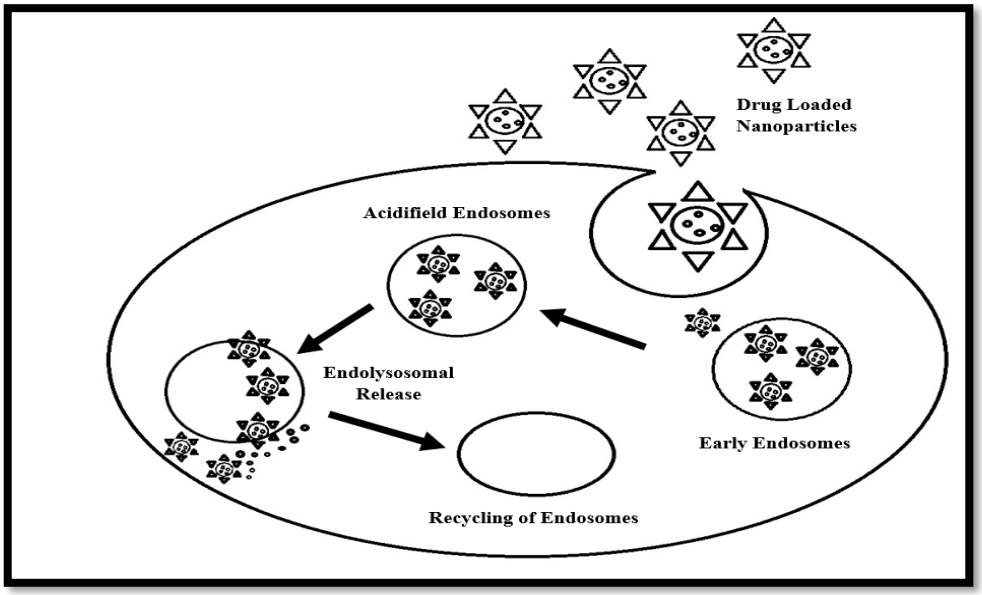


Fig 3 : Schematic representation of Engulfment of PLGA nanoparticles by cells

- **Methods to reduce limitations** : Several techniques for surface modification have been devised to make nanoparticles that are not detected by the RES in order to overcome these limitations. It is possible to coat nanoparticles with chemicals that provide a hydrophilic coating at their surface, thus masking their hydrophobicity. The most often used moiety for surface modification is polyethylene glycol (PEG), a non-ionic, hydrophilic polymer. The "PEGylation" extends their blood circulation half-life by several orders of magnitude. Furthermore, PEG has outstanding biocompatibility. For surface modification, poloxamer, poloxamines or chitosan have also been investigated. These groups have the ability to obstruct the hydrophobic and electrostatic interactions that facilitate opsonin binding to particle surfaces. Using

surface modification to target tumors or organs to promote more selective cellular binding and internalization via receptor-mediated endocytosis is another use. Targeting ligands are frequently grafted onto the surface of nanoparticles through a linkage on PEG chains. For ligands to retain their affinity for binding to receptors, they must be conjugated on nanoparticles in an appropriate manner. Extension of ligands away from the surfaces of the nanoparticles is necessary to prevent shielding by the PEG chains, since a sufficient PEG coating is necessary to prevent identification by the RES. The way that nanoparticles interact with cells and are absorbed is also significantly influenced by their surface charges. Because of the ionic connections that are formed between positively charged particles and negatively charged cell membranes, positively charged nanoparticles appear to allow a greater level of internalization. Furthermore, while negatively and neutrally charged nanoparticles prefer to colocalize with lysosomes whereas positively charged nanoparticles appear to be able to escape from lysosomes following internalization and demonstrate perinuclear localization. The negative charges of PLGA nanoparticles can be changed to neutral or positive charges by altering their surface, such as by coating them with chitosan or PEGylating the PLGA polymer [25].

- **Methods of formulation development** : The production of PLGA nanoparticles can be accomplished through two distinct groups of methods: top-down and bottom-up. A monomer is used as the starting point for bottom-up processes such as precipitation polymerization, interfacial polymerization, and emulsion or microemulsion polymerization. The pre-formed polymer is used to create the nanoparticles in top-down methods such as emulsion evaporation, emulsion diffusion, solvent displacement, and salting out.

  Nanoparticles can be made using various techniques. The structural organization may vary depending on the preparation method used. The medication is either adsorbed on the surface of a matrix called a "nanosphere" or entrapped inside the core of a "nanocapsule."

  The multiple emulsification-solvent evaporation method is the most widely utilized process for creating PLGA nanoparticles. Hydrophobic pharmaceuticals can be encapsulated using this process, which involves dissolving the chemical and polymer in an organic solvent such as dichloromethane. In order to prepare the emulsion oil (O) in water (W), or O/W, water and a surfactant (such as polysorbate-80 or poloxamer-188) are added to the polymer solution. The process of homogenization or sonication

produces nanoscale droplets. Following centrifugation, the solvent is then removed or evaporated, and the nanoparticles are gathered.

This method was modified to create the double emulsion W/O/W, which was then utilized to encapsulate hydrophilic medications such as proteins, nucleic acids, and peptides. The nanoprecipitation approach, commonly known as the interfacial deposition method, is another way to create nanoparticles. In short, the medication and polymer are dissolved in an organic solvent (like acetone) and then gradually added to water. After centrifugation, the organic solvent evaporates and the pellets are gathered. There are other methods, like the spray-drying approach. Drug loading into nanoparticles can be accomplished in two ways: (i) by incorporating the drug into the particles while they are being made, and (ii) by having the drug adsorb on the particles after they have been made [26].

- **Physicochemical properties** : By measuring the surface charge, the efficacy of surface modification can be calculated. One technique involves measuring the nanoparticles' zeta potential (ζ) by tracking the movement of charged particles under the influence of an electrical potential. The zeta potential values might be positive, neutral, or negative depending on the polymer and surface modification. Using a technique known as "dynamic light scattering," photon correlation spectroscopy may determine both the average particle size and the polydispersity index. This method is based on the way that the particles' Brownian motion scatters light. The size and shape of the nanoparticles can be determined using imaging methods like atomic force microscopy, transmission electron microscopy, and scanning electron microscopy. The nanoparticles are usually between 100 and 250 nm in size [27].
- **Pitfalls** : High drug loading—that is, the percentage of loaded drug relative to the total quantity of nanoparticles—and high encapsulation efficiency—that is, the percentage of loaded drug relative to the total amount of drug employed for the formulation—are requirements for nanoparticles. Because nanoparticles are colloidal systems, it is difficult to determine the exact drug content. Consequently, ultracentrifugation is the most appropriate method for separating nanoparticles from non-encapsulated or non-adsorbed medication.

  Poor loading is one of the main drawbacks of PLGA-based nanoparticles. Indeed, although PLGA-based nanoparticles frequently exhibit excellent encapsulation efficiencies, drug loading is typically poor (about 1%), meaning that one milligram of the active component is present for every 100 milligram polymer of nanoparticles. This

modest drug loading poses a significant challenge to the design of PLGA-based nanoparticles for several medications.

A secondary significant drawback is the rapid release of medication from nanoparticles. This effect is explained for the majority of nanoparticles based on PLA. As a result, the medication may lose its effectiveness if it is unable to reach the intended tissue or cells. Understanding the drug release mechanisms is crucial due to the usage of nanoparticles in sustained release drug delivery. Five mechanisms exist for drug release, (i) drug desorption bound to the surface; (ii) diffusion through the polymer matrix; (iii) diffusion through the polymer wall of nanocapsules; (iv) matrix erosion of nanoparticles; and (v) a combined erosion–diffusion process. The polymer that is employed and the loading efficiency determine the drug release processes.

"Nanotoxicology," a brand-new subfield of nanotechnology, has surfaced. It's true that there are a lot of moving parts involved in how nanocarriers interact with biological systems. As might be expected, these components' behaviour within the body is altered by the size and surface characteristics of nanocarriers. More information is required to comprehend their structure-property correlations. While some nanomedicines were not evaluated, others obtained regulatory approvals attesting to their biocompatibility. Regulations and toxicology research are required to completely define the biocompatibility of nanocarriers in humans. In vitro experiments typically yield positive outcomes. Regretfully, these outcomes frequently deviate greatly from actuality in vivo. Similarly, animal models that are frequently employed in preclinical research are in no way representative of the clinical setting. The pharmaceutical industry and patients both must consider the financial implications of bringing a novel medicine delivery method to market. It can be costly to produce GMP PLGA with precise characteristics. Scaling-up is another barrier to the commercialization of nanoparticles. Numerous processes used in experimental manufacturing, including sonication, ultracentrifugation, dialysis, and others, cannot be replicated in an industrial setting (Fig 4) [28].

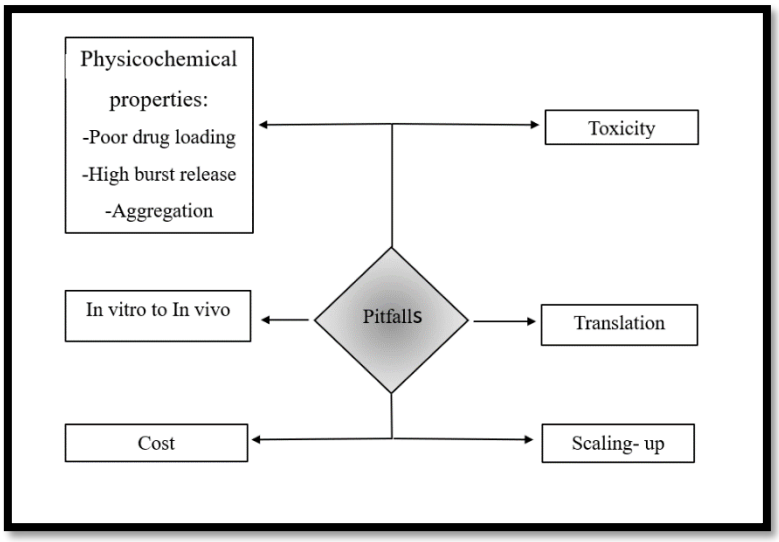


Fig 4 : Pitfalls of PLGA Nanoparticles

- **Encapsulation of small hydrophobic drugs** : The most common method for creating PLGA nanoparticles loaded with hydrophobic poorly soluble drugs is nanoprecipitation. Drug release and effective response of PLGA nanoparticles are influenced by (i) the surface modification, (ii) the preparation method, (iii) the particle size, (iv) the molecular weight of the encapsulated drug, and (v) the ratio of lactide to glycolide moieties.
- **Encapsulation of proteins** : Protein administration separately is extremely difficult because most proteins cannot diffuse across some biological barriers, their short half-life in vivo, their susceptibility to gastrointestinal degradation by digestive enzymes, and the epithelial barriers of the gastrointestinal tract limit their oral bioavailability. Recombinant proteins are mostly administered via invasive methods these days, like subcutaneous injections, and because of their quick breakdown and excretion, multiple injections are usually necessary to obtain therapeutic levels. The potential solution to address all of these issues and provide certain additional advantages has emerged from the encapsulation of these therapeutic proteins in PLGA nanoparticles. When proteins are incorporated into a polymer matrix, they are protected from enzymatic and hydrolytic breakdown in vivo, their integrity and activity are maintained, their bioavailability may be enhanced, and in certain situations, the therapeutic protein may be targeted to the desired location.

  Since proteins are typically hydrophilic macromolecules, one of the most often used techniques for encasing proteins into PLGA nanoparticles is the double emulsion W/O/W solvent evaporation process. There are certain difficulties with encasing

proteins in PLGA nanoparticles, such as instability issues. For instance, the protein dissolved in the aqueous phase in the first step of the W/O/W formulation procedure may aggregate or become denatured at the interface between water and organic solvent, adsorb to the hydrophobic polymer, or unfold due to the shear stress used to form the primary emulsion. Protein species that are denatured or aggregated not only lack therapeutic potential but also run the risk of causing unanticipated adverse consequences including toxicity or immunogenicity. In an effort to address these issues, a great deal of research has been done on formulation process optimization as a means of enhancing protein stability during traditional W/O/W procedures.

The environment's acidification brought on by the breakdown products (lactic acid and glycolic acid) and the presence of PLGA carboxylic acid end groups should also be taken into account. These elements may interact with the encapsulated protein's positive charges to modify or even prevent its release. The therapeutic protein may aggregate or lose some of its action as a result of being exposed to an acidic pH . Many stabilizing compounds have also been tried to improve protein stability, including sodium bicarbonate, trehalose, and pluronic F68 .

- **Encapsulation of nucleic acids** : Nucleic acids have the ability to either suppress gene expression (RNAi mediators) or boost gene expression by introducing a gene that is either under expressed or absent into cells (cDNA). The huge size and negative charge of nucleic acids cause them to be fragile and have low inherent transfection effectiveness. Polymer-based nanoparticle delivery has been explored as an alternative to viral delivery. Nucleic acids can be adsorbed by electrostatic interactions on the surface of nanoparticles by adding cationic polymers to the matrix or entangling into polymeric matrix. Either modified nanoprecipitation or W/O/W solvent evaporation is used to generate PLGA-based nanoparticles loaded with nucleic acids. The techniques utilized to create DNA-loaded nanoparticles or siRNA-loaded nanoparticles are largely the same throughout all investigations on nucleic acid delivery based on PLGA nanoparticles. The W/O/W technique uses extreme circumstances that may cause the breakdown of nucleic acids. Acetylated bovine serum albumin was added as an emulsifier to an aqueous phase containing nucleic acid, and this improved the encapsulation efficiency of hydrophilic molecules and stabilized the initial emulsion (W/O). A gentle formulation method called modified-nanoprecipitation involves dissolving polymers and nucleic acid in the same organic solvent (such DMSO), then adding the combination dropwise to an aqueous surfactant solution. Pre-complexing a

nucleic acid with a cationic moiety like DOTAP improved nucleic acid solubility in organic solvent. To expand the capabilities of nanoparticles, other polymers can be included in the PLGA matrix either as a component of the matrix or as a surface coating. To increase encapsulation efficiency, cationic polymers such chitosan or polyethyleneimine (PEI) could be used added. When PEI or chitosan moieties were present, encapsulation efficiencies increased from 43% to 80% and from 28% to 44%, respectively. Furthermore, cationic moieties improved endosomal escape and/or encouraged contact between positively charged nanoparticles and negatively charged cell membranes. For an encapsulation effectiveness greater than 70%, the volume and PLGA concentration needed to be adjusted. It should be noted that although the nucleic acid loading is quite low, ranging from 0.1 to 1 mg per 100 mg of nanoparticles, the encapsulation effectiveness can reach up to about 80% [30].

- **Cancer treatment with PLGA based nanoparticles** :-
  - Passive targeting by EPR effect and active targeting of specific receptors - Chemotherapeutic medicines cause harm to healthy tissues, which results in systemic toxicity and unfavourable effects. These effects significantly restrict the maximum dosage that anti-cancer medications can be taken, hence limiting their therapeutic efficacy. Recently, nanomedicine has attracted a lot of interest as potential solution to these issues. Currently, studies are being conducted on PLGA-based nanoparticles for applications such as cancer imaging and cancer treatment. Generally speaking, active or passive targeting is how nanoparticles can enter tumors. The technique of passive targeting leverages the size of nanoparticles and the distinct anatomical and pathological irregularities present in the tumor vasculature. Within the interstitial area, nanoparticles have the ability to extravagate and aggregate. This adds to an “enhanced permeability”. Tumor tissue is not efficiently drained due to the absence or inefficiency of lymphatic channels. As a result, there is "enhanced retention." The term "Enhanced Permeability and Retention" (EPR) effect refers to the combination of these two phenomena. This effect is regarded as the gold standard in the design of novel anti-cancer drug delivery systems. Targeting ligands are grafted at the surface of the nanoparticles in active targeting. The ligand is selected to bind particular receptors that are not expressed by normal cells but are overexpressed by tumor cells or the tumor vasculature. The consistency of the receptor's expression on all targeted cells plays a significant role in the selection

of targeting ligands. There are two distinct cellular targets: tumoral endothelial cells and cancer cells. Numerous overexpressed receptors, such as the transferrin receptor, the folate receptor, glycoproteins, the epidermal growth factor receptor (EGFR), or integrins, have been studied in order to target cancer cells. Targeting ligands must identify specific receptors, such as matrix metalloproteinases (MMPs), integrins (αvβ3, α5β1), vascular cell adhesion molecule-1 (VCAM-1), and vascular endothelial growth factor receptors (VEGFR-1 and VEGFR-2), in order to target tumoral endothelium. The tumor may die if its endothelium is destroyed, due to a shortage of nutrients and oxygen. Targeted nanocarriers thus sought to directly destroy angiogenic blood vessels and destroy tumor cells naturally. The anti-tumoral efficaciousness of actively targeted nanocarriers is due to improved cellular internalization rather than an increased tumor accumulation. Targeting endocytosis-prone surface receptors plays a crucial role in designing the delivery system. Thus, in addition to the nanoparticles' precise targeting, one of the main benefits of targeted nanoparticles is their receptor-mediated internalization [31].

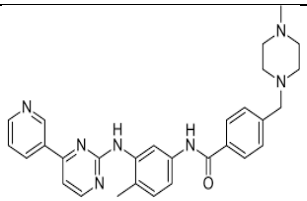
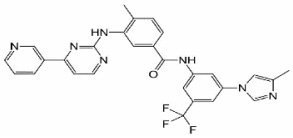
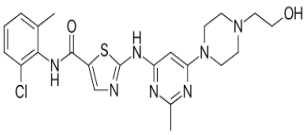
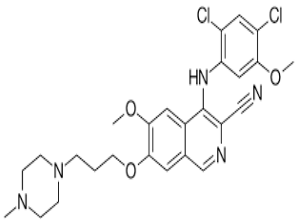
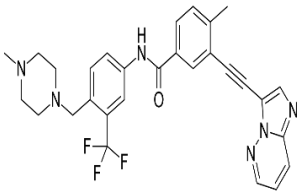
Tyrosine Kinase Inhibitors for the treatment of Leukemia

The clonal hematopoietic stem cell disease known as chronic myeloid leukemia (CML) is defined by a reciprocal translocation involving the fusion of the breakpoint cluster region (BCR) on chromosome 22q11.2 with the Abelson oncogene (ABL) on chromosome 9q34. The BCR-ABL1 fusion oncoprotein results in shortening chromosome 22 and is being called the Philadelphia (Ph) chromosome. The constitutive tyrosine kinase activity of this protein promotes myeloproliferative and hematological transformation. More than 90% of CML patients have the 210-kd protein, which is the main isoform of BCR-ABL.

Before the development of tyrosine kinase inhibitors (TKIs), patients with CML had only three treatment options: interferon-α (IFN-α), cytoreductive drugs, and allogeneic stem cell transplantation. The survival rate of patients with CML has significantly increased with the use of imatinib, the first discovered Tyrosine kinase inhibitor (TKI); however, over time, 20% to 30% of patients develop resistance to TKIs, which is typically caused by mutations in the BCR-ABL1 kinase domain. Some patients experience treatment failure despite BCR-ABL1 inhibition, indicating the activation of alternative resistance mechanisms. Additionally, 5% to 10% stop therapy

due to poor tolerability. This has resulted in the development of newer TKIs, such as Nilotinib, Dasatinib, Bosutinib and Ponatinib (Table 1), each of which has different potency and adverse effect profiles [32].

Table 1 : Different types of TKIs and their kinetic profiles

Tyrosine Kinase Inhibitor	Molecular structure	Kinase targets	Half life, hour	Absolute oral bioavailability	Metabolism	Elimination
Imatinib (1st line drug)		ABL, PDGFR, c-KIT	18	98%	Major: CYP3A4, minor: CYP1A2, CYP2D6, CYP2C9, and CYP2C19	~81% in feces, mostly as metabolites
Nilotinib (1st or 2nd line drug)		ABL, PDGFR, c-KIT, ARG, EPHB4	17	50-82%	Major: CYP3A4	~93% in feces, mostly as parent drug
Dasatinib (1st or 2nd line drug)		ABL, PDGFR, c-KIT, FGR, FYN, HCK, LCK, LYN, SRC, YES, EPHB4	3-5	14-34% (animal studies)	Major: CYP3A4	~85% in feces, mostly as metabolites
Bosutinib (Resistance or intolerance to prior therapy)		ABL, FGR, LYN, SRC	22.5	23-64% (animal studies)	Major: CYP3A4	~91% in feces
Ponatinib		Native/mutant BCR-ABL, T315I, VEGFR, PDGFR, FGFR, EPH receptors, SRC family kinases	24	unknown	Major: CYP3A4 Minor: CYP2C8, CYP2D6, CYP3A5 esterases and/or amidases	~87% in feces

- **Mechanism of action of TKIs** : TKI can block BCR-ABL downstream signal transduction pathways. BCR-ABL activates a wide range of signaling pathways; these pathways can be targeted for intervention when BCR-ABL itself is not fully inhibited. It's also possible that other oncogenic stimuli than BCR-ABL activate these pathways. Reciprocal translocation of chromosome 9 results in the aberrant formation of chromosome 22, or the Philadelphia chromosome, and plays a crucial role in the pathophysiology of CML. An oncogene that encodes the BCR-ABL1 fusion oncoprotein is formed when the Abelson gene (ABL) on chromosome 9 fuses with the BCR gene on chromosome 22. Tyrosine kinase activity is constitutively up-regulated in this protein, and it phosphorylates substrates to activate downstream molecular pathways including mTOR, JAK/STAT, PI3K/AKT, RAS/MEK, and Src kinases (Fig 5). These in turn dysregulate hematopoietic cells' adhesion, proliferation, transformation, and apoptotic activity. The BCR-ABL protein has a complex, coiled molecular structure that contains two spatial domains: the kinase domain, which contains the ATP, substrate, and allosteric myristate-binding pockets, and the Src-homology 2 (SH2) and SH3 domains, which bind adaptor proteins. Drug research against CML may use these various binding sites as well as downstream pathways as therapeutic targets [33].

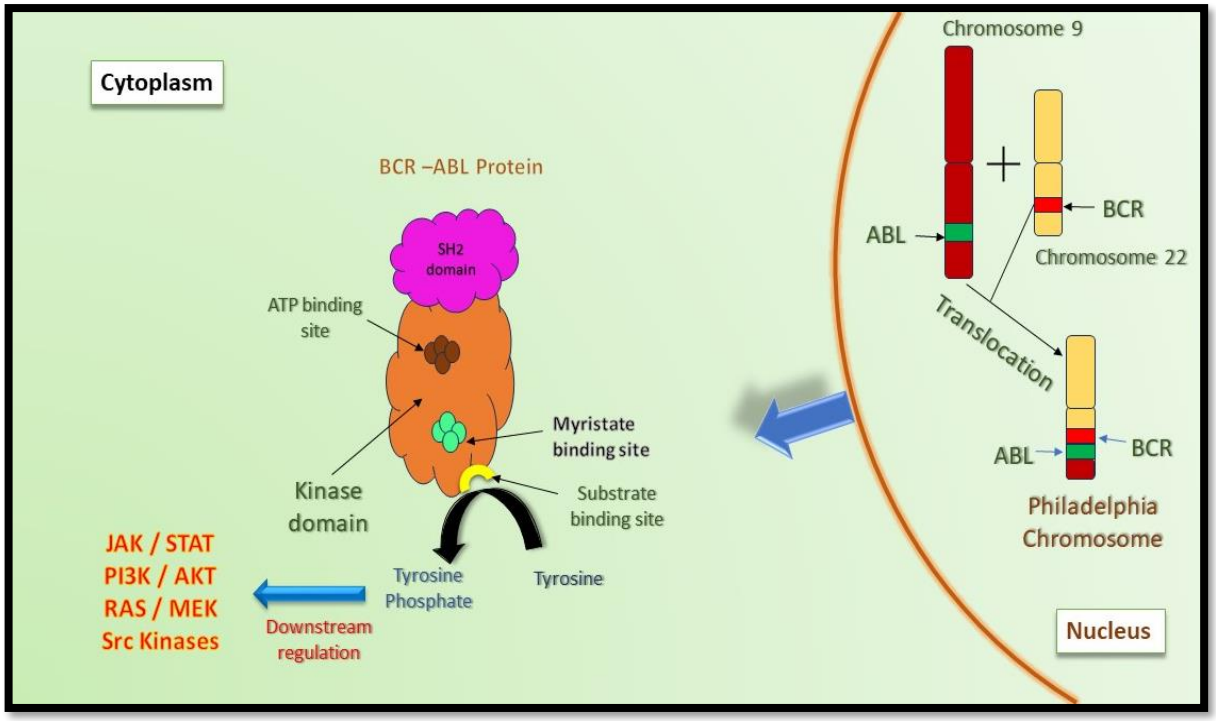


Fig 5 : Mechanism of Action of TKIs

Nilotinib

A more potent analog of imatinib, Nilotinib with a molecular weight of 529.5 g/mol, was approved by the US FDA in 2007 for the treatment of patients with CML who were either intolerant or resistant to imatinib. Nilotinib ($C_{28}H_{22}F_3N_7O$) is a member of (trifluoromethyl)benzenes, a member of pyrimidines, a member of pyridines, a member of imidazoles, a secondary amino compound and a secondary carboxamide. Nilotinib is a transduction inhibitor that targets BCR-ABL, c-kit and PDGF, for the potential treatment of various leukemias, preferably chronic myeloid leukemia (CML).

Chronic myelogenous leukemia (CML) is caused by the BCR-ABL oncogene. Nilotinib inhibits the tyrosine kinase activity of the BCR-ABL protein. Nilotinib fits into the ATP-binding site of the BCR-ABL protein with higher affinity than imatinib, over-riding resistance caused by mutations.

In vitro profiling has shown that Nilotinib is effective against the majority of imatinib-resistant Abl kinase domain mutations; however, clinically, five kinase domain mutations remain of major concern: T315I (gatekeeper mutation), F359V, E255K/V, and Y253H. These mutations are most commonly discovered during first line or second-line nilotinib therapy, and their presence is a contraindication to nilotinib use.

A phase III, randomized, open-label, multicentre study called Evaluating Nilotinib Efficacy and Safety in Clinical Trials—Newly Diagnosed Patients randomly assigned 846 patients with CML in a 1:1:1 ratio to receive either 400 mg once daily (imatinib) or 300 mg twice daily (BID) of Nilotinib. At a year, Nilotinib's MMR (Maternal Mortality Ratio) rates (43 % for the 300 mg dose and 43 % for the 400 mg dose) were almost twice as high as imatinib's (22 %) ($P < 0.001$).By 12 months, Nilotinib had far greater rates of Complete Cytogenic Remission (80% for the 300-mg dose and 78% for the 400-mg dose) than imatinib (65%). Patients receiving imatinib experienced a higher frequency of gastrointestinal and fluid-retention problems, while patients receiving Nilotinib experienced a higher frequency of dermatologic events and headaches. Nilotinib as a first line therapeutic agent is approved for the treatment of patients with newly diagnosed CML. Nilotinib use is causing vascular events such as peripheral arterial occlusive disease (PAOD), coronary artery disease (CAD), cerebrovascular disease (CVA), hyperglycemia, and hypercholesterolemia. These are growing concerns associated with the drug [34].

Novel strategies for targeted delivery of TKIs

Many methods, including dendrimers, polymeric nanoparticles, magnetic nanoparticles, and lipid-based delivery systems like liposomes, solid lipid nanoparticles (SLN), and nanostructured lipid carriers (NLC), can be used to target the distribution of tyrosine kinase inhibitors and also to exhibit release of the drug from the formulation in a controlled manner.

Some drugs of the TKIs class have limited permeability, while others have high permeability. These drugs are poorly soluble. TKIs therefore fall under the Biopharmaceutical Classification System (BCS) classes II or IV. These medications' physicochemical characteristics, such as solubility, dissolution rate, and permeability, make their formulations challenging. As a result, in recent years, a number of strategies have been researched to create innovative and effective TKI delivery methods. By using innovative techniques, notably nanostructured systems, the challenges faced by conventional formulations—such as dose-dependent adverse effects, variable bioavailability, and non-specific tissue distribution—have been overcome.

Co-delivery of TKIs with chemotherapeutic medicines or biopharmaceuticals such as oligonucleotides and monoclonal antibodies can be performed as a way to reduce drug resistance resulting from the efflux mechanism. This makes the combination of two TKIs advantageous when incorporated into novel targeted delivery methods like as NLCs, PEGylated liposomes, and nanoparticles. Even though a large number of TKI delivery systems are being researched, further in vivo tests are necessary to verify the therapeutic advantages of these formulations over free medications [35].

CHAPTER –II
OBJECTIVES

Chapter - II

Objectives of the study

1. Overcome the Challenge of Nilotinib Solubility:

Improve the solubility of Nilotinib to enhance its oral absorption and therapeutic efficacy.

2. Explore Lipid-Based Pharmaceutical Delivery Systems:

Investigate lipid-based formulations as a potential solution to increase the bioavailability of Nilotinib.

3. Targeted Delivery of Kinase Inhibitors:

Utilize PLGA polymeric nanoparticles based delivery systems to achieve targeted delivery, reduce dose-dependent side effects, and improve therapeutic efficacy.

4. Overcome Formulation Challenges:

Address the formulation challenges posed by the physicochemical properties of Nilotinib, such as solubility and dissolution rate.

5. Develop Sustained-Release PLGA Nanoparticles:

Prepare Nilotinib-loaded PLGA nanoparticles with improved drug loading for sustained-release delivery directly into blood cells.

6. Fill the Gap in Existing Research:

Contribute to the existing literature by being the first to report on the development of sustained-release PLGA nanoparticles containing Nilotinib, with an emphasis on improving drug loading and reducing polymer load.

Aim of the study

In this study my aim is to prepare Nilotinib loaded PLGA nanoparticles (NNPs) with improved drug loading as an alternative controlled-delivery system of Nilotinib directly into blood and exhibit sustained release of that particular drug.

Plan of Work

1. Literature Review:

Conduct a comprehensive literature review to gather relevant information on Nilotinib, PLGA nanoparticles, and drug delivery systems.

2. Drug Calibration:

Calibrate the drug to establish a standard reference for the amount of Nilotinib to be used in the experiments.

3. Formulation Development:

Develop the formulation of Nilotinib-loaded nanoparticles (NNPs) by optimizing the conditions for encapsulation.

- Preparation of Blank NPs: Prepare blank nanoparticles (without drug) to serve as controls in the study.
- Preparation of NNPs: Prepare the Nilotinib-loaded PLGA nanoparticles according to the optimized formulation.

4. Physicochemical Characterization:

Characterize the physicochemical properties of the Nilotinib-loaded and blank nanoparticles using various techniques:

- FTIR (Fourier-Transform Infrared Spectroscopy): Analyze the chemical structure and interactions.
- SEM (Scanning Electron Microscopy): Observe the surface morphology of the nanoparticles.
- TEM (Transmission Electron Microscopy): Investigate the internal structure and size of the nanoparticles.
- Drug Loading: Determine the amount of drug encapsulated in the nanoparticles.
- DLS (Dynamic Light Scattering): Measure particle size and zeta potential.
- In vitro Drug Release: Study the release profile of Nilotinib from the nanoparticles over time using PBS pH 7.4 buffer.

This plan outlines the systematic approach to developing and characterizing Nilotinib-loaded PLGA nanoparticles for sustained drug delivery.

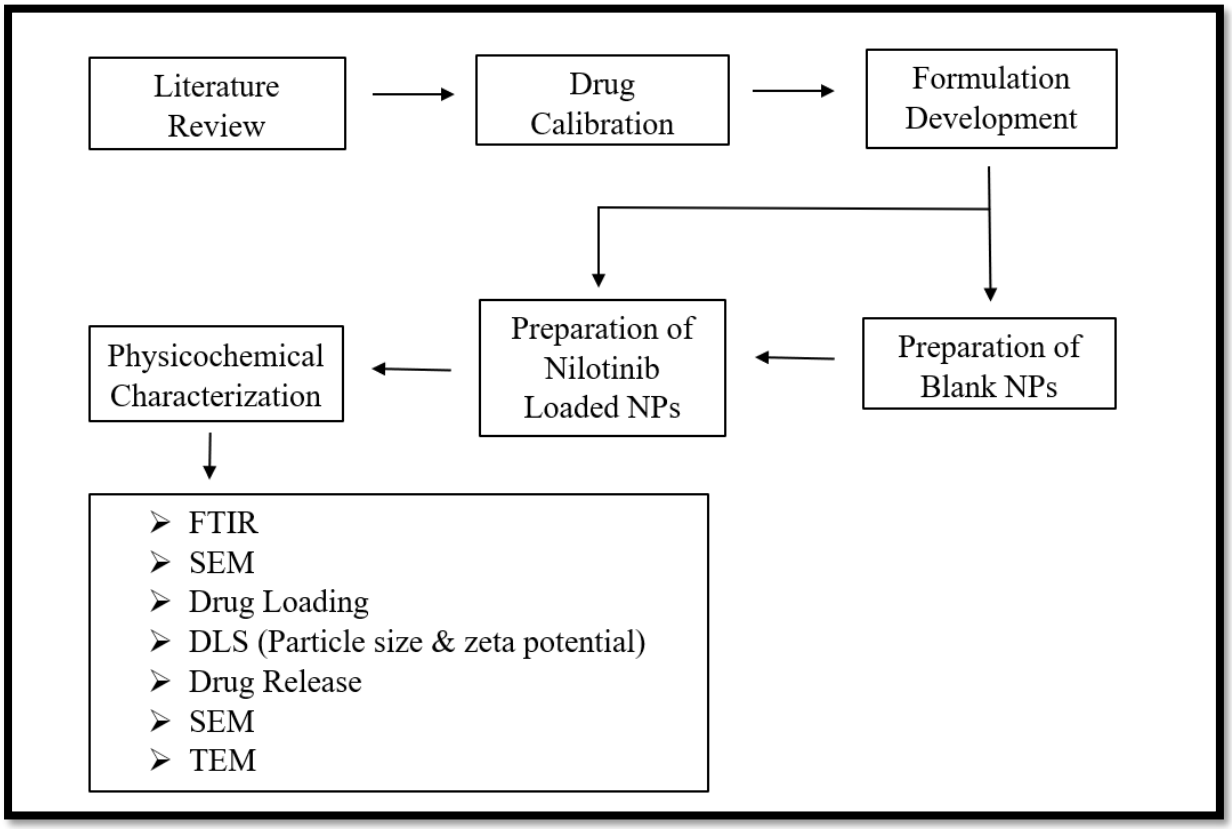


Fig 6: Schematic Representation of Workplan

CHAPTER – III
LITERATURE REVIEW

Chapter - III

Literature Review

- Lanier OL et al. (2022) – Hematological cancers, including leukemia, lymphoma, and multiple myeloma, have long been treated with conventional therapies such as chemotherapy and radiotherapy. While these traditional methods have been the cornerstone of cancer treatment, they often come with significant limitations, including severe side effects and incomplete remission in many patients. Immunotherapy harnesses the body's immune system to target and destroy cancer cells more selectively and effectively than conventional treatments. Among the various types of immunotherapies, checkpoint inhibitors have gained prominence for their ability to block the proteins that prevent immune cells from attacking cancer, thereby restoring the immune system's ability to fight against the disease. Vaccines, another promising approach, work by stimulating the immune system to recognize and attack specific cancer antigens, providing both treatment and preventive benefits. Monoclonal antibodies, designed to recognize and bind to specific proteins on cancer cells, have been used to deliver targeted treatments that spare healthy cells, reducing the side effects commonly associated with traditional chemotherapy. This review critically examines the characteristics and symptoms of hematological cancers and assesses the current treatment options. It delves into the mechanisms and applications of five common immunotherapies—checkpoint inhibitors, vaccines, cell-based therapies, monoclonal antibodies, and oncolytic viruses—highlighting their potential and limitations in the treatment of blood cancers. This literature review contextualizes the discussion of immunotherapies within the broader treatment landscape of hematological cancers, emphasizing the transformative impact of these new approaches [36].
- Wang J et al. (2022) – Cancer remains a disease with limited treatment options, despite advancements in medical research and technology. The challenges of metastasis and recurrence drive the need for innovative treatments. Traditional methods have significant limitations, leading to the development of cancer nanomedicine, which uses nanotechnology for more precise and less harmful treatments. Nanoparticles, which can be modified for targeted drug delivery, offer potential for more effective blood cancer therapies while minimizing side effects. However, there are still experimental

challenges to address before nanoparticle treatments can be widely used in clinical settings. This review highlights recent progress in using nanoparticles to deliver therapeutic drugs to treat blood cancers like leukemia, myeloma and lymphoma, and it discusses future directions for research in therapeutic nanomedicine [37].

- Danhier F et al. (2012) – Poly(lactic-co-glycolic acid) (PLGA) is a widely used biodegradable polymer in drug delivery systems, recognized for its favorable properties. Its biodegradability and biocompatibility make it an attractive choice for formulating polymeric nanoparticles. PLGA's approval by the FDA and European Medicines Agency underscores its safety and effectiveness, particularly for parenteral administration. The polymer is versatile, accommodating both hydrophilic and hydrophobic drugs, and offers protection against drug degradation while enabling sustained release. Furthermore, PLGA nanoparticles can be surface-modified for improved biological interactions and targeted delivery to specific organs or cells. These characteristics make PLGA an ideal candidate for drug delivery in various biomedical applications, including vaccination, cancer therapy, and inflammation treatment. This review highlights the reasons behind PLGA's selection for nanoparticle design and explores how its specific properties are leveraged for targeted drug delivery in different therapeutic contexts [38].
- Astete CE et al. (2006) – Poly(lactide-co-glycolide) (PLGA) nanoparticles can be engineered with various physical characteristics, such as size, size distribution, morphology, and zeta potential, by adjusting specific parameters during synthesis. This versatility has made PLGA a prominent material in nanoparticle research. The aim of this review is to provide a detailed, quantitative, and comprehensive overview of the top–down synthesis techniques used for PLGA nanoparticle production. It also explores the commonly employed methods for characterizing these nanoparticles. Numerous examples are discussed with a thorough understanding of the critical parameters involved in synthesis and how they can be manipulated to achieve desired nanoparticle properties. This review serves as a valuable resource for researchers seeking to optimize PLGA nanoparticle formulations for various applications by controlling their physicochemical characteristics [39].
- Shen J et al. (2020) – Leukemia, a malignancy originating from a hematopoietic stem cell clone, significantly disrupts the normal function of the hematopoietic system. Traditional drug therapies for leukemia often fall short due to their limited specificity

and stability, leading to suboptimal therapeutic outcomes. However, the advancement of nanotechnology has introduced nonviral nanoparticles as a promising solution for more effective leukemia treatment. These nanoparticles offer versatile modification capabilities, allowing for targeted drug delivery to specific lesion sites and controlled drug release. This targeted approach not only enhances the efficacy of the drugs but also reduces adverse side effects. This review highlights and summarizes the latest research on the use of nanoparticles for delivering various therapeutic drugs in leukemia, illustrating their potential as a transformative approach in the treatment of this challenging disease [40].

- Giles FJ et al. (2009) – This literature review discusses the impact of tyrosine kinase inhibitors (TKIs) on chronic myeloid leukemia (CML) treatment, emphasizing the revolutionary role these agents have played in improving patient outcomes. Imatinib, nilotinib, and dasatinib are highlighted as key TKIs that offer targeted therapeutic options, with each agent having a unique kinase inhibition profile that influences both efficacy and safety. While imatinib and nilotinib share a similar range of kinase targets, dasatinib exhibits a broader spectrum of activity, including potent inhibition of BCR-ABL and other off-target kinases. These differences in kinase targeting contribute to the distinct clinical safety and efficacy profiles of each drug, which physicians must consider when selecting the best treatment for individual patients. The review provides a detailed comparison of the kinases inhibited by these TKIs and examines their implications for treatment outcomes in CML [41].
- Pophali PA et al. (2016) – The literature review outlines the evolution of treatment for chronic myeloid leukemia (CML), starting with imatinib mesylate, the first approved tyrosine kinase inhibitor (TKI). While imatinib shows effective responses in about 60% of patients, some face issues with drug intolerance or resistance. The development of newer TKIs, including nilotinib, dasatinib, bosutinib, and ponatinib, has expanded treatment options, offering greater potency and different adverse effect profiles. These newer TKIs also show promise in achieving critical milestones, such as early and optimal molecular responses, and exhibit a lower incidence of BCR-ABL kinase domain mutations. However, randomized phase III trials have not demonstrated a significant survival benefit of these newer TKIs over imatinib when used as frontline therapy. Concerns about cost and specific side effects, like vascular disease with nilotinib and ponatinib and pulmonary hypertension with dasatinib, have tempered their

use as initial treatments. The review suggests that while new TKIs are beneficial in cases of imatinib failure or intolerance, their frontline use should consider patient-specific factors such as age, comorbidities, risk stratification, and cost. Future advancements may focus on combination therapies and agents targeting quiescent CML stem cells for potential disease eradication [42].

- Radmoghaddam ZA et al. (2022) – The literature review addresses the critical challenges in cancer therapy, including insufficient drug loading, poor solubility, premature drug leakage, and the unintended targeting of healthy cells. Traditional chemotherapy and other conventional treatments are associated with severe side effects, prompting the search for more effective alternatives. Nanomedicine has emerged as a promising solution, with various nanoparticles (NPs) being engineered to overcome these issues. Among these, lipid-based nanoparticles, particularly solid lipid nanoparticles (SLNs), have shown significant potential in cancer treatment. Tyrosine kinase inhibitors (TKIs), which are effective in inhibiting cancer cell growth, are commonly used in various cancers. The review focuses on the use of lipid-based NPs as nano-drug delivery systems for TKIs, highlighting the efficiency of SLNs in delivering TKIs and discussing their advantages and disadvantages in different drug delivery contexts [43].
- Sinha B et al. (2013) – The literature review examines recent advancements in the development of Poly-lactide-co-glycolide nanoparticles (VNPs) for improved pulmonary drug delivery. These nanoparticles, with sizes ranging from 207 to 605 nm, were engineered using a multiple-emulsification technique and were made porous with the addition of an effervescent mixture to enhance their suitability for pulmonary administration.

  The study assessed the pulmonary deposition of VNPs using a specialized inhalation chamber, revealing that the particles exhibited a maximum drug loading capacity of 30% (w/w) and a zeta potential of approximately -20 mV. The release profile of the drug from VNPs demonstrated an initial burst release of 20% within the first 2 hours, followed by a sustained release over 15 days.

  A key finding was that porous VNPs, characterized by a lower mass median aerodynamic diameter (MMAD) compared to nonporous particles, achieved superior initial drug deposition (~120 μg/g of tissue). Furthermore, the drug remained detectable in the lungs for up to 7 days with porous particles, compared to 5 days with nonporous

particles. These results indicate that porous nanoparticles with reduced MMADs are more effective in pulmonary deposition and maintaining drug presence in the lungs. Overall, the study highlights the enhanced pulmonary delivery and sustained drug retention capabilities of porous VNPs, demonstrating their potential for improved therapeutic efficacy in lung-targeted treatments [44].

CHAPTER – IV
MATERIALS & INSTRUMENTS

Chapter – IV

Materials and Instruments

- Chemicals used in the study

Table 2 : List of different chemicals or materials used in the study

Serial Number	Chemical Name	Source
1	Nilotinib	Hetero Drugs, Hyderabad, Telangana
2	Acid-terminated Poly Lactic-co-glycolic acid (ratio, 75:25; Molecular weight, 4000-15000 Da)	Sigma-Aldrich Co, St Louis, MO, USA
3	Polyvinyl alcohol (Molecular weight, 85000- 124000 Da) (hot water soluble)	SD Fine – Chemicals limited, Mumbai, India
4	Dichloromethane (DCM)	Merck Lifescience Pvt. Ltd. (Bengaluru, India)
5	Disodium hydrogen phosphate	Merck Lifescience Pvt. Ltd. (Bengaluru, India)
6	Potassium dihydrogen phosphate	Merck Lifescience Pvt. Ltd. (Bengaluru, India)
7	Sodium Chloride	Merck Lifescience Pvt. Ltd. (Bengaluru, India)
8	Ethanol	Merck Lifescience Pvt. Ltd. (Bengaluru, India)
9	Milli-Q water Millipore	Corp. Billerica, MA, USA
10	Hydrochloric Acid	Merck Lifescience Pvt. Ltd. (Bengaluru, India)
11	Sodium Hydroxide	Merck Lifescience Pvt. Ltd. (Bengaluru, India)

- List of Instruments and equipment in the study

Table 3 : List of various Instruments used in the study

Serial Number	Name	Source
1	Bath sonicator	Trans- O- Sonic, Mumbai, India
2	Cold centrifuge	HERMLE Labortechnik GmbH, Germany
3	Digital pH meter (EUTECH)	Thermo Fisher Scentific India Pvt. Ltd., Hiranandani Business Park, Mumbai, India
4	Digital weigh balance	Sartorius Corporate Administration, Otto- Brenner – StraBe 20, Goettingen, Germany
5	Disposable syringe (Dispo Van)	Hindustan Syringes and Medical Devices Ltd., Ballabgarh, Faridabad, Haryana, India
6	FTIR instrument	Bruker FTIR, Tensor – II, Bruker Optic GmbH, Karlsruhe, Germany
7	Normal Freezer	LG double door, Yeouido – dong, Seoul, South Korea
8	High speed homogenizer	IKA Laboratory Equipment, Model T10B Ultras- Turrax, Staufen, Germany
9	Incubator shaker	BOD-INC-1S, Incon, India
10	Laboratory Freeze Dryer (lyophilizer)	Instrumentation India, Kolkata, India
11	Magnetic stirrer	Remi Sales & Engineering Ltd, Ganesh Chandra Avenue, Bando House, Dharmatala, Kolkata, India
12	0.22 µ membrane filter	Merck Lifescience Pvt. Ltd. (Bengaluru, India)
13	Particle size and zeta sizer	Zetasizer nano ZS 90, Malvern Zetasizer Limited, Malvern,UK

Serial Number	Name	Source
14	Scanning electron microscope	SEM Joel JSM- 7600F, Tokyo, Japan
15	Transmission electron microscope	TEM, JEOL JEM – 2010, JEOL, USA
16	UV-VIS spectrophotometer	
17	Vortex mixture	Remi Sales & Engineering Ltd, Ganesh Chandra Avenue, Bando House, Dharmatala, Kolkata, India

CHAPTER – V
METHODOLOGY

Chapter - V

Methodology

- **Preparation of calibration curve of Nilotinib in Ethanol : Water (1:1) & Ethanol : PBS (pH-7.4) (1:1) Solution**
  - **Preparation of buffer solution**

Phosphate buffer saline pH 7.4 was prepared according to I.P protocol. 2.38 g of disodium hydrogen phosphate (Na_2HPO_4), 0.19 g of potassium dihydrogen phosphate (KH_2PO_4) and 8g of sodium chloride (NaCl) were dissolved in 1000 ml double distilled water. pH was adjusted to 7.4 using pre-calibrated pH meter (Eutech Instruments), 0.1N Sodium Hydroxide (NaOH) solution and 0.1N Hydrochloric acid (HCl) solution [44].

- **Preparation of stock solution**

2 mg of the drug was dissolved in 2 ml of ethanol and water in a 1:1 ratio in an Eppendorf tube and 2 mg of the drug was dissolved in 2 ml of ethanol and PBS pH 7.4 in a 1:1 ratio in an Eppendorf tube using vortex mixer. The concentration of both stock solutions was 1000 µg/ml [45].

- **Preparation of standard solution**

0.10 ml of stock solution was taken in the Eppendorf tube and diluted with ethanol and PBS pH-7.4 solution (1:1) and ethanol and water solution (1:1) respectively to make the 100µg/ml solution.

0.10 ml of the secondary stock solution (100µg/ml) were taken in Eppendorf separately and diluted with ethanol and PBS pH-7.4 solution (1:1) and ethanol and water in (1:1) respectively to make the concentration 10µg/ml.

For both ethanol and PBS pH-7.4 solution (1:1) and ethanol and water (1:1) in 0.1, 0.2, 0.3,0.4, 0.5, 0.6, 0.7 ml from 10µg/ml solution were taken in Eppendorf separately and diluted with the respective solvent to prepare 1 ml solution of 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml, 5µg/ml, 6µg/ml and 7µg/ml [45].

- **Determination of Absorption Maximum of Nilotinib**

For the determination of absorption maxima, about 1mg of the drug was dissolved in 10 ml of ethanol and PBS pH-7.4 solution (1:1) and ethanol and water solution (1:1) respectively using a vortex mixer. The solution was then scanned under UV-VIS double-beam spectrophotometer using ethanol and PBS pH-7.4 solution (1:1) and ethanol and water solution (1:1) respectively as blank. A single characteristic peak at

263 nm in ethanol and PBS pH-7.4 solution (1:1) and ethanol and water solution (1:1) respectively were obtained, which was close to the published lambda max (λ_{max}) of Nilotinib [44].

- **Preparation of the Standard**

  To prepare the standard curve in all the cases, first, the blank solution was taken into UV-VIS double-beam spectrophotometer at lambda max-263nm, and the absorbance of the blank was set to zero. Various concentrations of drug solution in ethanol and PBS pH-7.4 solution (1:1) (1,2,3, 4,5,6,7 µg/ml) were taken and their absorbances were measured in UV-VIS double beam spectrophotometer at lambda max-263 nm from lower concentration to upper concentration up to 7 µg/ml. The same procedure was followed in the case of the drug in ethanol and water (1:1) with their respective standard solutions. All the absorbances for their respective solvent systems were noted down against their concentration. Values were then taken into MS Excel; a graph was prepared using concentration at the X axis and absorbance at the Y axis [46]**.**

- **Preparation of PLGA nanoparticles containing Nilotinib**

  In this experimental study, the synthesis of nanoparticles was carried out using a double emulsion solvent evaporation technique. Specifically, 50mg of PLGA (75:25) and 2.5mL of Ethanolic solution containing 5mg of Nilotinib were dissolved in a 3mL organic phase consisting of dichloromethane (DCM). The organic phase containing the drug and polymer was subjected to homogenization at 20000 rpm using an IKA Laboratory Equipment, Model T10B Ultra Turrax in Staufen, Germany, followed by vigorous emulsification with a 2.5% aqueous PVA solution to form a o/w emulsion. The resulting primary emulsion was added dropwise to a 75ml 1.5% aqueous PVA solution and homogenized at 20000 rpm for 5 minutes to form a double emulsion (w/o/w). The double emulsion was sonicated while keeping ice-cold water in the bath for 30 minutes to reduce the particle size. Subsequently, the solution was allowed to stir on a magnetic stirrer overnight at room temperature to remove the organic solvents, resulting in the formation of nanoparticles during the evaporation of the solvents and solidification of the polymers. The larger particles were separated by centrifugation of the nanoparticle aqueous suspension at 5000 rpm for 10 minutes, followed by centrifugation of the nanoparticles at 16000 rpm for 45 minutes, and the supernatant was collected using a cooling centrifuge. To remove excess PVA associated with the nanoparticles, the pellets obtained after centrifugation were washed three times with Milli-Q water and

precipitated by centrifugation at 16000 rpm. The pellet was dispersed in 2-3ml of double-distilled water and subsequently frozen in a deep freezer (So Low, Environmental Equipment, Ohio, USA) and lyophilized in a freeze dryer (Laboratory Freeze Dryer, Instrumentation India, Kolkata, India) to obtain the dry powdered nanoparticles [44].

Physicochemical characterization of developed formulation

- **Drug excipient interaction study using Fourier Transform Infrared Spectroscopy (FTIR)** :

  Fourier Transform Infrared Spectroscopy (FTIR) is a highly valuable analytical technique commonly employed to investigate drug-excipient interactions in pharmaceutical formulations (Ewing et al., 2015). The present study utilized Fourier-transform infrared (FTIR) spectroscopy analyze the interaction between the drug and the selected excipients. The analysis was performed on blank nanoparticles (i.e., nanoparticles without any drug), drug (Nilotinib), PLGA, PVA, Nilotinib-loaded-PLGA nanoparticles, and a physical mixture of drug and excipients. The sample was mixed with high-quality KBr at a 1:100 ratio in each case and then pressed into pellets. Subsequently, they were analyzed for their spectral response over a range of 4000-600 cm-1 using a Bruker FTIR instrument (Bruker FTIR, Tensor-II, Bruker Optic GmbH, Karlsruhe, Germany) [45].

- **Determination of particle size and zeta potential :**

  In a Dynamic Light Scattering (DLS) study, the size distribution of particles in a solution is determined by measuring the intensity autocorrelation function of scattered light calculate the zeta potential. Electrophoretic mobility measurements can also be conducted he hydrodynamic diameter and zeta potential of developed nanoparticles were measured ng the DLS method. For this purpose, approximately 2 mg of Nilotinib-NPs are weighed and dispersed Milli-Q water (Millipore Corp., MA, USA) using a bath sonicator (Trans-o-sonic. Mumbai, India) for 30 minutes. Finally, the particle size and zeta potential were analyzed using the Malvern Zetasizer Nano-ZS 901 (Malvern Instruments, Malvern, U.K.). This analytical technique allows for the accurate determination of the size and charge of nanoparticles, making it an essential tool in the field of nanotechnology [46].

- **Determination of surface morphology by scanning electron microscopy (SEM) and High-resolution transmission electron microscopy (TEM) :**

In the realm of nanotechnology, the determination of nanoparticle surface morphology is crucial to understanding their properties and potential applications. Advanced microscopy techniques such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are employed to achieve this. In this study, lyophilized nanoparticles were subjected to scanning electron microscopy (SEM) after being coated with a thin layer of platinum. The transmission electron microscope (TEM) was used to investigate the internal morphology of the nanoparticles and drug distribution. For this purpose, a nanoparticle suspension in Milli-Q water was allowed to air dry on a standard carbon-coated copper grid (mesh) for 5-6 hours. Subsequently, image analysis was performed using a high-resolution Transmission Electron Microscope (JEOL HR, Tokyo, Japan). The findings of this study contribute to the understanding of nanoparticle surface morphology and pave the way for further research in this area.

- **Percentage of drug loading and encapsulation efficiency** :

The percentage of drug loading and encapsulation efficiency are fundamental parameters in drug delivery systems. They are crucial in determining the effectiveness of the encapsulation process and the quantity of drug that has been successfully loaded into the nanoparticles. To determine drug loading, Nilotinib-NP (2 mg) and blank nanoparticles (2 mg) were weighed into a centrifuge tube. Then, 2 mL of ethanol: water (1:1) solvent combination was added to each tube. The nanoparticles-containing tubes were incubated in an incubator shaker for 4 hours at 37°C. After that, the mixture was sonicated for 30 minutes, and the continuous phase was separated by centrifugation at a speed of 16000 rpm using a cold centrifuge (Hermle refrigerated centrifuge, Siemensstr, Wehingen, Germany). Finally, UV spectrophotometric analysis was performed at 263 nm to determine the drug release after collecting the supernatants from the Nilotinib-NP/blank nanoparticle solutions. The UV absorbance of the supernatant from blank nanoparticles was subtracted from the Nilotinib loaded nanoparticles to obtain the absorbance for Nilotinib only. Equations 1, 2 and 3 were utilized to compute the percentage of drug loading and entrapment efficiency [46].

1. Theoretical drug loading (%) =
   {Amount of drug /(Amount of drug+Excipients)} x 100
2. Drug loading (actual)% =
   (Amount of drug in nanoparticles / Amount of nanoparticles obtained) x 100
3. Entrapment efficiency (%) =
   {Drug loading (actual) (%) / Amount of drug loading (theoretical) (%)} x 100

- **In vitro drug release study :**

  An in vitro drug release study is a crucial step in understanding how drugs are released from nanoparticles in simulated physiological conditions in a lab setting. This assessment is essential for optimizing drug delivery systems by determining the controlled release behaviour of drug loaded nanoparticles. The pre-weighed freeze-dried Nilotinib-NP (1 mg/ml, 2 ml) was resuspended in different buffers at 37°C with slow agitation (60 shaking/minute) in an incubator shaker. The buffers used were phosphate-buffered saline (PBS) (pH 7.4). After centrifugation (at 16,000 rpm for 10 minutes in a cold centrifuge), the supernatant was collected, and the pellet was resuspended with a fresh buffer. At different intervals, ranging from 1 hour to 30 days, the supernatants were collected, and the volume removed was replaced with mL of fresh buffer each time. The bottom pellets were resuspended using a vortexing technique, and the samples were placed back in the incubator shaker until the next sampling. To determine the drug concentration in the collected samples, a UV-VIS spectrophotometric analysis was conducted at 263 nm [46].

CHAPTER – VI
RESULTS

Results

➢ UV Absorption spectra of Nilotinib

For the determination of absorption maximum (λ_{max}) we take 1mg of drug and dissolve in ethanol and water (1:1) solvent in Eppendorf tubes and scan the prepared solution in double beam UV-Visible spectrophotometer from 400 to 200 nm, the (λ_{max}) was found at 263 nm.

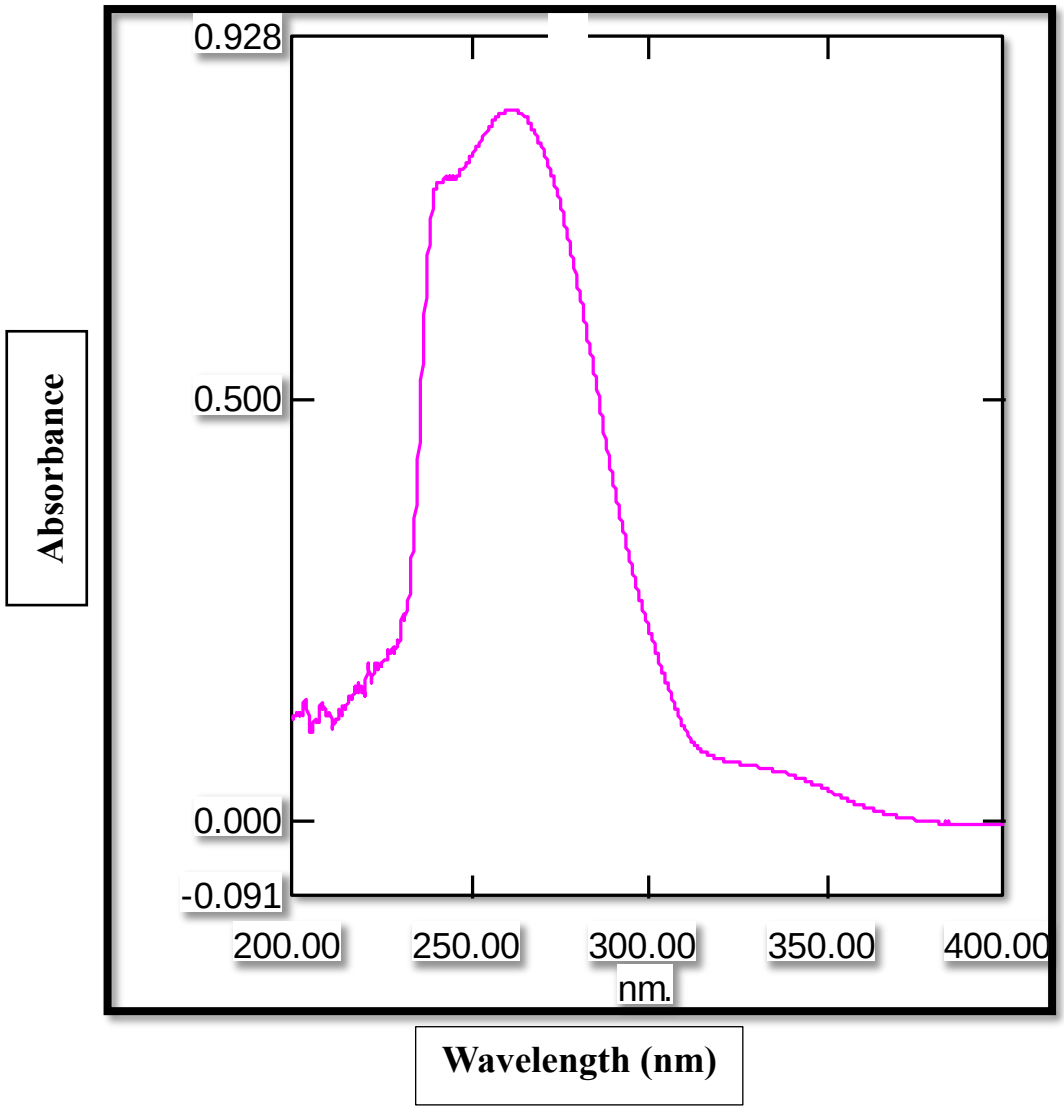


Fig 7 : Absorption maximum (λ_{max}) of Nilotinib at 263 nm in ethanol : Water (1:1)

For the determination of absorption maximum (λ_{max}) we take 1mg of the drug and dissolve it in ethanol and PBS pH 7.4 (1:1) solvent in Eppendorf tubes and scan the

prepared solution in double beam UV-visible spectrophotometer from 400 to 200 nm, the (λ_{max}) was found at 263 nm.

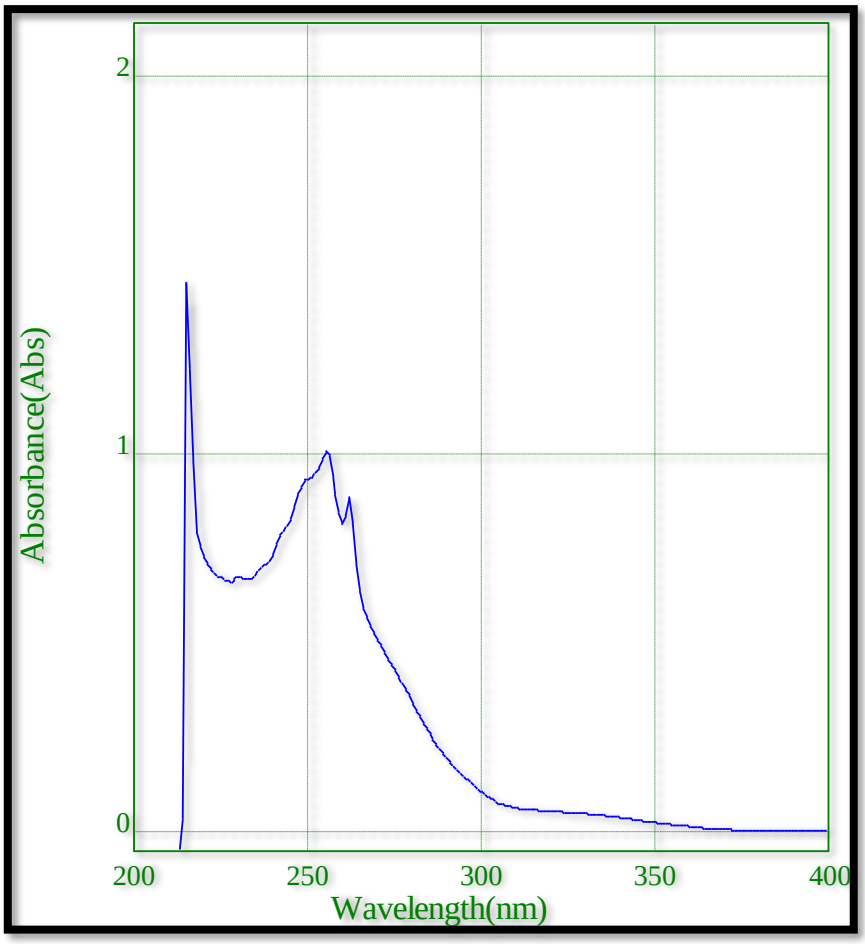


Fig 8 : Absorption maximum (λ_{max}) of Nilotinib at 263 nm in ethanol : PBS pH 7.4 (1:1)

➢ The Calibration curve of Nilotinib

Two different calibration curves were prepared – one in ethanol: water (1:1) and one in ethanol: PBS (pH 7.4) (1:1) for studying nanoparticle entrapment efficiency and in vitro-drug permeation study. Each reading was made in triplicate, and the average value was taken. The dilutions prepared for the standard curves were as follows.

Table 1: Absorbances of Nilotinib against various concentrations in ethanol: water (1:1)

Concentration (µg/ml)	Abs 1	Abs 2	Abs 3	Average
1	0.078	0.098	0.098	0.091333
2	0.204	0.196	0.187	0.195667
3	0.319	0.324	0.319	0.320667
4	0.426	0.402	0.399	0.409
5	0.525	0.523	0.47	0.506
6	0.621	0.639	0.632	0.630667
7	0.695	0.683	0.706	0.694667

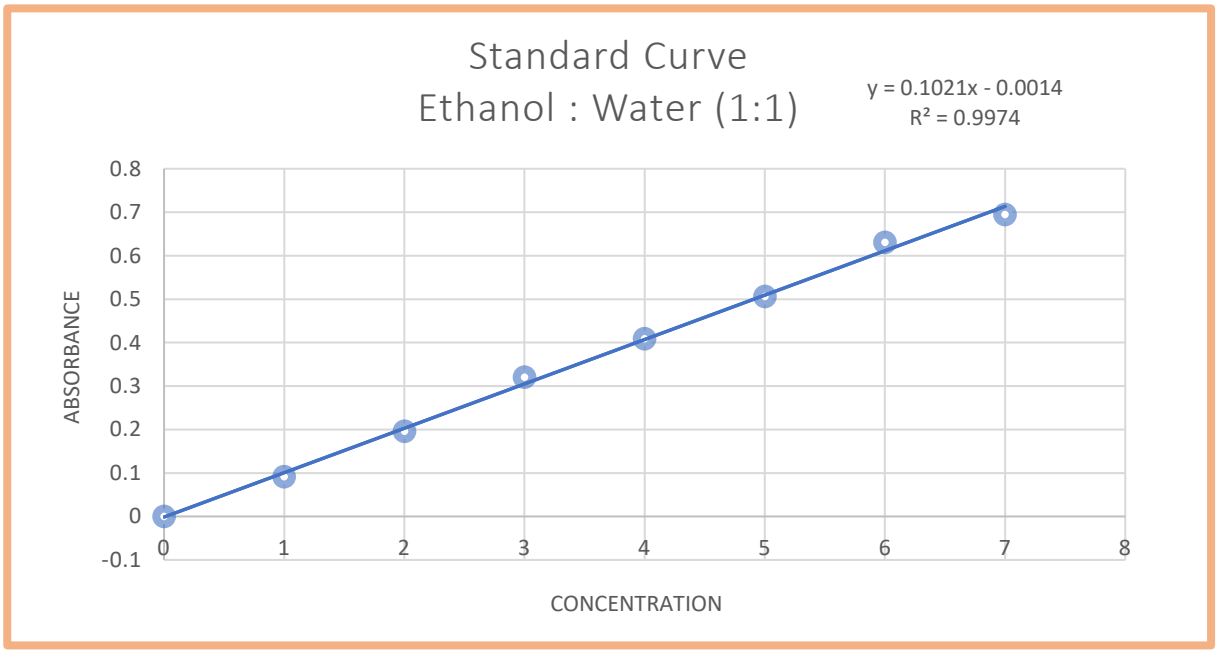


Fig 9 : Standard curve of Nilotinib in Ethanol: water (1:1) with R^2 of 0.9974

Table 2: Absorbance of Nilotinib against various concentrations of Ethanol:PBS pH 7.4 (1:1)

Concentration (µg/ml)	Abs 1	Abs 2	Abs 3	Average
1	0.068	0.081	0.077	0.075333
2	0.132	0.132	0.133	0.132333

3	0.189	0.19	0.192	0.190333
4	0.262	0.265	0.267	0.264667
5	0.319	0.318	0.31	0.315667
6	0.362	0.365	0.364	0.363667
7	0.432	0.431	0.432	0.431667

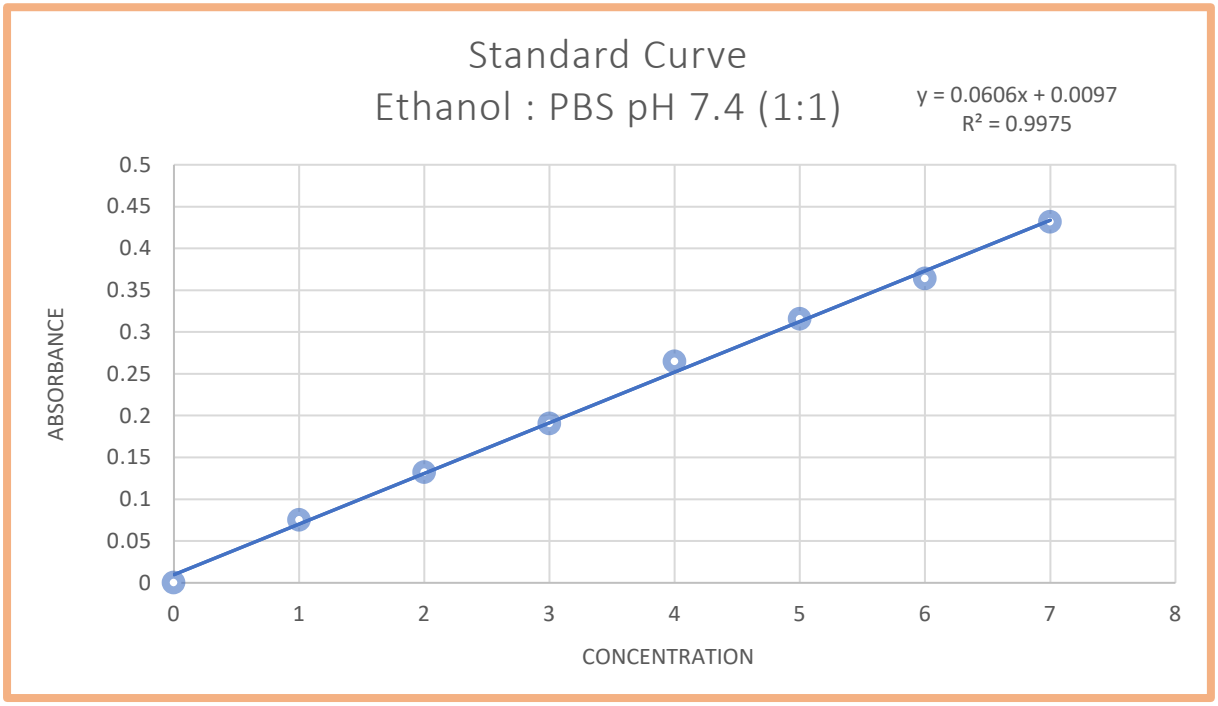


Fig 10 : Standard curve of Nilotinib in Ethanol: PBS pH 7.4 (1:1) with R^2 of 0.9975

- **Study of Drug – Excipient interaction through Fourier Transform Infrared (FTIR) Spectroscopy**

  In the present study we evaluated the drug excipient interaction using FTIR spectroscopy. FTIR spectra assess the drug excipient interaction at the level of functional groups by determining their vibrational patterns. Here the spectra of the drug, the individual excipients (Poly Lactic – co – Glycolic Acid & Poly Vinyl Alcohol), blank nanoparticles (without drug) and Nilotinib loaded nanoparticles have been depicted in the pictures from the figure 10 to 15.

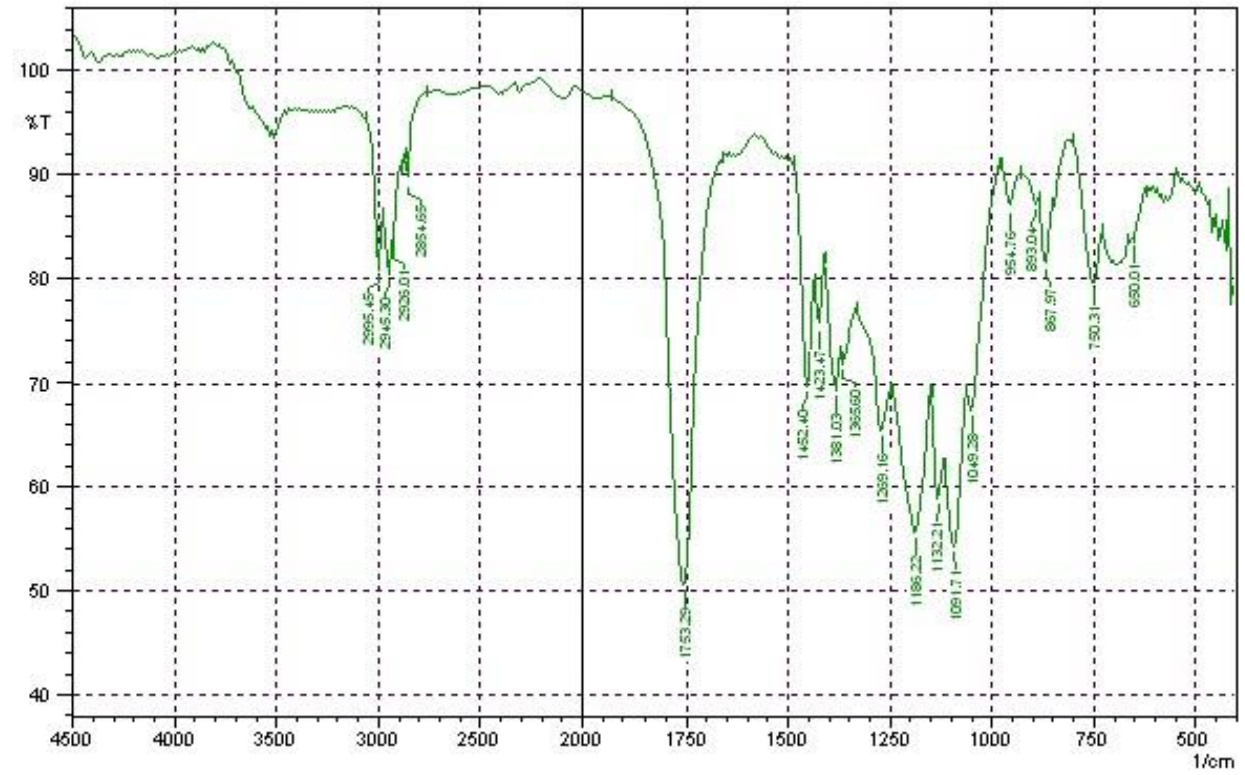


Fig 11 : FTIR graph of Poly Lactic – co – Glycolic Acid (PLGA)

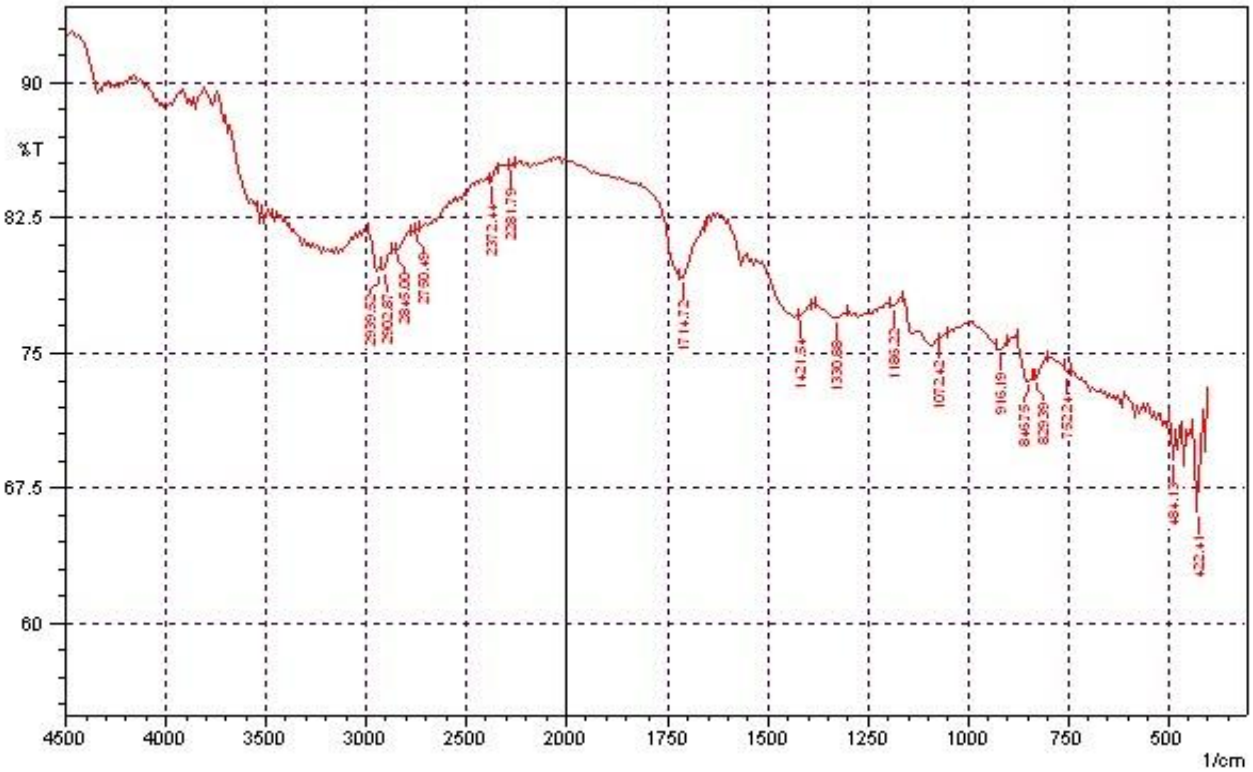


Fig 12 : FTIR graph of Poly Vinyl Alcohol (PVA)

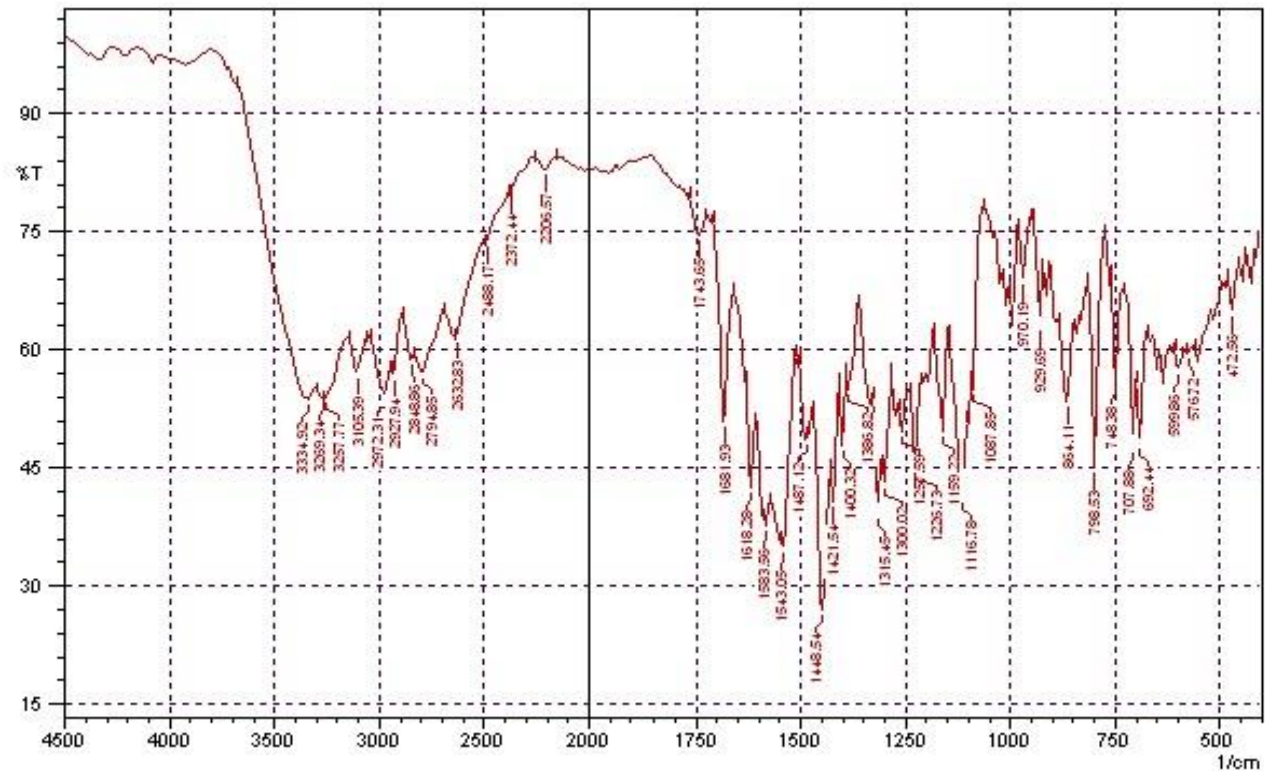


Fig 13 : FTIR graph of Nilotinib

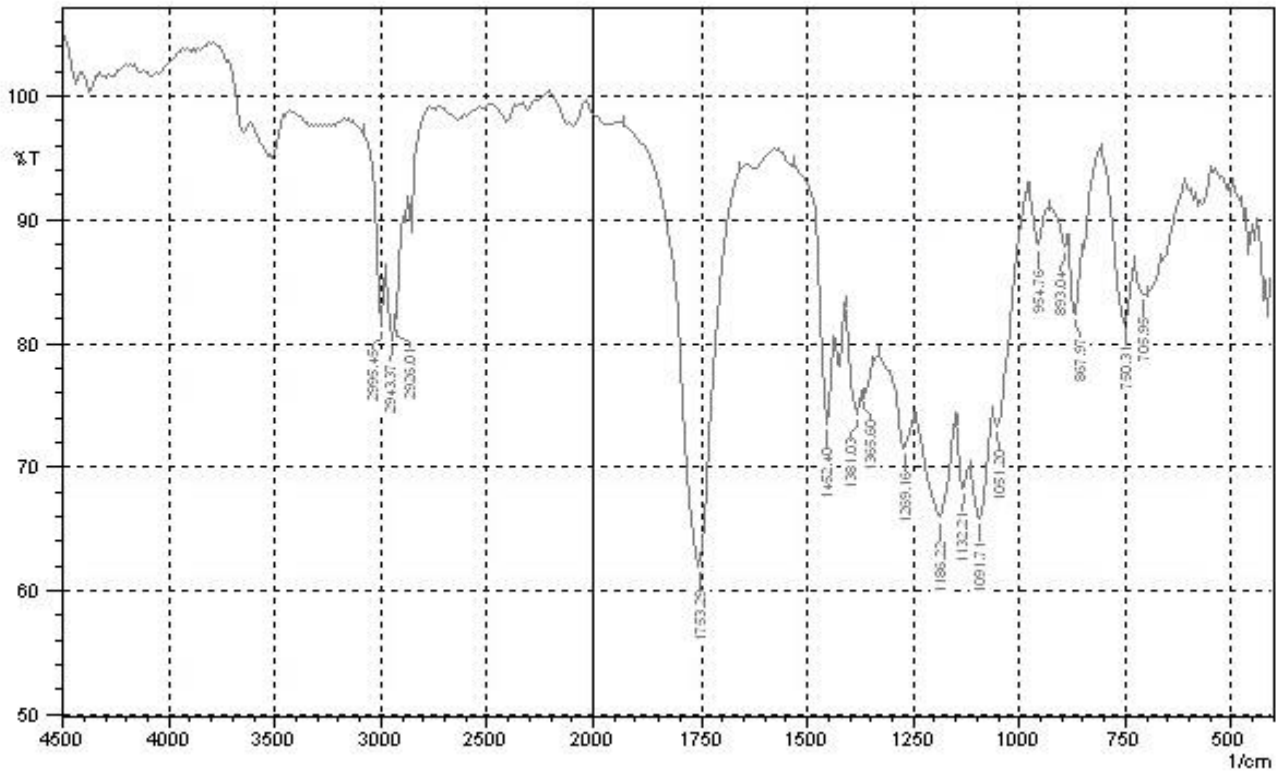


Fig 14 : FTIR graph of Physical mixture of PLGA, PVA, Nilotinib

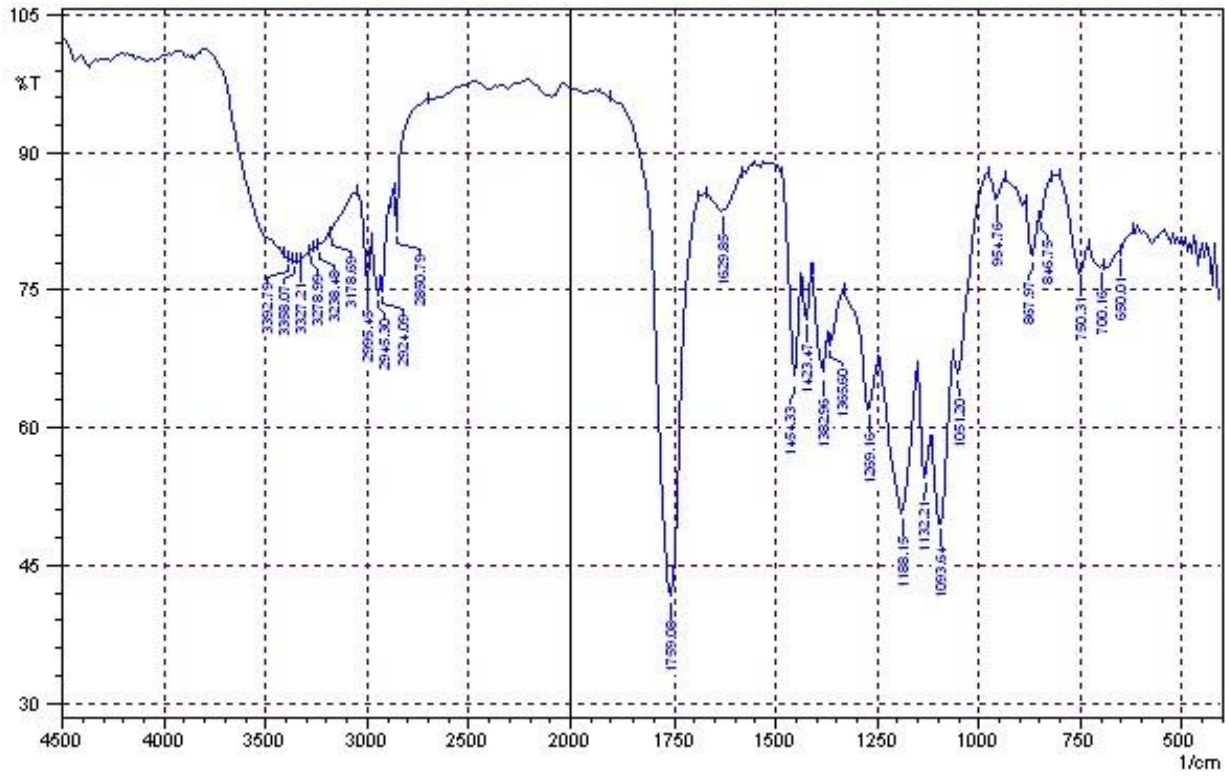


Fig 15 : FTIR graph of Blank Nanoparticles

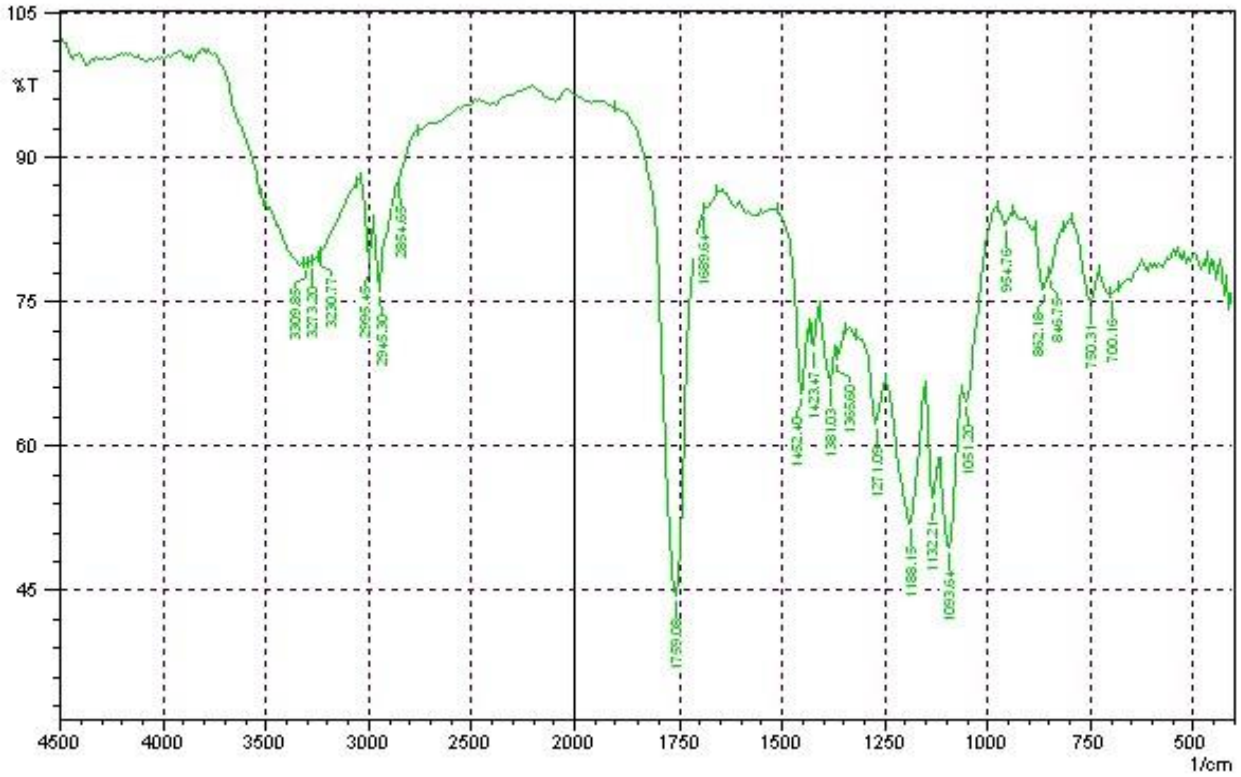


Fig 16 : FTIR graph of Nilotinib loaded Nanoparticles

➢ Particle Size & Zeta potential measurement study

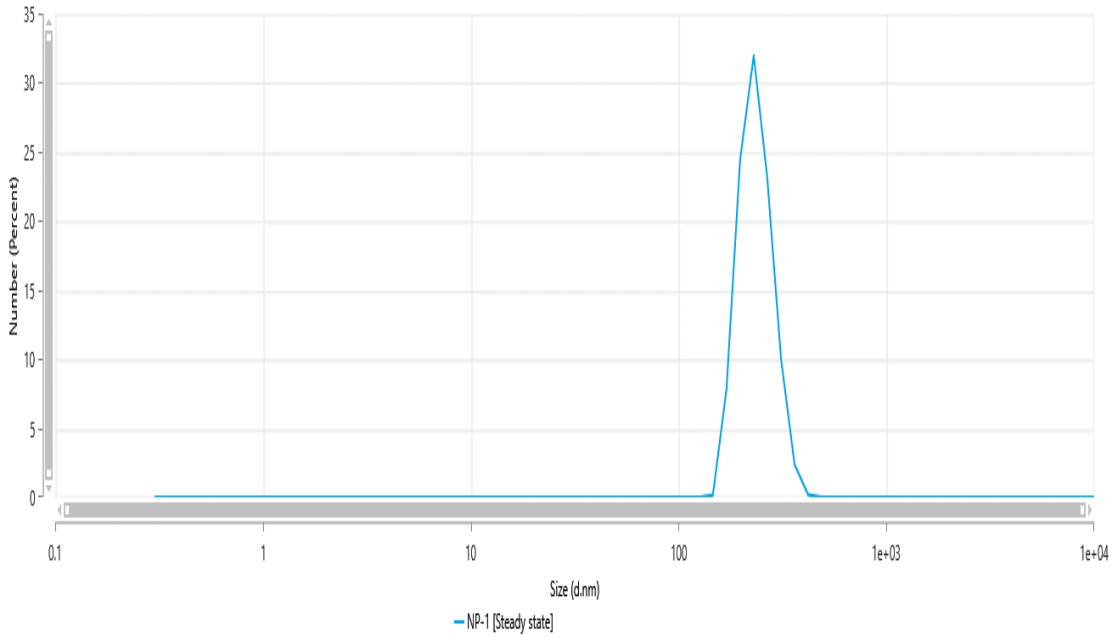


Fig 17 : Particle size distribution graph of Nilotinib loaded nanoparticles (Formulation)

Table 6: Z-average value and polydispersity index of the formulation

Experimental Study	Liposomes
Z average value	280.2 nm
Polydispersity index	0.873

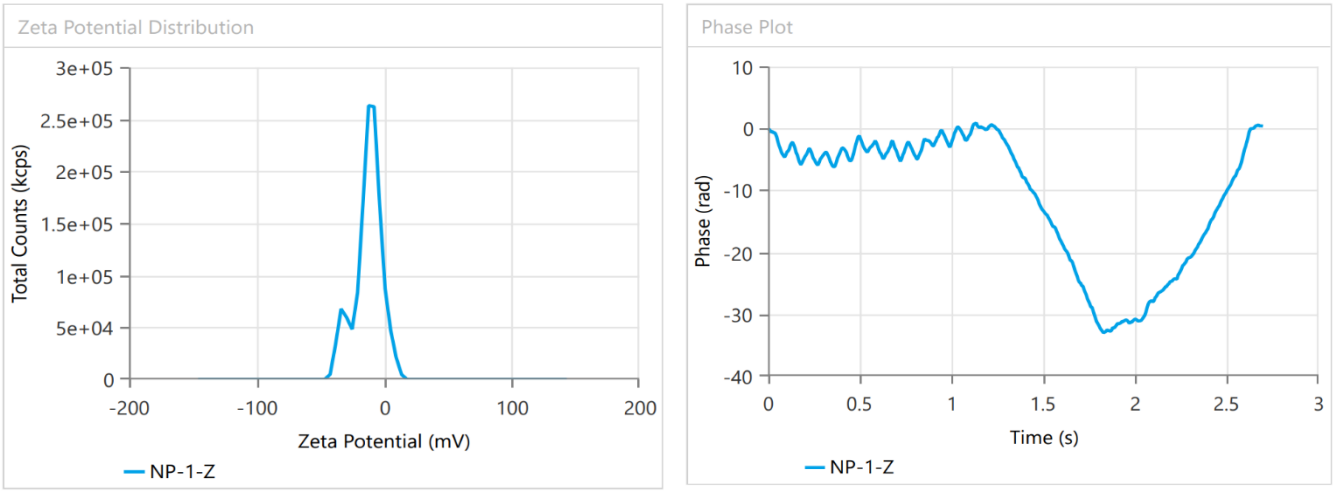


Fig 18 : Zeta potential graph of the formulation (-12.5 mV)

- **Surface Morphology study of Lyophilised formulation by scanning electron microscopy (SEM) and High-resolution transmission electron microscopy (HR-TEM)**

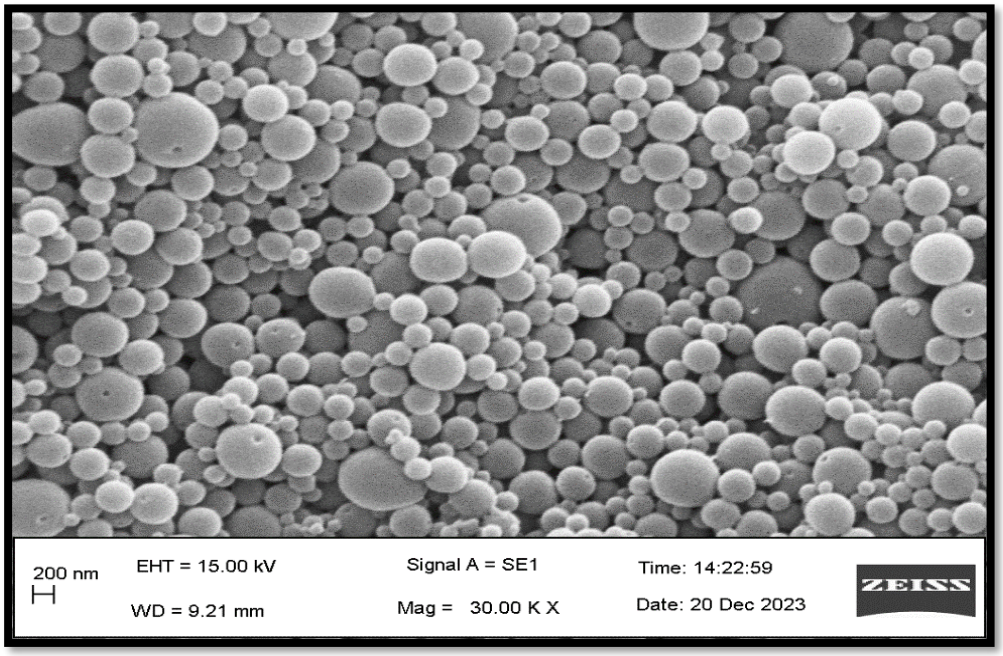


Fig 19 : SEM image of Blank Nanoparticles (without drug) at 30000x magnification

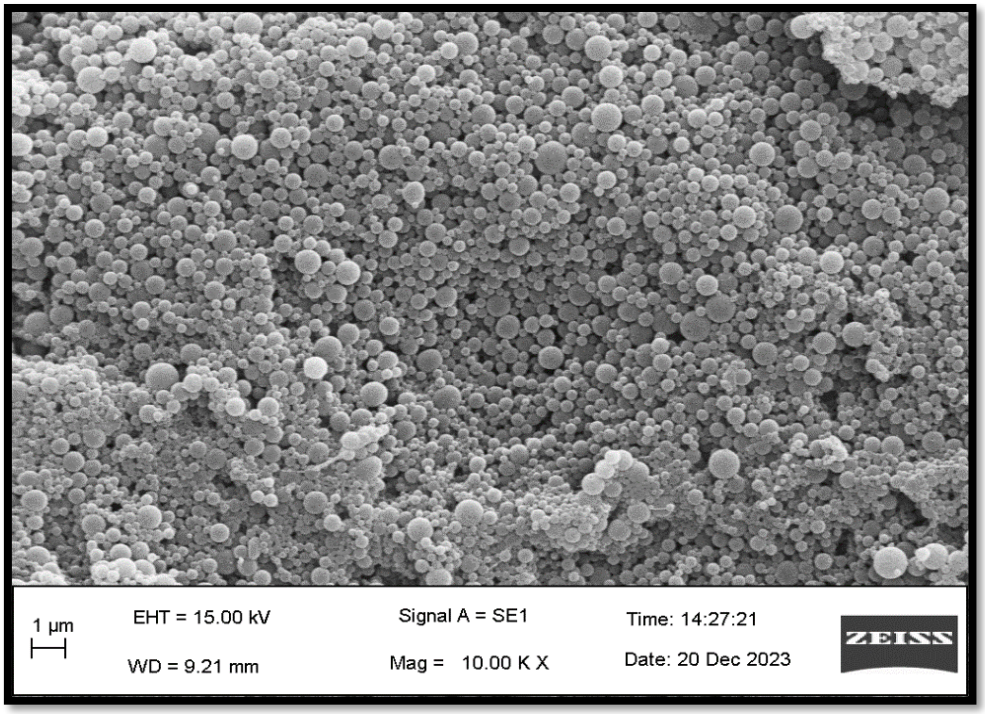


Fig 20 : SEM image of Blank Nanoparticles (without drug) at 10000x magnification

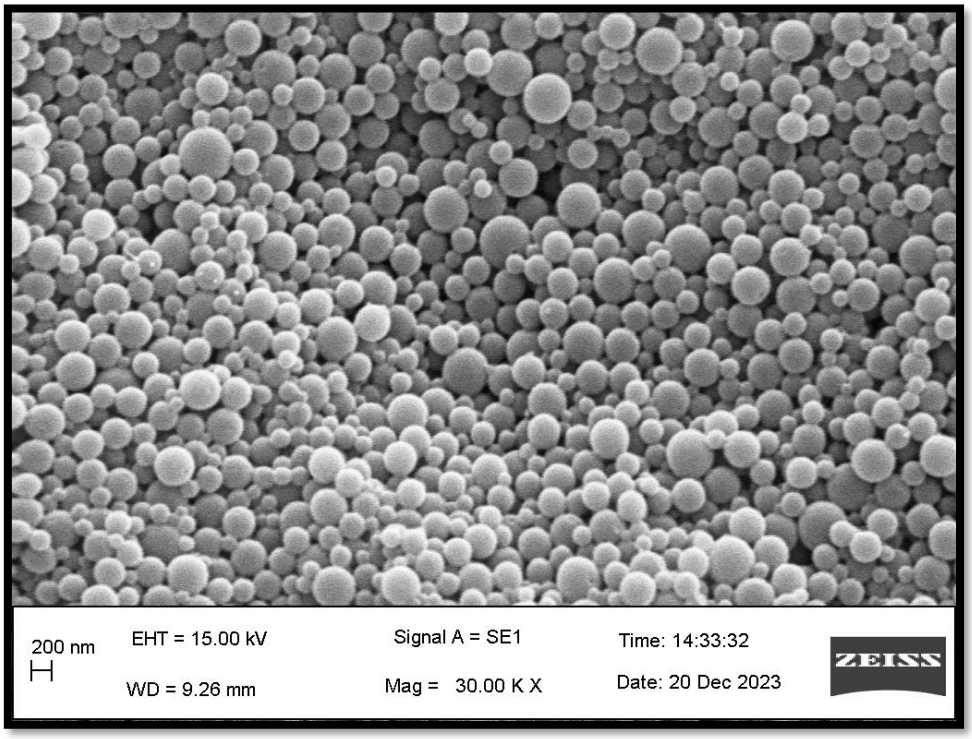


Fig 21 :SEM image of Nilotinib loaded Nanoparticles at 30000x magnification

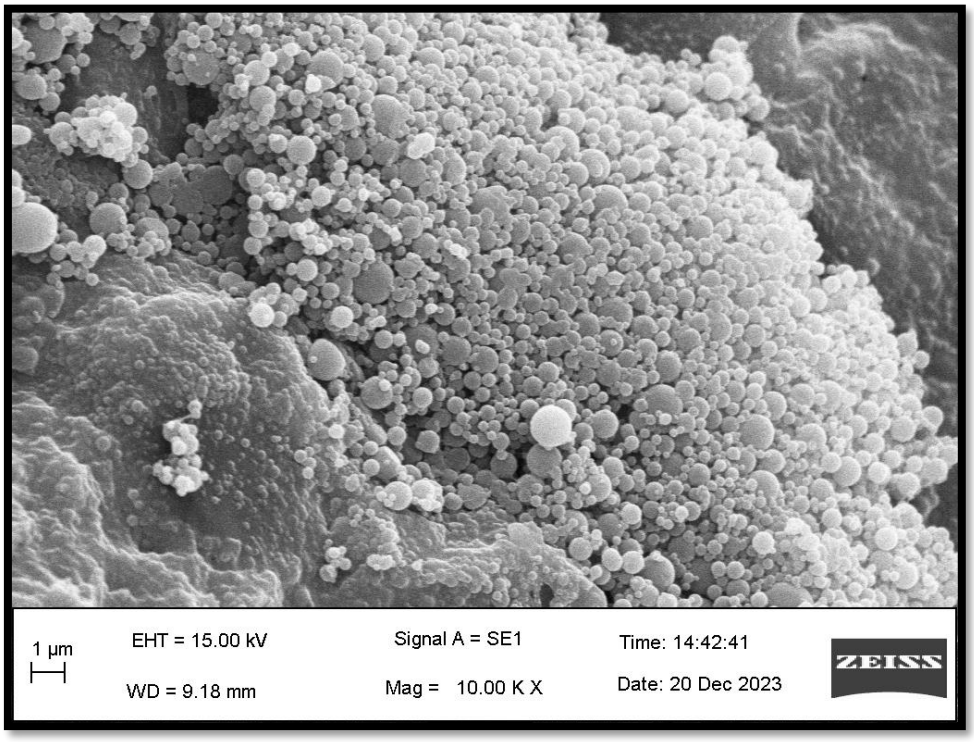


Fig 22 :SEM image of Nilotinib loaded Nanoparticles at 10000x magnification

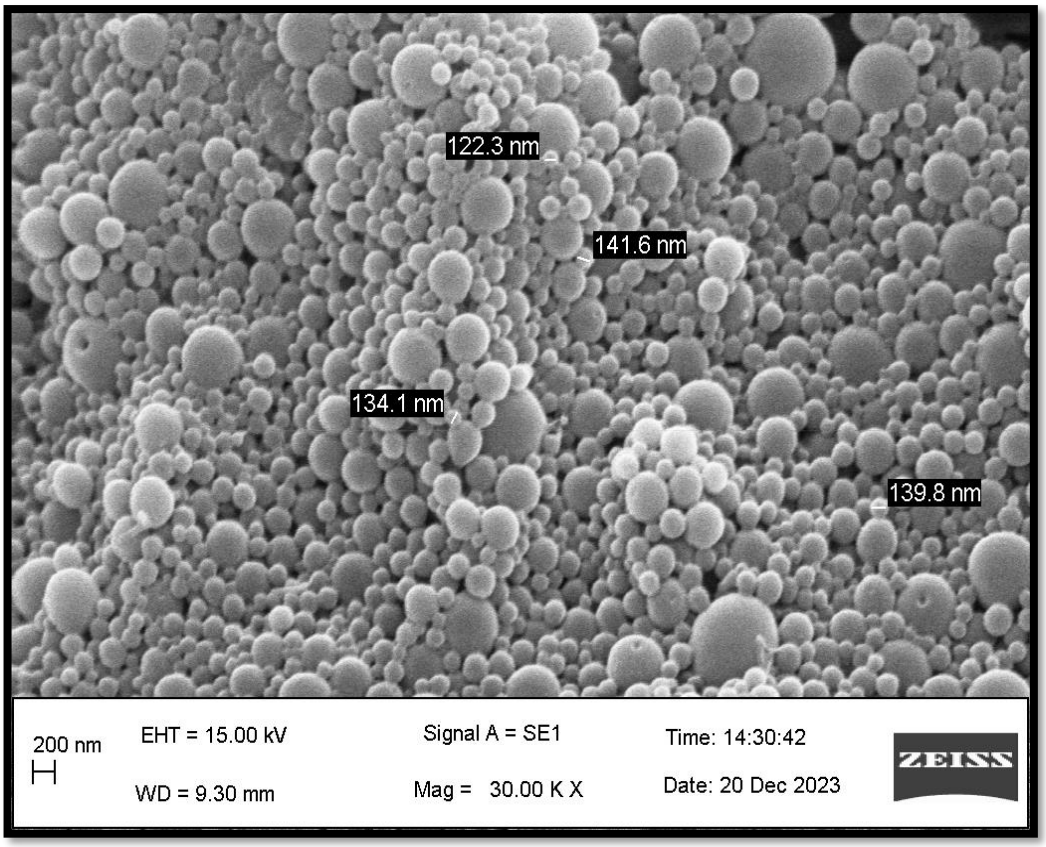


Fig 23 :SEM image of Nilotinib loaded Nanoparticles at 30000x magnification with diameter

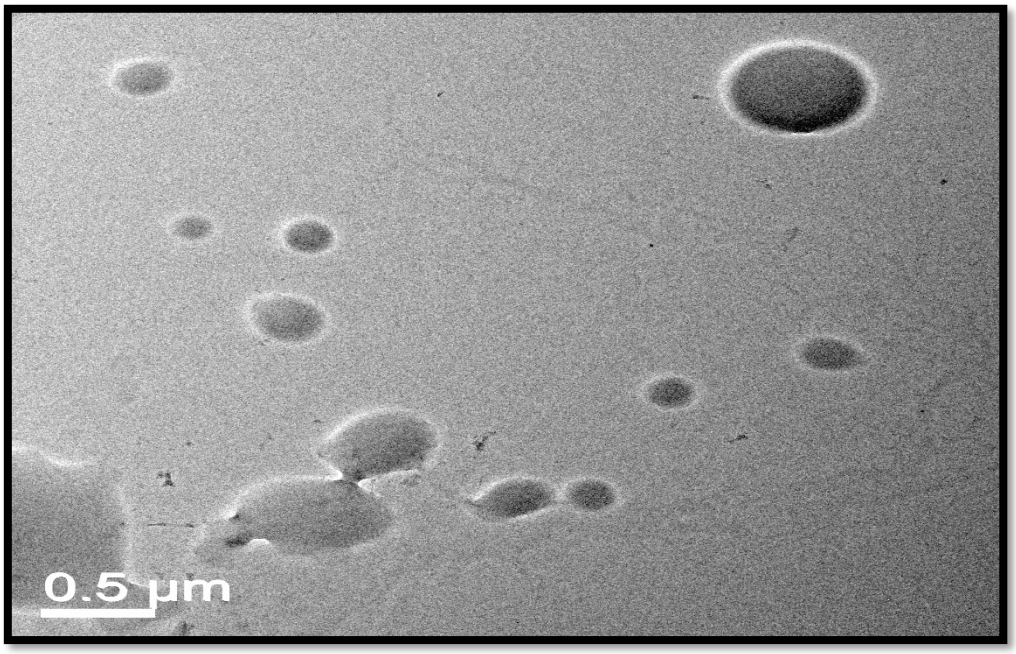


Fig 24 :TEM image of Nilotinib loaded Nanoparticles

➢ Drug Loading and Entrapment Efficiency of Nilotinib loaded nanoparticles

The composition of experimental formulation along with respective drug loadings and entrapment efficiencies were given in the following table.

Table 7: Theoretical and practical drug loading with entrapment efficiency of formulation

Formulation	Amount of drug taken (mg)	Amount of PLGA taken (mg)	Theoretical drug loading (%)	Practical drug loading (%)	Entrapment efficiency
PLGA Nanoparticle loaded with Nilotinib	5	50	9.09	3.862	42.48

➢ In vitro Drug release kinetic study from Nilotinib loaded nanoparticles

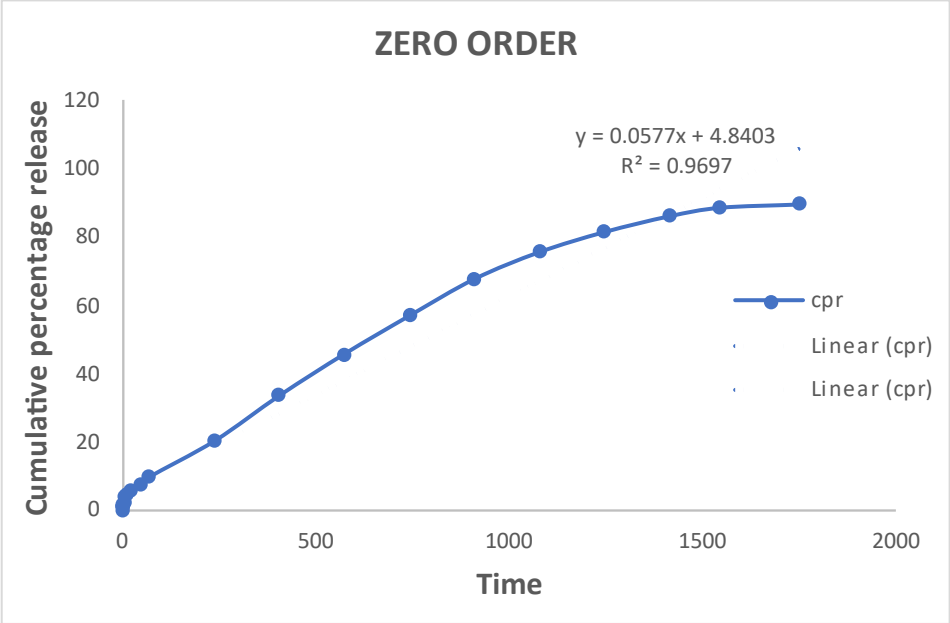


Fig 25: Zero order release kinetics

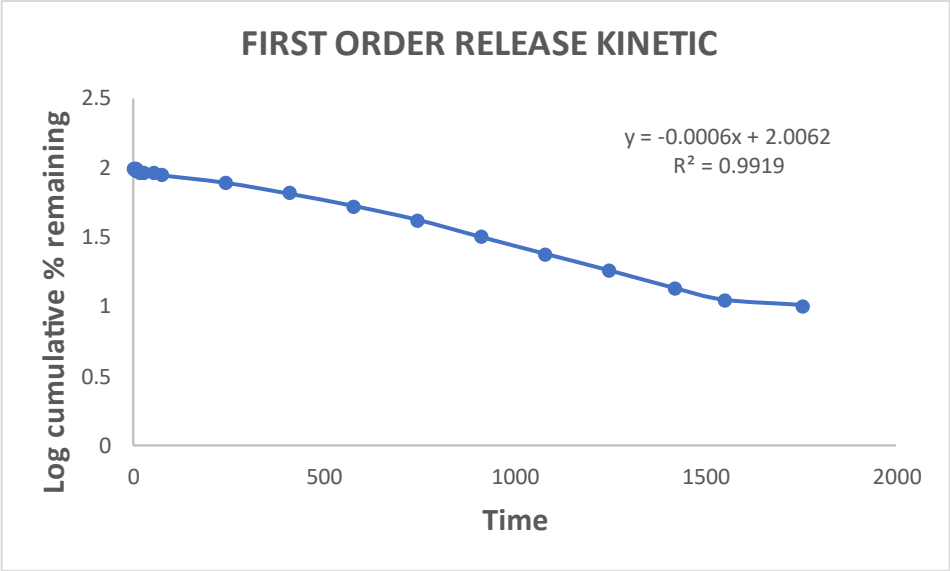


Fig 26: First order release kinetics

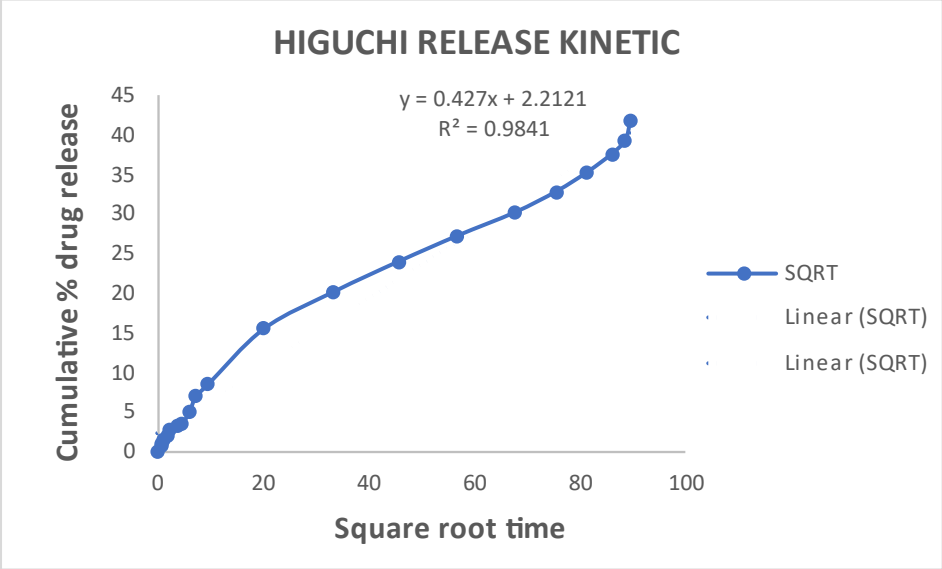


Fig 27: Higuchi release kinetics

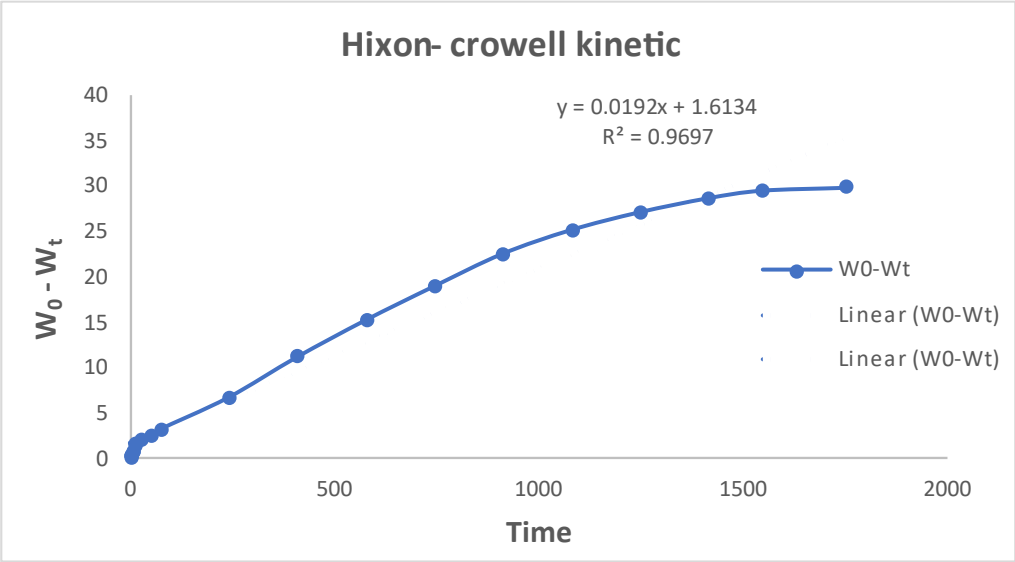


Fig 28: Hixon crowell kinetics

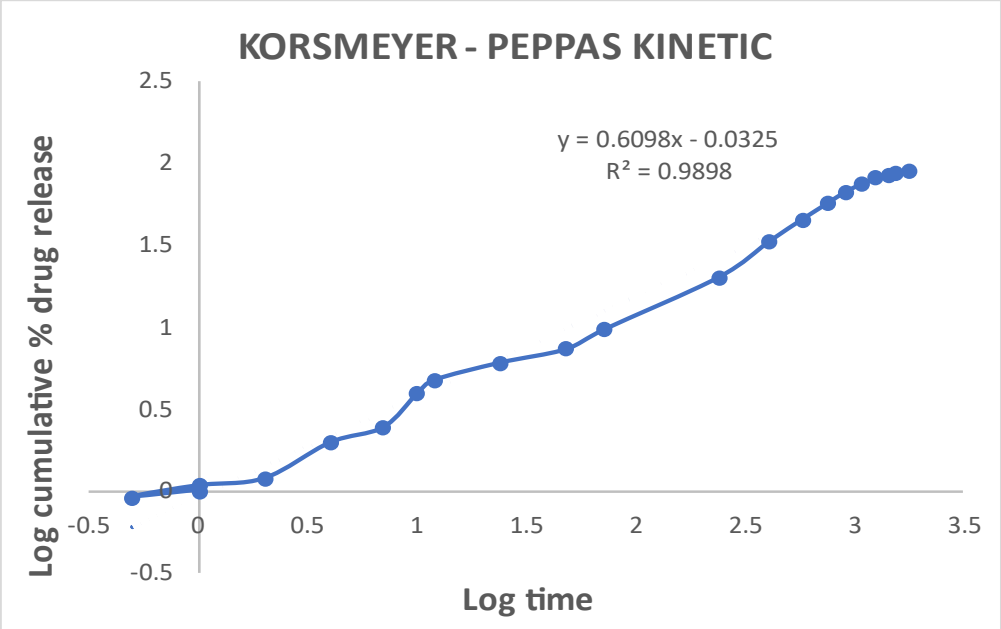


Fig 29: Korsmeyer peppas release kinetics

CHAPTER – VII
DISCUSSIONS

Chapter – VII

Discussions

- **The UV Absorption Spectrum of Nilotinib in different solvent systems**

  UV scanning of Nilotinib in PBS pH 7.4 and ethanol water solution (1:1) yielded lambda max values at 263nm, which comes under the reported peak of Nilotinib at 260 – 265nm. This confirms the authenticity and purity of the product being used and yields the reference wavelength for spectrophotometric calculation in further studies.

- **The Calibration curve of Nilotinib in ethanol : water (1:1) and ethanol : PBS pH 7.4 (1:1)**

  Two different calibration curves were prepared one in ethanol water (1:1) and another one in ethanol PBS pH7.4 (1:1) to study in-vitro drug release and Nanoparticles entrapment efficiency. Each reading was made in triplicate, and the average values were taken. The correlation coefficient (R^2) values were 0.9974 (ethanol: water) and 0.9975 (Ethanol: PBS pH7.4). These values favour the accuracy of the calibration curves used for further analysis.

- **Drug – Excipients interaction study by Fourier transform Infrared Spectroscopy (FTIR)**

  The FTIR (Fourier Transform Infrared) spectrum provides valuable information about the molecular composition and functional groups present in a sample. Drug-excipient interactions were investigated using FTIR spectroscopy. The stability of the drug in the formulation, drug release pattern from it and other physicochemical properties such as surface charge, shape, size etc related to the formulation depends on drug – excipients interaction. In this study, FTIR analysis was performed to detect any chemical interactions between the drug molecule and excipients. The results demonstrate that PLGA exhibited peaks at 3600 cm^{-1}, 2945 cm^{-1} and 1753 cm^{-1} for the O-H stretching, asymmetric stretching of -CH_2 and C=O stretching band of the carboxylic acid group respectively. Similarly Nilotinib showed characteristic peaks at 3335 cm^{-1}, 2795 cm^{-1},1682 cm^{-1},1543 cm^{-1} ,1448 cm^{-1}, 1116 cm^{-1}, 798 cm^{-1} and 708 cm^{-1} for C-N stretching for imidazole ring, C-H stretching vibration of aromatic ring, C=O stretching vibration in the amide moiety, C=C stretching of aromatic ring, C=C stretching of imidazole ring, C–N stretching of ring, N-H bending, C-F bending of molecule respectively. Additionally, FTIR spectra of PVA showed peaks at 3450 cm^{-1} for intermolecular

bonded O-H stretching vibrations, 2939 cm^{-1} for asymmetric stretching of -CH_2 and 1715 cm^{-1} due to water absorption. Furthermore, blank (without drug) nanoparticles exhibited the presence of all characteristic peaks of the polymer PLGA. Similarly, Nilotinib loaded nanoparticles showed IR peaks at 2945 cm^{-1} , 1759 cm^{-1} , 1381 cm^{-1} and 1271 cm^{-1} for C-H stretching, C=O stretching, C-H bending, and C-O stretching due to ester group respectively. In the case of Nilotinib loaded nanoparticles, they shows no drug peak, indicating complete drug encapsulation within PLGA nanoparticles because blank nanoparticles and Nilotinib loaded nanoparticles show similar FTIR graph. Furthermore, physical mixture of excipients (PLGA, PVA) and Nilotinib FTIR graph show all the major peak of PLGA, PVA and Nilotinib, this suggest the presence of drug in the formulation. Some minor shifts seen in the peaks of formulation corresponding to PLGA and PVA this could be due to physical interaction between the functional groups of the excipients and Nilotinib, most likely due to formation of Van der Waal's force of attraction or dipole – dipole interaction or weak hydrogen bond formation. This physical interaction might help in the formation of spherical nanostructure.

- **Particle size and Zeta potential analysis**
  - **Particle size and size distribution study** : A particle size and size distribution study of the nanoparticle formulation was carried out by Dynamic Light Scattering Spectroscopy (DLS). The principle of the particle size determination by DLS is the measurement of rate of fluctuation of the intensity of scattered light due to Brownian motion of particles. From the result it was observed that the Z average value of the sample is 280.2 nm which suggest that the average hydrodynamic diameter of the particles in the sample is 280.2 nm. This means that most of the particles in the sample have a size close to 280.2 nm which means the particles in your sample are relatively small, around the nanoscale, which is typical for many nanoparticle applications in fields such as drug delivery. PDI value was found to be 0.873 which suggests that the formulation has a narrow dispersion of size in the sample.
  - **Zeta Potential study** :The zeta potential value indicates the degree of electrostatic repulsion between adjacent, similarly charged particles in a dispersion. Particles with a zeta potential greater than +10 mV or less than -10 mV are considered stable. The negative zeta potential value of -12.5 mV of the

formulation suggests that the particles in formulation have strong electrostatic repulsion, preventing them from coming together and aggregating. Therefore, the colloidal system is likely to be very stable with particles having strong negative charges that help to maintain dispersion and prevent aggregation.

- **Study of Morphology of formulation by SEM and TEM**
  - Size, shape and external morphology of the nanoparticle formulations were assessed by Scanning Electron Microscopy (SEM). From the result it was found that lyophilised nanoparticles loaded with Nilotinib had smooth surface and were in 100-200 nm which is in nano size range. The surface of the polymeric vesicles had no leakage and the nanoparticles were uniformly distributed.
  - To further investigate the nanoparticles' interior structure, Transmission Electron Microscopy (TEM) study has been employed. TEM images revealed a dark structure, indicating a homogeneous drug distribution throughout the particles.
- **Drug loading and Entrapment efficiency study**

  The theoretical drug loading was 9.09(%), whereas the practical drug loading was found to be 3.862%. The entrapment efficiency was found to be 42.48%. The drug, Nilotinib is lipophilic in nature, and thus it will be entrapped into the polymeric core in nanoparticles.
- **In Vitro Drug Release Study**

  In-vitro drug release study data were collected over 1752 hours, and the cumulative release percentage of drug from the formulation was plotted against time to determine the drug release pattern in various media. The graph clearly showed the sustained release of the drug from the formulation.

  The drug release pattern was also correlated with the established models of release kinetics, namely Zero order kinetics, First order kinetics, Higuchi kinetics, Hixson-Crowell kinetics and Korsmeyer-Peppas kinetics. Considering the R^2 values (0.9697, 0.9919, 0.9841, 0.9697,0.9898) in all the above-mentioned kinetics models in PBS media, it has been found that the drug release pattern from the formulation followed first order release model.

CHAPTER – VIII
CONCLUSIONS

Chapter - VIII

Conclusions

Based on the findings of this study, Nilotinib-loaded nanoparticles were successfully developed and characterized in vitro, demonstrating promising potential for improved drug delivery in the treatment of chronic myeloid leukemia. FTIR analysis showed minimal interaction between the drug and excipients, supporting the suitability of the chosen materials for this nanoformulation. The nanoparticles exhibited a uniform size distribution within the nano range, smooth surface morphology, and spherical shape, as confirmed by size analysis and internal uniform drug distribution throughout the spherical nanostructure of the formulation as shown in TEM images. The high zeta potential values suggest good stability of the formulations, and the moderate to high drug entrapment efficiency indicates effective drug encapsulation. The optimized formulation demonstrated a sustained release profile, which could potentially reduce drug side effects, decrease dosing frequency, and increase dosing intervals. However, further in vivo studies are necessary to assess the therapeutic efficacy and safety of these nanoparticles. If successful, this novel nanoparticle formulation could offer a significant advancement over current treatments for chronic myeloid leukemia, providing a more effective and safer option for patients.

CHAPTER – IX
REFERENCES

Chapter – IX

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CERTIFICATE OF APPROVAL

This is to certify that **SRIJITA GOPE** bearing **Registration No: 163663 of 2022-23** has carried out the research work entitled "**NILOTINIB - LOADED POLY LACTIDE – CO - GLYCOLIDE NANOPARTICLES FOR CHRONIC MYELOID LEUKEMIA TREATMENT: DEVELOPMENT AND THEIR PHYSICOCHEMICAL CHARACTERIZATIONS** " independently with proper care and attention under my supervision and guidance in the Pharmaceutics Research Laboratory in the Department of Pharmaceutical Technology, Jadavpur University. She has incorporated her findings into this thesis of the same title, being submitted by her. In partial fulfilment of the requirements for the degree of **MASTERS OF PHARMACY** from Jadavpur University. I appreciate her endeavour to do the project and her work has reached my **gratification.**

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DECLARATION OF ORIGINALITY AND COMPLIANCE OF ACADEMIC ETHICS

I hereby declare that this thesis contains a literature survey and original research work by the undersigned candidate as part of her Master of Pharmaceutical Technology studies. All information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all materials, and the results are original to this work.

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