DEVELOPMENT AND IN VITRO CHARACTERIZATIONS OF SUBMICRON SIZE NILOTINIB-LOADED LIPID PARTICLES INTENDED FOR THE TREATMENT OF CHRONIC MYELOID LEUKEMIA

Submitted by

ADARSH YADAV

B. Pharm

EXAMINATION ROLL NO. - M4PHC24002

REGISTRATION NO. – 163655 of 2022- 2023

Under the guidance of

Prof. (Dr.) Biswajit Mukherjee

Division of Pharmaceutics
Department of Pharmaceutical Technology
Faculty of Engineering and Technology
Jadavpur University
Kolkata- 700032

Thesis submitted in partial fulfillment of the requirements for the DEGREE OF MASTER OF PHARMACY

Department of Pharmaceutical Technology
Faculty of Engineering and Technology
Jadavpur University
Kolkata- 700032

Session: 2022- 2024

CERTIFICATE OF APPROVAL

This is to certify that **ADARSH YADAV**, bearing **Registration No: 163655 of 2022-23** has carried out the research work entitled "**DEVELOPMENT AND IN VITRO CHARACTERIZATIONS OF SUBMICRON SIZE NILOTINIB-LOADED LIPID PARTICLES INTENDED FOR THE TREATMENT OF CHRONIC MYELOID LEUKEMIA"** independently with proper care and attention under my supervision and guidance in the Pharmaceutics Research Laboratory in the Department of Pharmaceutical Technology, Jadavpur University. He has incorporated his findings into this thesis of the same title, which he is submitting in partial fulfilment of the requirements for the degree of **MASTERS OF PHARMACY** from Jadavpur University. I appreciate his endeavour to do the project, and his work has reached my gratification.

Head of the Department
Prof. (Dr.) Amalesh Samanta
Department of Pharmaceutical Technology
Faculty of engineering and technology
Kolkata-700032

Project Guide
Prof. (Dr.) Biswajit Mukherjee
Department of Pharmaceutical Technology
Faculty of engineering and technology
Kolkata-700032

Dean
Prof. Dipak Laha
Faculty of Engineering and technology
Jadavpur University
Kolkata-700032

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 his 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.

Name: ADARSH YADAV

Examination Roll Number: M4PHC24002

Registration Number: 163655 of 2022- 2023

Signature with Date

ACKNOWLEDGEMENT

I would like to express my deep gratitude to my project guide Prof. (Dr.) Biswajit Mukherjee, Department of Pharmaceutical Technology, Jadavpur University, for his valuable guidance, encouragement, support, and inspiration throughout the work. His unremitting advice, affection, and help will be responsible for the successful completion of this work.

I express my heartfelt thanks to my laboratory seniors, especially Rima Chandra, Alankar Mukherjee for their support. I also thank Dr. Ramkrishna Sen, Ashique Al Hoque, Manisheeta Ray, Mrinmoy Barman, Ratna Roy, Sandipan Mallick, Pintu Rana, and many others for their valuable support and cooperation, which made my work fruitful.

I would also like to extend my gratitude to my fellow classmate Srijita Gope, who helped me in various ways during my research work.

I extend my special thanks to my mother, Indu Yadav, and father, Yogendra Yadav, for their support, love, and encouragement. I would like to extend my special thanks to Jyoti Bala for her support, love and encouragement. I would also like to acknowledge the IACS(Kolkata) and the Department of Physics (Jadavpur University), Kolkata, for their assistance in providing me with instrument facilities for my research work.

I want to thank UGC (University Grants Commission) for providing me with a scholarship throughout my academic sessions.

Last but not least, I would like to express my gratitude towards my parents and friends for their kind cooperation and encouragement, which have helped me in every step of my academic career.

Dedicated to my guide and my family

LIST OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

AIM OF THE RESEARCH WORK

AIMS

This study focuses on the preparation and evaluation of a liposomal formulation of nilotinib, aimed at improving its therapeutic efficacy and reducing side effects in the treatment of Chronic Myeloid Leukemia (CML). Lipid nanoparticle-encapsulation is explored to enhance drug delivery, targeting, and bioavailability. We focus on the sustained release of the drug from the formulation to get lower drug toxicity and more extended drug action.

The main aim of the research work is

- To develop a stable lipid nanoparticles formulation of nilotinib.
- To prepare of standard curve of nilotinib.
- To determine the stability of the particle using a zeta sizer.
- To determine the average particle size of the liposomal formulation by DLS (Dynamic Light Scattering).
- To determine the chemical interaction between the drug and excipient or between excipients by FTIR (Fourier Transform Infrared Spectroscopy).
- To determine the surface morphology of the formulation by FE-SEM (Field emission scanning electron microscope).
- To determine drug loading and entrapment efficiency, check how much of the drug is incorporated into the prepared liposomal formulation.
- To determine an In-vitro drug release study for the evaluation of release kinetics of the lipoformulation prepared formulation.

CHAPTER 2

INTRODUCTION

INTRODUCTION

Nanotechnology as a drug delivery is a promising approach for the efficient delivery of drugs inside the body. The major aim of technology is to release the active and efficient molecules of drugs for site-specific action with accurate doses. Using nanotechnology, it may be possible to improve the delivery of poorly water-soluble drugs; targeted delivery of drugs in a cell- or tissue-specific manner; transcytosis of drugs across tight epithelial and endothelial barriers; delivery of large macromolecule drugs to intracellular sites of action; co-delivery of two or more drugs or therapeutic modality for combination therapy; real-time read on the *in vivo* efficacy of a therapeutic agent. (1)

Lipid nanoparticles were the first compounds investigated as drug carriers. They are colloidal carriers, usually with a size range of 80–300 nm. Lipoformulations have been reported to show a strong potential for effective drug delivery to the site of action by increasing the solubility of drugs and improving their pharmacokinetic properties, such as the therapeutic index of chemotherapeutic agents, rapid metabolism and a significant reduction of harmful side effects. (2)

2.1 CHRONIC MYELOID LEUKEMIA

Chronic myeloid leukemia (CML) is a type of cancer that affects the blood and bone marrow. It's characterized by the uncontrolled growth of myeloid cells, which are a type of white blood cell. In CML, this abnormal cell growth is primarily driven by a genetic abnormality called the Philadelphia chromosome, which results from a translocation between chromosomes 9 and 22. This translocation leads to the formation of a fusion gene called BCR-ABL1, which produces a protein that promotes cell growth and division. (3)

BCR is a ubiquitously expressed cytoplasmic protein with multiple functionalities. ABL1 is also ubiquitously expressed and has several functions, including inhibition of cell cycle progression and proliferation, integrin signaling, and DNA repair. While ABL1 kinase activity is tightly controlled in physiological conditions, the chimeric BCR/ABL1 protein is constitutively active and re-localized to the cytoplasm. BCR/ ABL1 activates numerous downstream pathways leading to increased proliferation, reduced apoptosis, abnormal adhesion and migration, and genetic instability. The introduction of BCR/ABL1 tyrosine kinase inhibitors (TKIs) revolutionized the treatment of CML and started the era of targeted therapies in oncology. (4)

2.1.1 TYPES OF CHRONIC MYELOID LEUKEMIA (5)

Chronic myeloid leukemia (CML) is mostly categorized according to the stage at which it is progressing.

A. Chronic Phase CML: This is the first stage of the illness, marked by a larger percentage of mature myeloid cells in the blood and bone marrow and a more indolent history. Initially, patients in this stage may not show any symptoms at all, and the illness is frequently found by chance when doing routine blood testing.

B. Accelerated Phase CML: In this stage, the disease starts to advance more quickly. Spleen enlargement, fever, weight loss, and increased tiredness are some of the intensifying symptoms that patients may encounter. There may be an increase in the proportion of immature white blood cells, or blast cells, in the bone marrow and blood, which suggests a higher chance of developing acute leukemia.

C. Blast Phase (or Blast Crisis) CML: This is the most advanced stage of CML, characterized by the rapid expansion of blast cells in the blood and bone marrow. Patients in the blast phase often have a worse prognosis and can need more intense care, such as stem cell transplantation or chemotherapy.

2.1.2 EPIDEMIOLOGY

In 2018, 15% of all adult instances of leukemia were diagnosed with CML, or one new case for every 100,000 persons worldwide. India has an annual incidence of CML ranging from 0.8 to 2.2 per 100,000 persons, according to recent research. In India, this is the most prevalent kind of leukemia, constituting between 30% - and 60% of all cases. Around 91- 96% of CML patients, the Ph' chromosome is present. (6)

The worldwide prevalence of CML is not well known but has been estimated to be 10-12/ 100,000 inhabitants, with a steady increase due to the dramatic improvement in survival of these patients. (7)

There is no evidence of a genetic inclination in any particular person, and there are few case reports of familial CML. It has been determined that certain lifestyle choices, such as smoking and having a high body mass index, may increase the risk of CML. Though not perfect, there has been consistent evidence linking de novo CML to chemical exposure to benzene, organic solvents, alkylating agents, topoisomerase II inhibitors, or other chemotherapeutic agents. (4)

2.1.3 ETIOLOGY

The ABL gene is the human homologue of the v-ABL oncogene carried by the Abelson murine leukemia virus (A-MuLV), and it encodes a nonreceptor tyrosine kinase. Human ABL is a ubiquitously expressed 145-kd protein with 2 isoforms arising from alternative splicing of the first exon. Thus, the normal ABL protein is involved in the regulation of the cell cycle, in the cellular response to genotoxic stress, and in the transmission of information about the cellular environment through integrin signaling. Overall, it appears that the ABL protein serves a complex role as a cellular module that influences decisions in regard to cell cycle and apoptosis. (8)

The 160-kd BCR protein, like ABL, is ubiquitously expressed. The first N-terminal exon encodes a serine-threonine kinase. The only substrates of this kinase identified so far are BAP-1 and BCR itself. The center of the molecule contains a region with DBL-like and Pleckstrin-homology (PH) domains that stimulate the exchange of guanidine triphosphate (GTP) for guanidine diphosphate (GDP) on Rho guanidine exchange factors, which in turn may activate transcription factors such as NF-kB. The C-terminus has GTPase activity for RAC, a small GTPase of the RAS superfamily that regulates actin polymerization and the activity of an NADPH oxidase in phagocytic cells. In addition, BCR can be phosphorylated on several tyrosine residues, especially tyrosine 177, which binds GRB-2, an important adapter molecule involved in activating the RAS pathway. (9)

2.1.4 BCR-ABL1 ONCOGENE

ABL proteins are nonreceptor tyrosine kinases essential for signal transduction and cell growth regulation. The N-terminal segment of ABL includes SH2 and SH3 domains, which regulate tyrosine kinase function. SH2 is crucial for phosphor-tyrosine binding, while SH3 negatively regulates tyrosine kinase activity. The C-terminal part contains DNA-binding domains, nuclear localization signals, and an actin-binding site. Structural alterations in ABL and BCR contribute

to leukemogenicity transformation in BCR-ABL. (10) The N-terminal coiled-coil motif of BCR enhances tyrosine kinase activity and F-actin binding. The serine-threonine kinase domain of BCR activates ABL-mediated signaling pathways. The N-terminal fusion of BCR to ABL disrupts the SH3 regulatory domain, making ABL constitutively active. BCR-ABL proteins (p210 and p190) exhibit higher tyrosine phosphokinase activity than normal ABL (p145). (11)

The fusion of the ABL1 and BCR genes resulting from the reciprocal translocation t (9;22) (q34; q11.2) gives rise to the BCR-ABL1 oncogene that encodes for the constitutively active BCR-ABL1 tyrosine kinase. BCR-ABL1 can transform hematopoietic stem cells but not committed progenitors lacking self-renewal capacity. The breakpoint within ABL1 at 9q34 occurs in nearly all patients upstream of exon 2, with some variability in the specific site either upstream of exon 1b, downstream of exon 1a, or more frequently between the two. Breakpoints within BCR localize to three main breakpoint cluster regions. In most patients with CML and in one-third of those with Ph-positive acute lymphoblastic leukemia (ALL), the breakpoint maps to the major breakpoint cluster region (M-BCR), which spans BCR exons 12–16 (formerly called b1–b5), giving rise to a fusion transcript with either e13a2(b2a2) or e14a2(b3a2) junctions that translates into a 210-kDa protein (p210BCR-ABL1). In two-thirds of patients with Ph-positive ALL and rarely in CML, the breakpoints within BCR localize to an area of 54.4 kb between exons e2′and e2, termed the minor breakpoint cluster region (m-BCR), which translates to a 190-kDa protein (p190BCR-ABL1). A third breakpoint cluster region (μ-BCR) has been identified, giving rise to a 230-kDa fusion protein (p230BCR-ABL1), associated with a more indolent CML course and with a phenotype more akin to chronic neutrophilic leukemia. The translation product is a protein of 230 kd termed p230 BCR–ABL. The expression of p190 BCR–ABL in CML may be associated with monocytosis, and the expression of p230 BCR–ABL may be associated with the chronic neutrophilic leukemia variant and with thrombocytosis. (12)

2.1.5 PATHWAYS OF BCR–ABL SIGNALLING

I. **Ras and the MAP kinase pathways:** BCR-ABL can activate Ras through autophosphorylation at tyrosine 177, creating a docking site for Grb-2. Grb-2, binding to SOS, stabilizes Ras in its active form. Shc and Crkl also activate Ras by binding to BCR-ABL, though Crkl's role is primarily in fibroblasts and not myeloid cells. MAP Kinase Pathways Activated Ras recruits Raf, leading to a kinase cascade through Mek1/2 and Erk, ultimately activating gene transcription. Ras activation is critical in Ph-positive leukemia,

evidenced by the rarity of activating mutations in this leukemia. This suggests that the Ras pathway is constitutively active due to BCR-ABL. (13)

II. **Jak-Stat pathway:** The first evidence for the Jak-Stat pathway's involvement came from studies on v–ABL–transformed B cells. Constitutive phosphorylation of Stat transcription factors (Stat1 and Stat5) was observed in BCR-ABL-positive cell lines and primary CML cells. Stat5 activation contributes to malignant transformation, primarily by providing anti-apoptotic signals through the transcriptional activation of Bcl-xL. Unlike physiological activation, BCR-ABL can directly activate Stat1 and Stat5 without Jak proteins' prior phosphorylation. (14) Stat6 activation is specific to P190-BCR-ABL proteins, which may explain the predominantly lymphoblastic phenotype in certain leukemias. BCR-ABL transforms growth factor-dependent cell lines to become factor-independent by activating both the Ras and Jak-Stat pathways. There is evidence of an autocrine loop in BCR–ABL–induced secretion of growth factors, specifically IL-3 and G-CSF, and increased expression of growth factor receptors like the oncostatin M β receptor. The Jak-Stat pathway, particularly through Stat5, plays a significant role in the anti-apoptotic mechanisms and growth factor independence of BCR-ABL-positive cells, contributing to leukemogenesis. (15)

III. **PI3 kinase pathway:** BCR-ABL forms complexes with PI3 kinase, CBL, CRK, and CRKL, resulting in PI3 kinase activation. The serine-threonine kinase AKT, downstream of PI3 kinase, is implicated in anti-apoptotic signaling. AKT phosphorylates and inactivates the pro-apoptotic protein BAD, preventing it from binding to anti-apoptotic proteins like BclXL, thus promoting cell survival. BCR-ABL may mimic IL-3 survival signals through PI3 kinase, promoting cell survival in a similar manner. BCR-ABL affects phospho-inositol metabolism by activating inositol phosphatases Ship-1 and Ship-2, which respond to growth factor signals, creating a balance akin to normal growth factor stimulation. In summary, BCR–ABL–positive cells rely on PI3 kinase and Akt for proliferation and survival, with BCR-ABL mimicking normal growth factor signals to maintain anti-apoptotic mechanisms. (16)

IV. **Myc pathway:** Myc activation by BCR-ABL depends on the SH2 domain. Overexpression of Myc can partially rescue transformation-defective SH2 deletion mutants, while a dominant-negative Myc mutant suppresses transformation. The role of Myc in Ph-positive cells are likely mirrors its function in other tumors, acting as either a proliferative or apoptotic signal depending on the context. In CML cells, the apoptotic effects of Myc are probably counterbalanced by other mechanisms, such as the PI3 kinase pathway. In

summary, Myc plays a critical role in the proliferation and survival of BCR-ABL-positive cells, with its activation linked to the SH2 domain of BCR-ABL and its function modulated by other cellular pathways to prevent apoptosis. (17)

2.1.6 SYMPTOMS OF CML: (18)

1. **Abnormal Growth and Distribution**: The myeloid progenitor cells increase in number and are found in places outside the bone marrow (extramedullary locations). This expansion happens because the cells' ability to multiply has been altered, leading to an imbalance between self-renewal and differentiation. This means more progenitor cells are produced while the number of stem cells decreases.

2. **Loss of Regulation**: As these progenitor cells continue to grow, they become less responsive to the body's normal growth-regulating signals. This loss of regulation means they can proliferate without control.

3. **Defective Adherence**: Normally, progenitor cells stick to the bone marrow structure, but in CML, they do not adhere properly, allowing them to enter the bloodstream prematurely. This adherence is usually mediated by integrins, which are proteins on the cell surface that help the cells attach to their environment. In CML, this process is disrupted.

4. **Resistance to Death**: CML cells can avoid the normal process of cell death (apoptosis), partly due to the activity of a protein called p210 BCR-ABL. This protein helps the cells survive without the usual growth signals and makes them resistant to treatments like chemotherapy.

5. **Influence of Cytokines**: Certain proteins, called cytokines, are overproduced in CML patients and contribute to the abnormal growth of progenitor cells. For example, high levels of interleukin-1β in advanced CML can stimulate further cell proliferation.

2.1.7 DISEASE TRANSFORMATION IN CML:

Transformation of chronic myeloid leukemia (CML) to more advanced stages is often marked by resistance to treatment, high white blood cell counts, increased blasts in blood and bone marrow, abnormal platelet counts, unexplained fever, enlarged spleen, extramedullary disease, weight loss, and bone/joint pain. Cytogenetic and molecular changes occur in 50-80% of patients during the transition to the accelerated and blast phases.

Cytogenetic Changes: (19)

- Minor changes: Monosomies of chromosomes 7, 17, and Y; trisomies of chromosomes 17 and 21; and translocation t(3;21)(q26;q22).
- Major changes: Trisomy 8 (most common), isochromosome i(17q) (almost exclusive to myeloid blast phase), trisomy 19, and double Ph chromosome.

Molecular Abnormalities: (20)

- Correspond to cytogenetic changes and include abnormalities in genes such as p53, RB1, c-MYC, p16INK4A, RAS, and the AML–EVI-1 fusion protein from t(3;21) translocation.
- p53 abnormalities (20-30% of blast phase patients) are linked to myeloid transformation and associated with aberrant methylation.
- RB1 abnormalities are more associated with lymphoid transformation.
- Up to 50% of patients with lymphoid transformation have a homozygous deletion of p16INK4A.
- Altered methylation, including transcriptional silencing of the calcitonin gene and changes within the m-BCR region, is also noted during the progression from chronic to blast phase.

2.1.8 DIAGNOSIS AND MONITORING OF CML IN PATIENTS WITH BCR-ABL

Cytogenetic analysis detects the Philadelphia (Ph) chromosome in 90% of CML patients and is the standard diagnostic test, useful for identifying additional abnormalities (clonal evolution). However, it is labor-intensive, examining only 20-25 metaphase cells per sample. In the remaining 10% of patients without a detectable Ph chromosome, molecular analysis can identify BCR-ABL fusion products in about half. (21)

Genomic PCR and Southern blot analysis determine the exact DNA breakpoints of fusion products, while RT-PCR and Northern blot detect BCR-ABL transcripts at the RNA level. The p210BCR-ABL protein is identified using specific antibodies in immunoprecipitation or Western blot analysis. (22)

Monitoring treatment involves PCR and fluorescence in situ hybridization (FISH) for BCR-ABL. Quantitative RT-PCR is used post-stem cell transplantation, though its use with interferon-α therapy is debated. FISH analyses both metaphase and interphase cells quickly and quantifiably from peripheral blood samples, avoiding marrow aspiration. Despite its advantages, FISH has up

to a 10% false positive rate and is ineffective if fewer than 10% of cells are Ph+. Hyper-metaphase FISH analyses up to 500 metaphase cells per sample without false positives but cannot use blood samples. A double-color probe FISH for blood samples shows improved sensitivity and specificity. CML patients on interferon-α undergo bone marrow cytogenetic studies every 6-12 months and peripheral blood interphase FISH every three months until less than 10% of cells are Ph-positive, followed by hyper-metaphase FISH for further monitoring. (23)

2.1.9 CURRENT THERAPIES IN THE TREATMENT OF CML

A. TYROSINE KINASE INHIBITORS

TKIs are the standard treatment for newly diagnosed CML patients across all disease phases. Six TKIs are currently approved: imatinib, dasatinib, nilotinib, bosutinib, ponatinib, and radotinib (available only in South Korea). These drugs are categorized into three generations based on their development and efficacy. Each TKI has unique properties regarding potency, activity against BCR/ABL1 mutants, off-target effects, pharmacokinetics, and adverse effect profiles. (12)

I. **First-generation TKI:**

- **Imatinib:** Revolutionized CML therapy since 2001, transforming it from a fatal condition to a manageable chronic disease. Initial studies showed its superiority over older treatments like IFN-α and cytarabine. Dose optimization studies suggested a median effective dose of around 600 mg daily. (14)

II. **Second-generation TKIs:**

- **Dasatinib:** Effective in patients who failed imatinib. Recommended doses are 100 mg daily for CML-CP and 140 mg daily for AP/BP-CML. Phase 3 studies showed its superiority over imatinib in achieving molecular and cytogenetic responses. (24)
- **Nilotinib:** Initially successful in imatinib-resistant patients. Approved for frontline therapy of CML-CP with 300 mg twice daily. Notable for improving progression-free survival but associated with cardiovascular adverse events like hyperglycemia and hyperlipidemia. (25)
- **Bosutinib:** Initially approved for third-line therapy. Frontline trials showed mixed results, with a reduced dose (400 mg daily) later proving more effective in achieving molecular responses but not significantly impacting overall survival. (26)

III. **Third-generation TKI:**

- **Ponatinib:** Designed to target the BCR/ABL1T315I mutant, which is resistant to first and second-generation TKIs. Potent but less selective, leading to inhibition of multiple kinases and associated cardiovascular toxicity. Dose adjustments are recommended to balance efficacy and reduce toxicity, as demonstrated in the OPTIC trial. (27)

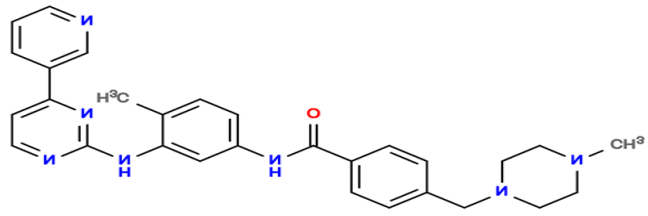
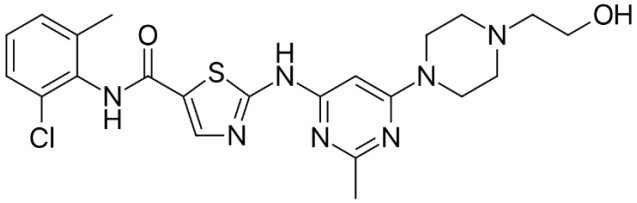
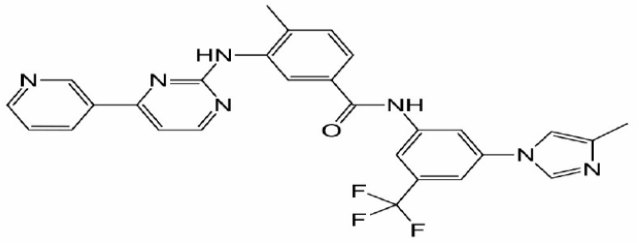
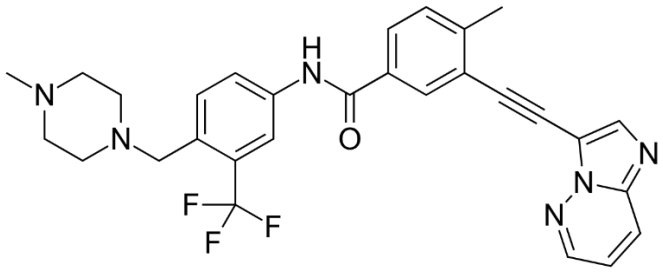
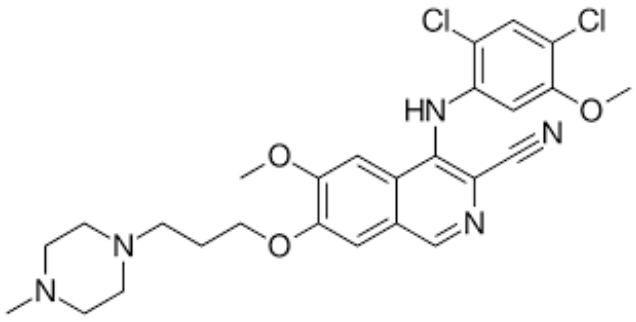
B. INTERFERON-α (IFN-α)

IFN was that standard of care drug therapy prior to the introduction of TKIs, and continues to be an option for the rare patients who are intolerant of all TKIs. It remains the safest option to maintain responses in women who plan to become pregnant. There is also data showing that combining IFN-α with TKIs can improve the depth of molecular responses. (28)

C. HEMATOPOIETIC STEM CELL TRANSPLANT (HSCT): (29)

- Recommended for patients progressing to the accelerated phase (AP) or blast phase (BP) and those with TKI resistance. Better outcomes if performed during the second chronic phase.
- **Donor Stem Cell Source:** Peripheral blood is preferred for AP/BP-CML; bone marrow is preferred for chronic phase (CP-CML) to reduce graft-versus-host disease.
- **Pre-Transplant Therapy:** Imatinib before HSCT may lower chronic graft-versus-host disease and relapse risks. Post-transplant TKI therapy is recommended for high relapse-risk patients despite potential myelosuppression.

Table 8: List of tyrosine kinase inhibitors along with their structure, half-life and daily dose

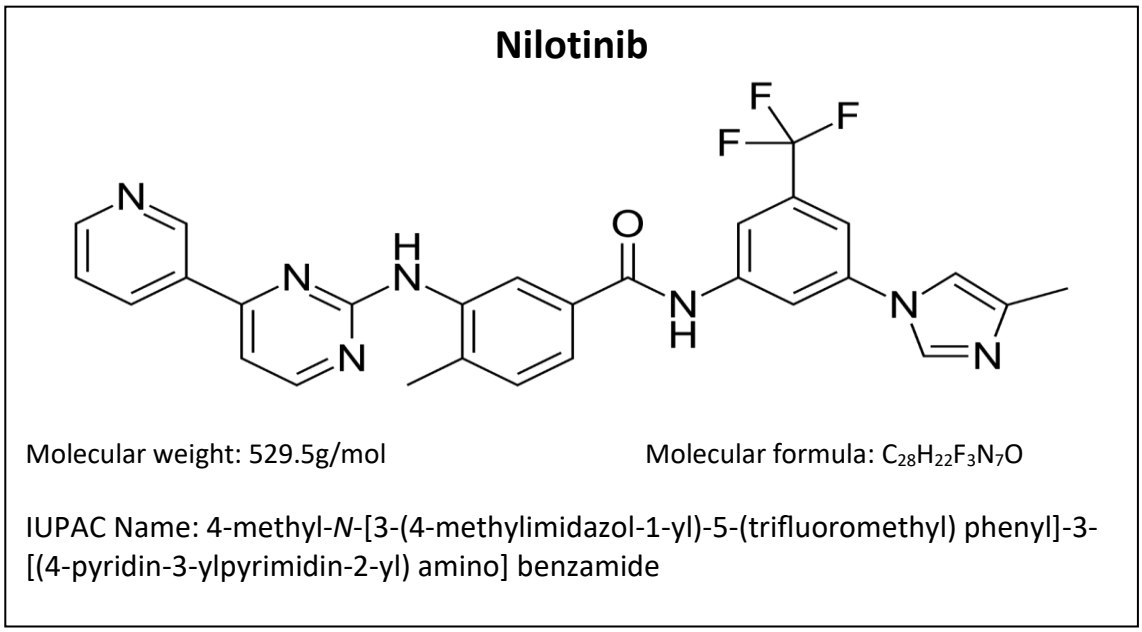
TKI	Structure	Half Life	Daily dose
Imatinib		18 hours	400 mg daily
Dasatinib		3-5 hours	100 mg once daily
Nilotinib		17 hours	300 mg twice daily
Ponatinib		12-66 hours	45 mg once daily
Bosutinib		22 hours (oral)	400 mg once daily

2.2 NILOTINIB

Nilotinib (Tasigna) is an orally administered, selective inhibitor of BCR-ABL tyrosine kinase, including most imatinib-resistant BCR-ABL mutants. Novartis developed Nilotinib. It was developed based on the structure of the ABL-imatinib complex to address imatinib intolerance and resistance. It was approved for medical use by the FDA in October 2007. Nilotinib is a member of (trifluoromethyl)benzenes, a member of pyrimidines, a member of pyridines, a member of imidazole, a secondary amino compound, and a secondary carboxamide. It has a role as an antineoplastic agent, a tyrosine kinase inhibitor, and an anti-coronaviral agent. (30)

2.2.1 PHYSICOCHEMICAL NATURE

Nilotinib is a white to slightly yellowish powder. It is practically insoluble in water but shows solubility in organic solvents such as dimethyl sulfoxide (DMSO) and ethanol. The melting point of nilotinib is typically in the range of 236-238°C. Nilotinib has a relatively high hydrophobicity, which is reflected in its partition coefficient (log P value). This property affects its solubility and absorption characteristics. It is practically insoluble in water, with better solubility in organic solvents. Nilotinib is chemically stable under normal storage conditions but should be protected from light and moisture to prevent degradation. (31)



Molecular weight: 529.5g/mol Molecular formula: $C_{28}H_{22}F_3N_7O$

IUPAC Name: 4-methyl-*N*-[3-(4-methylimidazol-1-yl)-5-(trifluoromethyl) phenyl]-3-[(4-pyridin-3-ylpyrimidin-2-yl) amino] benzamide

Figure 2: Structure of Nilotinib with is IUPAC name, molecular weight and molecular formula

2.2.2 MECHANISM OF ACTION

Nilotinib is a Kinase Inhibitor. The mechanism of action of nilotinib is as a BCR-ABL Tyrosine Kinase Inhibitor, Cytochrome P450 2C8 Inhibitor, Cytochrome P450 2D6 Inhibitor, Cytochrome P450 2B6 Inducer, and Cytochrome P450 2C8 Inducer, and UGT1A1 Inhibitor, and P-Glycoprotein Inhibitor. (32)

2.2.3 PHARMACODYNAMIC PROFILE

Nilotinib is an aminopyrimidine derivative that inhibits the tyrosine kinase activity of the chimeric protein BCR-ABL. Nilotinib acts via competitive inhibition at the triphosphate (ATP)-binding site of BCR-ABL protein in a similar manner to imatinib. However, nilotinib has a higher binding affinity for the ABL kinase. Nilotinib binds to an inactive protein conformation in which the P-loop of ABL occupies a region that ATP would occupy in the active conformation. Once bound to the ATP-binding site, nilotinib inhibits tyrosine phosphorylation of proteins involved in BCR-ABL-mediated intracellular signal transduction. The inhibitory activity of nilotinib in CML lines is markedly higher than that of imatinib. In imatinib-sensitive CML cell lines, the activity of nilotinib is 10–60 times that of imatinib. (33)

2.2.4 PHARMACOKINETIC PROFILE

During the dose-escalation portion of the phase I trial, it was noted that plasma concentrations peaked at 600 mg, with no significant increase at higher doses. This suggested saturation of a carrier and prompted the institution of a twice-daily dosing regimen. Indeed, a dose of 400 mg twice daily resulted in higher plasma levels compared with 800 mg taken as a single dose. At the recommended dosage of nilotinib 400 mg twice daily, the mean peak serum concentration of 3.6 μmol/L was achieved after a median time of 3 hours. The serum trough concentration at steady state was 1.7 μmol/L at this dosage. More than 90% of an administered dose is eliminated in the feces within 7 days. The apparent plasma half-life of nilotinib is 17 h, and some 98% of the drug is protein-bound. Its absorption is increased by food, particularly fatty meals. (25)

The concurrent administration of a single dose of nilotinib and ketoconazole in healthy volunteers led to a threefold increase in nilotinib's systemic exposure, indicating that the CYP3A4 inhibitor influenced its metabolism. In a similarly designed study where single doses of nilotinib and midazolam were given together, there was a 30% increase in midazolam's systemic exposure, suggesting that nilotinib may act as a weak CYP3A4 inhibitor. (34)

2.2.5 DRUG INTERACTIONS

The enzyme CYP3A4 of the cytochrome P450 family metabolizes nilotinib. As such, co-administering inhibitors of CYP3A4 with ketoconazole or grapefruit juice has been demonstrated to result in increased exposure to nilotinib. On the other hand, medications that increase CYP3A4 expression, such as rifampicin, considerably reduce exposure to nilotinib. (31)

By itself, nilotinib inhibits CYP2C8, CYP2C9, CYP2D6, CYP3A4, and UGT1A1. Consequently, care should be used when administering nilotinib with medications that are metabolized by these enzymes, such as vitamin K-antagonists. Combining nilotinib with drugs that also prolong the QT interval (such as amiodarone or sotalol) can increase the risk of cardiac arrhythmias. Low levels of potassium or magnesium can exacerbate nilotinib's cardiac effects. (35)

2.2.6 TOXICITY

Nilotinib demonstrates a generally favorable toxicity profile based on phase II and III studies. Common non-hematological side effects included rash and headache, with severe rash being rare. Hematological toxicities were more frequent with second-line nilotinib, with notable rates of severe anemia, neutropenia, and thrombocytopenia. Biochemical abnormalities like elevated lipase, ALT, bilirubin, and hyperglycemia were typically self-limited. Preclinical studies indicated QTc prolongation, necessitating frequent ECG monitoring, but long-term follow-up showed no severe QTc prolongation or Torsade de Pointes. (25)

However, there was an increased incidence of peripheral artery occlusive disease (PAOD) and other cardiovascular events in nilotinib-treated patients compared to those on imatinib. The risk of atherosclerosis was noted, with higher cholesterol and LDL levels in nilotinib patients. The prospective analysis confirmed higher rates of PAOD in nilotinib-treated patients. Monitoring for PAOD and cardiovascular risk factors is recommended. Overall, nilotinib is considered safe, with most severe adverse events manageable through dose adjustments, though caution is advised for patients with cardiovascular risks. (36)

2.3 LIPID NANOPARTICLES

Liposome/lipo-formulations, also known as lipid vesicles or sometimes referred to simply as vesicles, are microscopic spherical structures composed of one or several lipid bilayers entrapping a small volume of the aqueous phase. The aqueous core can entrap a Hydrophilic drug, whereas

the lipophilic surface can entrap hydrophobic drugs. The size of the liposome/lipo-formulation ranges from 30nm to micrometer, and the phospholipid bilayer is 4-5nm thick. (37) They are particularly notable for their applications in drug delivery, gene therapy, and as adjuvants in vaccines. Lipid nanoparticles (lipo-particles) are usually solid particles containing drugs. Both give outstanding stability to the encapsulated drug from physiological degradation, extend the half-life of the drug, control the release of drug molecules, and provide sustained release, and lipid nanoparticles and liposome/lipo-formulations are excellent biocompatible and safe. They can selectively deliver the drug to the target site through passive or active targeting, thus decreasing the systemic side effects, elevating the maximum tolerated dose, and improving the therapeutic benefits. (38)

2.3.1 PHYSIOCHEMISTRY OF LIPOFORMULATIONS

Liposome/lipo-formulations are primarily made of phospholipids, which are amphiphilic molecules with hydrophilic heads and hydrophobic tails. In aqueous solutions, these phospholipids naturally form bilayers due to their dual affinity, with the hydrophilic heads facing the water and the hydrophobic tails facing inward, creating a lipophilic inner compartment that acts as a barrier. Hydrophobic interactions and van der Waals forces hold the bilayers together, while hydrogen bonds and polar interactions with water stabilize them. The final lipid structure depends on the lipids' nature, concentration, temperature, and shape and can encapsulate ions or molecules present during formation. (39). Lipo-particles are lipid particles that encapsulate drugs.

2.3.2 TYPES OF LIPOFORMULATIONS (40)

Liposome/lipo-formulations can be classified by preparation method (e.g., reverse-phase evaporation or vesicle extrusion), size (small, intermediate, or large), and lamellarity (uni-, oligo-, or multilamellar vesicles).

1. Small Unilamellar Vesicles (SUVs): These are single-layered liposome/lipo-formulations with diameters typically ranging from 20 to 100 nm.
2. Large Unilamellar Vesicles (LUVs): These are larger single-layered vesicles, with diameters ranging from 100 nm to several micro meters.
3. Multilamellar Vesicles (MLVs): These liposome/lipo-formulations have multiple lipid bilayers, like an onion, with diameters typically ranging from 100 nm to several micro meters. Lipid nanoparticles are solid lipid nano size particles, contains drugs.

2.3.3 ADVANTAGES OF LIPOFORMULATIONS (41)

- Lipo-formulations increase the efficacy and therapeutic index of the drug
- Lipo-formulations increase stability via encapsulation
- Lipo-formulations are non-toxic, flexible, biocompatible, and completely biodegradable for systemic and non-systemic administration
- Lipo-formulations reduce the toxicity of the encapsulated agent
- Lipo-formulations reduce the exposure to toxic chemicals in the human body
- Lipo-formulations have the flexibility to couple with site-specific ligands to achieve activity.

2.3.4 DISADVANTAGES OF LIPOFORMULATIONS (42)

- The solubility of the drug sometimes decreases when incorporated into the liposome/lipo-formulation
- A liposome/lipo-formulation has a shorter half-life
- The tendency of phospholipids to undergo oxidation is higher
- There may be leakage or fusion of encapsulated drug
- The production cost of liposome/lipo-formulations is high, although lipo-particle is somewhat low.

2.3.5 COMPONENTS OF LIPOFORMULATIONS USED IN THIS STUDY

Cholesterol: It is a lipid molecule with the chemical formula $C_{27}H_{46}O$. Cholesterol has a steroid nucleus composed of four fused hydrocarbon rings (A, B, C, and D rings). This core structure is common to all steroids and consists of three six-membered cyclohexane rings (A, B, and C) and one five-membered cyclopentane ring (D). At the 3-position of the A-ring, cholesterol has a hydroxyl (OH) group, which is characteristic of all sterols. This hydroxyl group makes cholesterol an alcohol and imparts slight polarity to the molecule. Cholesterol has a branched aliphatic hydrocarbon tail attached at the 17-position of the D-ring. This hydrophobic tail influences the molecule's solubility and its interaction with other lipid molecules. Cholesterol is amphipathic due to its hydrophilic hydroxyl group and hydrophobic hydrocarbon body. However, it is mostly hydrophobic and is insoluble in water but soluble in organic solvents such as chloroform and ethanol. Cholesterol is one of the main components of liposome/lipo-formulations and can be used in almost all liposome/lipo-formulations and even in lipo-particles. The addition of cholesterol can promote the packing of lipid chains and bilayer formation, modulate the fluidity and rigidity of the membrane, and further affect drug release, stability of lipoparticles, and kinetics of exocytosis. The effects of cholesterol on bilayer

properties are concentration-dependent. Low (2.5 mol %) and high (>30 mol %) concentrations of cholesterol showed little effect on the properties of the lipid bilayer. (43)

Soya Lecithin: Soya lecithin, also known as soy lecithin, is a complex mixture derived from soybean oil. Lecithin generally refers to various triglycerides, Phospholipids, and glycolipids. The primary components of soya lecithin are phospholipids, which include Phosphatidylcholine (PC) {major component that contributes to the emulsifying properties}, Phosphatidylethanolamine (PE), Phosphatidylinositol (PI), Phosphatidylserine (PS). Along with phospholipids, soya lecithin contains triglycerides, which are fatty acid esters of glycerol. It may also contain small amounts of carbohydrates, sterols, and free fatty acids. For liposome/lipo-formulation or lipo-particle formulation, lecithin sources other than soybean carry certain drawbacks. Therefore, lecithin from these sources is less stable when compared to soybean lecithin and other plant-derived lecithin. It has the MW-758.1 with a total polar surface area (TPSA) is 111 $Å^2$. Due to its less polar surface area, it is lipophilic in nature, and thus, the lipophilic drug will be encapsulated into the lipid bilayer encapsulate into the lipid bilayer. (44)

CHOLESTEROL **SOYA LECITHIN**

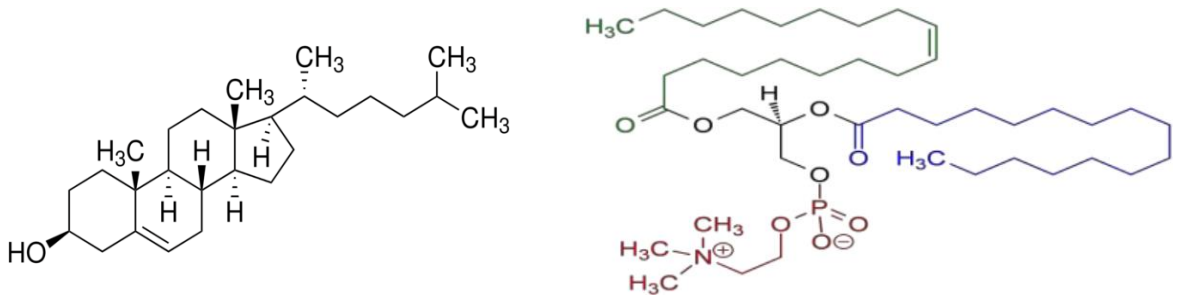


Figure 3: Structure of Cholesterol and soya lecithin

Cholesterol and soya lecithin both are biocompatible and biodegradable in nature. None of them have any serious toxic effects when given as a drug delivery system. There is inadequate evidence of human carcinogenicity of cholesterol. There are no such reported serious interactions in-between cholesterol, soya lecithin, and drug (Nilotinib), which can inhibit the release of drug from the formulation.

2.3.6 METHOD OF PREPARATION OF LIPOFORMULATIONS

The general method of preparation involves four steps for the preparation of lipo particles(45).

- Drying down lipids from organic solvents
- Dispersion of lipids in aqueous media
- Purification of resultant lipo-particle/ liposome/lipo-formulations
- Analysis of the final product

THIN FILM HYDRATION METHOD (BANGHAM METHOD)

It involves dissolving all lipids and hydrophobic drugs in an organic solvent using a round-bottom flask. The solvent is gently evaporated under reduced pressure, forming a thin film. This film is then hydrated with an aqueous buffer solution at a temperature above the lipid's transition temperature (Tm). The buffer solution can contain hydrophilic drugs to be encapsulated in the lipo-particle/liposome/lipo-formulation's aqueous core. A slower rate of hydration improves drug encapsulation efficiency. The extrusion method ensures stable lipo-particles or liposome/lipo-formulations with more encapsulation efficiency over sonication. (44)

2.3.7 EVALUATION OF LIPOPARTICLES

The characterization of the liposomal or lipo-particle formulation guarantees their consistent in vitro and in vivo performances. Physical, chemical, and biological factors are the three steps that make up the lipoparticle characterization process. Size, shape, surface, and drug release profile are examples of physical characterization characteristics. The purification and potency of different lipophilic ingredients are studied as part of chemical characterization, and the efficacy and suitability of a formulation for therapeutic use are established with the use of biological characterization measures. (40)

1 **Lipoparticle size and size distribution:** The key characteristics of lipoparticles are its vesicle size and size distribution. The drug release may be impacted by variations in lipoparticle size. Drug release will occur quickly as a result of the smaller lipoparticles' drugs being exposed to the particle surface. During lipoparticle dispersion storage and transit, smaller particles tend to congregate. Atomic force microscopy (AFM), transmission electron microscopy (TEM), and scanning electron microscopy (SEM) may all be used to examine the size and shape of lipoparticle particles. (46)

2 **Surface charge of lipoparticle (Zeta potential):** The way that lipoparticles interact with their biological surroundings is determined by their zeta potential. Zeta potential is used to assess the colloidal stability of lipoparticles this possibly serves as an indirect indicator of

surface charge. Zeta potential values are measured to offer an evaluation of the lipoparticle's storage stability. High values, either positive or negative, are recommended to preserve optimal stability and avoid particle aggregation. The total charge that a lipid vesicle accumulates in a certain medium is known as its zeta potential. It quantifies the force of attraction or repulsion between lipid vesicles in particular and other particles in general. (47)

3 **Lipoparticle shape:** The internalization, targeting, degradation, and transportation of drugs are all significantly impacted by the structure of lipoparticles. It has been noted that carrier form affects the rate at which drug-delivery vehicles interface with macrophages. A variety of electron microscopy methods are employed to evaluate the lipoparticles' shape. Field emission scanning electron microscopy (FESEM) may be used to evaluate the lipoparticle surface morphology because it provides high-resolution images of the lipoparticle surface and provides information on the formulation's overall composition, including the presence or absence of aggregates or lumps in the sample. (48)

4 **Lamellarity (for liposome/lipoformulations):** P-NMR and freeze-fracture electron microscopy (also known as cryo-TEM) can be used to measure the average number of bilayers present in liposome/lipoformulations. In a subsequent method, the signals with the outer leaflet of the outermost bilayers are recorded both before and after the addition of a broadening agent, like manganese ions. The use of freeze-fracture electron microscopy to examine the structural characteristics of aqueous lipid dispersions has grown in popularity in recent years. (49)

5 **Drug loading:** An effective nano liposomal system is defined by its high loading capacity, which lowers the quantity of matrix material that needs to be administered. The solid-state drug solubility in the polymer determines drug loading and entrapment efficiency. This solubility is influenced by the molecular weight, composition, drug-polymer interaction, and presence of the end functional group in the polymer. (46)

6 **Entrapment efficiency:** Since the results shown in experiments are typically dose-dependent, it is essential to measure the amount of material entrapped in lipoparticles/ liposome/lipoformulations. One could assume that the amount of material left is 100% encapsulated after unincorporated material is removed using separation procedures, but storage may cause the preparation to vary. In general, two methods may be used, i.e., mini-column centrifugation and protamine aggregation. (49)

7 **Drug release:** A precisely calibrated in vitro diffusion cell may be used to evaluate the drug release process from the lipoparticle/liposome/lipoformulations. Using in vitro tests to predict drug pharmacokinetics and bioavailability prior to expensive and time-consuming in vivo research can help lipoparticle/ liposome/lipoformulation-based formulations. The dilution-induced drug release in the buffer and plasma predicted the pharmacokinetic performance of lipoparticle/ liposomal formulations. The drug's bioavailability was evaluated using an additional assay that measured intracellular drug release caused by lipoparticle/ liposome/lipoformulation degradation in the presence of a mouse liver lysosome list. A number of methods are applied to determine the in-vitro release of drugs from the lipoparticle/ liposome/lipoformulation, like the Reverse dialysis bag technique, Dialysis bag diffusion technique, and Shaking incubator technique. (47)

CHAPTER 3

LITERATURE REVIEW

LITERATURE REVIEW

C. Has & P. Sunthar (2019): When it comes to delivering medications and other macromolecules into the bodies of humans and animals, liposome/lipoformulations, also known as lipid vesicles, provide an adaptable platform. While lipoformulations mediated encapsulation has been used for decades, with significant advancements and commercialization of liposomal formulations occurring in the late 1990s or early 2000s, research on novel approaches to lipoformulations and drug encapsulation is still ongoing. The methods for creating lipoparticles have become more sophisticated and reliable due to advancements in adjacent domains like electrohydrodynamics and microfluidics, as well as an increasing knowledge of the mechanics behind lipid assembly from colloidal and intermolecular forces. Better techniques that can scale up to an industrial production level have resulted from this. In this study, we discuss a number of cutting-edge techniques that have emerged in the past ten years and weigh their benefits against traditional approaches. This resource will be helpful to researchers who are just starting to investigate liposomal formulations since it provides general guidance for selecting the best approach. We have also included the known mechanics underlying the preparation techniques whenever possible.

Faisal Farooque et al., (2021): The first nanomedicine to be approved for use in clinical settings was liposome/lipoformulations. These are the spherical vesicles with a bilayer of phospholipids enclosing a middle empty aqueous region. Because of their extraordinary potential to stop medication deterioration and lessen unwanted effects, liposome/lipoformulations are being utilized more and more for targeted drug administration. For targeted drug delivery, the pharmaceuticals can be integrated into the liposome/lipoformulations' aqueous space (hydrophilic drugs) or phospholipid bilayer (hydrophobic drugs). Given the significance of liposome/lipoformulations as a drug delivery mechanism, this review study attempts to investigate their specifics. Six sections make up the entirety of the paper. This is an introduction section. Classification of liposome/lipoformulations is the subject of the second section. The final section goes into detail about the liposome/lipoformulations' structural elements. The preparation techniques for liposome/lipoformulations are covered in the fourth section of the publication. The

topic of liposome/lipoformulation characterization is covered in the fifth section. The use of liposome/lipoformulations is covered in the sixth section, and closing observations are covered in the last section. The literature demonstrates several liposome/lipoformulation kinds that are grouped according to their size, composition, number of lipid layers, and technique of manufacture. They have been utilized recently in the synthesis of several nanoscale medications, and a recent example of this may be seen in the suggested medication for black fungus, liposomal amphotericin B. However, because of the expensive and time-consuming procedures involved in their creation and fabrication, their application and development remain a problem. Thus, more investigation and advancement are necessary.

Greg L. Plosker and Dean M. Robinson (2008): In phase I/II study, nilotinib, an oral BCR-ABL tyrosine kinase inhibitor, demonstrated good clinical effectiveness in treating chronic myeloid leukemia (CML) with Philadelphia chromosome positivity that is either imatinib-resistant or -intolerant. The trial's phase I component determined the dose schedule for the trial's phase II, which had many arms. Nilotinib was tested in three of these arms, or phase II studies, for each of the three stages of CML. Major cytogenetic response (primary end-point) was attained in 48% of the 280 patients who received nilotinib in the phase II study for patients with chronic-phase CML and had a follow-up period of at least six months. Imatinib-resistant and intolerant individuals did not have significantly different major cytogenetic response rates, and nilotinib was beneficial for patients with BCR-ABL mutations (except T315I). In the phase II study using nilotinib, the haematologic response rate (primary endpoint) was 47% in patients with accelerated-phase CML (n = 119). 26% of patients experienced a complete haematologic response, whereas 21% either showed no signs of leukemia or went back to the chronic phase of CML. A significant secondary outcome in the study, major cytogenetic response, happened in 29% of participants. 39% of patients in the phase II study (n = 135) with CML in blast crisis had a haematologic response with nilotinib, indicating encouraging outcomes. Most nilotinib-related adverse events that have been recorded have been mild to moderate in severity. In 29% of the patients, thrombocytopenia and grade 3 or 4 neutropenia were seen.

Michael W. N. Deininger et al. (2000): Among all human cancers, chronic myeloid leukemia (CML) has perhaps been researched the most. A significant advancement in the study of cancer biology was made in 1960 with the identification of the Philadelphia (Ph) chromosome as the first consistently observed chromosomal aberration linked to a particular kind of leukemia. It took another ten years to demonstrate that the translocation involved the ABL proto-oncogene normally

on chromosome 9 and a previously unidentified gene on chromosome 22, which was later dubbed BCR for breakpoint cluster region. It took thirteen years to realize that the Ph chromosome is the product of a t(9;22) reciprocal chromosomal translocation. Following the definition of the pathogenetic principle as the dysregulated ABL tyrosine kinase activity, the initial animal models were created. With the introduction of ABL-specific tyrosine kinase inhibitors that specifically suppress the proliferation of BCR-ABL-positive cells both in vitro and in vivo, all of this information is being applied from the bench to the bedside before the end of the millennium. We will also discuss the molecular biology and physiological functions of BCR and ABL, as several features of CML pathogenesis may be related to the altered function of the two genes implicated in the Ph translocation.

Tomasz Sacha et al. (2018): Second-generation tyrosine kinase inhibitor nilotinib was created to circumvent imatinib resistance in a variety of BCR-ABL mutations. Compared to imatinib, it causes faster and deeper molecular responses in a larger percentage of patients receiving first-line therapy for newly diagnosed chronic myeloid leukemia. It also provides the opportunity to try a cessation trial and achieve a lasting medication-free remission. Within the framework of what is thought to be the ideal therapeutic outcome, we talk about the function of nilotinib in the current regimen for treating chronic myeloid leukemia.

Valentina R. Minciacchi et al. (2021): For a long time, chronic myeloid leukemia (CML) has served as a "model disease." We outline the history of CML here, concentrating on molecular pathways related to signaling, metabolism, and the bone marrow microenvironment. The journey starts with the first discovery of leukemia and the description of the Philadelphia Chromosome and ends with the current goal of achieving treatment-free remission after targeted therapies. We present current approaches for combination treatments targeted at eliminating the CML stem cell, which should be the last stop on this arduous journey.

Martin Höglund et al. (2015): When it comes to complete coverage of the target population and accurate diagnosis, national and regional population-based registries are essential resources for epidemiological research. More thorough reporting from chronic myeloid leukemia (CML) registries may potentially offer supplementary information on treatment outcomes to that from clinical trials. A crude annual incidence of 0.7–1.0/100,000, a median age at diagnosis of 57–60 years, and a male/female ratio of 1.2–1.7 are consistently reported in reports from many European CML registries. Over time, the incidence of CML has remained consistent. Variations in the reported prevalence of CML around the globe may be the result of methodological problems, but

it is impossible to rule out the possibility that there is a real difference between certain geographic regions and/or ethnic groupings. Although the exact frequency of CML is unknown, estimates range from 10–12/100,000 people, and this number has been steadily rising due to the patients' remarkable improvements in survival. Although the relative survival for patients over 70 years of age is still lower, CML patients' overall survival is similar to that of big clinical trials, according to current population-based research. Research on the impact of socioeconomic characteristics, the healthcare environment, and the potential elevated risk of secondary malignancy in CML patients is still underway.

Hamdi Nsairat et. al. (2022): Liposome/lipoformulations' great biocompatibility, biodegradability, and low immunogenicity make them the most often employed nanocarriers for a variety of hydrophilic and hydrophobic compounds that may be biologically active. Moreover, liposome/lipoformulations have demonstrated improved drug solubility, regulated dispersion, and surface modification potential for focused, extended, and sustained release. Liposome/lipoformulations can be thought of as having developed from typical, long-circulating, immune-targeting liposome/lipoformulations to stimuli-responsive and actively targeted liposome/lipoformulations based on their composition. Numerous liposomal-based drug delivery methods are presently authorized for use in clinical settings to treat a variety of illnesses, including cancer, fungal infections, and viral infections; other liposome/lipoformulations are in the advanced stages of clinical studies. The structure, content, production techniques, and clinical uses of liposome/lipoformulations are covered in this article.

Elias Jabbour et al. (2017): A myeloproliferative tumor, chronic myeloid leukemia (CML), affects 1-2 persons out of every 100,000 adults. It makes up around 15% of adult newly diagnosed cases of leukemia. A balanced genetic translocation, t (9;22) (q34; q11.2), including the fusing of the breakpoint cluster region (BCR) gene on chromosome 22q11.2 with the Abelson gene (ABL1) from chromosome 9q34, is the hallmark of CML. The Philadelphia chromosome is the name given to this rearrangement. This translocation results in the molecular production of a BCR-ABL1 fusion oncogene, which is translated into a BCR-ABL1 oncoprotein. Frontline therapy: For the first-line treatment of individuals with recently diagnosed CML in chronic phase (CML-CP), the US Food and Drug Administration has approved four tyrosine kinase inhibitors (TKIs): imatinib, nilotinib, dasatinib, and bosutinib. Due to the availability of efficient salvage therapy, clinical trials using second-generation TKIs indicated quicker and deeper responses; nevertheless, this has not translated into increased long-term survival. Salvage treatment: Second- and third-generation TKIs

are available as second-line alternatives for patients who do not respond to front-line therapy. Despite their strength and selectivity, second and third-generation TKIs have distinct pharmacological profiles and response patterns about several patient and illness factors, including patients' comorbidities, disease stage, and BCR-ABL1 mutation position. Patients with the T315I "gatekeeper" mutation show resistance to all TKIs on the market right now, with the exception of ponatinib. For individuals with CML-CP who have not responded to at least two TKIs and for all patients in the late stages of the disease, allogeneic stem cell transplantation is still a viable treatment option.

Anandamoy Rudra et al., (2010): Several techniques were used to study the drug-excipient interaction: Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), field emission scanning electron microscopy for surface morphology, energy dispersive X-ray (EDX) analysis for elemental analysis, zeta potential, and size distribution using a Zeta-sizer and particle size analyzer, and dialysis membrane for in vitro drug release. Additionally, research was done on liposome/lipoformulation accumulation in vivo in tissues. The medication and excipients did not appear to undergo any chemical reaction. The EDX analysis verified that liposome/lipoformulations exhibit PE conjugation. The liposome/lipoformulations that included doxorubicin (DOX-L) and PE-conjugated doxorubicin (DOX-PEL) had a smooth surface, were uniformly dispersed, and had a negative surface charge. They were found in the nano-size range of 32–37 nm. For DOX-L and DOX-PEL, the loading efficiencies were 49.25% ± 1.05% and 52.98% ± 3.22%, respectively. According to an in vitro drug release study, doxorubicin was released from DOX-L and DOX-PEL in nine hours at a rate of 69.91% ± 1.05% and 77.07% ± 1.02%, respectively. Research using fluorescence microscopy revealed that the liver, lungs, and kidneys had a good distribution of liposome/lipoformulations. According to available data, PE-conjugated nanoliposome/lipoformulations were able to distribute the medication across different organs and release it continuously. This may be applied to attach certain antibodies to PE and target cells or tissues.

CHAPTER 4

MATERIALS & METHODOLOGY

4.1 MATERIALS AND INSTRUMENT USED

4.1.1 CHEMICALS- Nilotinib**,** Cholesterol, Soya-L-α-lecithin (SLE), Butylated Hydroxy Toluene (BHT), Chloroform, Ethanol, Potassium dihydrogen phosphate, Disodium hydrogen phosphate, Sodium chloride are required for development of the formulation.

Table 9: List of the chemicals required for the preparation of drug lipo particles with their source

NAME	SOURCE
Nilotinib	Hetero Drug, Hyderabad, Telangana, India
Cholesterol	HiMedia Laboratories Pvt. Ltd. (Mumbai, India)
Soya-L-α-lecithin (SLE)	HiMedia Laboratories Pvt. Ltd. (Mumbai, India)
Butylated Hydroxy Toluene (BHT),	Qualigens Fine Chemicals (Mumbai, India)
Chloroform	Merck Lifescience Pvt. Ltd. (Mumbai, India)
Potassium dihydrogen phosphate	Merck Specialities Pvt. Ltd. (Mumbai, India)
Disodium Hydrogen Phosphate	E Merck Ltd. (Mumbai, India)
Sodium chloride	Merck Lifescience Pvt. Ltd. (Mumbai, India)
HPLC water	Merck Lifescience Pvt. Ltd. (Mumbai, India)
Ethanol	Changshu Hongsheng Fine Chemicals Co. Ltd., (Changshu city)
Hydrochloride acid	Merck Lifescience Pvt. Ltd. (Mumbai, India)
Sodium chloride	Merck Lifescience Pvt. Ltd. (Mumbai, India)

Table 10: List of the equipment used for the preparation of liposomal formulation and evaluation with their source

NAME	SOURCE
Rotary vacuum evaporator	Rota-vap Super-fit model-PBU-6, Mumbai, India
Low temperature circulating bath	Instrumentation India, Kolkata, India
Vacuum Desiccators	Tarson, Kolkata, India
Bath type sonicator	Tarson-O-Sonic, Mumbai, India
UV-Vis's double -beam spectrophotometer	UV 2450, Shimadzu, Japan (Department of Pharmaceutical technology, Jadavpur University)
Vacuum Freeze dryer	Instrumentation India, Kolkata, India
Digital balance	Sartorius, Goetingen, Germany
High speed Ultra cold Centrifuge (Model Z 32 HK)	Hermle Labortechnik GmbH, Wehingen, Germany
Fourier-Transform Infrared Spectroscopy (FTIR)	R. C. Patel Institute of Pharmaceutical Education & Research, Shirpur, Maharashtra -425 405
Magnetic stirrer	Remi Equipment, Mumbai, India
pH meter	Eutech Instruments, Mumbai, India
Vortex mixture (Model CM100)	Remi Equipments, Mumbai, India
Distillation Plant	Sicco, Kolkata, India
All glass apparatus	Borosil, Mumbai, India
Pipette & Micro Tips, Centrifuge Tube	Tarsons Products Pvt. Ltd., Kolkata, India.
Field emission scanning electron microscopy (FE-SEM)	Department of physics, PG Science building, Jadavpur University, Kolkata, India.
DLS (Dynamic light scattering)	Department of Central Scientific Service, Indian Association for the Cultivation of Science, Kolkata.
Zeta sizer	Department of Central Scientific Service, Indian Association for the Cultivation of Science, Kolkata.

4.2 METHODOLOGY

4.2.1 PREPARATION OF CALIBRATION CURVE OF NILOTINIB IN ETHANOL: WATER (1:1) AND 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 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.

- **Preparation of stock solution**

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

- **Preparation of standard solution**

0.10 ml of stock solution was taken in 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.

4.2.2 DETERMINATION OF ABSORPTION MAXIMUM OF NILOTINIB

For the determination of absorption maxima, about 1mg 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 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 was obtained, which was close to the published lambda max of Nilotinib.

4.2.3 PREPARATION OF THE STANDARD CURVE

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.

4.2.4 PREPARATION OF NANOSIZED LIPOSOMAL FORMULATION

Out of several methods of liposome/lipoformulation preparation, in my work, I have used the conventional lipid layer hydration method (also called a thin-film hydration method) to develop nanosized liposome/lipoformulations. Both Blank liposome/lipoformulations and drug-loaded liposome/lipoformulations were prepared.

A. **PREPARATION OF THIN FILM**- In a 250 ml round-bottom flask, dissolve 150 mg soy lecithin, 75 mg cholesterol, and a pinch of BHT in 10 ml chloroform. In this phase, 10 mg of the medicine are added because it is soluble in ethanol. The same amounts of cholesterol, BHT, soy lecithin, and chloroform were used as a blank. A rotary vacuum evaporator operating at RPM-45, a water bath temperature of 50°C, and a circulator temperature of 4°C was used to form the thin film. Vacuum evaporation was maintained until the solvent had completely evaporated. After that, the thin film inside the round-bottom flask were placed in a desiccator for the whole night to allow any remaining solvent to evaporate
B. **HYDRATION-** On day 2, the thin film was hydrated using 75 ml of PBS pH 7.4 and a rotating water bath set to 45 minutes at 45 RPM and 50°C. Lipid layer in the buffer system forms vesicles which are primarily multilamellar.
C. **SONICATION-** The mixture inside of the round-bottom flask was then sonicated in the bath sonicate for 45 minutes in order to produce unilamellar vesicles. After three hours in the dark, the liposome/lipoformulation dispersion was refrigerated for the whole night.

D. **CENTRIFUGATION-** To separate the liposome/lipoformulation vesicles from the unentrapped drug and the lecithin and cholesterol that were not involved in the liposome/lipoformulation preparation, centrifugation was performed in a High-speed Ultra Cold Centrifuge. To centrifuge, the first liposome/lipoformulation dispersion was divided into two separate centrifuge tubes (50 ml) such that the weight of both tubes remained equal. The first cycle was performed at 4000 RPM, 4°C for 10 min (Washing phase), the supernatant was kept and the pellet was discarded. Then, again centrifuge at 16000 RPM, 4°C for 45 min, pellet was dispersed in new double distilled water and stored in a petri-dish that was sealed with parafilm and kept in a refrigerator at -20°C.

E. **LYOPHILIZATION-** The following day, dried liposome/lipoformulations were obtained through lyophilization, which was carried out for 14 hours using a freeze dryer. They were preserved at -20°C in Eppendorf tubes that were parafilm-sealed.

4.3 EVALUATION

1. DRUG LOADING

Take 2mg of blank and drug-encapsulated freeze-dried liposome/lipoformulations in Eppendorf tubes and then add 2ml solvent added to each tube. They were then vortexed for 3 min, sonicated for 30 min, and centrifuged for 15 min. During this process entrapped drug dissolved in solvent and undissolved lipid and cholesterol precipitated as a pellet. Supernatant containing drug (for drug-loaded liposome/lipoformulation) and without the drug (for blank liposome/lipoformulation) were taken in fresh Eppendorf tubes. Absorbance of supernatant of blank liposome/lipoformulation and drug-loaded liposome/lipoformulation were measured at 263 nm against solvent.

The absorbance of drug = Absorbance of supernatant of blank liposome/lipoformulation - Absorbance of supernatant of drug-loaded liposome/lipoformulation

The percentage of drug loading and drug loading efficiency was calculated using the following formula:

- Theoretical drug loading (%) = [(Amount of drug taken to prepare Liposome/lipo-formulation) / Amount of SLE + CHL + BHT + Drug] * 100
- Practical drug loading (%) = [(Amount of drug in Liposome/lipo-formulation)/(Amount of Liposome/lipo-formulation obtained)] *100
- Drug loading efficiency (%) = (Practical drug loading /Theoretical drug loading) *100

2. DRUG EXCIPIENT-INTERACTION STUDY USING FTIR SPECTROSCOPY

The pure drug Nilotinib, cholesterol, soya lecithin, physical mixture of drug with cholesterol, soya lecithin, and lyophilized formulation with and without drug were mixed separately, with infrared (IR) grade potassium bromide (KBr) in the ratio 1:100. Corresponding pellets were prepared by applying 5.5 metric ton pressure with a hydraulic press. The pellets were scanned in an inert atmosphere over a wave number range of 4000-400 cm^{-1} in a FTIR instrument.

3. SURFACE MORPHOLOGY OF LIPOSOME/LIPOFORMULATION BY FIELD EMISSION SCANNING ELECTRON MICROSCOPY (FE-SEM)

The external morphology of Nilotinib nanoliposome/lipo-formulation was analysed by freeze-dried particles were spread onto metal stubs and platinum coating was done by using ion-sputtering device. The coated particles were then vacuum dried and examined under FE-SEM as it uses a field emission gun (FEG) as its electron source. Unlike a conventional tungsten filament, the FEG generates a narrow, highly coherent beam of electrons by applying a strong electric field to a sharp tungsten or carbon nanotube tip this results in a smaller electron spot size and higher brightness, allowing for better resolution and image quality. FE-SEM is done at Department of Physics, PG Science building, Jadavpur University, Kolkata, India.

4. PARTICLE SIZE DISTRIBUTION STUDY

The size distribution of reconstituted lyophilized liposome/lipo-formulations was determined by Dynamic Light Scattering and analysed by DTS software at Department of Central Scientific Service, Indian Association for the Cultivation of Science, Kolkata. The principle of the particle size determination by DLS is the measurement of the rate of fluctuation of the intensity of scattered light due to Brownian motion of particles. Determination of the intensity fluctuation yields the value of Brownian motion of the particles due to thermally induced collisions between the particles which are converted into particle-size by using DTS software. The mean particle diameter (Z average) was calculated by the software from the measured particle size distribution. The freshly prepared lyophilized formulation was suspended in double distilled water and poured into the cuvette and analysed by the instrument.

5. POLYDISPERSITY INDEX (PDI)

Polydispersity index is a number which is a measure of size distribution of particles in a given sample. This value may be equal to one or greater or less than one. Generally, for monodispersed sample the value is 0.05 and the values greater than 0.7 indicate that the sample has very broad size distribution and is probably not suitable for dynamic light scattering technique. Polydispersity index of the different formulations was measured by the instrument Zeta sizer nano ZS (ZETASIZER, Nano ZS 90, Malvern Instrument Ltd. UK).

6. ZETA POTENTIAL MEASUREMENT

Zeta potential is a measure of the magnitude of the electrostatic or charge repulsion or attraction between particles in a liquid suspension. This parameter ensures the dispersion stability of the nanoliposome/lipo-formulation formulation. Zeta potential of the different formulations was determined by the instrument ZETASIZER, Nano ZS 90, Malvern Instrument Ltd. UK.

7. INVITRO DRUG RELEASE STUDY FROM THE NANOLIPOSOME/LIPOFORMULATION FORMULATION THROUGH DIALYSIS MEMBRANE

In a 250 ml conical flask, 50 ml of phosphate buffer saline (pH 7.4) was taken. The weighted amount (5mg) of lyophilized formulation was reconstituted in 1ml of PBS. Then it was taken into dialysis bag (Himedia dialysis membrane-60, Mumbai, India). Two ends of the dialysis bag were tightly bound with cotton threads. The dialysis bag was hanged inside the conical flask with the help of stand so that portion of dialysis bag with the formulation could dip into the buffer solution. The flask was kept on magnetic stirrer. Stirring was maintained with the help of magnetic bead at room temperature. Sampling was done by withdrawing 1 ml from released media with the help of micropipette and 1 ml of fresh solvent was added at definite time intervals. Samples were analysed in a spectrophotometer at the wavelength of 263 nm. With the help of standard curve, the drug concentration was measured.

CHAPTER-5

RESULTS

5.1 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 and another solvent ethanol and PBS pH 7.4 (1:1) 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.

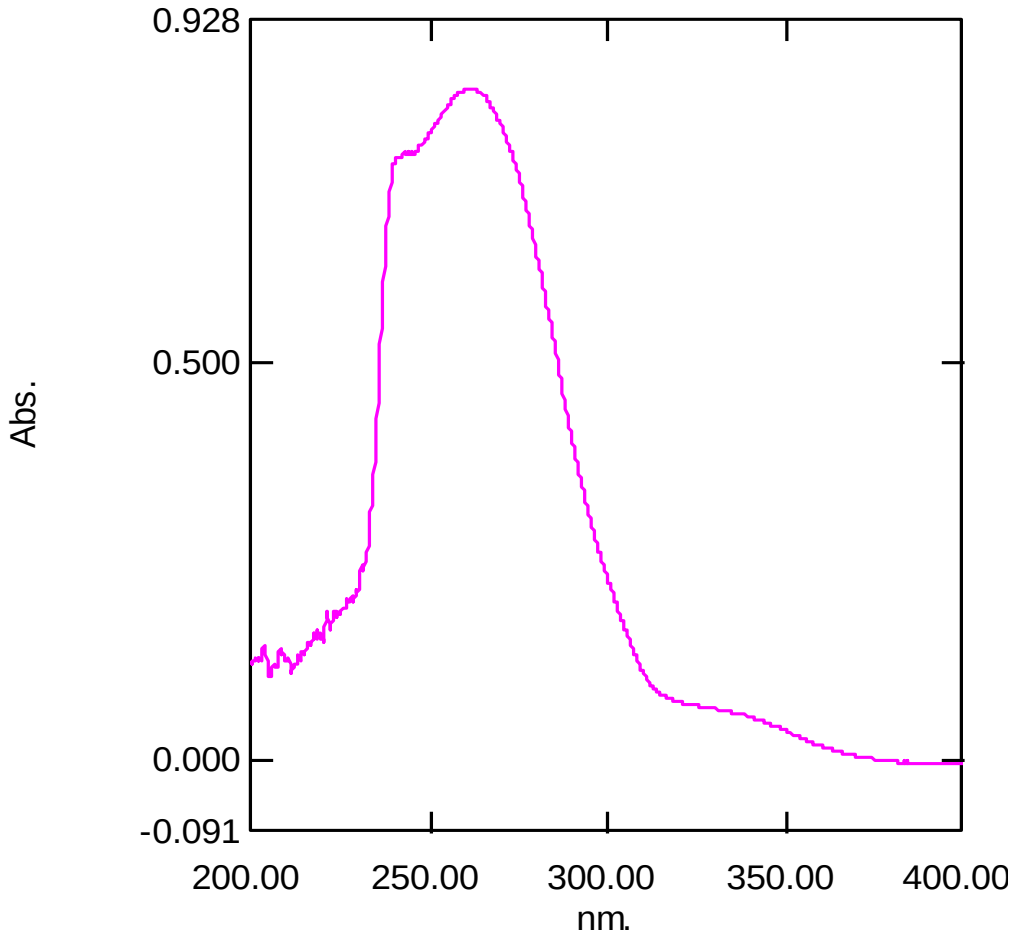


Figure 3: Lambda max of Nilotinib in Ethanol and water solution (1:1)

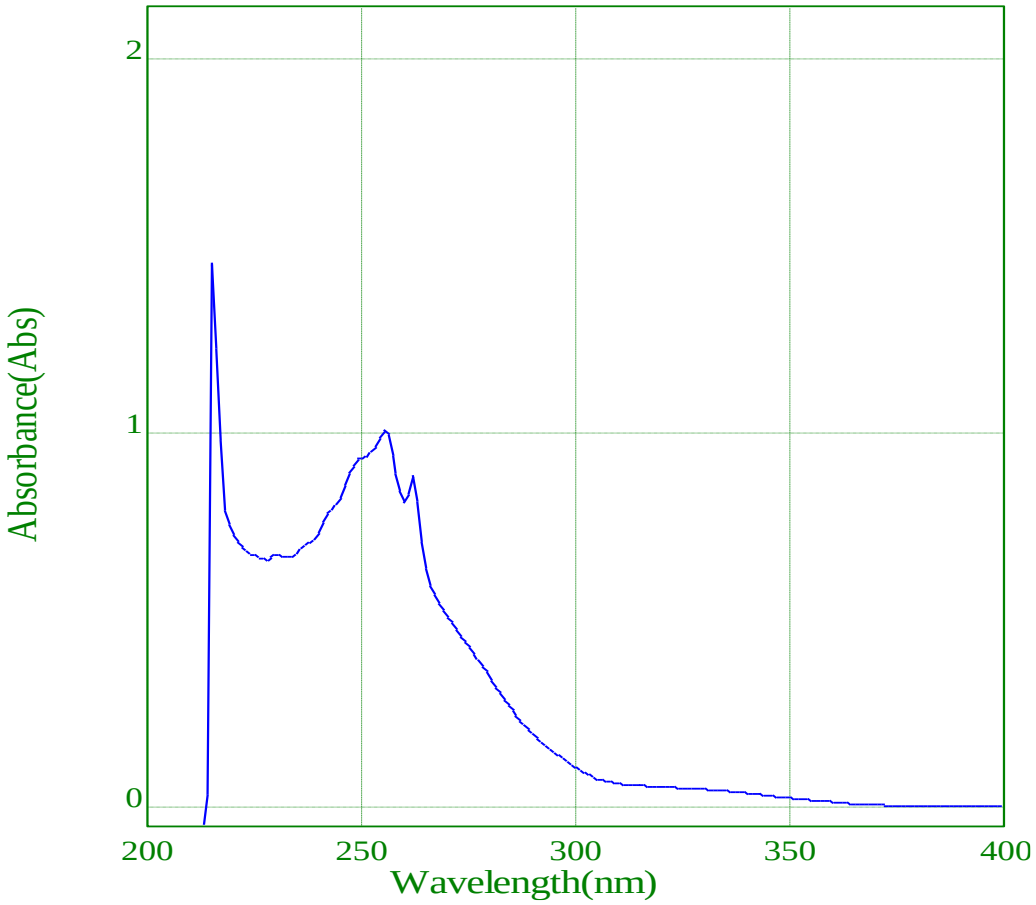


Figure 4: Lambda max of Nilotinib in Ethanol and PBS pH 7.4 (1:1) solution

5.2 THE CALIBRATION CURVE OF NILOTINIB

Two different calibration curves were prepared – one in Ethanol: PBS pH 7.4 (1:1) & one in Ethanol: water (1:1) for studying liposome/lipo-formulation 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 11: Absorbances of Nilotinib against various concentrations in ethanol: water (1:1)

Conc (µg/ml)	A1	A2	A3	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

Table 12: Absorbance of Nilotinib against various concentrations of ethanol and PBS (1:1) pH 7.4

Conc (µg/ml)	abs 1	abs 2	abs 3	Avg Abs
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

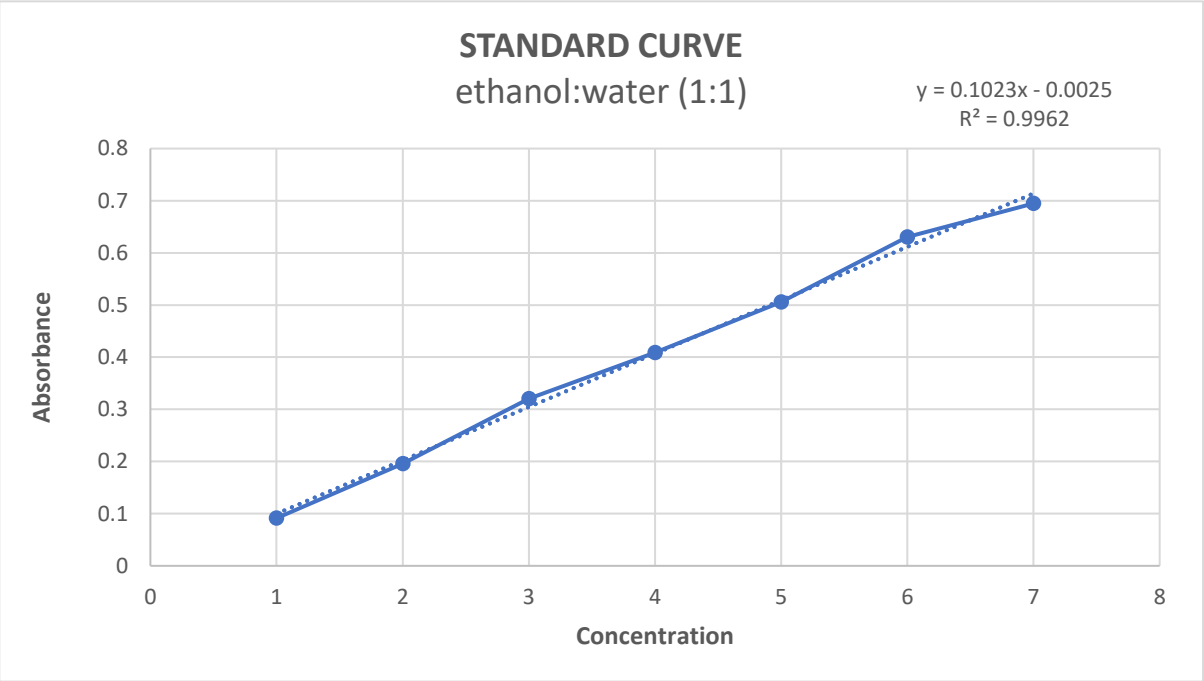


Figure 5: Standard curve of Nilotinib in Ethanol: water (1:1) with R^2 of 0.9962

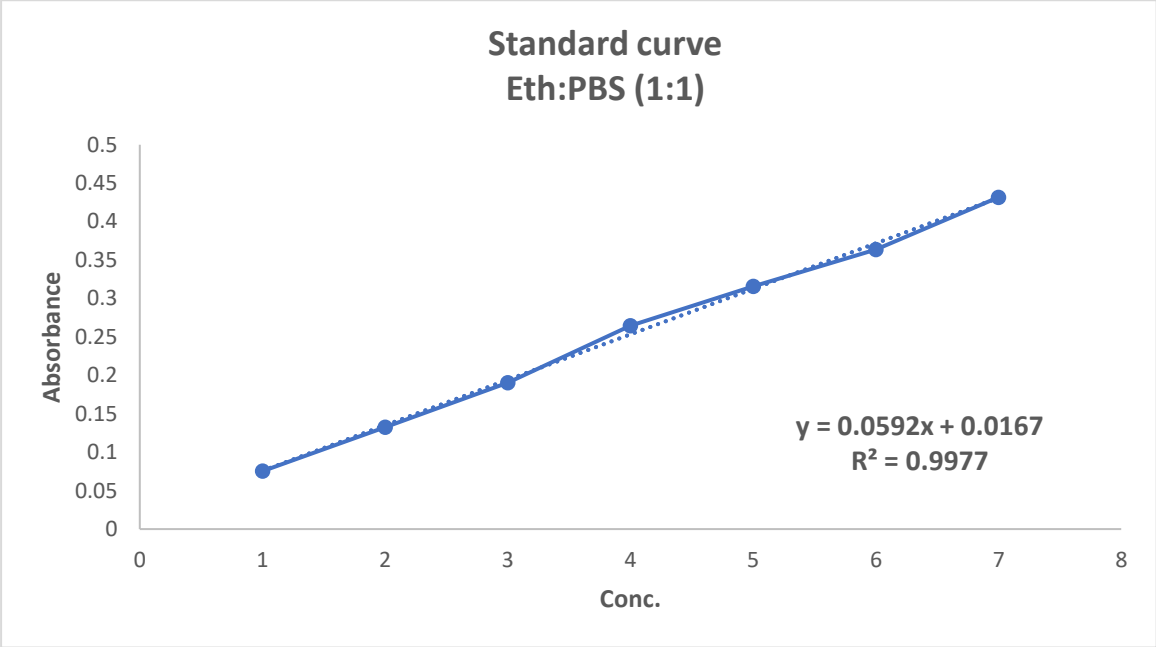


Figure 6: Standard curve of nilotinib in ethanol and PBS (1:1) with R^2 of 0.9977

5.3 STUDY OF DRUG-EXCIPIENT INTERACTION THROUGH FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY

In the present study we evaluated the drug excipient interaction by FTIR spectroscopy. FTIR spectra asses the drug excipient interaction at the level of functional groups by determining their vibrational patterns. Here the spectra of drug, the individual excipients (Cholesterol & Soya Lecithin), blank liposome/lipo-formulations (without drug) and drug loaded liposome/lipo-formulation have been depicted in the pictures from the figure 7 to 12.

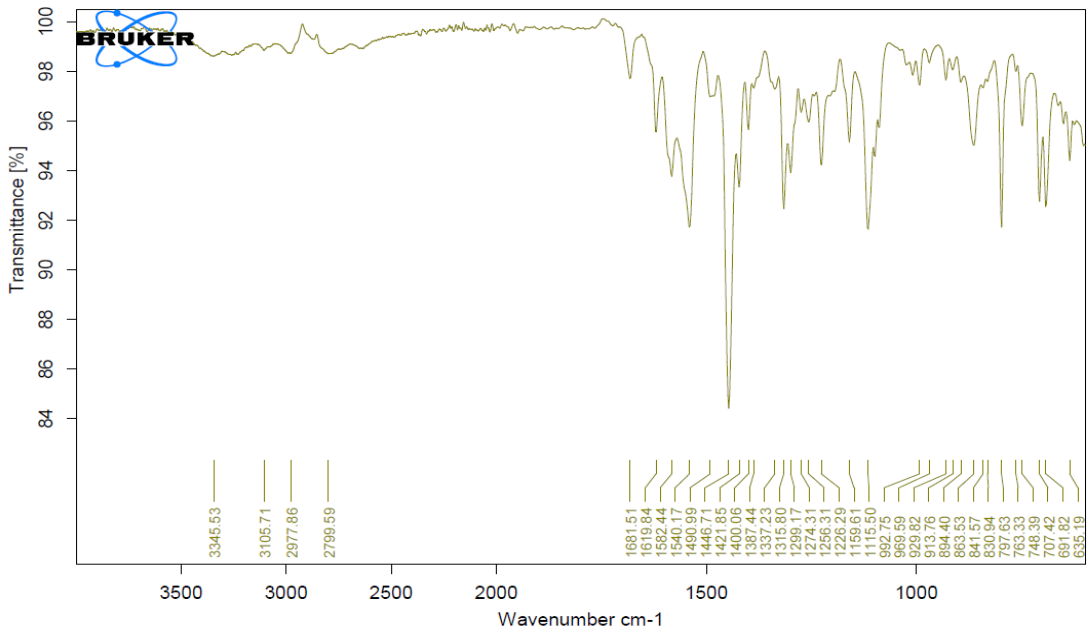


Figure 7: FTIR graph of the drug Nilotinib

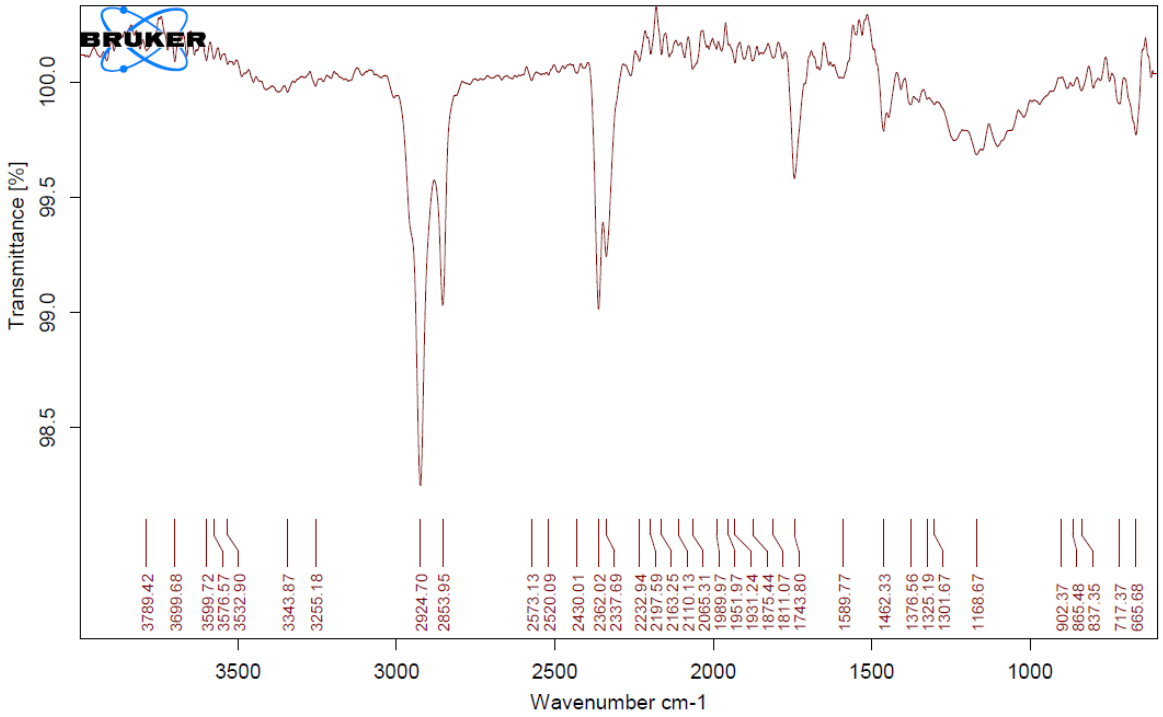


Figure 8: FTIR graph of Cholesterol

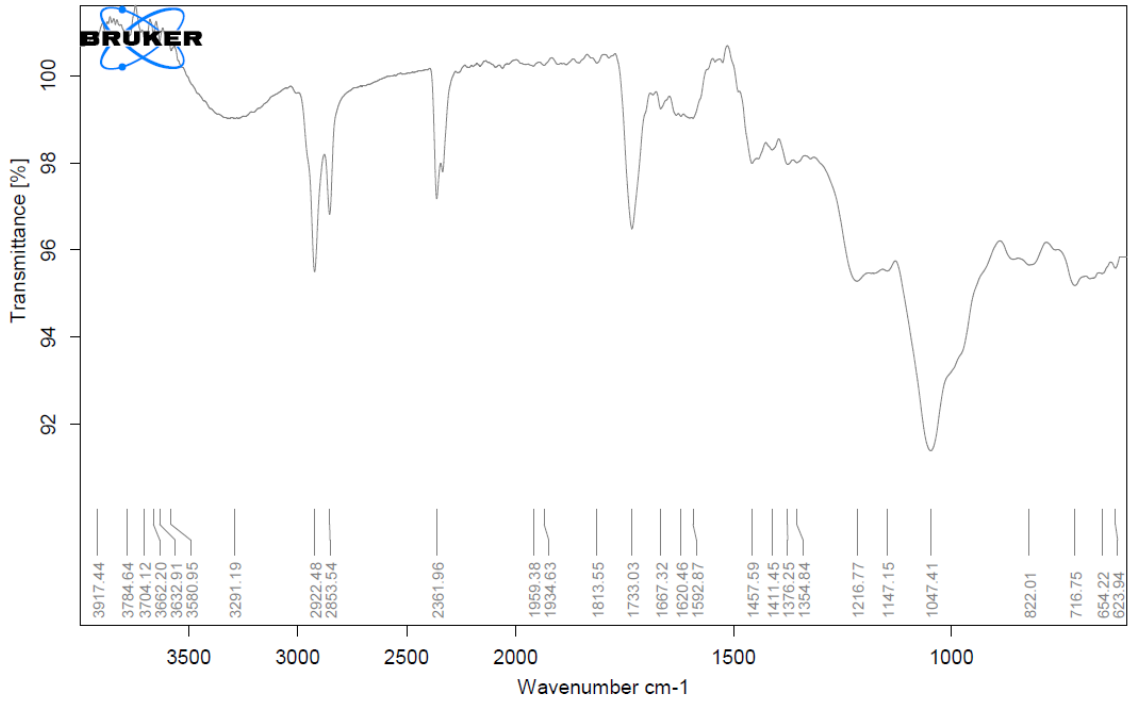


Figure 9: FTIR graph of Soya Lecithin

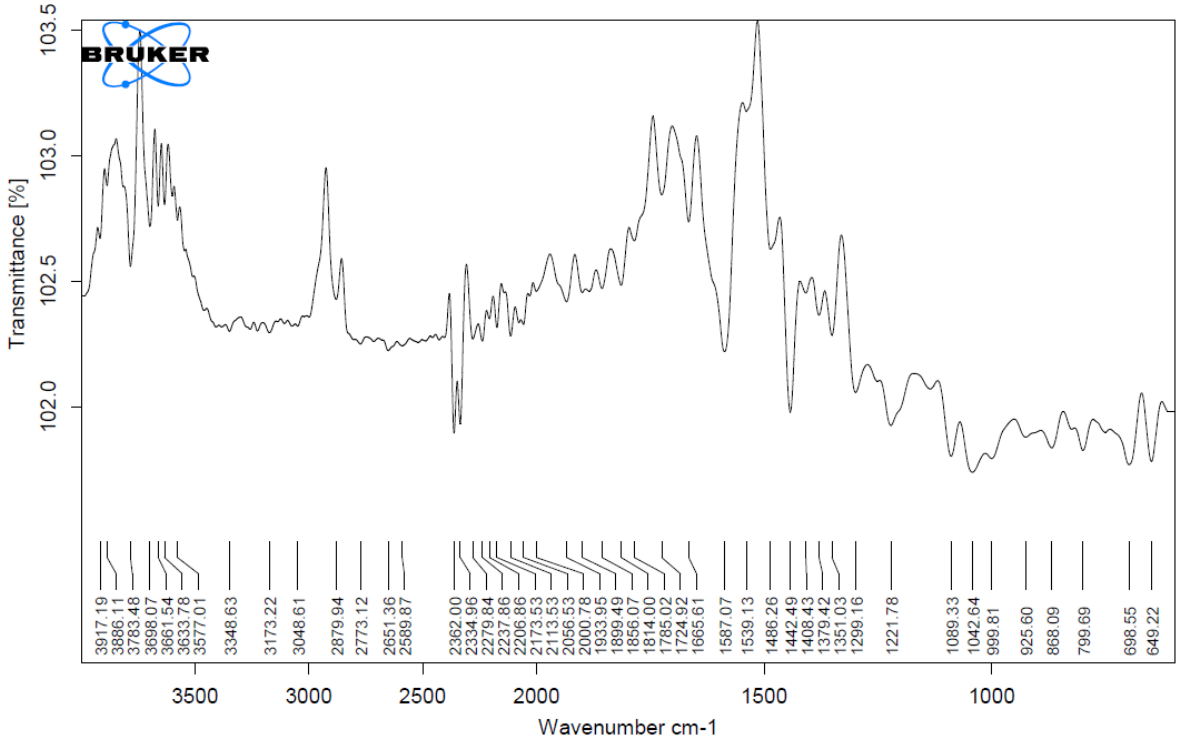


Figure 10: FTIR graph of physical mixture of cholesterol, soya lecithin and drug

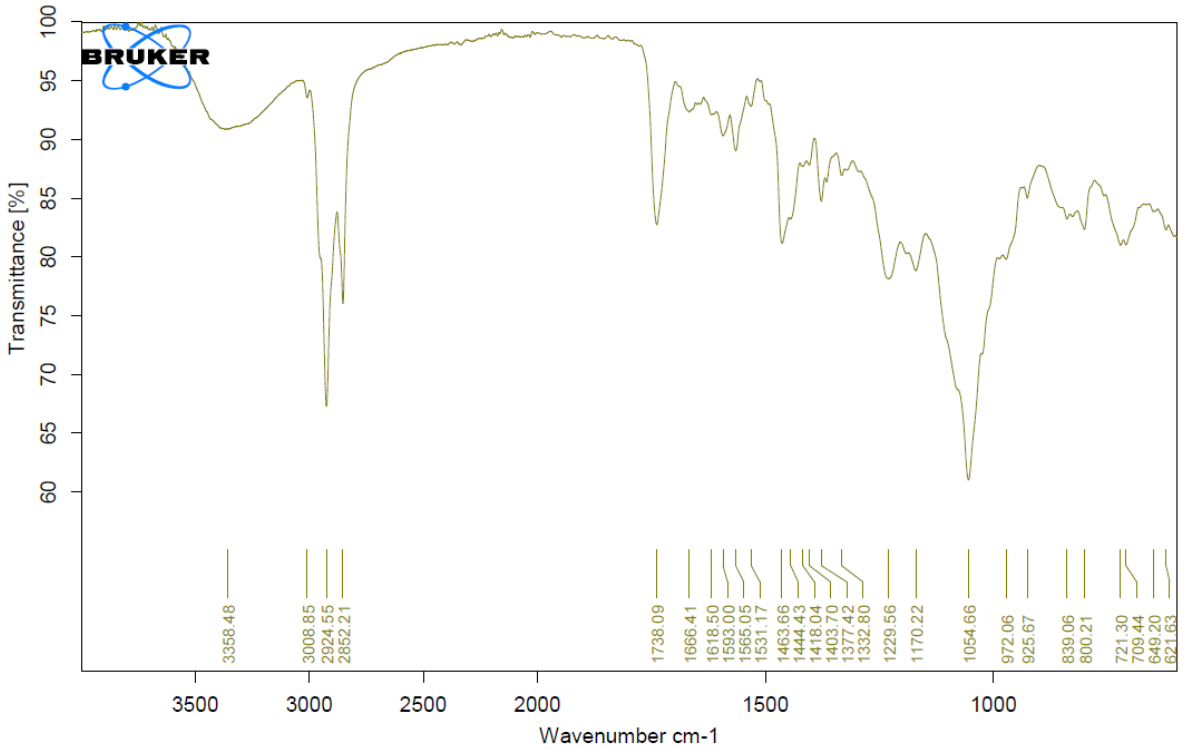


Figure 11: FTIR graph of blank liposome/lipoformulations

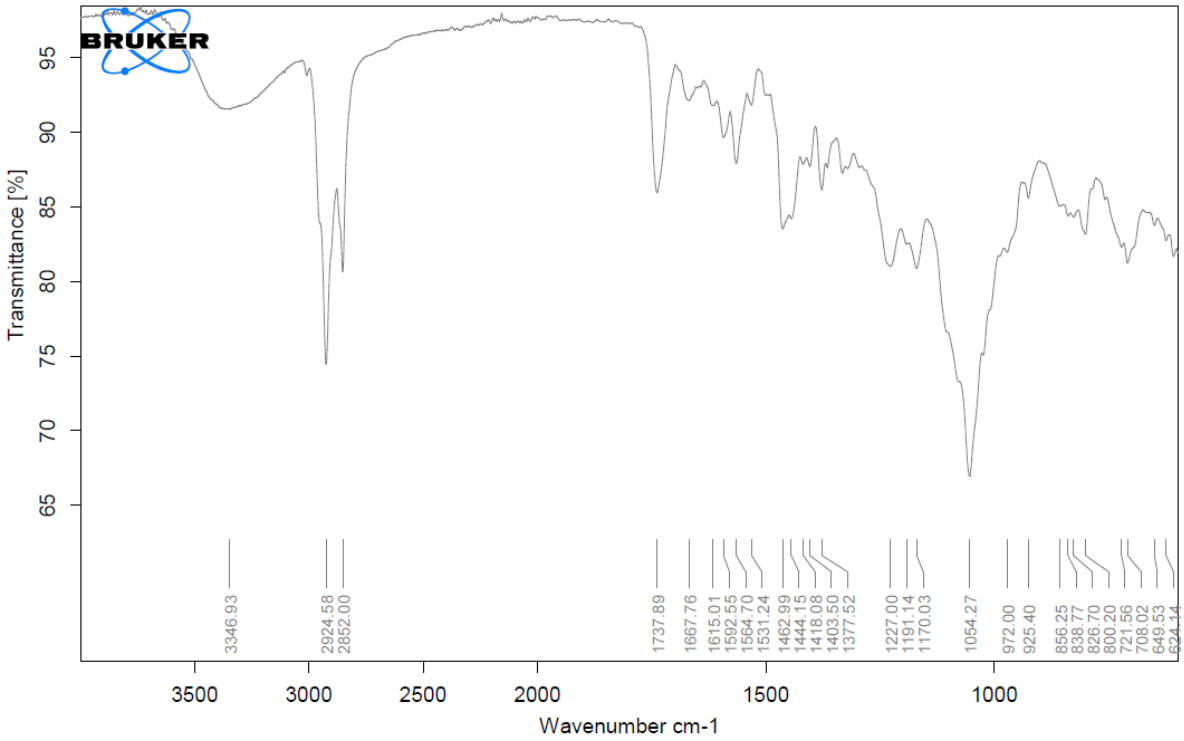


Figure 12: FTIR graph of liposomal formulation containing drug

5.4 DRUG LOADING AND ENTRAPMENT EFFICIENCY OF LIPOSOME/LIPOFORMULATION

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

Table 13: Theoretical and practical drug loading with entrapment efficiency of formulation

Formulation	Amount of drug taken (mg)	Amount of CHL taken (mg)	Amount of SLE taken (mg)	Theoretical drug loading (%)	Practical drug loading (%)	Entrapment efficiency
Liposome	10	75	150	9.09	5.81	63.93

5.5 SURFACE MORPHOLOGY STUDY OF LYOPHILISED LIPOSOME/LIPOFORMULATIONS BY FIELD EMISSION SCANNING ELECTRON MICROSCOPY

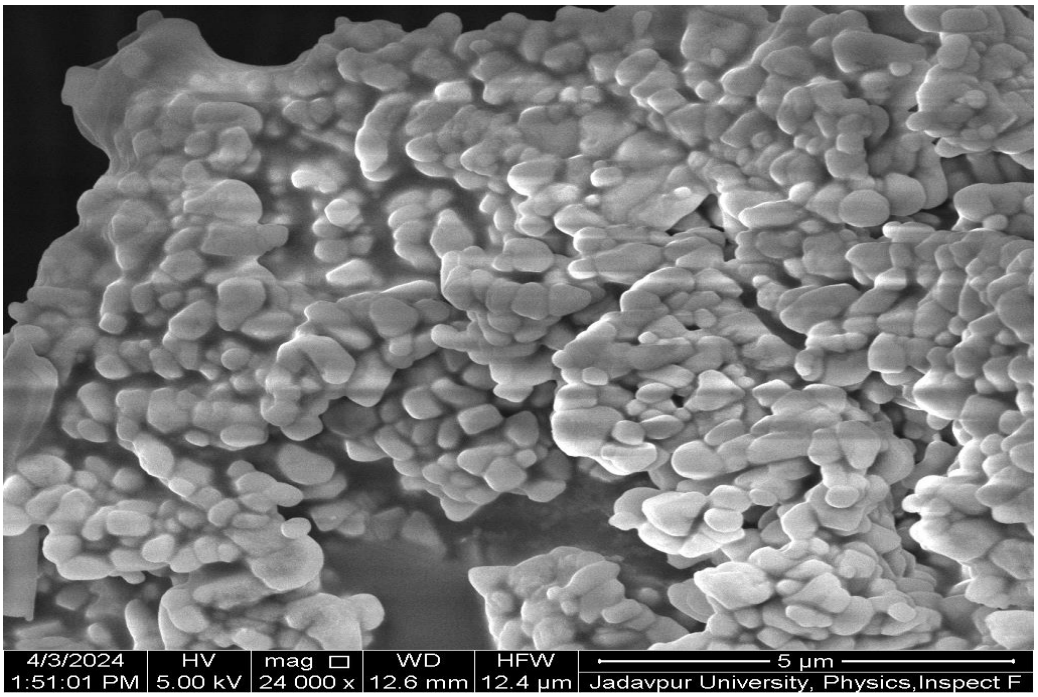


Figure 13: FE-SEM image of liposome/lipo-formulations at 24000x magnification

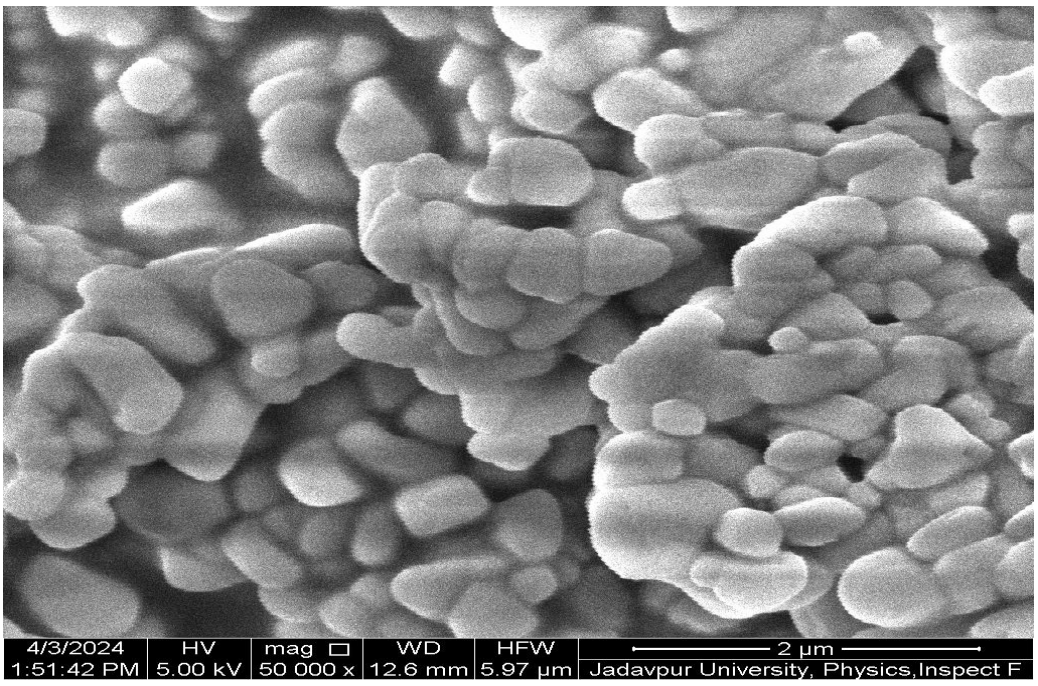


Figure 14: FE-SEM image of liposome/lipo-formulation at 50000x magnification

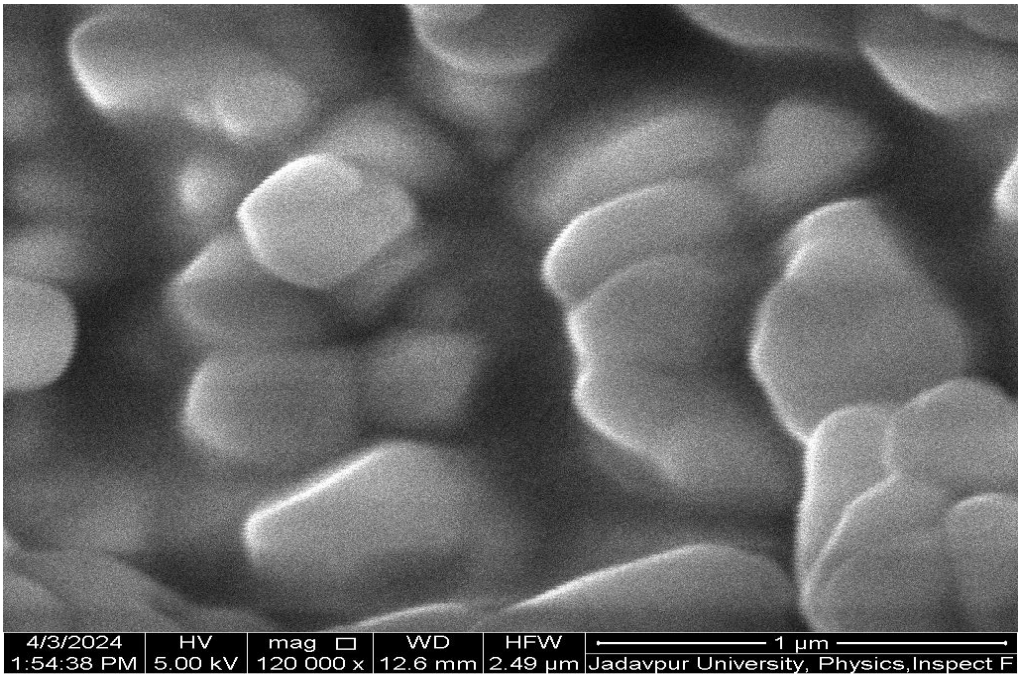


Figure 15: FE-SEM image of liposome/lipo-formulations at 120000x magnification

5.6 PARTICLE SIZE DISTRIBUTION STUDY

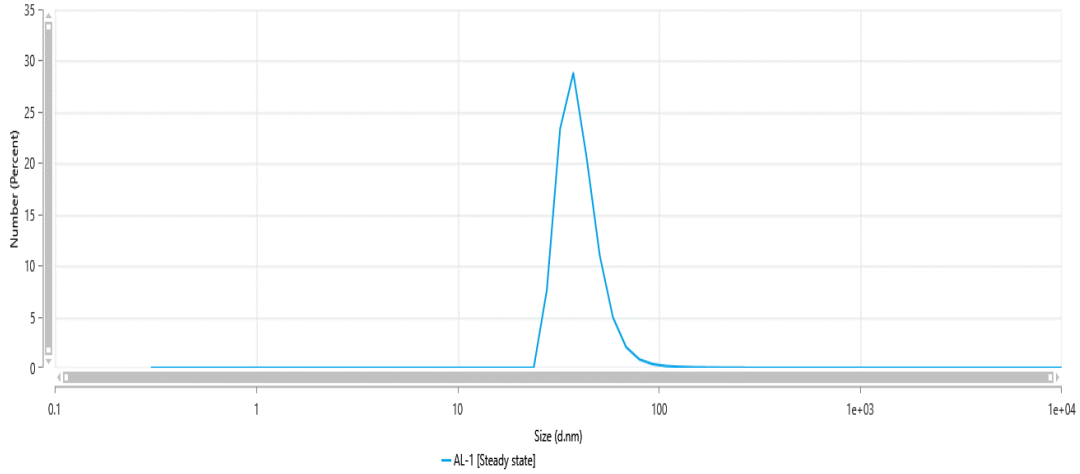


Figure 16: Particle size distribution graph of AL-1(Formulation)

Table 14: Z-average value and polydispersity index of the formulation

Experimental Study	Liposomes
Z average value	127.7 nm
Polydispersity index	0.3754

5.7 ZETA POTENTIAL STUDY OF FORMULATION

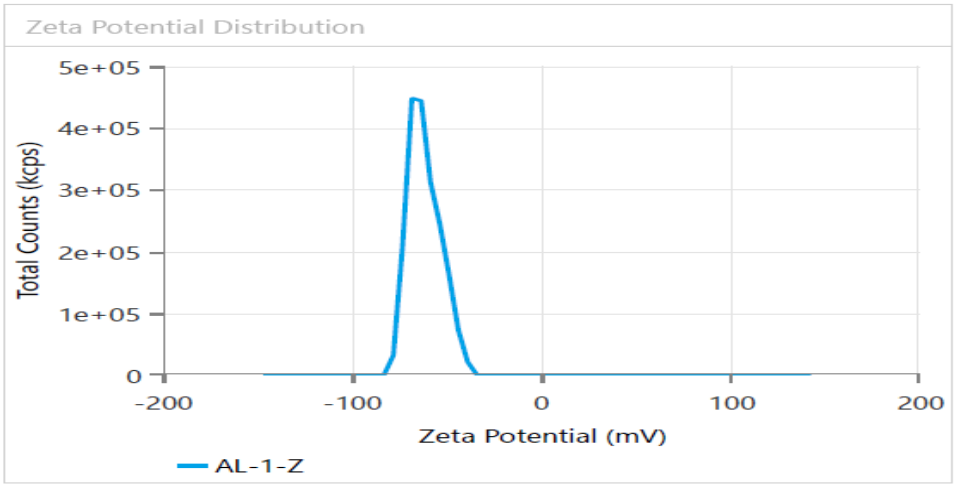


Figure 17: Zeta potential graph of AL-1(Formulation)

5.8 DRUG RELEASE KINETIC STUDY

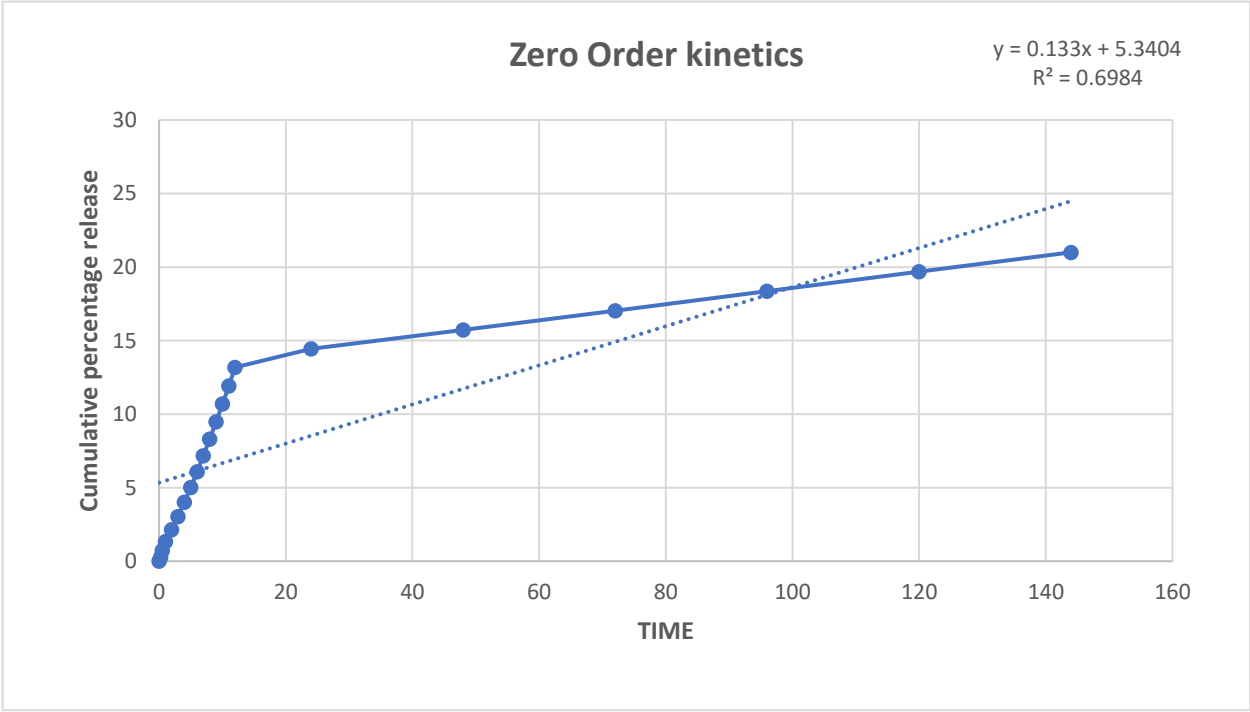


Figure 18: Graph of Zero order kinetics

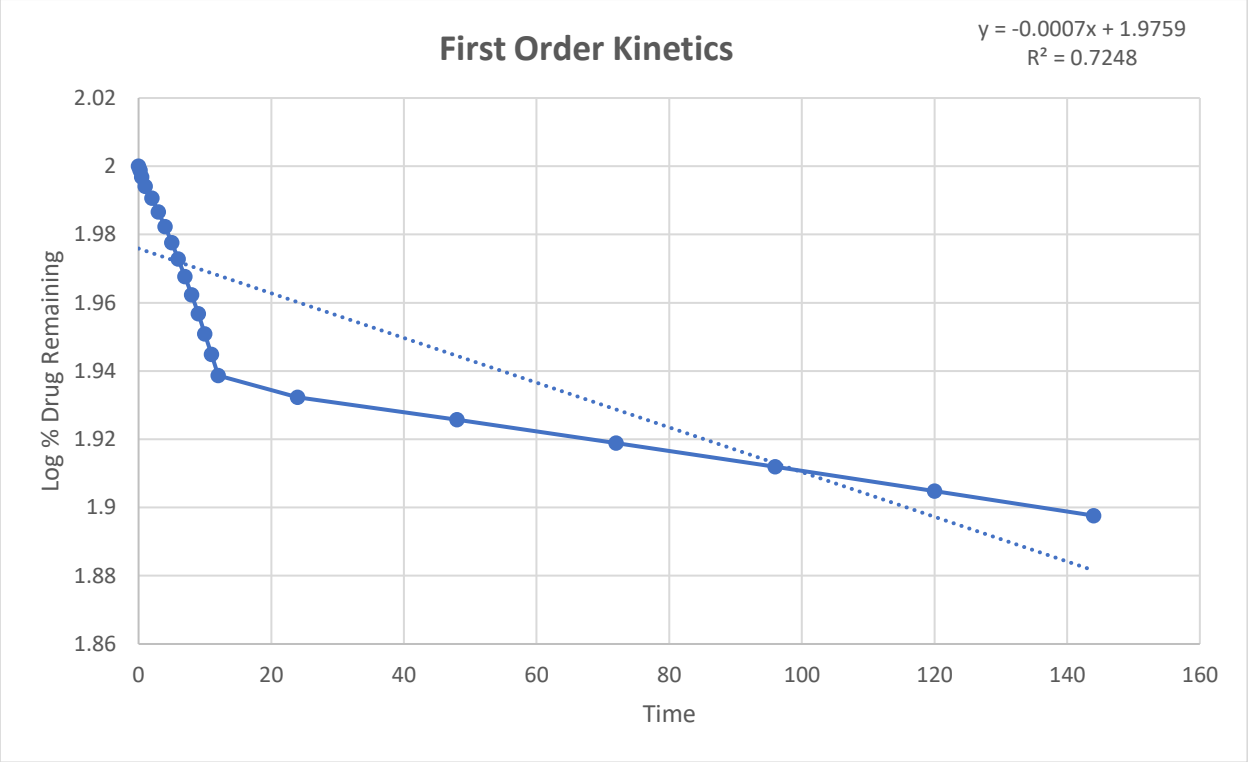


Figure 19: Graph of First order kinetics

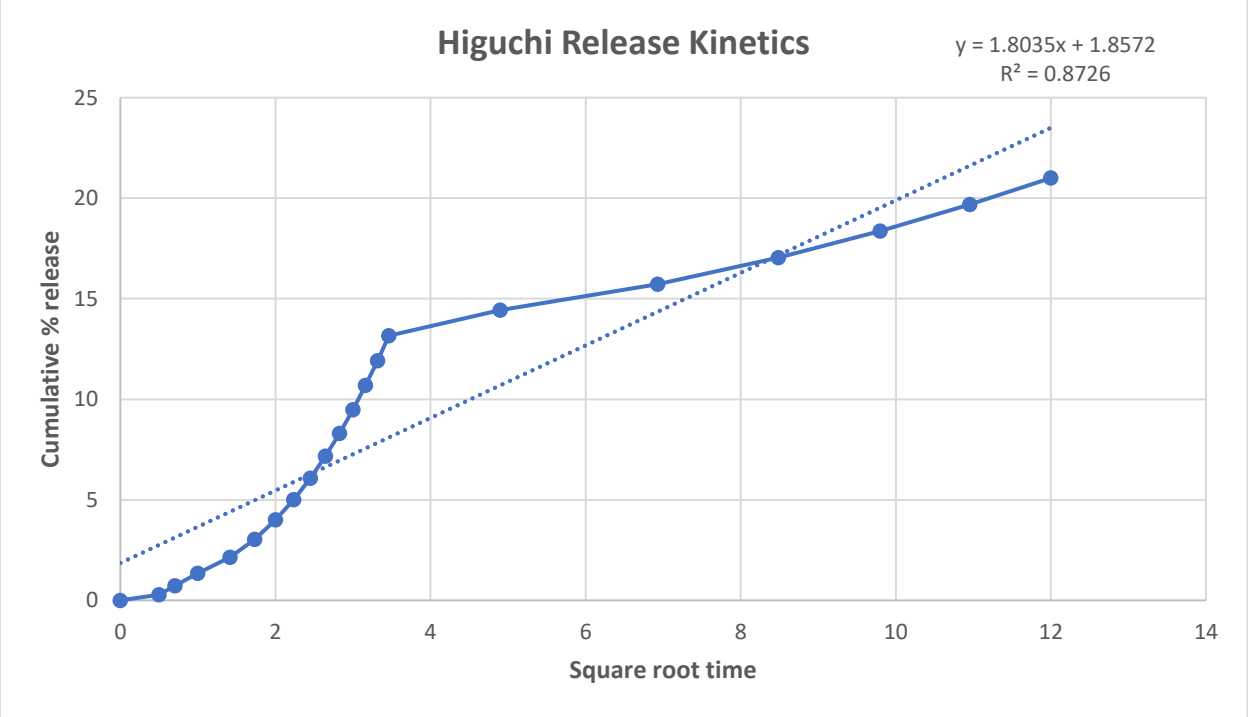


Figure 20: Graph of Higuchi Release kinetics

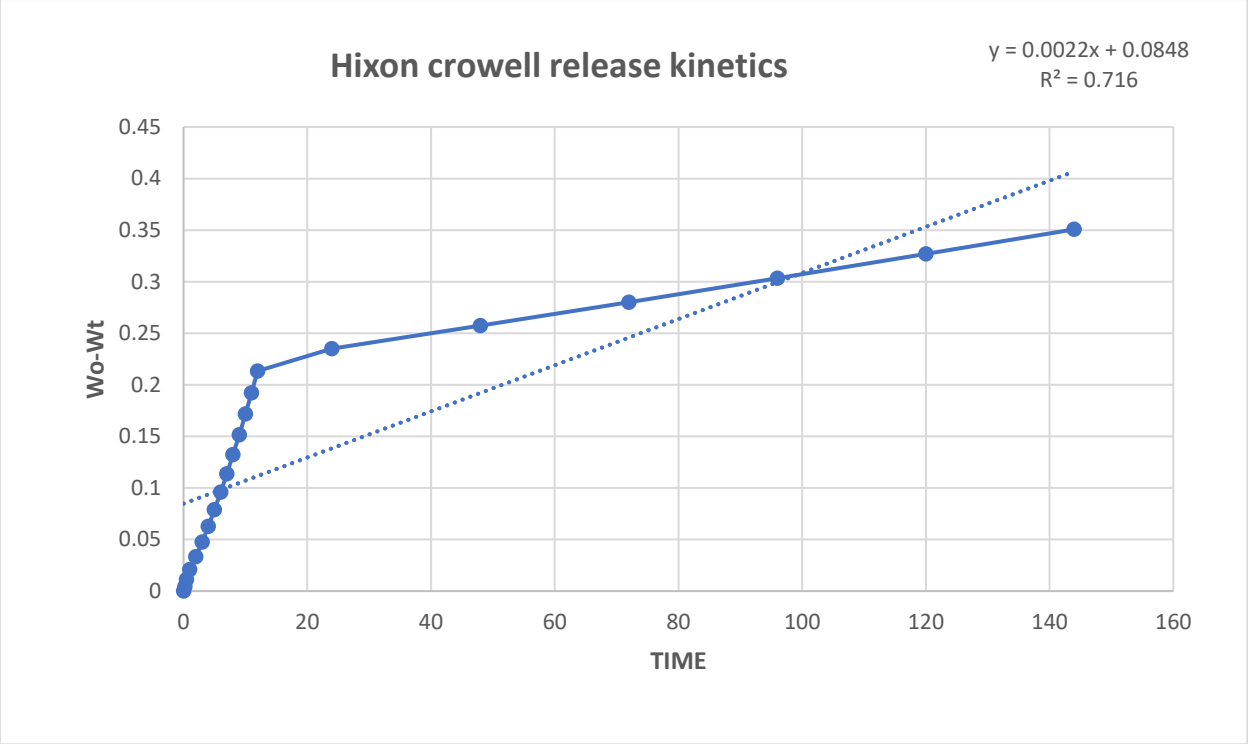


Figure 21: Graph of Hixon Crowell release kinetics

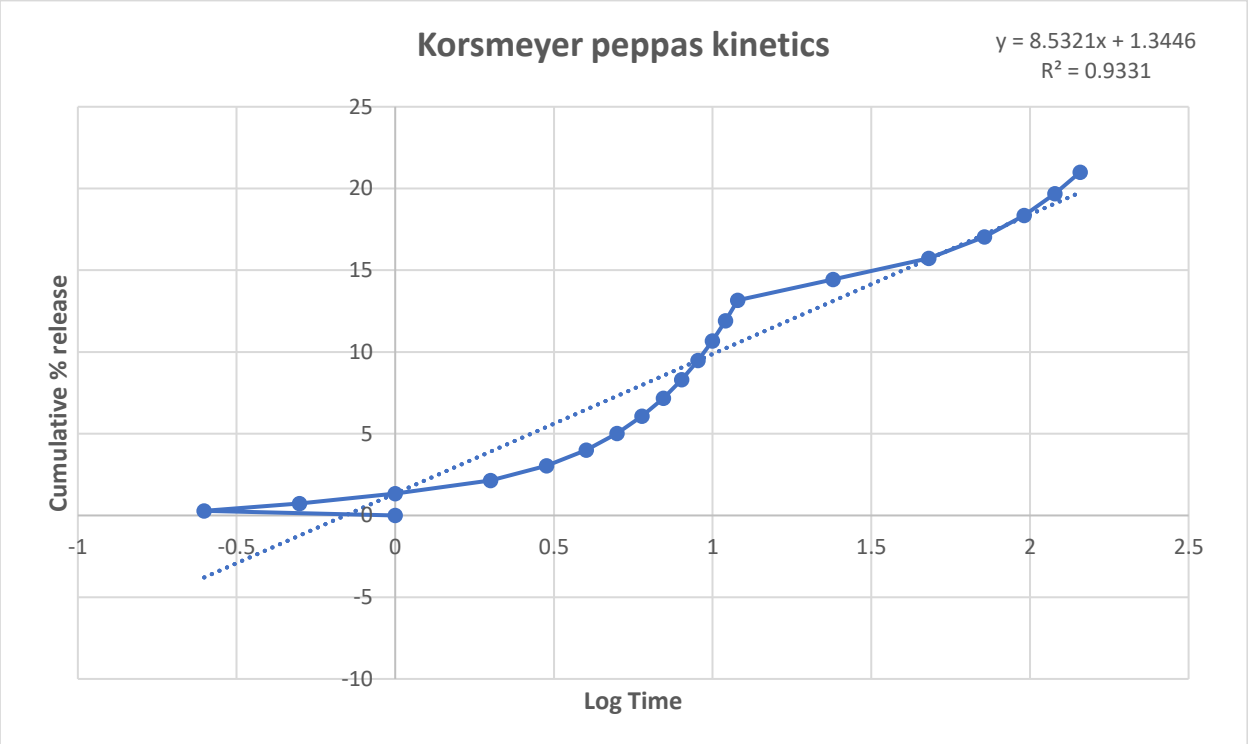


Figure 22: Graph of Korsmeyer Peppas release kinetics

CHAPTER-6

DISCUSSIONS

6.1 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 262nm, which comes under the reported peak of Nilotinib at 260 – 265nm. This confirms the authenticity and purity of the product being used and yielded the reference wavelength for spectrophotometric calculation in further studies.

6.2 THE CALIBRATION CURVE OFF NILOTINIB IN ETHANOL WATER (1:1) AND ETHANOL PBS pH7.4 (1:1)

Two different calibration curves were prepared one in ethanol water (1:1) and another in ethanol PBS pH7.4 (1:1) to study in-vitro drug release and liposome/lipo-formulation entrapment efficiency. Each reading was made in triplicate, and the average values were taken. The correlation coefficient (R^2) values were 0.9962 (ethanol: water) and 0.9977 (Ethanol: PBS pH7.4). These values favour the accuracy of the calibration curves used for further analysis.

6.3 DRUG EXCIPIENTS INTERACTION STUDY

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

The principle peaks of Nilotinib observed at wave numbers 3345 cm^{-1} for C-N stretching for imidazole ring , 2799 cm^{-1} for C-H stretching vibration of aromatic ring, 1681 cm^{-1} for C=O stretching vibration in the amide moiety, 1540 cm^{-1} for C=C stretching of aromatic ring,1446 cm^{-}

^{1} for C=C stretching of imidazole ring, 1115 cm^{-1} for C–N stretching of ring, 797 cm^{-1} N-H bending, 707 cm^{-1} C-F bending of molecule.

The liposome/lipoformulation without drug showed the peaks of liposome/lipoformulation at wave numbers 2924 cm^{-1} (Lecithin) for the N-H stretching suggesting the presence of amines or amides and 2852 (Cholesterol) for O-H stretching vibrations, indicative of free hydroxyl groups in alcohols, 2362 cm^{-1} (Lecithin) C≡N stretching indicating presence of nitrile and & 1733 cm^{-1} (lecithin) for C=O stretching for the carbonyl group, 1376 cm^{-1} (lecithin) for C-H bending vibrations indicate presence of alkene group. In case of drug loaded liposome/lipoformulation shows no drug peak, indicating complete drug encapsulation within liposomal vesicle because blank liposome/lipoformulation and liposome/lipoformulation with drug show similar FTIR graph. Furthermore, physical mixture of excipients (Cholesterol and soya lecithin) and liposome/lipoformulation FTIR graph show all the major peak of drug soya, lecithin and Cholesterol this suggest the presence of drug in the formulation. Some minor shifts seen in the peaks of formulation corresponding to cholesterol and soya lecithin this could be due to physical interaction between the functional groups of the excipients and drug, 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 formulation.

6.4 DRUG LOADING AND ENTRAPMENT EFFICIENCY STUDY

The theoretical drug loading was 9.09(%), whereas the practical drug loading was found to be 5.81%. The entrapment efficiency was found to be 63.93%. The drug, Nilotinib is lipophilic in nature, and thus it will be entrapped into the lipid core in liposome/lipoformulation.

6.5 STUDY OF MORPHOLOGY BY FE-SEM

Size, shape and external morphology of the nanoliposome/lipoformulation formulations were assessed by Field emission scanning electron microscopy (FE-SEM). From the result it was found that lyophilised nanoliposome/lipoformulations had smooth surface and were in nano size range. The surface of the lipid vesicles had no leakage and the liposome/lipoformulations were uniformly distributed.

6.6 VESICLE SIZE AND SIZE DISTRIBUTION STUDY

Particle size and size distribution study of the liposome/lipoformulation formulation was carried out by dynamic light 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 127.7nm which suggest that the average hydrodynamic diameter of the particles in your sample is 127.7 nm. This means that most of the particles in the sample have a size close to 127.7 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 0.3754 which suggest that formulation is narrow size dispersion of sample.

6.7 ZETA POTENTIAL STUDY

The value of the zeta potential indicates the degree of electrostatic repulsion between adjacent, similarly charged particles in a dispersion. Particles with a zeta potential greater than +30 mV or less than -30 mV are considered stable. The high negative value of -61.66 mV suggests that the particles have strong electrostatic repulsion, preventing them from coming together and aggregating. Therefore, your colloidal system is likely to be very stable with particles having strong negative charges that help maintain dispersion and prevent aggregation.

6.8 IN VITRO DRUG RELEASE STUDY

In-vitro drug release study data were collected over 144 hours, and the cumulative release percentage of drug from the formulation was plotted against time to determine the drug release pattern in various media. From the figures, it was seen that almost 80% of drug content were released from the formulation within 144 hours in PBS 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 First order kinetics, Higuchi kinetics, Hixson-Crowell kinetics and Korsmeyer-Peppas kinetics. Considering the R^2 values (0.7248, 0.8726, 0.716, 0.9331) in all the above-mentioned kinetics models in PBS media, it has been found that the drug release pattern from the formulation followed Korsmeyer-Peppas model which is a mathematical model used to describe drug release from polymeric systems, particularly when the mechanism of release is not well understood or when multiple processes (such as diffusion and erosion) are involved. It is especially useful for analysing the release of drugs from controlled-release formulations.

CHAPTER-7

CONCLUSIONS

CONCLUSIONS

In the present work, Nilotinib loaded nanoliposome/lipo-formulations were developed and their physio-chemical characterization was carried out invitro. From the result of liposomal size analysis, it was clear that all nanoliposome/lipo-formulation formulations were in nano size range and narrow size distribution. Surface morphology study showed that the surface of the nanoliposome/lipo-formulation was smooth and liposome/lipo-formulations were spherical in shape. The nanoliposome/lipo-formulations are uniformly distributed. Higher value of zeta potential indicated the higher stability of the formulation. The liposomal formulation shows good drug entrapment efficiency as well as the FTIR graph suggest that there is barely any interaction between the excipient and drug and the interaction which happens is also occur due to the physical interaction which might help in liposomal formulation. The optimized formulation showed sustained release profile, as shown in the release curve, which will reduce the drug's side effects, decrease the dosing amount, and increase the dosing interval. However, animal studies of the formulation need to be done to check the appropriate therapeutic activity and toxicological activity. After fulfilling the in-vivo investigation, if we get a required result, this will open a new era of treatment over the existing medications for the chronic myeloid leukemia.

CHAPTER-8

REFERENCES

1. Farokhzad OC, Langer R. Impact of nanotechnology on drug delivery. ACS Nano. 2009;3(1):16–20.
2. Aghebati-Maleki A, Dolati S, Ahmadi M, Baghbanzhadeh A, Asadi M, Fotouhi A, et al. Nanoparticles and cancer therapy: Perspectives for application of nanoparticles in the treatment of cancers. J Cell Physiol. 2020;235(3):1962–72.
3. Reckel S, Hamelin R, Georgeon S, Armand F, Jolliet Q, Chiappe D, et al. Differential signaling networks of Bcr-Abl p210 and p190 kinases in leukemia cells defined by functional proteomics. Leukemia. 2017;31(7):1502–12.
4. Osman AEG, Deininger MW. Chronic Myeloid Leukemia: Modern therapies, current challenges and future directions. Blood Rev [Internet]. 2021;49(March):100825. Available from: https://doi.org/10.1016/j.blre.2021.100825
5. Ma WX, Ma N, Chen XH, Zhang YY, Zhang WQ. An overview of chronic myeloid leukemia and its animal models. Sci China Life Sci. 2015;58(12):1202–8.
6. Kumar A, Tilak V, Ali A. Cytogenetic Analysis of Chronic Myeloid Leukemia in an Eastern Indian Population. Int J Clin Med Educ Res. 2023;02(02):37–42.
7. Höglund M, Sandin F, Simonsson B. Epidemiology of chronic myeloid leukaemia: an update. Ann Hematol. 2015;94(2):241–7.
8. Laneuville P. Abl tyrosine protein kinase. Semin Immunol. 1995;7(4):255–66.
9. Montaner S, Perona R, Saniger L, Lacal JC. Multiple signalling pathways lead to the activation of the nuclear factor κB by the Rho family of GTPases. J Biol Chem. 1998;273(21):12779–85.
10. Quintás-Cardama A, Cortes JE. Chronic myeloid leukemia: Diagnosis and treatment. Mayo Clin Proc. 2006;81(7):973–88.

11. Deininger MWN, Goldman JM, Melo J V. The molecular biology of chronic myeloid leukemia. Blood. 2000;96(10):3343–56.
12. Jabbour E, Kantarjian H. Chronic myeloid leukemia: 2018 update on diagnosis, therapy and monitoring. Am J Hematol. 2018;93(3):442–59.
13. Senechal K, Halpern J, Sawyers CL. The CRKL adaptor protein transforms fibroblasts and functions in transformation by the BCR-ABL oncogene. J Biol Chem. 1996;271(38):23255–61.
14. O'Brien SG, Deininger MWN. Imatinib in patients with newly diagnosed chronic-phase chronic myeloid leukemia. Semin Hematol. 2003;40(2 SUPPL. 2):26–30.
15. Danial NN, Pernis A, Rothman PB. Jak-STAT Signaling Induced by the v- abl oncogene . Science (80-). 1995;269(5232):1875–7.
16. Skorski T, Kanakaraj P, Nieborowska-Skorska M, Ratajczak MZ, Wen SC, Zon G, et al. Phosphatidylinositol-3 kinase activity is regulated by BCR/ABL and is required for the growth of Philadelphia chromosome-positive cells. Blood. 1995;86(2):726–36.
17. Sawyers CL, Callahan W, Witte ON. Dominant negative MYC blocks transformation by ABL oncogenes. Cell. 1992;70(6):901–10.
18. Minciacchi VR, Kumar R, Krause DS. Chronic myeloid leukemia: A model disease of the past, present and future. Cells. 2021;10(1):1–23.
19. Mitelman F. The cytogenetic scenario of chronic myeloid leukemia. Leuk Lymphoma. 1993;11(s1):11–5.
20. Stuppia L, Calabrese G, Peila R, Guanciali-Franchi P, Morizio E, Spadano A, et al. p53 Loss and point mutations are associated with suppression of apoptosis and progression of CML into myeloid blastic crisis. Cancer Genet Cytogenet. 1997;98(1):28–35.
21. Gore JM. Chronic myeloid leukemia and chronic lymphocytic leukemia. J Am Acad Physician Assist. 2014;27(2):45–6.
22. Yin X, Li Z, Lyu C, Wang Y, Ding S, Ma C, et al. Induced effect of zinc oxide nanoparticles on human acute myeloid leukemia cell apoptosis by regulating mitochondrial division. IUBMB Life. 2022;74(6):519–31.
23. Hochhaus A, Reiter A, Skladny H, Reichert A, Saussele S, Hehlmann R. Molecular monitoring of residual disease in chronic myelogenous leukemia patients after therapy. Recent Results Cancer Res. 1998;144(Cml):36–45.
24. Shah NP, Rousselot P, Schiffer C, Rea D, Cortes JE, Milone J, et al. Dasatinib in imatinib-resistant or -intolerant chronic-phase, chronic myeloid leukemia patients: 7-year follow-up of study CA180-034. Am J Hematol. 2016;91(9):869–74.

25. Deininger MW. Nilotinib. Clin Cancer Res. 2008;14(13):4027–31.
26. Cortes JE, Gambacorti-Passerini C, Deininger MW, Mauro MJ, Chuah C, Kim DW, et al. Bosutinib versus imatinib for newly diagnosed chronic myeloid leukemia: Results from the randomized BFORE trial. J Clin Oncol. 2018;36(3):231–7.
27. Zhou T, Commodore L, Huang WS, Wang Y, Thomas M, Keats J, et al. Structural Mechanism of the Pan-BCR-ABL Inhibitor Ponatinib (AP24534): Lessons for Overcoming Kinase Inhibitor Resistance. Chem Biol Drug Des. 2011;77(1):1–11.
28. Guilhot F, Chastang C, Michallet M, Guerci A, Harousseau J-L, Maloisel F, et al. Interferon Alfa-2b Combined with Cytarabine versus Interferon Alone in Chronic Myelogenous Leukemia. N Engl J Med. 1997;337(4):223–9.
29. Barrett AJ, Ito S. The role of stem cell transplantation for chronic myelogenous leukemia in the 21st century. Blood. 2015;125(21):3230–5.
30. Chaitanya G, Pawar AKM. Journal of Chemical and Pharmaceutical Research , 2015 , 7 (12): 219-225 Research Article Development and validation of UV spectrophotometric method for the determination of pazopanib hydrochloride in bulk and tablet formulation. 2015;7(12):219–25.
31. Ostendorf BN, le Coutre P, Kim TD, Quintás-Cardama A. Nilotinib. Recent Results Cancer Res. 2014;201:67–80.
32. Blay JY, Von Mehren M. Nilotinib: A novel, selective tyrosine kinase inhibitor. Semin Oncol [Internet]. 2011;38(1 SUPPL.):S3–9. Available from: http://dx.doi.org/10.1053/j.seminoncol.2011.01.016
33. Plosker GL, Robinson DM. Adis Drug Profile. Drugs. 2008;68(4):449–59.
34. Tanaka C, Yin OQP, Sethuraman V, Smith T, Wang X, Grouss K, et al. Clinical pharmacokinetics of the BCR-ABL tyrosine kinase inhibitor nilotinib. Clin Pharmacol Ther [Internet]. 2010;87(2):197–203. Available from: http://dx.doi.org/10.1038/clpt.2009.208
35. Haouala A, Widmer N, Duchosal MA, Montemurro M, Buclin T, Decosterd LA. Drug interactions with the tyrosine kinase inhibitors imatinib, dasatinib, and nilotinib. Blood. 2011;117(8):75–87.
36. Sacha T, Saglio G. Nilotinib in the treatment of chronic myeloid leukemia. Futur Oncol. 2019;15(9):953–65.
37. Jesorka A, Orwar O. Liposomes: Technologies and analytical applications. Annu Rev Anal Chem. 2008;1(1):801–32.
38. Has C, Sunthar P. A comprehensive review on recent preparation techniques of liposomes.

J Liposome Res [Internet]. 2020;30(4):336–65. Available from: https://doi.org/10.1080/08982104.2019.1668010

39. Bozzuto G, Molinari A. Liposomes as nanomedical devices. Int J Nanomedicine [Internet]. 2015;10:975–99. Available from: https://doi.org/10.2147/IJN.S68861
40. Vemuri S, Rhodes CT. Preparation and characterization of liposomes as therapeutic delivery systems: a review. Pharm Acta Helv. 1995;70(2):95–111.
41. Pradhan B, Kumar N, Saha S, Roy A. Liposome: method of preparation, advantages, evaluation and its application. J Appl Pharm Res [Internet]. 2015;3(3):01–8. Available from: https://japtronline.com/index.php/joapr/article/view/54
42. Mishra H, Chauhan V, Kumar K, Teotia D. Journal of Drug Delivery and Therapeutics A comprehensive review on Liposomes : a novel drug delivery system. J Drug Deliv Ther. 2018;8(6):400–4.
43. Farooque F, Wasi M, Mughees MM. Liposomes as Drug Delivery System: An Updated Review. J Drug Deliv Ther. 2021;11(5-S):149–58.
44. Nsairat H, Khater D, Sayed U, Odeh F, Al Bawab A, Alshaer W. Liposomes: structure, composition, types, and clinical applications. Heliyon [Internet]. 2022;8(5):e09394. Available from: https://doi.org/10.1016/j.heliyon.2022.e09394
45. Dwivedi C, Verma S. Preparation and Characterization of Liposomes with Application. J Sci Innov Res. 2013;2(2):486–510.
46. Ghosh S, Mukherjee B, Chaudhuri S, Roy T, Mukherjee A, Sengupta S. Methotrexate Aspasomes Against Rheumatoid Arthritis: Optimized Hydrogel Loaded Liposomal Formulation with In Vivo Evaluation in Wistar Rats. AAPS PharmSciTech. 2018;19(3):1320–36.
47. Mukherjee B, Patra B, Layek B, Mukherjee A. Sustained release of acyclovir from nano-liposomes and nano-niosomes: an in vitro study. Int J Nanomedicine [Internet]. 2007;2(2):213–25. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17722549%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2673966
48. Rudra A, Deepa RM, Ghosh MK, Ghosh S, Mukherjee B. Doxorubicin-loaded phosphatidylethanolamine-conjugated nanoliposomes: In vitro characterization and their accumulation in liver, kidneys, and lungs in rats. Int J Nanomedicine. 2010;5(1):811–23.
49. Edwards KA, Baeumner AJ. Analysis of liposomes. Talanta. 2006;68(5):1432–41.

CERTIFICATE OF APPROVAL

This is to certify that **ADARSH YADAV**, bearing **Registration No: 163655 of 2022-23** has carried out the research work entitled "**DEVELOPMENT AND IN VITRO CHARACTERIZATIONS OF SUBMICRON SIZE NILOTINIB-LOADED LIPID PARTICLES INTENDED FOR THE TREATMENT OF CHRONIC MYELOID LEUKEMIA**" independently with proper care and attention under my supervision and guidance in the Pharmaceutics Research Laboratory in the Department of Pharmaceutical Technology, Jadavpur University. He has incorporated his findings into this thesis of the same title, which he is submitting in partial fulfilment of the requirements for the degree of **MASTERS OF PHARMACY** from Jadavpur University. I appreciate his endeavour to do the project, and his work has reached my gratification.

29/8/24.

Head
Dept. of Pharmaceutical Technology
Jadavpur University
Kolkata-700 032, W.B. India

Head of the Department
Prof. (Dr.) Amalesh Samanta
Department of Pharmaceutical Technology
Faculty of engineering and technology
Kolkata-700032

29/08/2024

Project Guide
Prof. (Dr.) Biswajit Mukherjee
Department of Pharmaceutical Technology
Faculty of engineering and technology
Kolkata-700032

Dipak Laha 29.8.24

Dean
Prof. Dipak Laha
Faculty of Engineering and technology
Jadavpur University
Kolkata-700032

DEAN
Faculty of Engineering & Technology
JADAVPUR UNIVERSITY
KOLKATA-700 032

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 his 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.

Name: ADARSH YADAV

Examination Roll Number: M4PHC24002

Registration Number: 163655 of 2022- 2023

Adarsh Yadav.
29/08/2024

Signature with Date

ACKNOWLEDGEMENT

I would like to express my deep gratitude to my project guide Prof. (Dr.) Biswajit Mukherjee, Department of Pharmaceutical Technology, Jadavpur University, for his valuable guidance, encouragement, support, and inspiration throughout the work. His unremitting advice, affection, and help will be responsible for the successful completion of this work.

I express my heartfelt thanks to my laboratory seniors, especially Rima Chandra, Alankar Mukherjee for their support. I also thank Dr. Ramkrishna Sen, Ashique Al Hoque, Manisheeta Ray, Mrinmoy Barman, Ratna Roy, Sandipan Mallick, Pintu Rana, and many others for their valuable support and cooperation, which made my work fruitful.

I would also like to extend my gratitude to my fellow classmate Srijita Gope, who helped me in various ways during my research work.

I extend my special thanks to my mother, Indu Yadav, and father, Yogendra Yadav, for their support, love, and encouragement. I would like to extend my special thanks to Jyoti Bala for her support, love and encouragement. I would also like to acknowledge the IACS(Kolkata) and the Department of Physics (Jadavpur University), Kolkata, for their assistance in providing me with instrument facilities for my research work.

I want to thank UGC (University Grants Commission) for providing me with a scholarship throughout my academic sessions.

Last but not least, I would like to express my gratitude towards my parents and friends for their kind cooperation and encouragement, which have helped me in every step of my academic career.

Harsh Yadav
29/08/2024