PREPARATION AND EVALUATION OF LAMIVUDINE-LOADED LIPOSOME FOR THE TREATMENT OF AIDS

THESIS SUBMITTED IN THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF PHARMACY

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BY

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CERTIFICATE OF APPROVAL

This is to certify that **Sohini Chakraborty** bearing **Registration No: 160265 of 2021-22** has carried out the research work entitled **"PREPARATION AND EVALUATION OF LAMIVUDINE LOADED LIPOSOME THE TREATMENT OF AIDS "** 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, being submitted by him. 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.

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DECLARATION OF ORIGINALITY AND COMPLIANCE OF ACADEMIC ETHICS

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

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DEDICATED TO
ALMIGHTY

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CHAPTER 1

INTRODUCTION

INTRODUCTION

Nanotechnology refers to research and technology development at the atomic, molecular, and macromolecular scale, leading to the controlled manipulation and study of structures and devices with length scales in the 1- to 100-nano meters range scale. The small size, surface modification elevated solubility, and multifunctionality of nano- particles open many new research avenues for biologists. This rapidly growing field allows the opportunity to design and develop multifunctional nanoparticles that can target, diagnose, and treat diseases such ascancer, Aids and many other disease. When any drug is administered only a very small fraction of the dose actually goes into the relevant receptors or sites of action, and remaining portion of the dose is wasted by taken up into the wrong tissue or it is removed from the right tissue easily. Scientists are doing more and more experiment with nano formulations to a) Maximize drug activity, b) Minimize side effects. As a successful delivery liposome is getting more and more popularity and favourable approach for drug administration in human body. The advantage of liposomes are a) carry both water and oil soluble drugs, b)biocompatibility, c)nontoxicity d) Targetability e)Flexible f)non immunogenic g)protein stabilization .liposomal delivery is a fastest growing and improving delivery system as it is contributing in too many areas like –Drug delivery, chemistry, biophysics, cosmetics , structure and function of biological membrane.(Wong et al. 2010)

Development of drug delivery systems for brain delivery also into the central nervous system is one of the most difficult and Challenging research topics in pharmaceutical areas, mainly due to the presence of two anatomical and biochemical dynamic barrier -the blood–brain barrier (BBB) and blood –cerebrospinal fluid barrier (BCSFB) which separates the blood from the cerebral parenchyma thus limiting the brain uptake of the majority of therapeutic agents .BBB is a highly selective barrier contain selective semipermeable border of endothelial cell that prevents solute substances comes in contact with the extracellular fluid of the central nervous system .The blood brain barrier is formed by endothelial cells ,astrocytes ,Tight junction ,pericytes which are embedded in the basement membrane of the capillary. BBB contains continuous capillary system so there is no gap present between the endothelial cell of the capillary. This barrier only allows small molecules to pass through passive diffusion also selective and active transport of organic anions, and macromolecules such as glucose, Amino acid .The blood brain barrier do not allow the passage of pathogens, hydrophilic molecule and also large molecule into the brain while it allows hydrophobic molecule (O_2,CO_2, hormones) and

non-polar molecules**(Haumann et al. 2020**). BBB inhibits free paracellular diffusion of water soluble molecules by the formation of complex tight junction (TJ). Tight junctions are present between the endothelial cells which are composed of smaller subunits of transmembrane proteins such as occluding claudins and junctional adhesional molecule. This barrier also prevents the passage of peripheral immune factors-like signaling molecule, antibodies and immune cells into the CNS. Thus prevent the brain cell from the damage of peripheral immune activities. BCSFB is a fluid –brain barrier that is composed of membranes that separate blood from CSF at the capillary level and CSF from brain tissue.

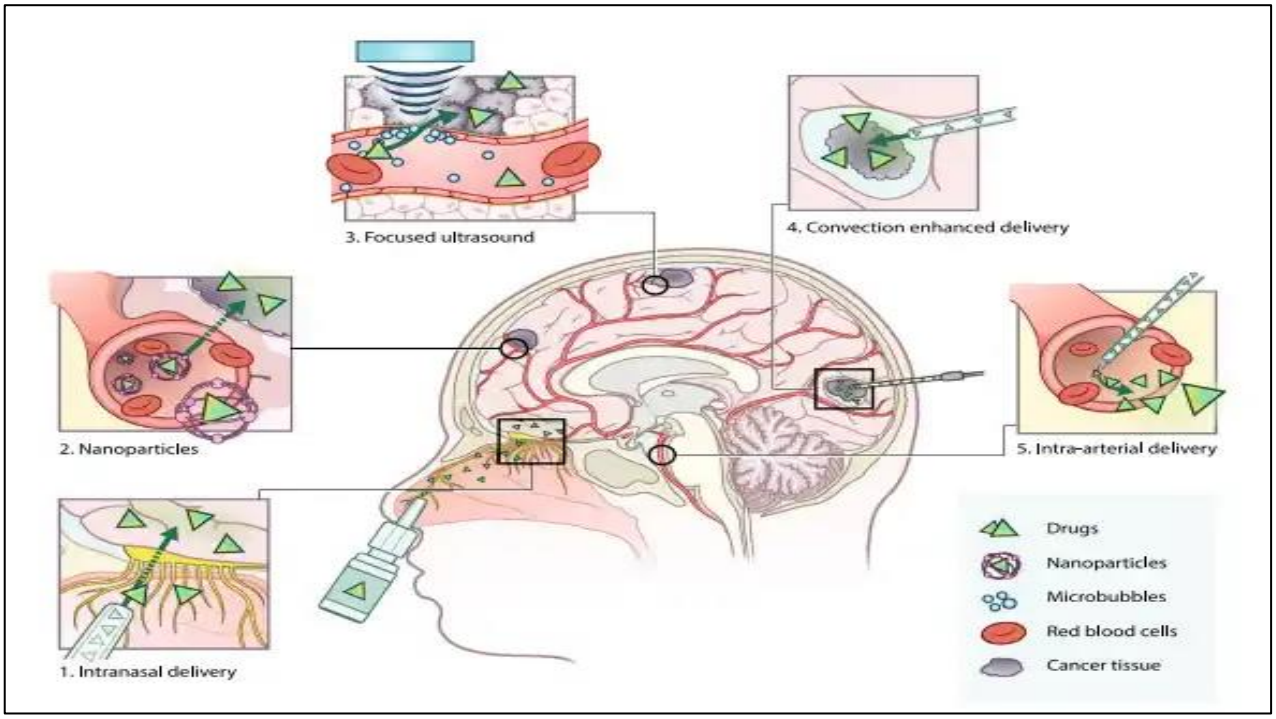

FIGURE 1- Overview of current drug delivery methods for the treatment of primary brain disease (Haumann et al. 2020) **Panel 1. Intranasal drug delivery:** drug is formulated in spray particles that enter the brain through the nasal cavity via the neuroepithelium. Here, the drug can enter without interference of the blood–brain barrier (BBB). **Panel 2**. **Nanoparticles**: nanoparticles encapsulate drugs to increase plasma half-life and allow entry to the brain parenchyma by the enhanced permeability and retention (EPR) effect, endocytosis, and receptor-mediated transcytosis. **Panel 3. Microbubble-mediated focused ultrasound:** microbubbles are intravenously administered and upon the application of focused ultrasound, microbubbles start to oscillate. The oscillation disrupts the BBB, temporarily opening it to allow drugs to enter the brain parenchyma. **Panel 4 Convection enhanced delivery (CED):** surgical placement of catheters in the brain to administer the drug directly in the tumor site. **Panel 5. Intra-arterial**

drug delivery: catherization of the blood vessel and injection of drugs directly in the vicinity of the tumor, sometimes in combination with hyperosmolar drugs that open the BBB.

Different strategies have been found to deliver the drug into the CNS by crossing the BBB. These strategies are Changing the drug molecule from polar to non-polar, disrupting the BBB, Formulation that helps to deliver the drug into the brain-that include: prodrug, liposomal analogue, chemical drug delivery system, Carrier mediated transport, Receptor mediated transport system. Two methods for disrupting the blood brain barrier are a) **Osmotic blood** – **brain barrier disruption** and **b) Biochemical blood brain –brain barrier disruption.** These methods have certain limitations- They are invasive procedures, have toxic side effects , low efficacy, and also cause brain damages or infection from the toxins and chemicals**(Daneman and Prat 2015)**. All of these afore mentioned techniques involve the circulatory system to penetrate the drug into CNS. They will increase the penetration of drug throughout the body. Sometime after manipulation in the drug design, many potential drug cannot penetrate in the brain parenchyma at therapeutic concentration, so the alternative strategy to enhance the penetration of drug into the CNS depends on some method that do not involve cardiovascular system. These methods do not need drug manipulation to increase BBB permeability –we can give the drug in intraventricular or intrathecal route and also in olfactory pathway. Nanomedicines (such as polymeric nanoparticles and nano liposomes) have becomea most suitable option for targeted drug deliveryinto the brain because they can cross the BBBby various targeting mechanisms (i.e., enhancedretention of nanoparticles in the brain–blood capillaries and opening the tight junction of brain endothelial cells).(Wong et al. 2010).The advantages of nano size delivery system are tiny size, enhance solubility , better permeability ,multifunctionality . These liposomal drugdelivery can theoretically yield high CNS drug concentration with minimal systemic side effects and toxicity. liposomal drug delivery is one of the emerging nano drug delivery techniques for improving cancer treatment, Neuro AIDS treatment. This term 'Liposome' has been derived from two Greek words – that is 'Lipos', that represents fat and 'Soma', known as body. It relates to the body's structural building block-phospholipids molecules. Thus a liposome can be defined as a spherical vesicle along with a membrane composed of phospholipids bilayers similar to that of the cell membranes Liposomes blended with lipid chains can be composed of originally derived phospholipids. Phospholipid molecules have a head group and a tail group. In the water, the head group are drawn to water and aligned to structure a water-facing surface. The combined structure forms a closed bilayer called as liposomes. They are employed as cell membrane models as well as vehicles for drug delivery. (Muthu and Singh 2009)

Liposome consist of one or more lipid bilayers which surrounds aqueous units. Due to their size and hydrophobic and hydrophilic character liposomes are promising systems for drug delivery. vesicles which have one or more phospholipid bilayer membranes can transport aqueous or lipid drugs, depending on the nature of those drugs..(Samad and Sultana 2007). The most advantageous features of liposomes are their ability to incorporate and deliver large of drug and the possibility to decorate their surface with different ligands. Targeted drug delivery of various drugs for the treatment of cancer, AIDS and braindisorders is the primary research area in which nanomedicines have a major role and need. (Lian and Ho 2001)

Human immunodeficiency virus (HIV) is a lentivirus from the *Retroviridae* family responsible for the acquired immunodeficiency syndrome (AIDS). At present, there are two known types of HIV, HIV-1 and HIV-2, HIV-1 is much more virulent, transmittable and prevalent, and the cause of the majority of HIV infections in the world. The pandemic strain of HIV -1 is closely related to a virus found in *chimpanzees* of the subspecies *Pan troglodytes troglodyte* lives in the forest of central Africa. HIV 2 is less transmittable and is largely confined to West Africa, southern Senegal Guinea, Liberia.(Lifson 1988)

HIV infection results in compromised immune defense by causing extensive destruction of T-helper cells, macrophages, dendritic cells and other cellular components associated with cell-mediated immunity .As a result, HIV-infected patients are substantially more vulnerable to opportunistic infections.(Marks 2002)

According to the 2007 update by the Joint United Nations Program on HIV/AIDS and World Health Organization, every day over 6800 individuals become newly infected and over 5700 patients die from AIDS. the sub-Saharan African region remains as the epicenter of the pandemic. 22.5 million people in these countries. The prevalence is also very high in the Caribbean Islands (1.0%), Latin America (0.5%), Eastern Europe and Central Asia (0.9%).HIV is a commonly transmitted via unprotected sexual activity ,blood transfusions, hypodermic needles, from mother to child through breast milk and exposure to blood, semen, and vaginal fluids. .

This virus destroy CD4+ lymphocytes and impair cell mediated immunity, increasing risk of certain infections and cancer. HIV infects vital cells in the human immune system, such as Helper T cells (specifically CD4+ T cells) macrophages, and dendritic cells. HIV infection leads to low levels of CD4+T cells, so cell mediated immunity is lost and the body becomes more susceptible to opportunistic infections, Leading to the development of AIDS (Miedema et al. 1988).The virus can enter the CNS compartment from the systemic circulation via two routes: i) through the blood-cerebro spinal fluid barrier (BCSFB) at the choroid plexus as

cell-free viral particles, and/or ii) through the blood-brain barrier (BBB) in form of infected monocytes. The second route is known as the "Trojan horse approach". The brain macrophages and microglial cells, upon infection are responsible for further production of HIV-1 virus, and can also release viral proteins such as glycoprotein 120 (gp120), Tat (transcriptional activator) and VPR (viral protein R). These viral proteins have been shown to be neurotoxic *in vitro* and trigger various harmful events such as activation of apoptotic pathways, cell-cycle arrest of neuronal cells and stimulation of the production of reactive oxidative species, glutamate, cytokines and other inflammatory factors from uninfected astrocytes(Kaul et al. 2005). Additionally, gp120 and Tat can render the BBB leakier which further promotes the permeability of HIV-infected monocytes. Once in the brain compartment the virus actively replicates make reservoir, resulting in showing neurological complications, latent infection and drug resistance. Current antiretroviral drugs (ARVs) often fail to effectively reduce the HIV viral load in the brain. This is due to the poor transport of many Anti-retroviral drug, in particular protease inhibitors, across the blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCSBF)(Kaul et al. 2005). Studies have shown that nanocarriers including polymeric nanoparticles, liposomes, solid lipid nanoparticles (SLN) and micelles can increase the local drug concentration gradients, facilitate drug transport into the brain via endocytotic pathways and inhibit the ATP-binding cassette (ABC) transporters expressed at the barrier sites. By delivering anti-retroviral drug with nanocarriers, significant increase in the drug bioavailability to the brain is expected to be achieved(Wong et al. 2010). Approximately 10 to 20% treated patients demonstrate minor cognitive/motor disorder (MCMD), which presents with symptoms such as cognitive and motor slowing, poor concentration and impaired memory, a more severe form of neurological complications collectively termed HIV-associated dementia (HAD) or AIDS dementia complex may develop (Wong et al. 2010).Breakdown of the BBB occurs in response to HIV or viral proteins Tat and gp 120.This disruption results in alterations in TJ protein expression, leading to enhanced paracellular compound flux across the BBB, leading to alteration in the expression and function of active efflux transport protein such as p-glycoprotein. The breakdown of BBB can lead to decreased concentration, memory, psycomotor speed, incoordination and tremor.

Lamivudine, commonly called 3TC, is an antiretroviral medication used to prevent and treat HIV/AIDS. It is also used to treat chronic hepatitis B when other options are not possible. It is effective against both HIV-1 and HIV-2 It is typically used in combination with other antiretrovirals such as zidovudine and abacavir. Lamivudine may be included as part of post-

exposure prevention in those who have been potentially exposed to HIV. Lamivudine is a nucleoside reverse transcriptase inhibitor (NRTIs). This class of antiretroviral disrupts the activity of the HIV enzyme reverse transcriptase . In 1996, lamivudine **was licensed in Europe as a treatment for HIV** when used in combination with other anti-HIV drugs. It was discovered by Bio Chem Pharma . Lamivudine is an analogue of cytidine. It is phosphorylated to active metabolites that compete for incorporation into viral DNA. They inhibit the HIV reverse transcriptase enzyme competitively and act as a chain terminator of DNA synthesis.(Vasconcelos et al. 2008)

Lamivudine is administered by mouth, and it is rapidly absorbed with a bio-availability of over 80%. While systemic exposure to lamivudine does not change with food intake, taking lamivudine with food slows the **absorption rate by 40%.** The solid tablet is preferred in children because bioavailability is 40% lower in children using the oral liquid form. The larger dose, 300 mg taken once daily, has shown to have larger trough and maximum serum levels and is less consistent throughout the day compared to the smaller 150 mg tablet taken twice daily. Lamivudine is a commonly used hydrophilic antiviral drug for treatment of acquired immunodeficiency syndrome. Lamivudine has a short biological half-life (4-6 hour) and requires frequent administration for a prolonged period of time (Jain et al. 2007b).

➢ COMPONENTS OF BLOOD BRAIN BARRIER

The blood–brain barrier was discovered in the late 19th century by Paul Ehrlich. The purpose of the blood–brain barrier is to protect against circulating toxins or pathogens that could cause brain infections, while at the same time allowing vital nutrients to reach the brain. Its other function is to help maintain relatively constant levels of hormones, nutrients and water in the brain – fluctuations in which could disrupt the finely tuned environment.

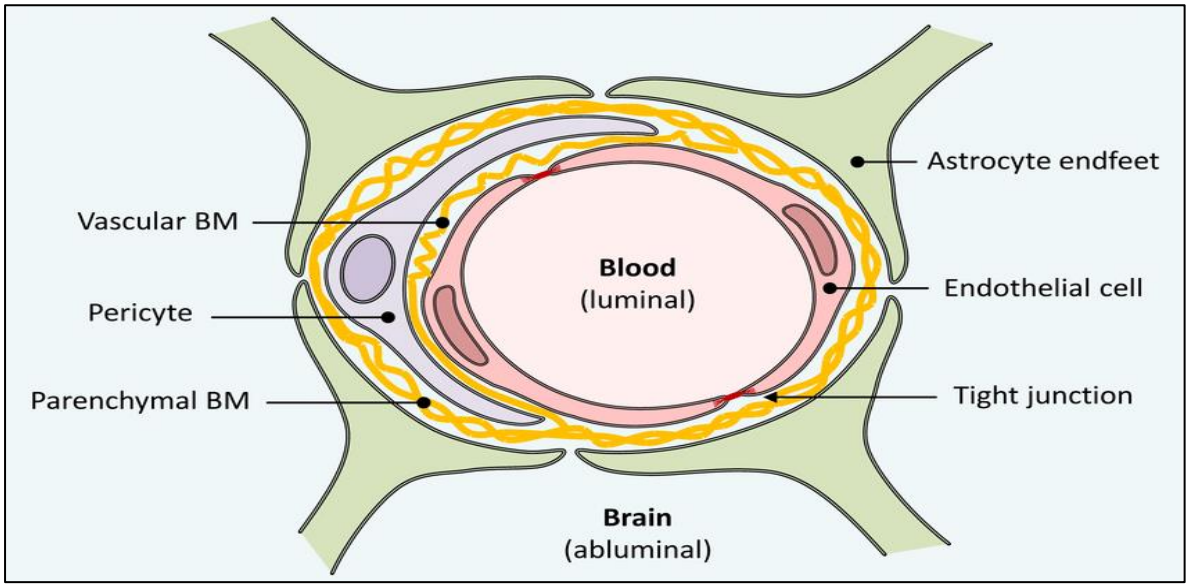

FIGURE 2. Anatomical structure of the blood–brain barrier (BBB). (Chen and Liu 2012a)

The blood–brain barrier is formed by **a)Endothelial cells of the capillary wall**,**b) Astrocyte end-feet ensheathing the capillary,** and **c)Pericytes embedded in the capillary basement membrane**. (Wong et al. 2010).

- **ENDOTHELIAL CELLS**

Endothelial cells (ECs) are mesodermally derived modified simple squamous epithelial cells that form the walls of blood vessels. The ECs lining of the cerebral blood vessels are the core anatomical structure of the BBB. In the CNS, ECs are sealed by both tight and adherens junctions, thereby restricting paracellular transport. These cells also display limited vesicular transport, effectively reducing the vesicle-mediated transcytoplasmic movement of solutes into the CNS.

ECs have distinct luminal and abluminal surfaces with compartment receptors, and efflux and influx transporters, which together regulate the vectorial transport of cargoes .The ECs of the CNS contain higher numbers of mitochondria than peripheral ECs, reflecting a greater energy expenditure to drive ion gradients which are critical for the activity of enzymes and transporter systems .They also present with a net negative surface charge, repelling negatively charged compounds.as well as an absence of fenestrations (small transcellular pores that allow free diffusion).

➢ JUNCTIONAL COMPLEXES OF THE ENDOTHELIAL CELLS

Tight junctions are located between ECs, forming continuous blood vessels by completely sealing the interendothelial cleft, and limiting paracellular permeability (gate function). The major tight junction proteins are claudins and occludins that localize to two-cell contacts. **Claudins** are a multigene family of 20–24 kDa membrane proteins, among 27 claudins known in humans, claudin-5 is the most enriched isoform in the brain endothelium. other claudins are present in kidney, intestine, inner ear, testis, retina. where they have an intrinsic role in regulating permeability. (Daneman and Prat 2015)

Occludin is a 65 kDa trans membraneous protein that was the first tight junction protein to be discovered, Occludin has four transmembrane domains, The first extracellular loop is involved in cell adhesion, occludin is not essential for the formation of tight junctions however, it plays a critical role in the regulation and maintenance of BBB function, Occludin has also been shown to regulate epithelial cell differentiation and signal transduction.

Junction adhesion molecules are 40kDa membrane protein that belong to the immunoglobulin superfamily. Establishing cell polarity, and are also involved in regulating leucocyte adhesion and migration. Adherens junctions are involved in the initiation and stabilization of the adhesion of ECs to one another, regulating paracellular permeability intracellular signaling contact inhibition during vascular growth, Cadherins are the major adherens junction proteins. They are calcium-dependent transmembrane proteins which are involved in cell adhesion through homotypic interactions, cadherin is abundant in the CNS endothelium, and is a critical player in cell survival, vessel assembly and stabilization.(Wong et al. 2010)

➢ BASEMENT MEMBRANE

The vascular tube is surrounded by two Basement Membranes, the inner vascular Basement Membrane and the outer parenchymal BM, The vascular BM is an extracellular matrix secreted by the ECs and PCs, whereas the parenchymal basement membrane is secreted by the astrocytic process. These BMs are comprised of different secreted molecules including type IV collagens, laminin, nidogen, heparin sulfate proteoglycans, and other glycoproteins. These Basement membranes provide an anchor for many signaling processes at the vasculature, but also provide an additional barrier for molecules and cells to cross before accessing the neural tissue.

Disruption of these Basement membranes by matrix matalloproteinases is an important component of BBB dysfunction and leukocyte infiltration that is observed in many different neurological disorders.

- **ASTROCYTES**

Astrocytes are a major glial cell type. The endfeet of the basal process almost completely ensheath the vascular tube, and contain a discrete array of proteins including dystroclycan, dystrophin, and aquaporin 4. The dysroglycan– dystrophin complex is important to link the endfeet cytoskeleton to the basement membrane by binding agrin . This linkage coordinates aquaporin4 into orthogonal arrays of particles, which is critical for regulating water homesostasis in the CNS. Astrocytes provide a cellular link between the neuronal circuitry and blood vessels. This neurovascular coupling enables astrocytes to relay signals that regulate blood flow in response to neuronal activity(Daneman and Prat 2015)

Astrocytes have been identified as important mediators of BBB formation and function. Astrocytes and pericytes also express angiotensin 1. In addition to contributing to neuronal functions such as synaptic plasticity and redox metabolism Astrocytes have also been shown to upregulate the expression of transporters such as P-glycoprotein (P-gp) and glucose transporter 1 (GLUT1), as well as facilitating their polarized localization. These cells also promote proteoglycan synthesis, ensuring increased EC charge selectivity. In addition, astrocytes contribute to ionic, amino acid and neurotransmitter homeostasis in the brain.

- **IMMUNE CELLS**

CNS blood vessels interact with different immune cell populations both within the blood as well as within the CNS. The two main cell populations within the CNS are perivascular macrophages and microglial cells. Perivascular macrophages are monocyte lineage cells that sit on the abluminal side of the vascular tube. These cells are derived from blood borne progenitors, and chimera experiments suggest that they are able to cross the BBB. Microglial cells are resident CNS parenchymal immune cells that are derived from progenitors in the yolk sac and enter the brain during embryonic development.

These cells are involved in regulating neuronal development innate immune response, and wound healing, and can act as antigen-presenting cells in adaptive immunity.In addition, different blood-borne immune cell populations, including neutrophils, T cells, and

macrophages, can interact with CNS vessels when activated and are thought to regulate BBB properties in responseto infection, injury, and disease by releasing reactive oxygen species that can increase vascular permeability.(Miedema et al. 1988)

- **TRANSPORT FUNCTION OF BBB**

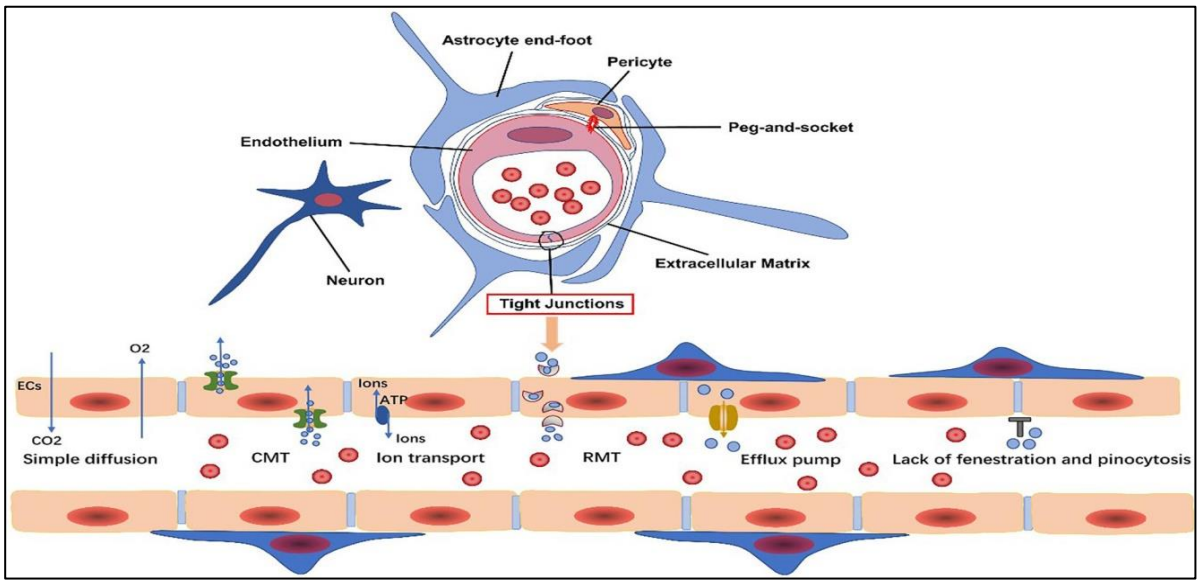

FIGURE 3- Transport across the BBB**(**(Fernandes et al. 2021)

The low permeability of the paracellular junctions allows the transport properties of the cells to control the movement of ions and molecules between the blood and the brain. Various modes of transport for physiological cargoes are employed at the BBB, and evidently these can also be utilized for drug delivery. In general, under physiological conditions substances may cross the BBB by **passive diffusion, carrier mediated transport, receptor mediated transport and Adsorptive transcytosis**. The possibility for substances to passively cross the BBB by diffusion is very limited due to BBB physiology . The exception are lipid soluble small molecules with an molecular weight of less than 400Da.Uptake of such substances is possible by lipid –mediated free diffusion across the BBB .High lipid solubility in turn is correlated to low hydrogen bonding .Here the structure of molecule possesses high affinity for specific carriers or receptor or receptor found at the BBB. For example water-soluble metabolic substrates such as glucose, are bound by specific transporters, Here the glucose transporter type 1 **(GLUT)** That has affinity for other hexose, including mannose and galactose. Due to the high energy demand of the brain, the abundance of GLUT 1 at the microvasculature is high relative to other transporters ,Which enables rates of glycolysis in the brain .Another example is the large neutral amino-acid transporter type 1**(LAT1),**which is a carrier for phenylalanine ,but also transports over 10 other large neutral amino acids. A drug that shows such an affinity for a carrier mediated transport system is **L-DOPA.**

There are two main types of transporters expressed by CNS ECs: **efflux transporters and nutrient transporters.** Active efflux transporters can be found at the BBB that mediate the asymmetric efflux of metabolites from the brain back to the blood circulation. The Classical active efflux transporter system within the BBB is P-glycoprotein(p-gp).This protein is encoded by the multidrug resistance gene 1**(MDR1).** Gases such as oxygen and carbon dioxide readily diffuse across the BBB following a concentration gradient. In addition, a wide range of lipid-soluble molecules can enter the brain by diffusion, depending on their lipid solubility, molecular weight and hydrogen bonding capacity (8–10 hydrogen bonds). The cerebral endothelium also expresses a number of specific solute transporters in order to facilitate the **carrier-mediated transport (CMT)** of carbohydrates (glucose), amino acids, monocarboxylic acids, hormones, fatty acids, nucleotides, ions, amines, choline and vitamins. **Receptor mediated transport** processes additionally occur at the BBB as mechanism regulating the crossing of endogenous substances. These peptide receptors may mediate processes such as transcytosis of ligands from blood to brain or from brain to blood or only uptake into the brain capillary endothelium. Examples are transport of insulin or transferrin. Insulin is transported across the BBB by binding to an endogenous BBB insulin receptor. Similarly for transferrin a specific receptor Tf receptor can be found at the BBB.(Chen and Liu 2012b)

Adsorptive transcytosis has been described for some compounds and nanoparticles, but it contributes significantly to the transport of endogenous substances across the BBB is still under investigation .Cationic molecules have a greater propensity to cross the BBB, probably via their interaction with the negatively charged EC membrane and proteoglycans in the basement membrane. Efflux mechanisms also contribute to barrier functions, with ATP- binding cassette (ABC) transporters. ABC transporters have been identified in humans and classified into seven families The BBB also has a major role in controlling the concentration of ions in the CNS, with the abluminal Na^{+}–K^{+}-ATPase regulating the influx of sodium and the efflux of potassium ions.

- **HIV-**

HIV (*human immunodeficiency virus*) is a virus that attacks cells that help the body fight infection, making a person more vulnerable to other infections and diseases. It is spread by contact with certain bodily fluids of a person with HIV, most commonly during unprotected sex (sex without a condom or HIV medicine to prevent or treat HIV), or through sharing injection drug equipment. If left untreated, HIV can lead to the disease AIDS (*acquired immunodeficiency syndrome*). The human body can't get rid of HIV and no effective HIV cure exists. So, once you have HIV, you have it for life. However, effective treatment with HIV

medicine (called antiretroviral therapy or ART) is available. If taken as prescribed, HIV medicine can reduce the amount of HIV in the blood (also called the viral load) to a very low level. If a person's viral load is so low that a standard lab can't detect it, this is called having an undetectable viral load. People with HIV who take HIV medicine as prescribed and get and keep an undetectable viral load can **live long and healthy lives** and **will not transmit HIV to their HIV-negative partners through sex.**

- **AIDS-**

AIDS is a chronic immune system disease caused by the human immunodeficiency virus (HIV). HIV damages the immune system and interferes with the body's ability to fight infection and disease. HIV can be spread through contact with infected blood, semen, or vaginal fluids. There's no cure for HIV/AIDS, but medications can control the infection and prevent disease progression. AIDS is the late stage of HIV infection that occurs when the body's immune system is badly damaged because of the virus.

- **A person with HIV is considered to have progressed to AIDS when** The number of their CD4 cells falls below 200 cells per cubic millimeter of blood (200 cells/mm3). (In someone with a healthy immune system, CD4 counts are between 500 and 1,600 cells/mm3.). Or They develop one or more opportunistic infections regardless of their CD4 count.

- **Structure of HIV virus-**

HIV is similar in structure to other retroviruses. It is roughly spherical with a diameter of about 120 nm. It is composed of two copies of positive-sense single-stranded RNA that codes for the virus's nine genes enclosed by a conical capsid composed of 2,000 copies of the viral protein. Virus contain many enzymes as reverse transcriptase, proteases, ribonuclease and integrase. A matrix composed of the viral protein p17 surrounds the capsid ensuring the integrity of the virion particle. This is, in turn, surrounded by the viral envelope, that is composed of the lipid bilayer taken from the membrane of a human host cell when the newly formed virus particle buds from the cell. The viral envelope contains proteins from the host cell and relatively few copies of the HIV envelope protein, which consists of a cap made of three molecules known as **glycoprotein (gp) 120,** and a stem consisting of three **gp41** molecules that anchor the structure into the viral envelope. The envelope protein, encoded by the HIV *env* gene, allows the virus to attach to target cells and fuse the viral envelope with the target cell's membrane releasing the viral contents into the cell and initiating the infectious cycle. As the sole viral protein on the surface of the virus, the envelope protein is a major target

for HIV vaccine efforts.

The RNA genome consists of at least seven structural landmarks **(LTR, TAR, RRE, PE, SLIP, CRS, and INS),** and nine genes **(*gag*, *pol*, and *env*, *tat*, *rev*, *nef*, *vif*, *vpr*, *vpu*, and sometimes a tenth *tev*, which is a fusion of *tat*, *env* and *rev*),** encoding 19 proteins. Three of these genes, *gag*, *pol*, and *env*, contain information needed to make the structural proteins for new virus particles. For example, *env* codes for a protein called gp160 that is cut in two by a cellular protease to form gp120 and gp41. The six remaining genes, *tat*, *rev*, *nef*, *vif*, *vpr*,and *vpu* (or *vpx* in the case of HIV-2), are regulatory genes for proteins that control the ability of HIV to infect cells, produce new copies of virus (replicate), or cause disease.

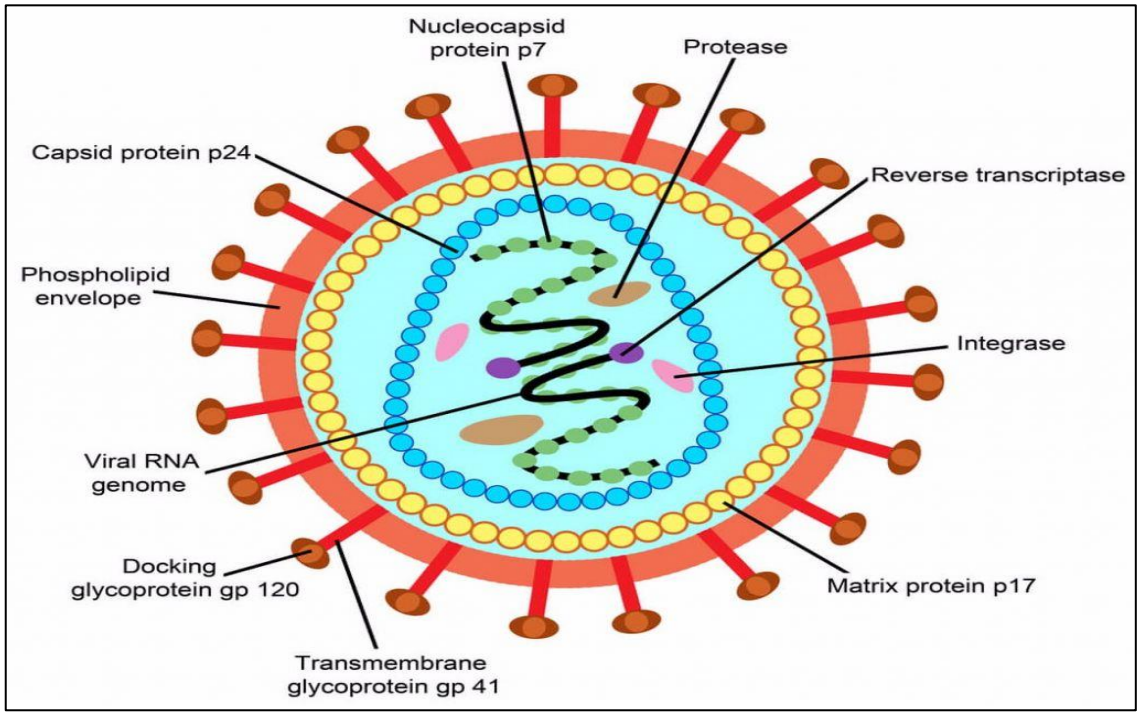

FIGURE 4-Structure of HIV Virus (Chen and Liu 2012a; Dawany 2023)

➢ Replication Cycle of HIV virus-

Host cells infected with HIV have a shortened life span asa result of the virus's using them as "factories" to producemultiple copies of new HIV. Thus, HIV continuously usesnew host cells to replicate itself. As many as 10 million to 10 billion virions (individual viruses) are produced daily. In the first 24 h after exposure, HIV attacks or iscaptured by dendritic cells in the mucous membranes and skin. Within 5 days after exposure, these infected cells make their way to the lymph nodes and eventually to the peripheral blood, where viral replication becomes rapid. CD4+ lymphocytes that are recruited to respond toviral antigen migrate to the lymph nodes. These become activated and then proliferate via complex interaction of cytokines released in the microenvironment of the lymphnodes. This sequence of events makes the CD4+ cells more susceptible to HIV infection, and it explains the generalized

lymphadenopathy characteristic of the acute retroviral syndrome seen in adults and adolescents. In contrast, HIV-infected monocytes allow viral replication but resist killing. Thus, monocytes act as reservoirs of HIV and as effectors of tissue damage in organs such as the brain.

The HIV life cycle includes six phases: **Binding and entry, Reverse transcription, integration, Replication, Budding, and Maturation.**

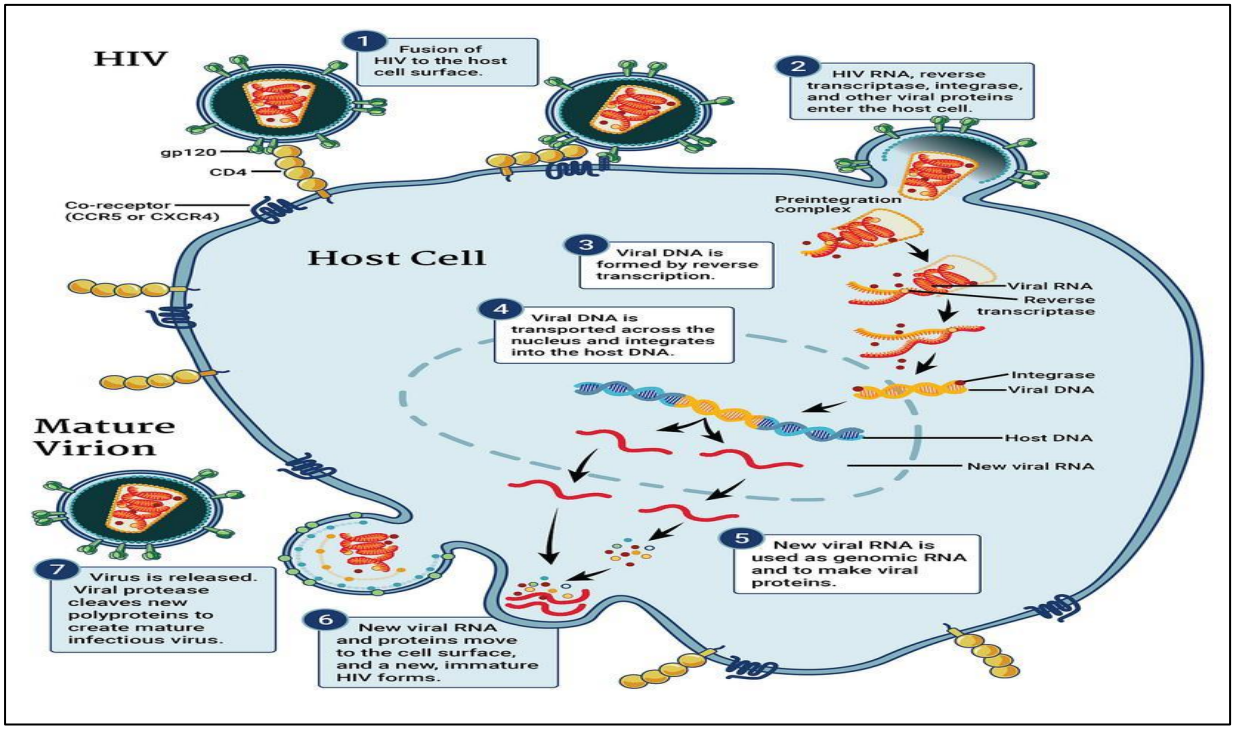

FIGURE 5-The replication cycle of HIV virus (Totonchy and Cesarman 2016)

- **Binding and Entry-**

The envelop proteins gp120 and gp41 bind to CD4 +cell receptors and coreceptors on the outside of CD4+ cells and macrophages. The chemokine receptors CCR5 and CXCR4 facilitate viral entry. T-cell tropic viruses require CXCR4 to bind, and macrotropic strains of the virus require CCR5.R5 is the most common virus transmitted during acute infection.

The joining of the proteins and the receptors and coreceptors fuses the HIV membrane with the CD4+ cell membrane, and the virus enters the CD4+ cell and macrophage. The HIV membrane and the envelope proteins remain outside of the CD4 +cell, Whereas the core of the virus enters the CD4+ cell.CD4+ cell enzymes interact with the viral core and stimulate the release of viral RNA and the viral enzymes reverse transcriptase, integrase, and protease.

- **Reverse Transcription-**

  The HIV RNA must be converted to DNA before it can be incorporated into the DNA of the CD4+cell.This incorporation must occur for the virus to multiply.The conversion of HIV RNA to DNA is known as reverse transcription and is mediated by the HIV enzyme reverse transcriptase.The result is the Production of a single strand of DNA from the viral RNA. The single strand of this DNA then undergoes replication into double-stranded HIV DNA.

- **Integration-**

  Once reverse transcription has occurred, the viral DNA can enter the nucleus of the CD4+cell.The viral enzyme integrase then inserts the viral DNA into the CD4+cell's DNA. This process is known as integration. The CD4+ cell has now been changed into a factory used to produce more HIV.

- **Replication-**

  The new DNA, which has been formed by the integration of the viral DNA into the CD4+cell, causes the production of messenger DNA that initiates the synthesis of HIV proteins.

- **Budding-**

  The HIV proteins and viral RNA, all the components needed to make a new virus, gather at the CD4+cell membrane to form new viruses.These new viruses push through the different parts of the cell wall by budding. Many viruses can push through the wall of one CD4+ cell. These new viruses leave the CD4+cell and contain all the components necessary to infect other CD4+ cells.

- **Maturation-**

  The new virus has all the components necessary to infect other CD4+cells but cannot do so until it has matured. During this process, the HIV protease enzyme cuts the long HIV proteins of the virus into smaller functional units that then reassemble to form a mature virus. The virus is now ready to infect other cell.

➢ Effects on Immune system-

The pathogenesis of HIV is basically a struggle between HIV replication and the immune responses of the patient, via cell-mediated and immune mediated reactions. The HIV virus directly and indirectly mediates CD4+T-cell destruction. There is destruction of mature CD4+cells,CD4+progenitor cells in bone marrow, the thymus, and peripheral lymphoid organs, as well as CD4+cells within the nervous system, such as microglia. The result of this destruction is failure of T-cell production and eventual immune

suppression.

There are many mechanisms of CD4+ cell depletion by HIV infection. Direct HIV – mediated cytopathic effects include single-cell killing as well as cell fusion, or syncytium formation. The syncytium is a fusion of multiple uninfected CD4+cells with one HIV-infected CD4+cell via CD4-gp120interaction.This fusion results in a multinucleated syncytium, or giant cell, which may ultimately serve as a means to produce many virions.

- **Table1-The spread of HIV outside lymphoid organs to the brain, spinal cord, lung, colon, liver,and kidney usually occurs late during illness**.

System	Cell
Hematopoietic	• T-cell(CD4+or CD8+) • Macrophages/monocytes • Dendritic cells • Fetal thymocytes and thymic epithelium • B-cells • NK cells • Megakaryotic Cells • Stem Cells
Central Nervous	• Microglia • Capillary endothelial cells • Astrocytes • Oligodendrocytes
Large Intestine	• Columnar epithelium
Other	• Kupfer cells(Liver) • Synovial Cells • Placental tophoblast cells

Different Clinical stages of the disease-

- **Clinical Stage 1-**
  During this stage HIV-infected patients may or may not have signs and symtoms of HIV infection. In HIV infected adults, this phase may last 8-10 years. The HIV enzyme-linked immunosorbent assay and western blot or immunofluorescence assay will be positive. The CD4+count is greater than 500 cells/μL in children over 5 years of age.
- **Clinical Stage 2-**
  In this stage minor signs and symtoms of HIV infection begin to apear. They maydevelop,Candidiasis,lymphadenopathy,molluscomcontagiosum,hepatospleno

megaly,herpeszoster,peripheralneuropathy.CD4+ falls in between 350-499/μL in children older than 5 years. Once patients are in this stage they remain in stage 2.

- **Clinical stage 3-**

  HIV–infected patients with weakened immune systems can develop infections. Cryptosporidiosis, lymph node, tuberculosis, fever, Pneumonia and other opportunistic infection is common. Patient may loose weight. Their viral load continues to increase, and the CD4+count falls to less than 200-349 cells/μL in children older than 5 years.

- **Clinical Stage 4-**

  Patient may develop pneumocystis jirovecci pneumonia, Cytomegalovirus infection, toxoplasmosis, meningitis, Kaposi sarcoma and other infections that commonly occur with a severely depressed immune system. The viral load is very high, and the CD4+ count is less than 200 cells/μL in children older than 5years.At this point in the disease course death can be imminent.

➢ LIPOSOME

British haematologist **Dr. Alec. D. Bangham** was the first person to introduce the term 'Liposomes' in 1961, at the Institute of Cambridge, England. The word liposome derives from two Greek words 'Lipos'means fat and 'soma'means body of structure. That means a structure in which a fatty envelop encapsulate internal aqueous compartments. In 1960,Alec Bangham first described how membrane molecule e.g phospholipid interact

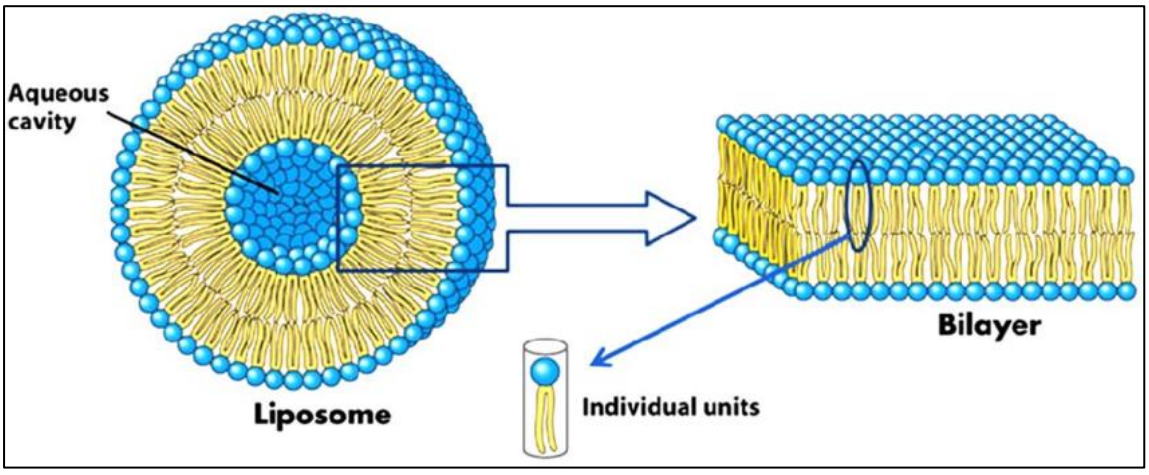

FIGURE-6 Structure of Liposome(www.researchgate.net/figure/Cartoon-diagram-of-liposome-the-individual-units-are-the-lipid-molecules_fig2_301219384/download**)**

with water to form unique structure of liposome. **Alec Bangham** found that phospholipids combined with water immediately formed a sphere because one end of

each molecule is water soluble while the opposite end is water insoluble. Water soluble medications can be added to the aqueous core and fatsoluble medications are incorporated into the phospholipids layer. Nanoliposomes are also able to enhance the performance of bioactive agents by improving their solubility and bioavailability in vitro. Recently the liposome formulations are targeted to reduce toxicity and increase accumulation at the target site

In my study, I have incorporated the drug into liposomes made up of soya lecithin and Cholesterol.

Liposomes are self-assembled bilayer (uni-lamellar) or multilayer (multi-lamellar) vesicles made up of Phospholipid and cholesterol, which have an aqueous core and a lipophilic surface. The aqueous core can entrap a Hydrophilic drug, whereas the lipophilic surface can entrap hydrophobic drugs. The size of the liposome ranges from 30nm to micrometer, and the phospholipid bilayer is 4-5nm thick. A liposome is being widely used for the delivery system of drugs, proteins, nucleic acids, and imaging agents, and the administration routes can be parenteral, pulmonary, oral, transdermal, ophthalmic & nasal route. (Rudra et al. 2010)

There are several reasons for selecting liposomes as a drug delivery system. It gives outstanding stability to the encapsulated drug from physiological degradation, extends the half-life of the drug, controls the release of drug molecules, and provides sustained release & liposomes are excellent biocompatible and safe. Liposomes 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.

DIFFERENT TYPES OF LIPOSOME

- **NIOSOMES-**

  The first niosome formulations were developed and patented in year of 1975.Niosomes result from the organized assembly of sufficiently insoluble surfactants in aqueous media. A niosome consists of drugs, cholesterol or its derivatives, non-ionic surfactants and, sometimes, ionic amphiphiles. The drugs, both hydrophilic and hydrophobic, can be encapsulated in niosomes. In niosomes, non-ionic surfactants are the main ingredient, rather than phospholipids, which is the primary component in liposomes. Non-ionic surfactants used in the niosomes are amphipathic, including terpenoids polysorbates Spans alkyl oxyethylenes (usually from C12 to C18) and so on. Squalene is a natural lipid. It is used to prepare niosomes with the advantage of enhancing the rigidity and stability of niosome formulations with minimal cytotoxicity in vitro and in vivo . Additionally, some

charged molecules or ionic amphiphiles, such as dicetyl phosphate (DCP) and phosphatidic acid (negatively charged molecules) also used.

- **TRANSFEROSOMES:**

Transferosome was first introduced in the early 1990s.It is an ultradeformable vesicle, elastic in nature. It can squeeze itself through a pore which is many times smaller than its size due to its elasticity. Transferosomes are applied to the skin and permeate through the stratum corneum lipid lamellar regions as a result of the hydration or osmotic force in the skin. Transferosomes are made up of a phospholipid compoent along with a surfactant mixture. The ratio of individual surfactants and total amount of surfactants control the flexibility of the vesicle. They can act as a carrier for low as well as high molecular weight drug e.g analgesic, anesthetic, corticosteroids, sex hormone, anticancer, insulin, gap junction protein and albumin. Peripheral drug targeting i.e transdermal immunization can also be achieved with this type of drug delivery system.(Pierre and Dos Santos Miranda Costa 2011)

- **ETHOSOMES**

Ethosomes are lipid vesicles containing phospholipid, alcohol (ethanol and isopropyl alcohol) in relative high concentration and water.Ethosomes are soft vesicles made of phospholipids and ethanol(in higher quantity)and water. The size range of ethosomes may vary from nanometers to micron. Ethosomes permeate through the skin layers more rapidly and possess significantly higher transdermal flux in comparison to conventional liposome. because of their high ethanol concentration, the lipid membrane is packed less tightly than the conventional vesicles, although it has equivalent stability, allowing a more malleable structure and improves the drug distribution ability in the stratum corneum lipids.(Jain et al. 2007a)

- **PROLIPOSOMES**

Proliposomes are defined as dry, free-flowing particles with a dispersed system that can immediately form a liposomal suspension when in contact with water. Compared with conventional liposomes, proliposomes exhibit more advantages in promoting drug absorption. Because of their solid properties, the physical stability of liposomes can be improved upon without influencing their intrinsic characteristics. Therefore, proliposomes would be a potential vehicle to help improve the oral absorption of hydrophobic drugs.

➢ CLASSIFICATION OF LIPOSOME

- **Based on structural parameters**

MLV-Multilamellar large vesicles>0.05µm

LUV-Large unilamellar vesicles-0.1µm

SUV-Small unilamellar vesicles-0.025-0.05µm

UV -Unilamellar vesicles (all size range)

OLV- Oligolamellar vesicles-0.1-1µm

MUV- Multivesicular vesicle

- **Based on method of preparation**

REV-Single or oligolamellar vesicle made by reverse phase evaporation method.

MLV-REV- Multilamellar vesicle made by reverse phase evaporation method.

SPLV- Stable plurilamellar vesicle.

FATMLV- Frozen & thawed multilamellar vesicle

DRV- Dehydration –rehydration vesicles.

VET-Vesicle prepared by extrusion method

SUVS-Sonicated unilamellar vesicles

EIV-Ether injection vesicle

EIV –Ethanol injection vesicle

- **Based on Composition and application**

Conventional liposome- Macrophage targeting,local depot,vaccination

Long circulating liposome –Selective targeting to pathological areas,circulating microreservoir.

Immuunoliposome-Specific targeting

Cationic liposome –Gene Delivery

Fusogenic liposome-Reconstituted sendai virus envelops

➢ Methods of preparation of liposome

All the methods of preparing liposomes involve 4 basic stages:

1) Drying down lipids from organic solvent
2) Dispersing lipid in aqueous media

3) Purifying the resultant liposome
4) Analyzing the final product

Method of liposome preparation and drug loading

1) Passive loading technique
2) Active loading technique

- **The passive loading technique involves three different methods**

- Mechanical dispersion method
- Solvent dispersion method
- Detergent removal method

- **The mechanical Dispersion method can be classified as**

- Sonication
- French pressure cell: extrusion
- Freeze thawed liposomes
- Lipid film hydration by hand shaking, non-hand shaking, or freeze-drying
- Micro-emulsification
- Membrane extrusion
- Dried reconstituted vesicles

- **The solvent dispersion method can be classified as**

- Ethanol injection
- Ether injection
- Reverse phase evaporation

- **The detergent removal method can be classified as**

- Dialysis
- Detergent removal of mixed micelles
- Gel-permeation chromatography
- Dilution

MECHANICAL DISPERSION METHOD

- **Preparation of lipid for hydration**

At first weighed amount of lipids are takenin a completely dried round bottom flask and dissolved in organic solvent to assure a homogeneous mixture of lipids.Usually chloroform or chloroform:nethanol mixtures are used as solvent.Once the lipids are thoroughly mixed in the organic solvent ,the solvent is removed to yield a lipid film.For small volumes of organic solvent(<1ml),the solvent may be evaporated using a dry nitrogen or argon stream in a fume hood.For larger volumes ,the organic solvent should be removed by rotary evaporation yielding a thin lipid film on the sides of a round bottom flask.The flask containing lipid film was kept in vaccum desiccators or pumps overnight to remove residual organic solvent .The alternative of chloroform is tertiary butanol or cyclohexane.(Németh et al. 2020)

- **Hydration of lipid film/cake**

Hydration of the dry thin film was done simply by adding an aqueous medium to the container of dry lipid..The lipid suspension should be maintained above the Tc during the hydration period.For high transition lipids,this is easily achieved by transferring the lipid suspension to a round bottom flask and placing the flask on a rotary evaporation system without a vaccum.Spinning the round bottom flask in the warm water bath maintained at a temperature above the Tc of the lipid suspension allows the lipid to hydrate in It's fluid phase with adequate agitation.A hydration time of 1 hour with vigorous shaking ,mixing or stirring is highly recommended.The vesicle suspension is allowed to stand overnight prior to size reduction which makes the sizing process easier and improve the homogeneisity of the size distribution.Suitable hydration medium include distilled water,buffer solution,saline and non-electrolytes such as sugar solutions.After the formation of a stable hydrated MLV suspension,the size of the particles can be reduced by a variety of technique ,including sonication or extrusion.(Chang and Yeh 2012)

- **Sonication**

Sonication is a simple method for reducing the size of liposomes and manufacturing of nanoliposome.Sonication typically produces small unilamellar vesicles(SUV) with diameters in the range of 15-50nm.Bath sonicator and probe sonicator both type of instruments are used for the size redution.Sonication tips also tend to release titanium particles into the lipid suspension which must be removed by centrifugation before use .For this reasons ,bath sonicators are most widely used for the preparation of SUV.The large particles can be removed by centrifugation.

- **French Pressure Cell Method-**

The method involves the extrusion of MLV at 20,000 psi at 4 degree through a small orifice.This method has several advantages over sonication method .The method is simple ,rapid, and reproducible and involves gentle handling of unstable materials. The disadvantages of the method are that the temperature is difficult to achieve,the working volumes are relatively small(about 50ml maximum) and the size of the resulting liposomes are somewhat larger than SUV prepared by the sonication method**.**

- **SOLVENT DISPERSION METHOD-**

  - **Ether injection method**

    A solution of lipids ,dissolved in diethyl ether or ether/methanol mixture is slowly injected to an aqueous solution of the material to be encapsulated at 55-65ºC or under reduced pressure.The subsequent removal of ether under vacuum leads to the formation of liosome. The main drawbacks of this method are –drugs to be encapsulated is

exposed to organic solvents and this method is not suitable for the encapsulation of thermolabile drugs.

- **Ethanol injection method**

The solution of lipid in ethanol is rapidly injected to a vast excess of buffer. The MLVs are immediately formed. The drawbacks of the method are that the population is heterogeneous(30-110nm),liposome are very dilute, It is difficult to remove all ethanol because it forms azeotrope with water and the possibility of various biologically active macromolecules to inactivation in the presence of even low amounts of ethanol.

- **Reverse phase evaporation method**

At first water in oil type emulsion is prepared by sonication of a two phase systems containing phospholipids in organic solvent (diethylether or isopropylether or mixture of isopropyl ether and Chloroform) and aqueous buffer.Then a viscous gel of lipid is prepared by evaporating organic solvents under reduced pressure.The liposomes are formed when residual solvent is removed by using rotary vaccum evaporator under reduced pressure.With this method high encapsulation efficiency up to 65% can be obtained in a medium of low ionic strength for example 0.01MNacl.It is used to encapsulate small and large macromolecules.The main disadvantage of the method is the exposure of the materials to be encapsulated to organic solvents and to brieeef period of sonication.

➢ Detergent removal method

The detergents at their critical micelles concentrations have been used to solubilise lipids. As the detergent is removed by dialysis the micelles become progressively richer in phospholipid and finally combine to form LUVs.The advantages of this method are very good reproducibility and production of liposome populations which are homogenous in size.The main drawback of this method is the retention of traces of detergent within the liposomes.A commercial device lipoprerp is a version of dialysis system available for the removal of detergents.

➢ Industrial production of liposomes

The various methods meant for the industrial production of liposomes are:

- **Detergent dialysis**

A pilot plant under the trade name of lipoprepr is available from Diachema AG, Switzerland. The production capacity at lipid concentration [80mg/ml] is 30ml liposome/minute. But when lipid concentration is 10-20mg/ml-100mg/ml then upto many litres of liposome can be produced.In USA, lipoprepr is marketed by Dianorm-

Geraete.

- **Microfluidization**

A method based on microfluidization /microemulsification/homogenization was developed for the preparation of liposomes.MICROFLUIDIZERR is available from microfluids corporation,Massachusetts,USA.A plot plant based on this technology can produce about 20 gallon/minute of liposome in 50-200 nm size range.The encapsulation efficiency up to 75% may be obtained.Aqueous dispersion of liposomes often have tendency to aggregate or fuse and susceptible to hydrolysis and /or oxidation.

- **Lyophilisation**

Freeze-drying [lyophilisation] involves the removal of water from products in the frozen state at extremely low pressure.The process is generally used to dry thermolabile products.This technique has a great potential as a method to solve long term stability problems with respect to liposomal stability.It is possible that leakage of entrapped materials may take place during the process of freeze –drying and on reconstitution .It was shown that liposomes when freeze –dried in the presence of adequate amount of trehalose(a Carbohydrate commonly found at high concentration in organism. retained as much as 100% of their original contents.It shows that trehalose is an excellent cryoprotectant for liposome.

➢ CHARACTERIZATION OF LIPOSOMES-

- **Table 2-Biological characterization**

Characterization parameters	Instrument for analysis
Sterility	**Aerobic/anaerobic culture**
Pyrogenicity	**Rabbit fever response**
Animal toxicity	**Monitoring survival rats**

- **Table 3-Chemical characterisation**

Characterization parameter	Instrument for analysis
Phospholipids concentration	HPLC/Barrlet assay
Cholesterol concentration	HPLC / cholesterol oxide assay
Drug concentration	Assay method
Phospholipids per oxidation	UV observance
Phospholipids hydrolysis	HPLC/ TLC
Cholesterol auto-oxidation	HPLC/ TLC

Anti-oxidant degradation	HPLC/TLC
PH	PH meter
Osmolarity	Osmometer

- **Table 4-Physical Characterization**

Characterization parameter	Instrument for analysis
Vesicle shape, and surface morphology	TEM and SEM
Vesicle size and size distribution	Dynamic light scattering ,TEM
Surface charge	Free flow electrophoresis
Electrical surface potential and surface PH	Zeta potential measurement and PH sensitive probes
Lamellarity	P31 NMR
Phase behavior	DSC, freeze fracture electron microscopy
Percent capture	Mini column centrifugation, gel exclusion
Drug release	Diffuse cell/ dialysis

➢ Advantages of liposome

- Liposomes increased the efficacy and therapeutic index of the drug.Liposome increased stability via encapsulation
- Liposomes are non-toxic, flexible, biocompatible, and completely biodegradable for systemic and non-systemic administration
- Liposomes reduce the toxicity of the encapsulated agent
- Liposomes reduce the exposure to toxic chemicals in the human body
- It has the flexibility to couple with site-specific ligands to achieve active

➢ Disadvantages of liposome

- Solubility of the drug sometimes decreases when incorporated into the liposome
- A liposome 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 liposomes is high

➢ APPICATION OF LIPOSOME

- **Liposome as immunological adjuvent-**

Advantage of use of liposomes as carrier for vaccine includes-

a) A non –immunogenic substance may be converted into immunogenic one.
b) Hydrophobic antigens may be reconstituted.
c) Multiple antigens may be incorporated into the liposome.
d) Adjuvants may be incorporated with antigens into the liposome.
e) Long duration of functional antibody activity may be achieved.
f) Toxicity and allergic reaction of antigens may be reduced by inclusion in liposome.

Liposomes have an adjuvant effect upon Protein antigens(Diphtheria Toxoid).The size and structure of liposome may be modulated as required which affect the immunogenicity of liposomal antigens.(Daraee et al. 2016)

- **Liposome as carrier of antigens**

Rate of uptake of liposome by reticulo-endothelial system must be minimized by using small neutral unilamellar liposome having higher Tc and cholesterol.

a) By coating the surface of liposome which would render the liposome recognizable by reticulo-endothelial system.

b) Coupling of molecules on the liposome surface which bind to their receptors on the surface of target cells.

c) Different type of antigen that can be incorporated into liposome include diphtheria toxoid, cholera toxin and herpes simplex virus type 1 antigen.**(Liu et al. 2021)**

- **Cancer Chemotherapy and neoplasia-**

Anticancer drugs are usually less selective. That is why they are toxic to normal cells.

Liposome have been used to entrap the drug successfully. This provide a lot of advantages.

A)Increase half-life of drug in circulation i.e.drug tends to deposit in the tissue .

B) Protects the metabolic degradation of drug.

C) Altered tissue distribution of drug with enhanced uptake in organs rich in mononuclear phagocytic cells. Example of such organs are liver, spleen, bone marrow and decrease uptake in kidney.

- **Arthritis-**

Most of the anti -arthritis drugs are steroid and destroyed by their peripheral effect.Scientist suggest that liposomes could be used in the treatment of local disease.Liposome delivery of steroid is very effective .

- **Diabetes-**

The feasibility of using liposome as potential delivery system for the oral delivery of insulin has been extensively studied.Alteration of blood glucose levels in diabetic animals was obtained by the oral administration of liposome encapsulated insulin.

- **Cell biological application-**

The most important use of liposome in cell biology is to manipulate the status of membrane lipid. It is clear that exchange of lipid cholesterol occurs between cells and liposome. Liposomes have been used to carry functional DNA and RNA into cells. Liposomes are used to insert regulatory molecule such as cAMP, cGMP, enzymatic factors in cells.

- **Lysosomal storage disease**

Lysosomal storage disease are caused due to genetically determined deficits of particular lysosomal hydrolytic enzyme such as Gaucher disease due to deficiency of β-glucosidase enzyme and Pomp's disease due to the deficiency of α-glucosidase enzyme. Liposomes have the potential for delivery of enzyme to the lysosomal system. A large number of lysosomal enzymes can be readily entrapped in liposome and in fact some enzymes have an affinity for the liposomal membrane.

- **Opthalmic delivery of drug-**

The effectiveness of liposome in ocular drug delivery depends on a number of factors including-

a) Drug encapsulation efficiency
b) Size of liposome.
c) Distribution of drug within liposome.
d) Stability of liposome in the conjunctival sac and ocular tissue.
e) Retention of liposome in the conjuctival sac.
f) Affinity of liposome exhibited towards the corneal surface.

Many drugs such as iodoxuridine, adrenaline, triamcinolone acetonide, Benzyl penicilin etc, can be encapsulated in the liposome for their ocular delivery.

- **Food nanotechnology-**

Another nonmedical application of liposome involves their utilization as carrier

systems to stabilize the bioactive materials against a range of environmental and chemical changes as well as to improve their bioavailability .Liposomes and nanoliposomes have been used in the food industry to deliver flavous and nutrients and, more recently ,have been investigated for their ability to incorporate antimicrobilals that could aid in the protection of foof products against microbial contamination.**(Reza Mozafari et al. 2008)**

CHAPTER 2

AIM OF THE RESEARCH WORK

AIM OF THE RESEARCH WORK

Nanotechnology refers broadly to a field of applied sciences and nanotechnology which is the control of matter on the molecular level in scales smaller than 1µm .They are made from natural/synthetic polymers and ideally suited to optimize drug delivery and reduced toxicity.The successful implementation of nanotechnology for drug delivery depends on their ability to penetrate through several anatomical barriers,sustained release of their content and their stability in nanometer size.

Human imunodeficiency virus is a retrovirus.Which causes irreversible destruction of the immune system,leading to the occurrence of AIDS..Throuh antiretroviral drugs are able to combat replication of the HIV by inhibiting reverse trascriptase and thereby viral DNA synthesis synthesis but a major limitation associated with the clinical use of these agents is their dose limiting toxicity and adequate distribution to specific sites in the body such as brain.Lamivudine is an oral medication that is used for the treatment of infections with the human immunodeficiency virus.Nanoliposome drug delivery is one of the emerging nanotechnologies,which facilitates drug delivery across the blood –brain barrier.By engineering the drug loading efficiency,pharmacokinetic properties and surface modification of the carrier nanoliposome drug delivery system usually aims to offer higher accuracy of delivery,more sustained release of drug and less systemic toxicity compared to the conventional drug delivery system.If lamivudine can be supplied as liposomal drug delivery system and the size of the liposome is within the nano range then targeting of drug to the nervous system can be achieved.Hence the present study is intended to develop lamivudine loaded nanoliposome and their physicochemical and in-vitro characterization .

To accomplish the above-mentioned objectives, the following studies will be performed-

- FTIR study to check drug-polymer and polymer-polymer interaction
- Drug loading and encapsulation efficiency of liposome to check how a large amount of drug is encapsulated into the liposome formulation.
- Electron microscopic study to check the liposome vesicle size and polymer cross-linking within the hydrogel.
- In vitro release study to check the sustained action of formulation and cumulative Drug release.

CHAPTER -3

LITERATURE REVIEW

LITERATURE REVIEW

various works were done from the middle of the 19th century for the development of liposome and nanoliposomes.some of the technological work for the development of liposomes and nanoliposomes are given below.

Bangham and Horne (1962) first observed that isolated and purified phospholipids of cellular origin dispersed in aqueous phase formed multilayered vesicles.This unique structures resembles with cellular organelles with lipid bilayers (Bangham,1993). In the 1960s and 1970s various liposome preparation methods were developed to study biological process of bacterial ,fungal and vertebrates membranes ,chemistry of biological membranes and of serum lipoproteins and membrane bound proteins and enzymes e.g .reconstitution of Na+k+ATPase,cytochrome oxidase,The muscle calcium transport pump,the glucose and anion transport system of erythrocytes.In the late 1970s number of workers established liposomes as drug carriers to improve the therapeutic index of drug by increasing % of drug molecules that reach the target tissue or decreasing the % of drug molecules that reach site of toxicity.

Rajendra et al. (2010) has made an attempt to provide for stable drug delivery system with or having improved therapeutic index for zidovudine in form of lyophilized liposomes.Liposome have been loaded by zidovudine (AZT) as a model drug using thin film hydration technique for targeted delivery of thi drug.Four different formulations were prepared with various concentration of egg phosphatidylcholine(EPC) and dipalmitoyl phosphatidylcholine (DPPC).A series of tests have been carried out to characterize the carrier vesicles in-vitro,including loading parameter, drug release kinetics,particle size distribution, Scanning electron microscopy (SEM) analysis.Liposome having acceptable loading parameters,released their drug content according to zero-order kinetics were selected for in vivo tissue distribution study.The AZT-loaded liposome showed preferential drug targeting to liver followed by lungs,kidney and spleen.AZT loaded liposome seem to be a promising delivery system for targeting the drug to reticuloendothelial system (RES).

Sancini et al.(2013) studied that functionalization with tat-peptide enhances blood-brain barrier crossing in-vitro of nanoliposomes carrying a curcumin-derivative to bind amyloid-β peptide.Production of abnormally high amounts of amyloid-β peptide in the brain plays a central role in the onset and development of Alzheimer's disease,a neurodegenerative disorder affecting millions of individuals worldwide.Here nanoliposomes decorated with a curcumin-

derivative,displaying high affinity for amyloid-β,were functionalized with a modified cell-penetrating TAT-peptide,with the aim of conferring on such nanoliposomes the ability to cross the blood-brain barrier. Confocal microscopy,mass spectrometry and radioactivity experiments with [3H]-sphingomyelin showed about 3-fold increase in the uptake of nanoliposomes by human brain capillary endothelial cells(hCMEC/D3) after the funtionalization withTAT peptide, with no alterations in cell viability.Moreover,TAT functionalization increased the permeability of curcumin nanoliposomes across a blood –brain barrier model made with the same cells.The similar permeabilities of curcumin derivative and [3H]-sphingomyelin suggested that nanoliposomes were trasported intact.Considering these results,nanoliposomes functionalized with the curcumin-derivative and TAT-peptide represents a promising tool for targeting amyloid-β directly in the brain parenchyma.

Mukherjee et al (2010) studied Doxorubicin-loaded phosphatidylethanolamine conjugated nanoliposomes for in-vitro characterization and their accumulation in liver,kidneys, and lungs in rats.From the study they found that PE-conjugated nanoliposomes released the drug in a sustained manner and were capable of distributing them in various organs .This may be used for cell/tissue targeting ,attaching specific antibodies to PE.

Subheet Jain et al (2007) Human immunodeficiency virus (HIV) is a retrovirus that causes irreversible destruction of the immune systemLamivudine is a commonlyused hydrophilic antiviral drug for treatment of acquired immunodeficiency syndrome (AIDS and hepatitis. Lamivudine has a short biological half-life (4-6 hour) .Transdermal route is, therefore, a better alternative to achieve constant plasma levels for prolonged periods of time, whichadditionally could be advantageous because of less frequentdosing regimens.

Ethosomescontain phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water. Unlike classical liposomes, ethosomes were shown to permeate through the stratum corneum barrier and were reported to possess significantly higher transdermal flux in comparisonto liposomes..The in vitro skin permeation of ethosomal zidovudine formulation was studied and was found to be 24-fold higher as compared with aqueous drug solution.The present study was aimed at investigating the possible mechanism of intracellular and intercellular delivery of ethosomes containing lamivudine as model drug using scanning electron microscopy (SEM), transmission electron microscopy (TEM), and fluorescence microscopy techniques and cell line studies. Lamivudine encapsulation in ethosomes could increase its skin permeation and intracellular uptake. This would enhance its antiviral efficiency and lead to better patient compliance.

Sipho Mdanda et al.*it is* reported that *35% of HIV-infected patients present with neurocognitive deficits at the time of death. The brain areas of neurodegeneration associated with HIV/AIDS was determined using tensor-based morphometry in conjunction with MRI and showed severe atrophy in the primary and association sensorimotor areas. Atrophy was primarily observed in the cortical white matter and is considered a marker of HIV cognitive impairment. The AZT and 3TC cocktail is currently used as a twice-daily pill in the treatment of HIV-positive pregnant women, infants, and children. During drug development studies, a preliminary requirement is to understand the drug pharmacokinetics and locali- zation patterns of drugs in target organs such as the brain.There is alsono information regarding the distribution and localization of AZT and 3TC in the brain. Therefore, the aims of this study are to develop a validated method for the quantitation of AZT and 3TC in the brain and to also determine the potential of the AZTand 3TC combination for the treatment of HAND by investigating its distribution patterns in the brain. The use of mass spectrometry imaging (MSI) in conjunction with LC-MS/MS will provide an insight into the ability of Combivir to penetrate through the blood–brain barrier (BBB), to determine if this combination can effectively target areas that are known to undergo neurodegeneration.

Krishna C et al Lamivudine (3TC) is a deoxycytidine nucleoside analogue that inhibits hepatitis B virus(HBV) replication and is used in the treatment of chronichepatitis B infection .In addition to the efficacy of antiviral drugs against HBV. Dextrans are glucose polymers,.In a series of studies showed that the plasma kinetics and tissue distribution of dextran carriers are dependent on the M_w of the polymer.Therefore, dextrans of different M_w may be useful for the delivery of drugs to different tissues after the systemic administration of the conjugate. For example, dextrans with M_w valuesof 20–70 kDa showed a high degree of selectivity for the liver when the liver/plasma area under the concentration–time curve (AUC) ratio was considered. The liver selectivity of these dextrans was attributed to their sizes, which restrict their passage through most vascular bed pores while allowing unrestricted passage through the substantially larger pore sizes of the liver sinusoids. The aim of the present study was to synthesize and characterize a conjugate of 3TC with dextran, ~25 kDa, intended for selective delivery of the anti-HBV drug to the liver. The conjugation of 3TC to dextran was achieved through a succinate linker, resulting in a macromolecular prodrug, potentially releasing 3TC and 3TC succinate (3TCS). In addition to in vitro characterization, the plasma pharmacokinetics and tissue disposition of the prodrug and parent drug were also studied in rats, a species that has recently been used as a model for humanHBV infection. This is the first

report of designing a macromolecular prodrug of anti- HBV drug lamivudine for targeted delivery to the liver.

Santra et al (2010) studied the development ,physicochemical and in-vitro evaluation of dexamethasone-containing liposomes. The purpose of this study was to develop dexamethasone containing liposome (DCL) based on different combination of cholesterol and soya-L-α-lecithin by lipid film hydration method.It was found that the average particle size of the liposome was 1000nm.It was found that drug loading of the formulations were between 1.39 %w/w In vitro drug release study shows that between 87% and 97% release of drug obtained from the different experimental liposome in 500 min.Drug release was found to follow krosemeyer kinetics.

Liang Han et al. Alcohol consumption is increasing, and alcohol abuse poses a serious threat on global public health, with approximately 5 % of deaths each year being associated with alcohol abuse. One-time excessive drinking causes acute alcohol intoxication, leading to central nervous system depression and metabolic abnormalities. In severe cases, excessive drinking results in respiratory and circulatory failure, and even death. Alcohol is mainly metabolized into acetic acid gradually by alcohol dehydrogenase and acetaldehyde dehydrogenase *in vivo*, and the acetaldehyde produced in the process is the major contributor to alcoholism . Therefore, there is an urgent need for drugs activating ALDH to enhance the conversion of acetaldehyde to acetic acid, thus relieving alcoholism. RU21 Hangover pill (RU21) is recognized as a safe, effective, non-toxic hangover product. Its active ingredients inhibit the oxidation of ethanol into acetal- dehyde and promote the decomposition of acetaldehyde, but it cannot quickly clean up the ethanol in the body. As a nucleoside analog, lamivudine acts as a clinical medicine for Hepatitis B and Acquired Immune Deficiency Syndrome (AIDS). Excessive alcohol intake induces damage to the central nervous system, and it has been reported that lamivudine protects the central nervous system by repairing neuronal damage. Via screening it is shown lamivudine exhibits an anti-alcoholism effect. Our results indicate that lamivudine decreases serum alcohol concentration dramatically, and potently activates acetaldehyde dehydrogenase(ALDH) to accelerate the conversion of acetaldehyde to acetic acid, which is finally metabolized by tricarboxylic acid cycle to be CO_2 and H_2O. Also lamivudine significantly improves symptoms post drinking, such as prolonging alcohol tolerance time and shortening sobering time, as well as reducing the death rate.

Heba F Salem et al. The drug delivery to brain is very challenging. The blood–brain barrier (BBB) is an example of both physical and chemical barriers, where tight junction among endothelial cells of BBB presents physical barrier and also endothelial cells of the BBB produce significant amount of adenosine triphosphate-driven drug efflux transporters, which present chemical barrier.

Glutathione receptors in the brain were largely localized in the white matter, more specifically in the neuroglial cells. Endocytic pathways are activated within the cells using a directing moiety or a ligand which is used as a vector .It has recently been shown that glutathione is coupled with micelles and nanoparticles and showed enormous potential in both targeting and enhancing the drug moieties into brain. They are easily coupled to different targeting moieties to improve the targeting of administered drugs to their site of action. In this study, glutathione–maleimide–polyethyleneglycol– distearoyl phosphatidyl ethanolamine was attached to nano-liposomes to deliver flucytosine. Moreover, in this study the ability of glutathione-modulated liposomes to target neuron cells of rat brain primary cell culture was evaluated.

Bhatia et al.(2004) formulated multilamellar liposomes of tamoxifen by thin film hydration method for topical delivery in order to avoid the side effects associated with oral administration,while parenteral administration is restricted due to its limited aqueous solubility.Prepared liposomes were characterized for morphological and micromeritic attributes,employing Malvern mastersizer and optical microscopy .The stability study was carried out to find out the liposome with desired property.They were compared the topical performances of liposomal tamoxifen citrate and non-liposomal system containing tamoxifen.They come into conclusion that liposomal tamoxifen has greater effect on skin than non liposomal system containing tamoxifen.

CHAPTER 4

MATERIALS AND EQUIPMENTS

MATERIALS AND EQUIPMENTS

- **Chemicals:** Lamivudine, Cholesterol, Soya-L-α-lecithin(SLE), Butylated Hydroxy Toluene(BHT), chloroform, , Potassium dihydrogen phosphate, Disodium hydrogen phosphate, Sodium chloride, , HPLC Water, Ethanol, Hydrochloride acid, Sodium Chloride(NaCl), were required to prepare Liposome .

- **Table 5: List of chemicals used for the preparation of Liposome with their source**

NAME	SOURCE
Lamivudine	Merck Lifescience Pvt. Ltd. (Mumbai, India)
Cholesterol	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)

➢ **Table 6: List of Equipment used in the liposome preparation and source**

NAME	SOURCE
Rotary vacuum evaporator	Rotavap Superfit 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
Advanced microprocessor UV-Vis single-beam spectrophotometer (Model Intech-295) Software-UV Professional V1.39.0	Model Intech-295, Gentaur GmbH, Aachen, Germany
Lyophilizer	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) (ECO-ATR, Model ALPHA, Software-OPUS 7.5)	Department of Chemistry, PG Science building, Jadavpur University, Kolkata , India
Magnetic stirrer	Remi Equipments, 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
Scanning electron microscopy	Center for Research in Nanoscience and Nanotechnology, University of Calcutta, Kolkata, India

Brookfield Viscometer	Department of chemistry, PG Science, Jadavpur University , Kolkata, India
Franz Diffusion cell	Borosil, Mumbai, India

Description of some materials used for the development of Liposome-

Description Of Lamivudine-

Racemic BCH-189 (the minus form is known as lamivudine) was invented **by Bernard Belleau while at work at McGill University** and Paul Nguyen-Ba at the Montreal-based IAF BioChem International, Inc. laboratories in 1988 and the minus enantiomer isolated in 1989.**.** Lamivudine was approved by the (FDA) on November 17, 1995, for use with zidovudine (AZT) and again in 2002 as a once-a-day dosed medication. Lamivudine, commonly called 3TC, is an antiretroviral medication used to prevent and treat HIV/AIDS. It is also used to treat chronic hepatitis B when other options are not possible.It is effective against both HIV-1 and HIV-2. Lamivudine was patented in 1995 and approved for use in the United States in 1995. It is on the World Health Organization's List of Essential Medicines.(Siegfried et al. 2006)

- **Mechanism of action lamivudine-**

  Lamivudine is an analogue of cytidine. It can inhibit both types (1 and 2) of HIV reverse transcriptase and also the reverse transcriptase of hepatitis B virus.Lamivudine inhibits viral DNA synthesis via reverse transcriptase DNA chain termination post phosphorylation .Once inside the cell ,Lamivudine is metabolised to the triphosphate form (lamivudine triphosphate) and monophosphate form(Lamivudine Monophosphate),during intracellular kinase phosphorylation. Both forms inhibit viral DNA synthesis.(Taylor, Fritz, and Parmar 2020)

  It is advantageous as an antiviral drug because Active lamivudine is primarily not recognised by human polymerase as a substrate,but actively compets with natural cytidine triphosphate to inhibit reverse transcriptase DNA synthesis seen in both HIV-1 and HBV infection**.(**Vasconcelos et al. 2008)

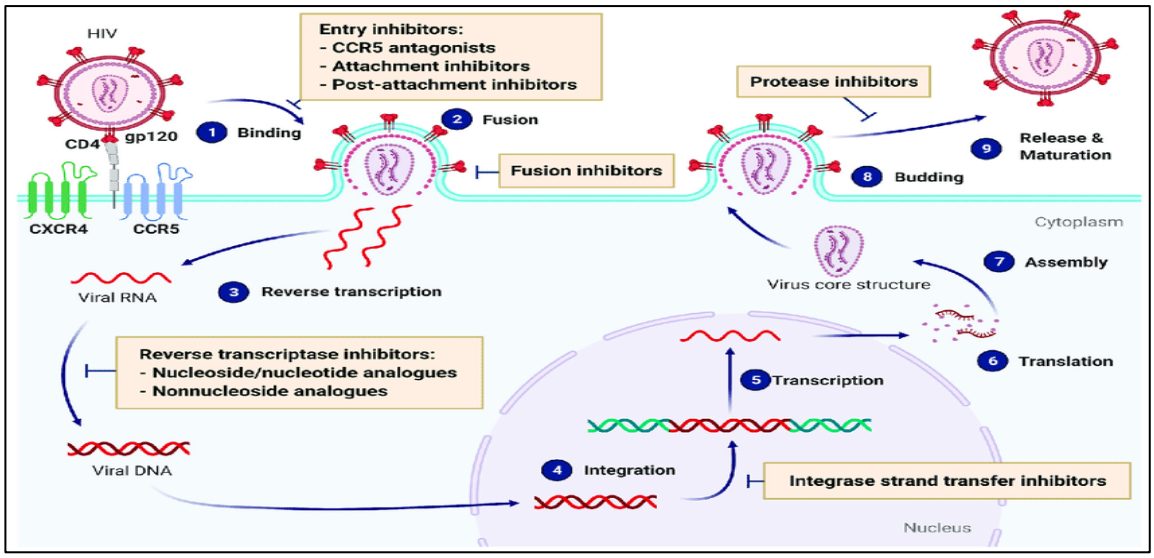

FIGURE 7-MOA of Lamivudine

(https://www.google.com/search?sca_esv=565026230&sxsrf=AB5stBgGqo04XRr5g3wLvztA77Xh3PB1qA:1694613186402&q=lamivudine+mechanism+of+action)

- **Chemistry of Lamivudine-**

Synonyms:3-TC

Molecular formula: C_{10} H_{15} N3 O_3 S

Molecular weight: 257.08

IUPAC name-3TC (-)-1-[(2*R*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine

Lamivudine is a monothioacetal that consists of Cytosine having a (2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl moiety attached at position 1. An inhibitor of HIV-1 reverse transcriptase, Intracellularly, lamivudine is phosphorylated to its active metabolites, lamiduvine triphosphate (L-TP) and lamiduvine monophosphate (L-MP). In HIV, L-TP inhibits HIV-1 reverse transcriptase (RT) via DNA chain termination after incorporation of the nucleoside analogue into viral DNA.

The lamivudine molecule contains a total of 27 bonds,There are 16 non-H bonds,3multiple bonds ,2 rotatable bonds ,3 double bonds.1 five-membered ring,1 six membered ring,1 Amidine derivative,1 primary amine,1 hydroxyl group,1 primary alcohol,1 ether,and 1 sulfide.It can be seen that substitution of NH2 functional group of lamivudine with CONH2, COPh, COOH, COCH3 analogues at positions 4 lead to a decrease in the binding affinity. The lower values of

the binding energy of lamivudine (CONH2, COPh, COOH, COCH3) analogues implies that they can block HIV-1 reverse transcriptase better than lamivudine. This confirms that the structural modification implemented is significantly related to their activity.(Otuokere, Ikpeazu, and Igwe 2017)

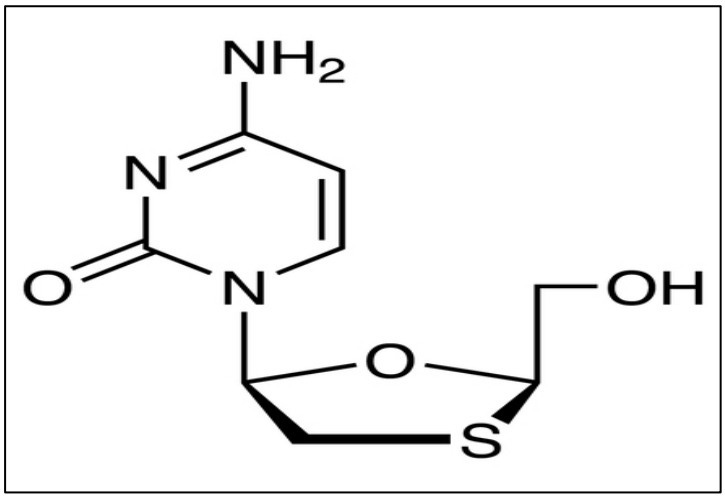

FIGURE 8: Structure Of Lamivudine(Otuokere, Ikpeazu, and Igwe 2017)

- **Physical and Chemical property**

Melting point-177ºC

Boiling point-475.4ºC

Storage temperature-2-8ºC

Solubility-Water Soluble

Form-powder

Colour-white to beige

BCS Class-1,3

Stability –Stable for 2 years from date of purchase as supplied.Solutions in DMSO may be stored at 20ºC for upto 1 month**.**

- **Pharmacokinetics:** Lamivudine is readily and rapidly absorbed via the oral route, with peak concentrations reached approximately 30 to 90 minutes following administration. Bioavailability is above 80% in adults, but some 10% lower in children. Timing of meals does not affect lamivudine absorption. . The volume of distribution of lamivudine is approximately 1.3 L/kg when given intravenously. The drug readily crosses the placenta and is present in breast milk.

Lamivudine is primarily eliminated via the renal route, with some 70% of the unchanged drug found in urine. Therefore, lamivudine dosing needs to be altered for patients with renal

insufficiency, but not reduced in patients with hepatic dysfunction. The elimination half-life of lamivudine approximates 5 to 7 hours, with an active 5′-triphosphate metabolite having a prolonged half-life of 10.5 to 15.5 hours. No interaction between lamivudine and cytochrome P-450 3A isozymes has been seen, reduced extraction ratio of the drug seen with trimethoprim, other than no important drug interactions have been described.(Moore et al. 1996)

- **Absorption-**

Lamivudine was rapidly absorbed after oral administration in HIV-infected patients. Absolute bioavailability in 12 adult patients was 86% ± 16% (mean ± SD) for the 150-mg tablet and 87% ± 13% for the oral solution. The peak serum lamivudine concentration (Cmax) was 1.5 ± 0.5 mcg/mL when an oral dose of 2 mg/kg twice a day was given to HIV-1 patients. When given with food, absorption is slower, compared to the fasted state.The solid tablet is preffered in children because bioavailability is 40% lower in children using the oral liquid form,300mg taken once daily, has shown to have larger trough and maximum serum levels.**(Yuen et al. 1995)**

- **Volume Of Distribution**

Apparent volume of distribution, IV administration = 1.3 ± 0.4 L/kg. Volume of distribution was independent of dose and did not correlate with body weight.

- **Protein Binding**

<36% bound to plasma protein.

- **Metabolism**

Lamivudine does not undergo metabolism via CYP450 pathway and minimally binds to plasma protein.Therefore,CYP450 inducers and inhibitors will not affect its metabolism,nor does it have many drug interactions with protein –bound medication.Metabolism of lamivudine is a minor route of elimination. In man, the only known metabolite of lamivudine is the trans-sulfoxide metabolite. This biotransformation is catalyzed by sulfotransferases.(Johnson et al. 1999)

- **Excretion**

The majority of lamivudine is eliminated unchanged in urine by active organic cationic secretion. 5.2% ± 1.4% (mean ± SD) of the dose was excreted as the trans-sulfoxide metabolite in the urine. Lamivudine is excreted in human breast milk and into the milk of lactating rats.**(Rao and Friedman 1988)**

- **Half Life**

5 to 7 hours (healthy or HBV-infected patients)

- **Clinical uses and recommended dosage**

The recommended oral dose of lamivudine in adult HBV-infected patients **is 100 mg once daily.** For HBV-infected children 2 to 17 years of age, the dose i**s 3 mg/kg (maximum dose 100 mg)** administered once daily.

Adult dosage for HIV-300 mg each day

Child dosage(ages 3 months to 17 years)-Dosage is based on child's weight

For children who weigh>20to <25kg-225 mg once daily,or 75 mg in the morning and 150 mg in the evening.

For children who weigh >25kg-300mg once daily,or 150 mg twice daily(Burger et al. 2007)

- **Toxicity and drug interactions**

a)Adverse reactions include pancreatitis, paresthesia, peripheral neuropathy, neutropenia, diarrhea, weakness, shallow breathing, muscle pain.Liver damage.(Heylen and Miller 1997)

b)Do not prescribe lamivudine/zidovudine, abacavir/lamivudine, or abacavir/lamivudine/zidovudine to patients taking emtricitabine

- **Resistance**

In HIV, high level resistance is associated with the M184V/I mutation in the reverse transcriptase gene as reported by Raymond Schinazi's group at Emory University. GlaxoSmithKline claimed that the M184V mutation reduces "viral fitness", because of the finding that continued lamivudine treatment causes the HIV viral load to rebound but at a much lower level, and that withdrawal of lamivudine results in a higher viral load rebound with rapid loss of the M184V mutation; GSK therefore argued that there may be benefit in continuing lamivudine treatment even in the presence of high level resistance, because the resistant virus is "less fit". The COLATE study has suggested that there is no benefit to continuing lamivudine treatment in patients with lamivudine resistance.[A better explanation of the data is that lamivudine continues to have a partial anti-viral effect even in the presence of the M184V mutation. (Fischer, Gutfreund, and Tyrrell 2001)

In hepatitis B, lamivudine resistance was first described in the **YMDD (tyrosine-methionine-aspartate-aspartate)** locus of the HBV reverse transcriptase gene. Tyrosine-methionine-aspartate-aspartate (YMDD)-motif mutants may emerge and elicit immune clearance during prolonged lamivudine treatment Twenty-three patients who

developed YMDD-motif mutants during the Asian lamivudine trial were included..(Yeh et al. 2000)

➢ Description of Cholesterol

Cholesterol from the ancient Greek chole-(bile) and stereos (solid) followed by the chemical suffix –Ol for an alcohol,is an organic molecule .It is a sterol and an essential structural component of animal cell membranes that is required to establish proper membrane permeability and fluidity .cholesterol is thus considered within the class of lipid molecules .In addition to its importance within cells,cholesterol also serve as a precursor for the biosysnthesis of steroid hormones,bile acids and vitamin D.Cholesterol is the principle sterol synthesized by animals,all cells,In vertebrates the liver typically produces greater amounts than other cells.(GRABLEY et al. 1992)

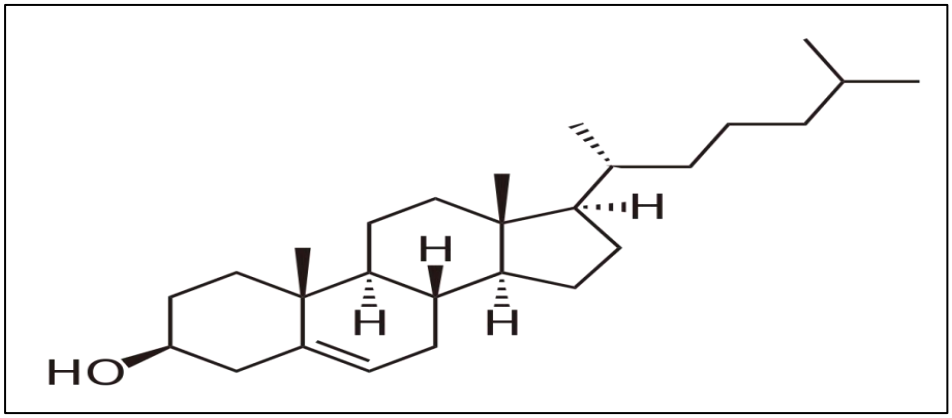

FIGURE 9: Structure of cholesterol(Shieh, Hoard, and Nordman 1981)

Chemical Formula- $C_{27}H_{46}O$

Appearance- White crystalline powder

Molecular weight -.386.6

Melting point- 148-150 ºC

Boiling point – 360ºC (decomposes)

➢ Solubility-

Solubility in water 0.095 mg/L (30ºC). Soluble in acetones, Benzene,Chloroform,Ethanol ,ethers ,hexane , isopropyl myristate and methanol

➢ Source-

Plant products such as flax seeds and peanuts contain cholesterol-like componds called phytosterols, which are believed to compete with cholesterol for absorption in the intestines.

Animal fats are complex mixture of triglycerides, with lesser amount of phospholipid and cholesterol. Major dietary source of cholesterol include cheese, egg yolks, beef, pork, poultry ,fish and shrimp. Human breast milk also contains some quantities of cholesterol.(Epand et al. 2003)

➢ Pharmacokinetics-

Cholesterol is oxidized by the liver into a variety of bile acids.These ,in turn are conjugated with glycine,taurine,glucuronic acid,or sulfate . A mixture of conjugated and non-conjugated bile acids,along with cholesterol itself,is excreted from the liver into bile.Approximately 95 % of the bile acids are reabsorbed from the intestines,and the rest are lost in the faeces.Cholesterol is eliminated from the body via faeces, urine and skin surface.Cholesterol is also eliminated in the milk of lactating females, primarily in the membranes of milk fat globules .

➢ Function-

- Cholesterol is required to build and maintain membranes.
- Within the cell membrane,cholesterol also functions in intracellular transport,cell signaling and nerve conduction.
- In the liver,cholesterol ,cholesterol is converted to bile,which is then stored in the gallbladder.Bile contains bile salts which solubilise fats in the digestive tract and aid in the intestinal absorption of fat molecules as well as the fat soluble vitamins,A,D, E, k.
- Cholesreol is an important precursor molecule for the synthesis of vitamin D and the steroid hormones including the hormones cortisole and aldosterne.

➢ Pharmaceutical use-

- Cholesterol is used as an emulsifying agent in the pharmaceutical formulation,also used as humectants.
- Cholesterol is used in liposome to encapsulate and deliver chemotherapeutic drugs to diseased tissues.
- Cholesterol C-14 is used clinically as an organ imaging agent .

➢ Description of soya-1-α-lecithin

Lecithin is a generic term to designate any group of yellow-brownish fatty substances occuring in animal and plant tissues composed of phosphoric acid,choline,fatty acids,glycerol,glycolipids,triglycerides(phosphatidylcholine,phosphatidylinositol).Lecithin was first isolated in 1846 by French chemist and pharmacist Theodore Gobley.(Shchipunov 2001**)**

- **Molecular weight**- 760.09g/mol

- **Molecular Formula**-$C_{42}H_{82}NO_8P$
- **Solubility-**Soluble in chloroform: 50mg/ml,clear,very faintly yellow.Soluble in hexane-ethanol,methanol,ether,toluene,mineral oils,fatty acids,.Sparingly soluble in benzene .Insoluble in water (CMC<0.001nM),cold acetone,cold vegetable and animal oils.

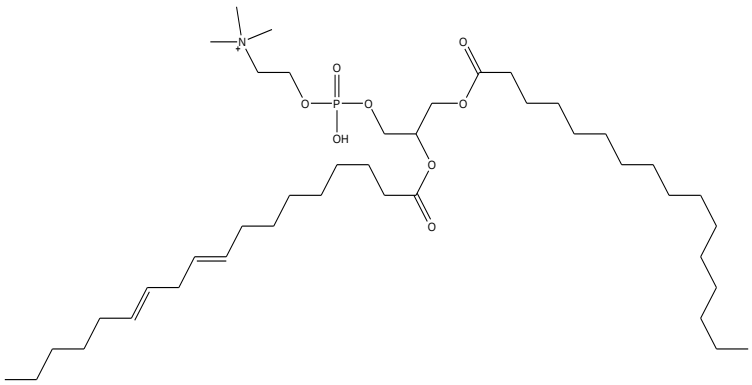

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FIGURE 10: structre of soya-1-α-lecithin(Scholfield 1981)

- **Source of lecithin**.

Lecithin can be obtained from plants, animals and marine sources like soy beans, eggs, milk, marine source, cotton seed and sunflower. The most concentrated natural and unrefined sources of lecithin are soybeans(1.48 to 3.08% lecithin),peanuts(1.11%),calf liver 0.85%),wheat (0.61%),oatmeal(0.65%)and eggs(0.39%) .The human spinal cord cord contains 6-10% lecithin and the human brain contains 4-6% lecithin in fresh substance.Soy oil has the highest lecithin and phophatide content of any oil.

- **Pharmacokinetics-**

Lecithin is absorbed into the mucosal cells of the small intestine ,mainly in the Duodenum and upper jejunum.

➢ Functional properties of Lecithin-

- Lecithin is a multi-functional surface-active agent.Each molecule has two faces.The fatty-acid portion of the molecule is attracted to fats(it is lipotropic) and the phosphoric acid portion is attracted to water (it is hydrotrophic).Because of this dual nature ,lecithin molecules tend to position themselves at the boundary between immiscible materials, such as oil and water. There they serve many useful functions through a surface modifying effect.

- Crystallization control is used to control the crystallization of sugar in fat systems and Viscosity modification.

CHAPTER-5

METHODOLOGY

METHODOLOGY

➢ Preparation Of Calibration Curve of lamivudine in PBS pH 7.2 and Water

• Preparation of Buffer solutions

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 8 g of Sodium Chloride (NaCl) were dissolved in 1000 ml double-distilled water, and the pH of the solution was adjusted to 7.4 using a pre-calibrated pH meter (Eutech. Instruments), 0.1N Sodium Hydroxide (NaOH) solution and 0.1N Hydrochloric acid (HCl) solution.

• Determination of absorption maxima of Lamivudine-

For the determination of absorption maxima, about 1 mg of drug was dissolved in 5ml of PBS pH7.2(while determining the absorption maxima of Lamivudine in PBS 7.2) and also taken 1mg of drug in 5ml water (while determing the absorption maxima of Lamivudine in water.The solution was then scanned under UV-VIS single-beam spectrophotometer (Model Intech-295, Gentaur GmbH, Aachen, Germany) using PBS pH7.2 and water respectively as blank. A single characteristic peak at 269nm in water and 270 nm in PBS(7.2)was obtained, which was close to the published lambda max of Lamivudine (Lamivudine-Wikipedia).

• Preparation Of Stock Solution-

For making the stock solution 1mg or 1000µg is taken in 1ml or 1000µl of water vortex for 1-2 minutes for dissolving the drug with water. From this main stock different concentrations is prepared respectively.

•Preparation Of Standard curve of Lamivudine in water and PBS 7.2-

From that stock solution a concentration of 10µg is prepared with 10 µg of API and 990µl of water in a Eppendorf Again votex for sometime. From the concentration of 10 µg/ml several concentraion like 1µg/ml ,2µg/ml,4µg/ml,6µg/ml,8µg/ml,12µg/ml Concentrations are prepared and 16µg/ml is prepared from 100µg/ml. To prepare the standard curve of Lamivudine in water first, the blank solution was taken into UV-VIS single-beam spectrophotometer at lambda max-269. and the absorbance of the blank(in this case water) was set to zero. Various concentrations of drug solution in Water (1,2,4,8,10,16, µg/ml) were taken and their absorbances were measured in UV-VIS single beam spectrophotometer at lambda max-269 nm from lower concentration to upper concentration up to 16 µg/ml.

The same procedure was followed in the case of the drug in PBS 7.2 with their respective standard solutions. From the concentration of 10 µg/ml several concentraion like 1µg/ml ,2µg/ml,4µg/ml,6µg/ml,8µg/ml,12µg/ml Concentrations are prepared and 16µg/ml is prepared from 100µg/ml. To prepare the standard curve of Lamivudine in PBS 7.2 first the blank solution (in this case PBS 7.2) was taken into UV-VIS single-beam spectrophotometer at lamda max-270. 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.

- **Determination of absorption maxima of Lamivudine-**

For the determination of absorption maxima, about 1 mg of drug was dissolved in 5ml of PBS pH7.2(while determining the absorption maxima of Lamivudine and also taken 1mg of drug in 5ml water (while determing the absorption maxima of Lamivudine.The solution was then scanned under UV-VIS single-beam spectrophotometer (Model Intech-295, Gentaur GmbH, Aachen, Germany) using PBS pH7.2 and water respectively as blank. A single characteristic peak at 269nm in water and 270 nm in PBS(7.2)was obtained, which was close to the published lambda max of Lamivudine (Lamivudine-Wikipedia).

- **Preparation and Development Of Liposome-**

Out of several methods of liposome preparation, in my work, I have used the conventional lipid layer hydration method (also called a thin-film hydration method) to develop nanosize liposomes. Both Blank liposomes and drug-loaded liposomes were prepared. In short , 125mg Soya lecithin,75mg Cholesterol, 10 mg lamivudine along with 1mg Butylated hydroxyl toluene(BHT) were dissolved in a solvent system (10ml chloroform) in 250 ml round bottom flask to prepare the drug-loaded liposome and for Blank liposome same quantities of Soya lecithin, cholesterol, BHT and chloroform was taken except lamivudine. The thin film was created using a Rotary vacuum evaporator at RPM-45, the Temperature of the Water bath 45°C, and the temperature of the circulator 4°C, and evaporation was continued under vacuum until the complete solvent got evaporated. The round bottom flask along with the thin film was then kept in a desiccator overnight for complete evaporation of any residual solvent. Hydration of the thin film was done on day 2 using 50ml PBS pH7.4 using the rotary water bath for 50min at rpm 45 and the temperature of the water bath 45°C. During hydration, lipid layer creates vesicles in the buffer system encapsulating the drug, and the vesicles created are mostly multi-lamellar. To create unilamellar vesicles, the content in the round bottom flask was then sonicated in bath sonicator for 45 min (3 cycles, 1 cycle for 15 min). The liposome dispersion was then kept in the dark place for 3 hours and then kept in the refrigerator overnight.

Centrifugation was done at day in High-speed Ultra cold Centrifuge (Model Z 32 HK) 3 to separate the liposome vesicles from the unentrapped drug and the lecithin and cholesterol which did not take part in a liposome preparation. To centrifuge, the first liposome dispersion was divided into two separate centrifuge tubes (50ml) in a manner that the weight of both the tubes remained equal. First cycle was carried out at 5000 rpm, 4°C for 15min, the supernatant was separated, the pellet was dispersed in fresh double distilled water, and it was centrifuged at 16000 rpm, 4°C for 45 min (Washing phase). Again supernatant was discarded, pellet was dispersed in 3 ml of double-distilled water, kept in petri-dish, sealed using parafilm and kept in a refrigerator at -20°C. The next day, Lyophilisation was done using Lyophilizer for 15h. where dried liposomes were obtained. They were kept in Eppendorf tubes, sealed using parafilm, and stored at -20°C.

PHYSICOCHEMICAL CHARACTERIZATION AND EVALUATION OF STAVUDINE NANOLIPOSOMAL FORMULATION-

- **Drug loading and entrapment efficiency study for liposome-**

Drug loading study was done using water and PBS 7.2 respectively. 2mg of blank and drug encapsulated freeze-dried liposome was taken in different Eppendorf tubes. 2ml water and PBS 7.2 was added in each Eppendorf tube respectively. They were then vortexed for 3 min. Then they were sonicated at a bath sonicator for 30 min (2 cycles, 15 min each) and then centrifuged for 15min at 4°C, 16000 rpm. During this process, the liposome vesicles will be disrupted, and the entrapped drug will come out and dissolve in the solvent (like in water and also in PBS). During centrifugation, the undissolved lipid and cholesterol will be precipitated as a plate. The supernatant containing the drug (for drug-loaded liposome) and without the drug (for blank liposome) were taken in fresh Eppendorf tubes. The absorbance of the supernatant of blank liposome and drug-loaded liposome was measured at 269 nm and 270nm respectively against the blank solvent (water and PBS).

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

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/ (Amount of SLE+ CHL+ BHT+ Drug) x 100
- Practical drug loading(%)= (Amount of drug in Liposome/Amount of Liposome obtained) x 100

- Drug loading efficiency(%)= (Practical drug loading /Theoretical drug loading) x 100

- **Liposome size and hydrogel polymer bonding study using Scanning Electron Microscopy (SEM)**

SEM study was done at the Centre for Research in Nanoscience and Nanotechnology (CRNN), University of Calcutta, Kolkata, India, to determine liposome vesicle size and hydrogel polymer interactions. All the freeze-dried samples were taken for SEM study. It was coated with electrically conductive metal like platinum, and the SEM images were obtained at various magnifications.

- . **Particle Size distribution Study-**

The size distribution of the reconstituted lyophilized liposomes was determined by Dynamic light scattering (DLS)(ZETASIZER,NanoZS 90,Malvern Instrument Ltd. UK) and analysed by DTS Software.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 these intensity fluctuations yields the value of Brownian motion of particle due to thermally induced collisions between the particles which are converted into particle size by using DTS oftware.The mean particle diameter (z average) was calculated by the software from the measured particle size distribution.The freshly prepared lyophilized formulations were suspended in double distiled water and poured into cuvette and analysed by the instrument .

- **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 formulation.Zeta potentials of the different formulations were determined by the instrument Zeta sizer nano ZS (Zetasizer,NanoZS 90, Malvern Instrument Ltd.UK)

- **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 may be greater or less than one.Generally for the nanoliposome sample the value is 0.05 and the values greater than 0.7 indicate that the sample has a very broad size distribution and is probably not suitable for the dynamic light scattering technique .(Dynamic Light Scattering Common Terms Defined @2011 Malvern

Instruments Limited).Polydispersity index of the formulation was measured by the instrument Zeta sizer nano ZS (Zetasizer , NanoZS 90, Malvern Instruument Ltd.UK)

- **Drug-Polymer interaction study using Infrared spectroscopy (IR)**

IR study was done for Cholesterol, soya lecithin, Blank liposome, Drug Loaded Liposome, at the Department of chemistry, PG Science building, Jadavpur University, Kolkata, India. The prepared formulation liposomes were given in the lyophilized form. The charts obtained were evaluated for any drug-polymer or polymer-polymer interaction.(Prakash et al. 2007)

- **In –vitro release study from the nanoliposomal formulation through dialysis membrane of lamivudine-**

Release study was done by using PBS 7.4.In a 250 ml conical flask , 50 ml of phosphate buffer saline,pH-7.2 was taken.The weighted amount (5mg) of lyophilised formulation was taken 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 glass rod so that portion of dialysis bag with the formulation could dip into the buffer solution.The flask was kept on a magnetic stirrer.Stirring was maintained with the help of a magnetic bead at room temperature.Sampling was done by withdrawing 1ml from the released medium with the help of micropipette and 1ml of fresh medium was added.Samples were analyzed in a spectrophotometer at the wavelength of 270 nm.With the help of standard curve,drug concentration was measured**.**

CHAPTER 6

RESULTS

RESULTS

The UV Absorption spectra of Lamivudine

The method for the determination of absorption Maxima (λ_{max}) of lamivudine was discussed in the previous section. After scanning the drug in a double beam UV-VIS Spectrophotometer from 400 to 200 nm, the λ_{max} was found at 269 nm with water and 270nm with PBS 7.2.

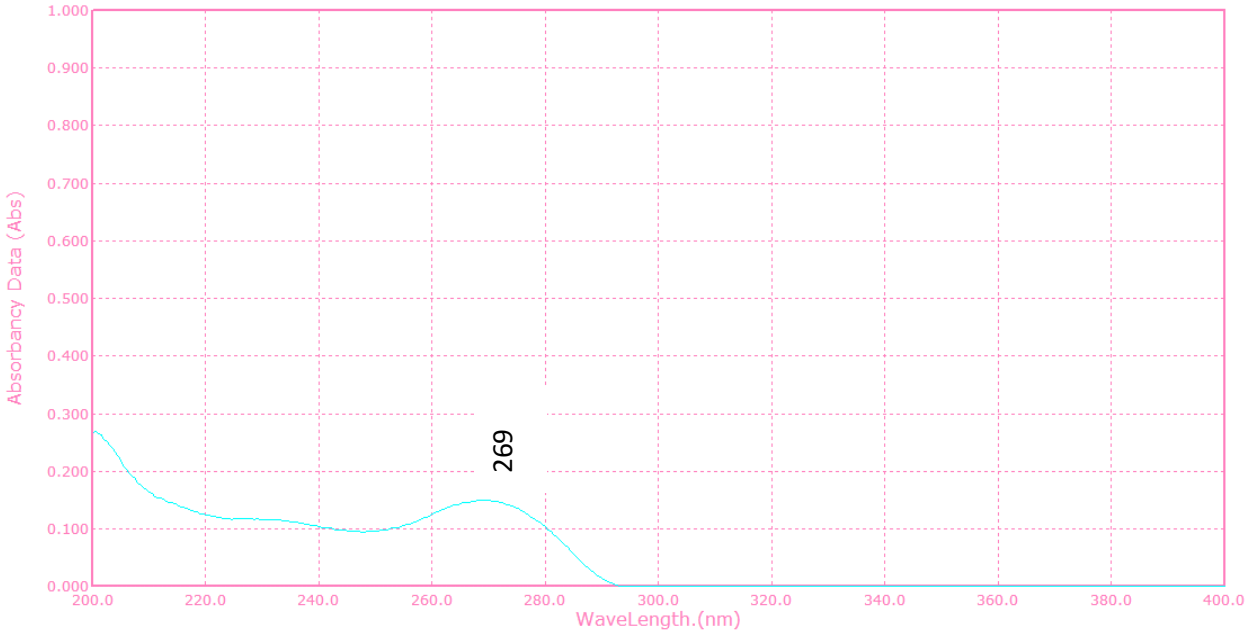

FIGURE- 11 Lamda max of lamivudine is found at 269 nm with water

The calibration curves of Lamivudine-

Two different calibration curves were prepared – one in PBS pH 7.2 & one in water for studying liposome 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 .

PBS pH 7.2– 1, 2,4,6,8,16,32 µg/mlWater – 1, 2, 4,6,8,16 µg/ml

The standard curves are depicted below with their respective absorbance value against concentrations.

Table7-The mean absorbance of Lamivudine against various concentrations in water –

CONCENTRATION (µg/ml)	MEAN ABSORBANCE
0	0
1	0.078
2	0.158
4	0.31
6	0.54
8	0.61
10	0.77
12	0.84
16	1.09

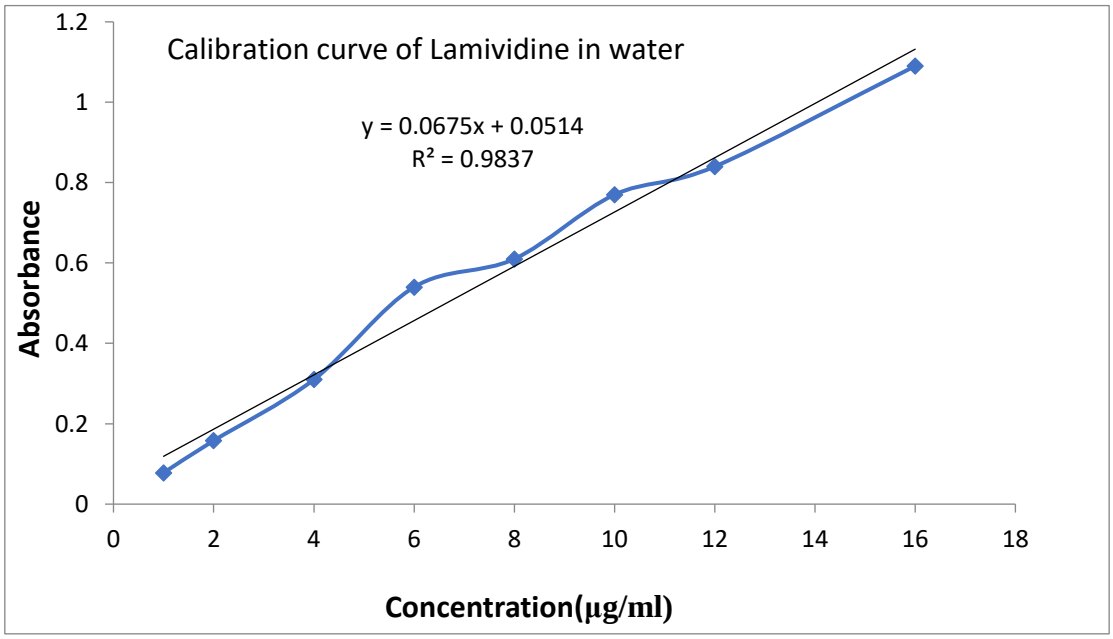

FIGURE 12. Calibration curve of Lamivudine in Water

Table 8-The mean absorbance of Lamivudine against various concentrations in PBS **(7.2)**

CONCENTRATION (µg/ml)	MEAN ABSORBANCE N=3
1	0.053
2	0.112
4	0.24
6	0.351
8	0.498
10	0.588
12	0.672
16	0.931
32	1.86

regression (R^2) values of the calibration curves suggest the accuracy of the experiments.

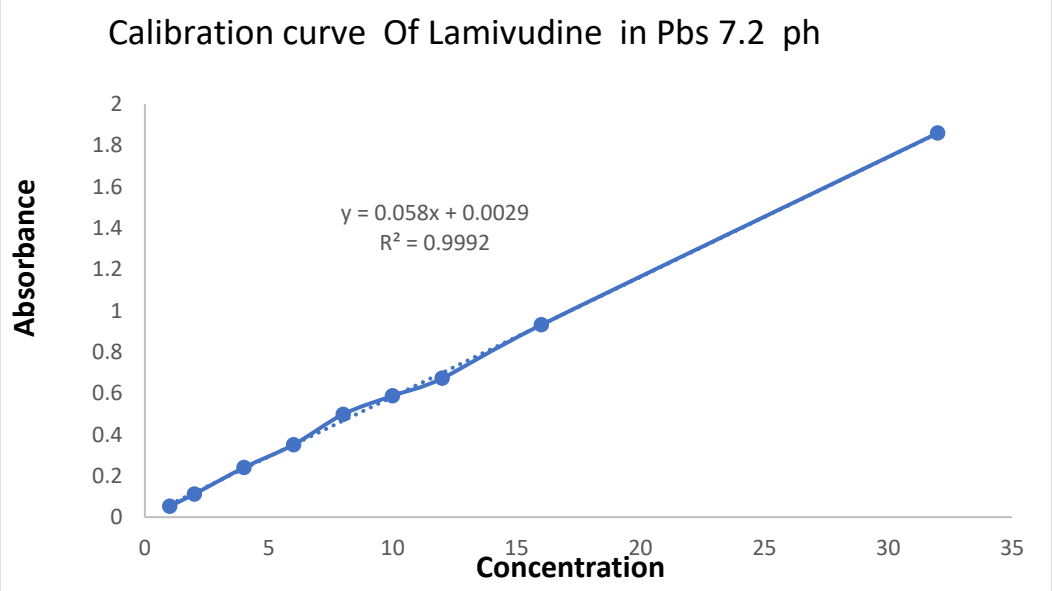

FIGURE 13 -Calibration curve of Lamivudine in PBS 7.2

➢ **Drug loading and entrapment efficiency of liposome**

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

Table 9: Composition, practical drug loading (%), entrapment efficiency (%) of the experimental liposome 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	2	75	150		2.43	1.56	43.73

➢ Surface morphology stydy of the liposomal formulation

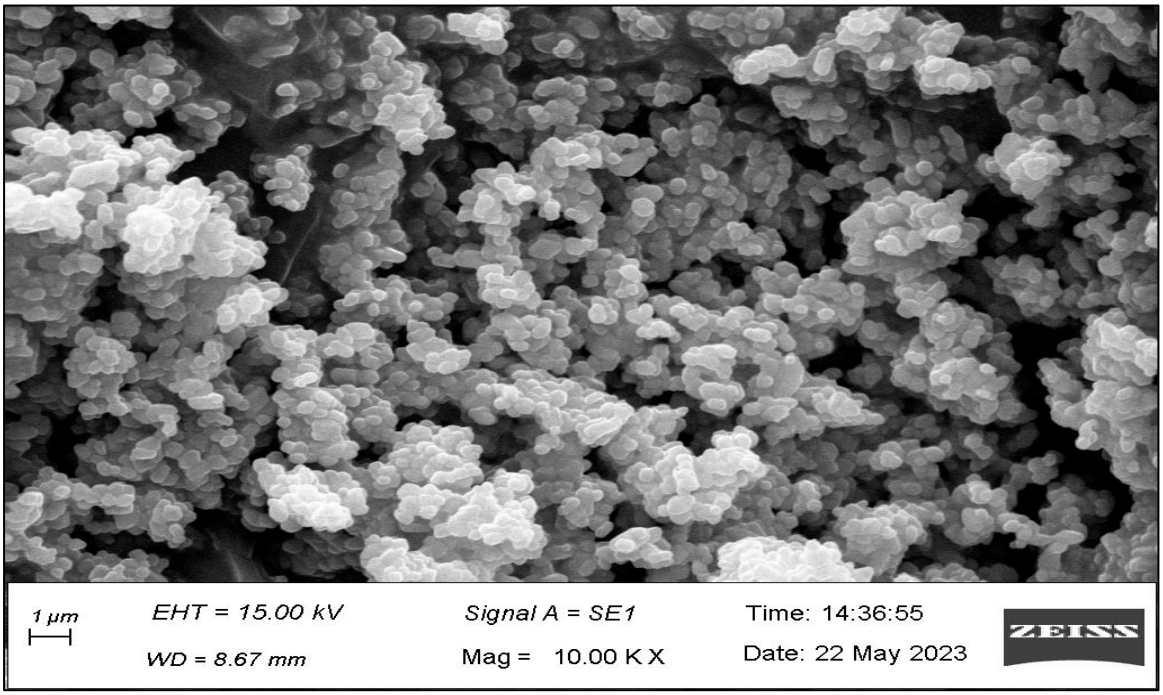

FIGURE 14: SEM image of lamivudine loaded liposome at 10x magnification

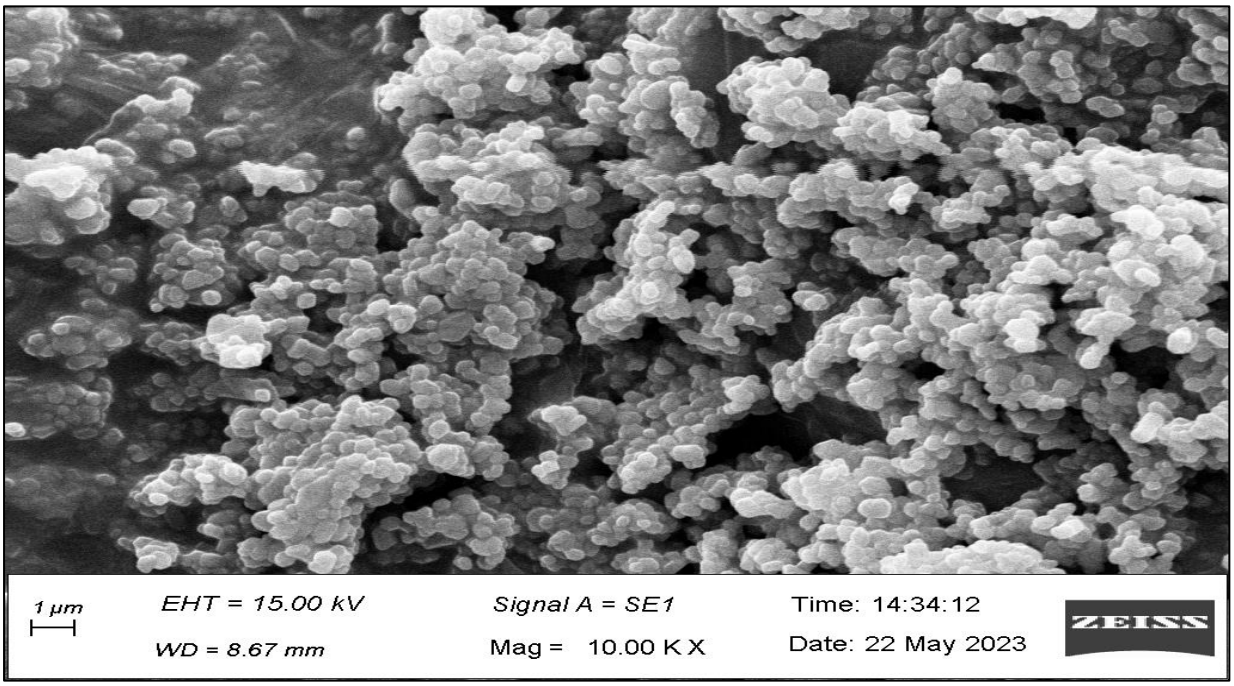

FIGURE 15 -SEM image of lamivudine loaded liposome at 10x magnification

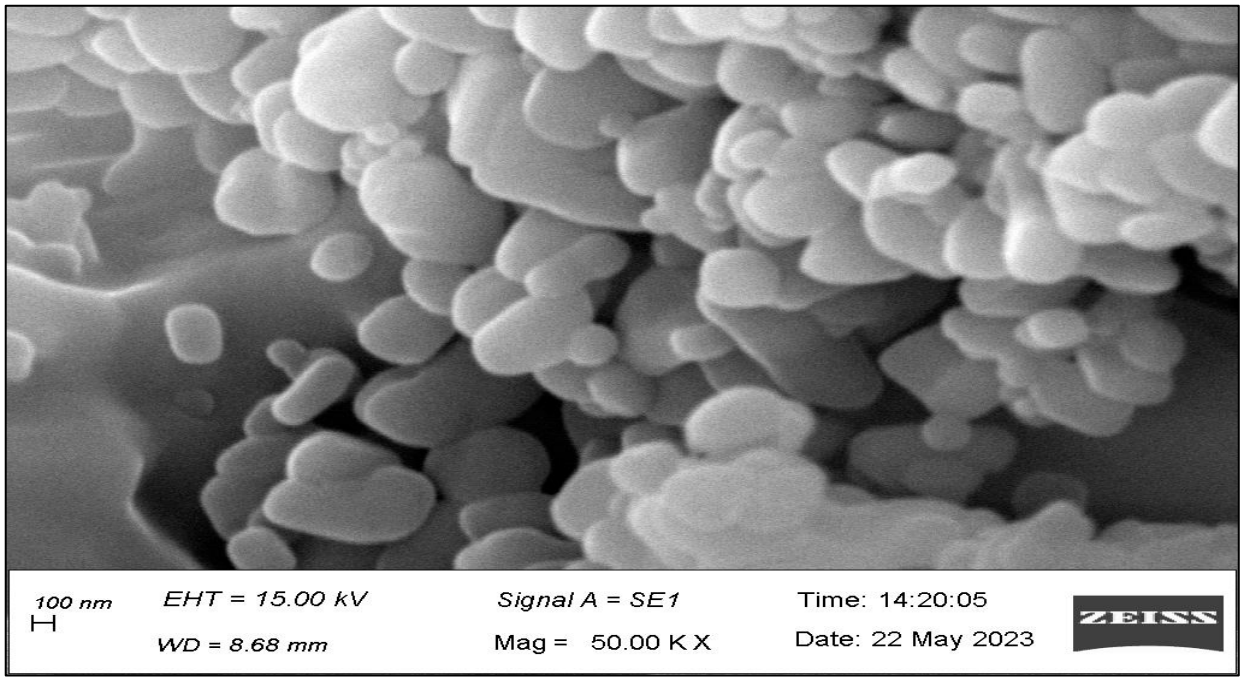

FIGURE16-SEM image of lamivudine loaded liposome at 30x magnification

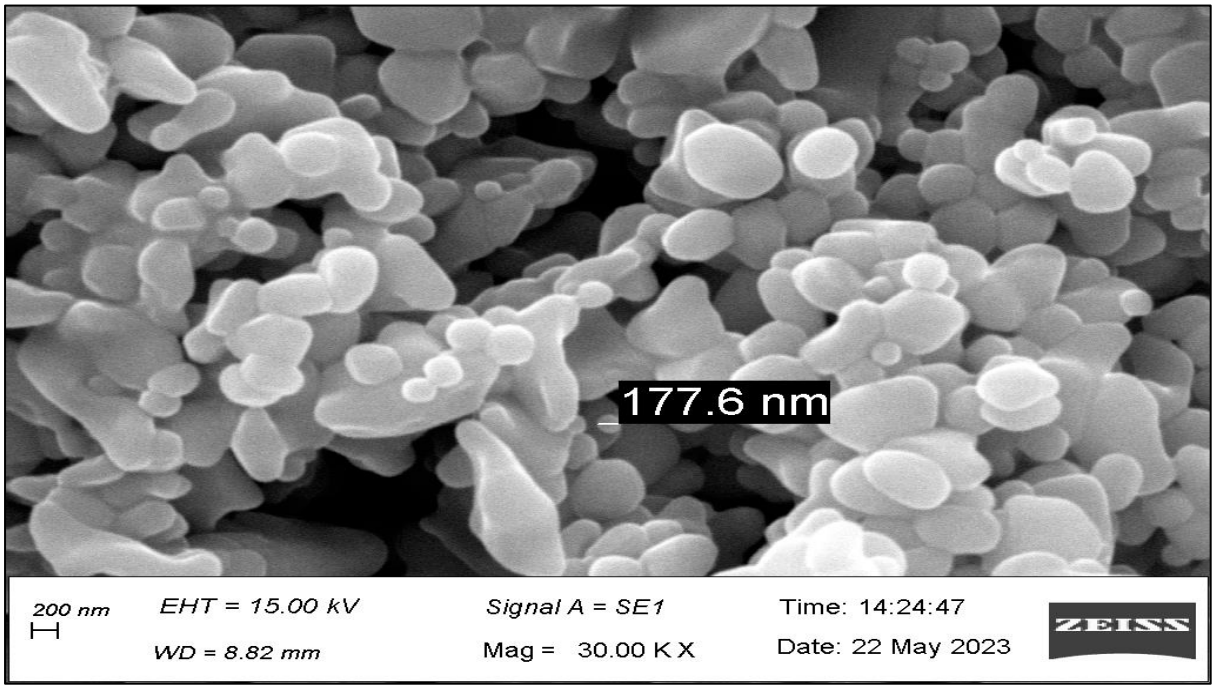

FIGURE 17 -SEM image of lamivudine loaded liposome at 50xmagnification

➢ Particle size distribution study-

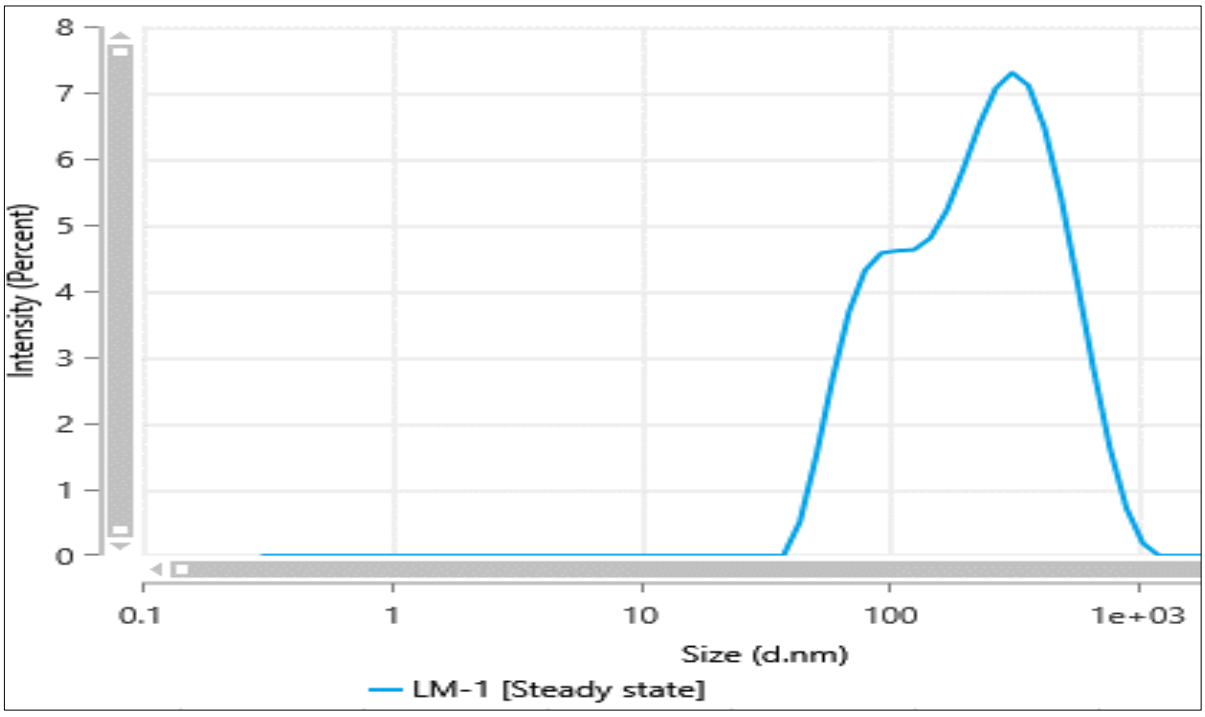

Figure18 :Particle size distribution of lamivudine liposomal formulation

➢ Zeta potential study –

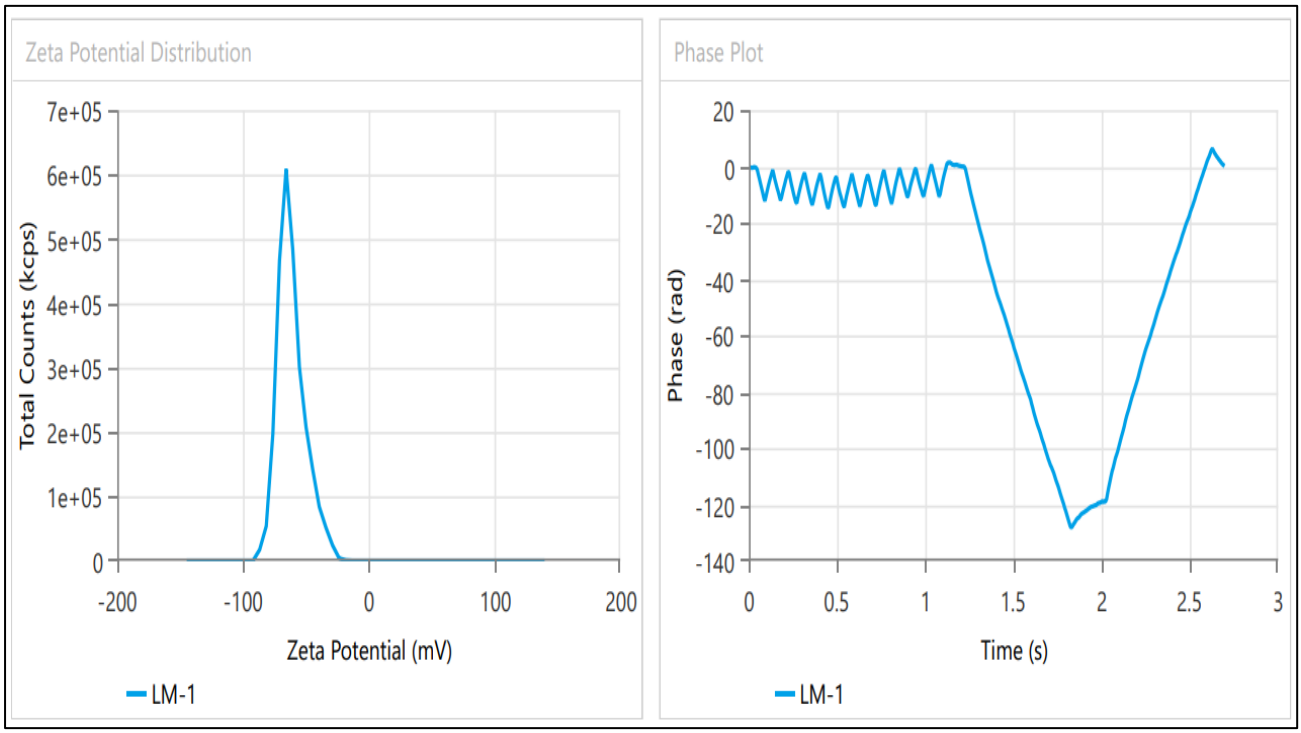

FIGURE 19- Zeta potential study of lamivudine-loaded liposomal formulation

Zeta potential value of the liposomal formulation of lamivudine is **-61.45mV**

➢ Table 10.-Z –Average value and Poly dispersity index of the formulation-

Z-Average value	**202.4**
PDI	0.4151

➢ FTIR STUDY OF FORMULATION AND COMPONENTS-

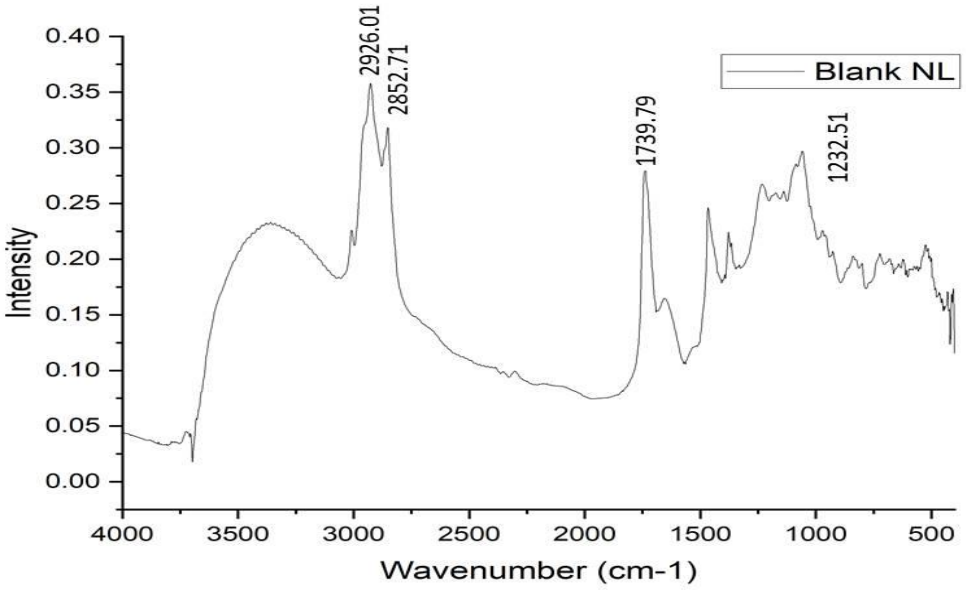

FIGURE 20 : FTIR spectrum of blank liposome

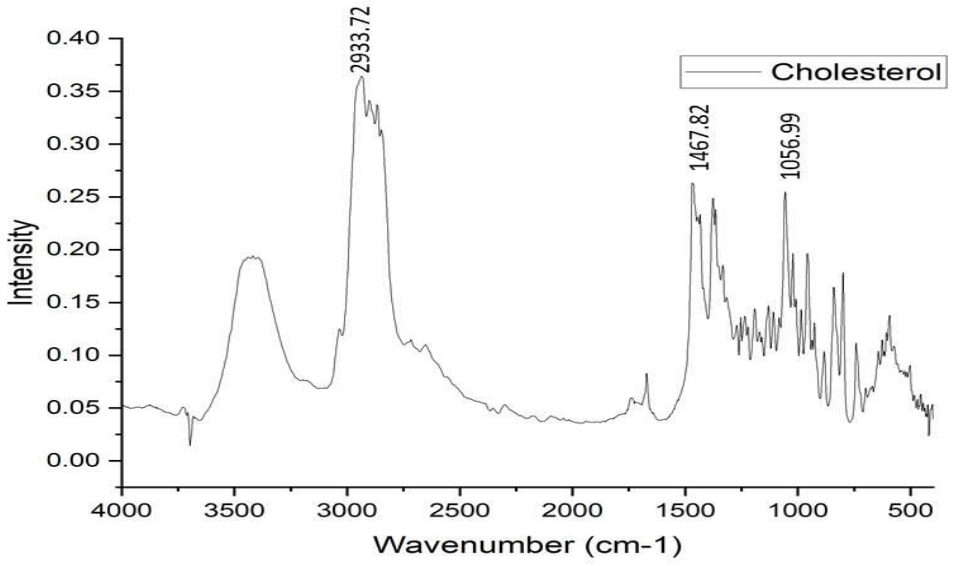

FIGURE 21: FTIR spectrum of Cholesterol

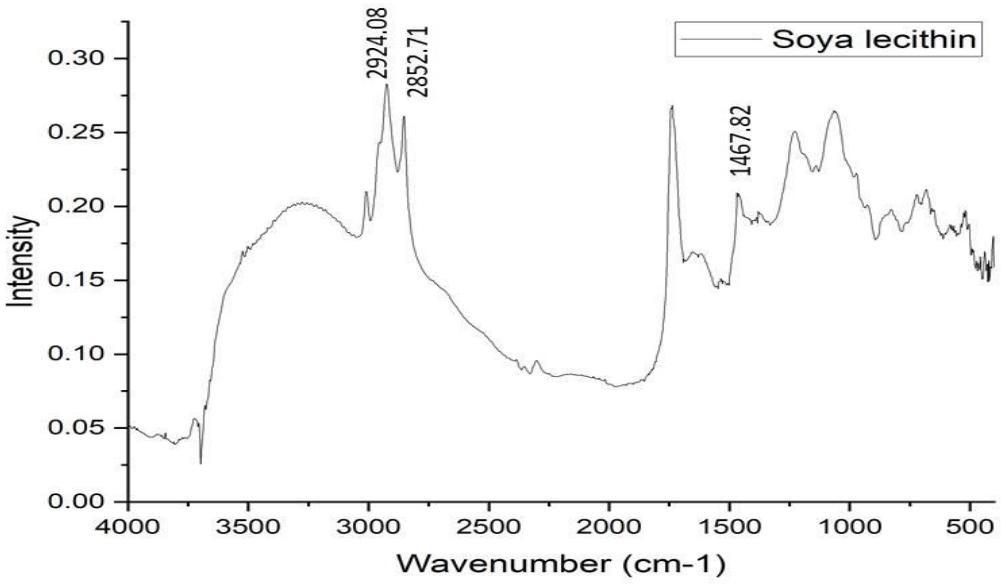

FIGURE 22: FTIR spectrum of Soya lecithin

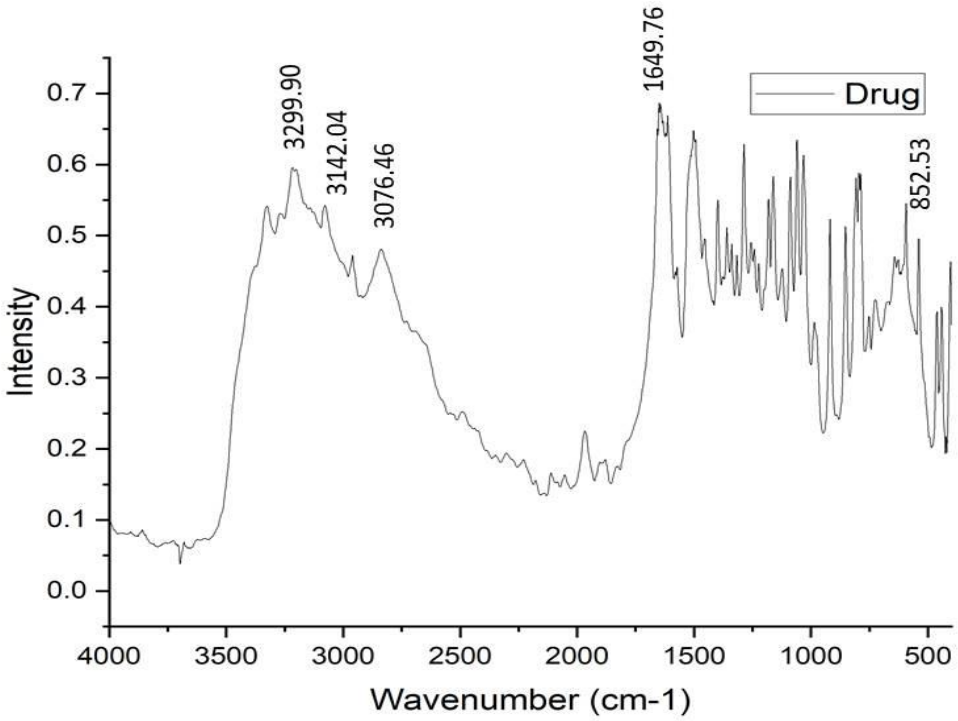

FIGURE 23: FTIR spectrum of the drug (Lamivudine)

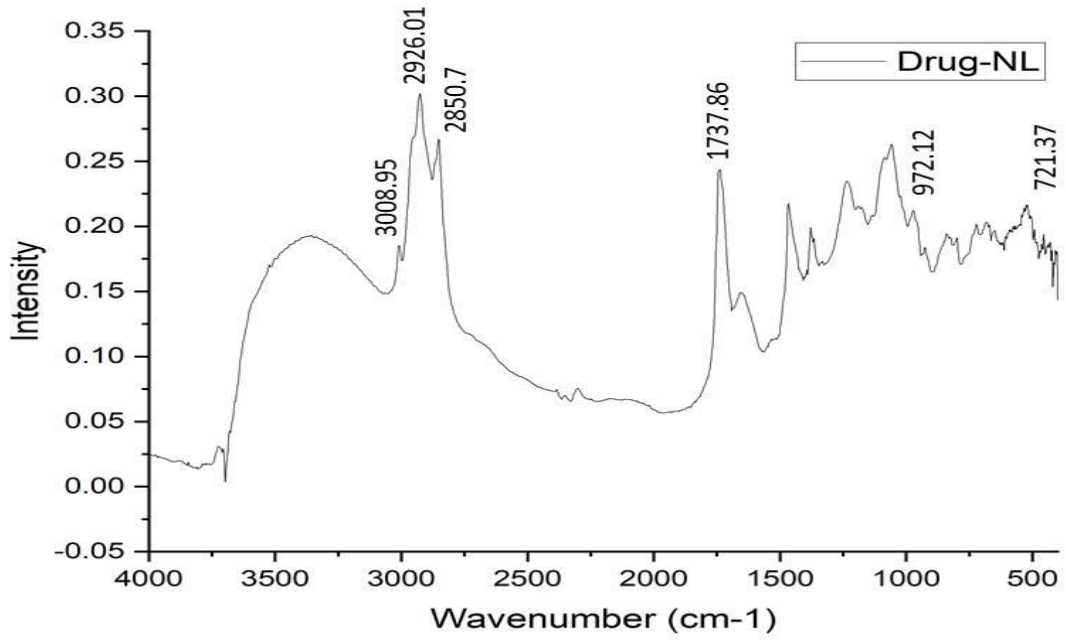

FIGURE 24 : FTIR spectrum of the drug-loaded nanoliposome (Lamivudine-loaded nanoliposome)

In-Vitro drug release study and analysis of drug release kinetics

After performing an in-vitro drug release study in PBS pH7.2as receiver fluid, various drug release kinetics models (zero-order, first-order, Higuchi, Hixon-Crowell, Korsmeyer Peppas) were prepared, and the preferred model with highest co-relation coefficient value was chosen as the actual release pattern of this formulation.

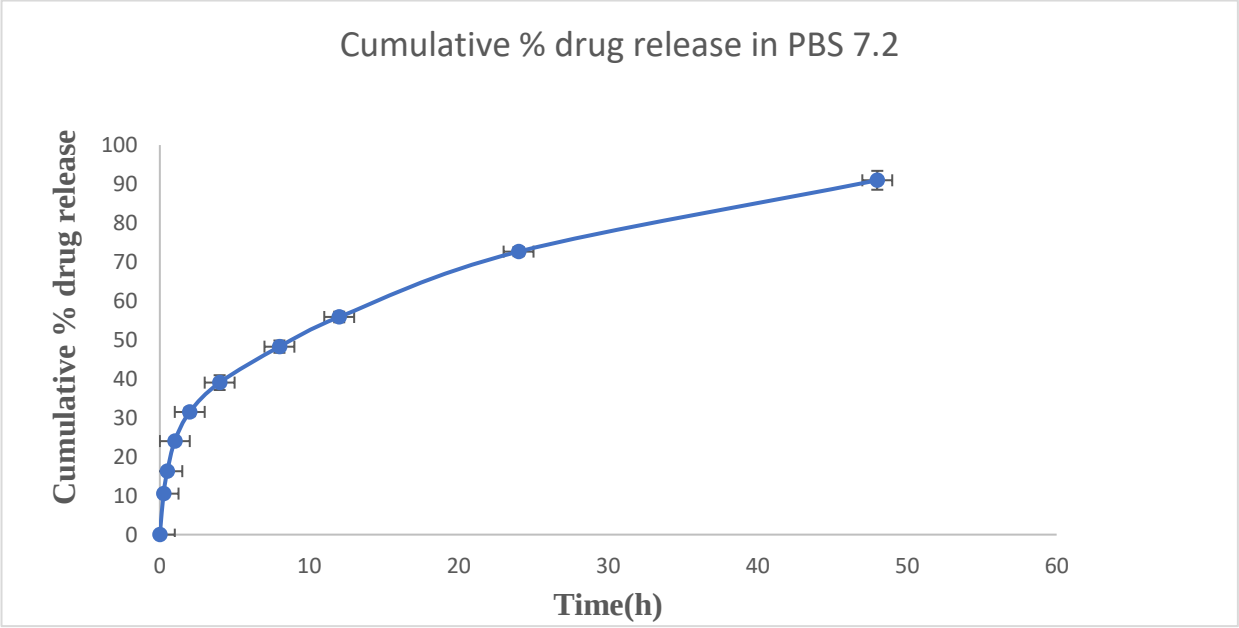

Figure 25: Cumulative % drug release in PBS pH7.2

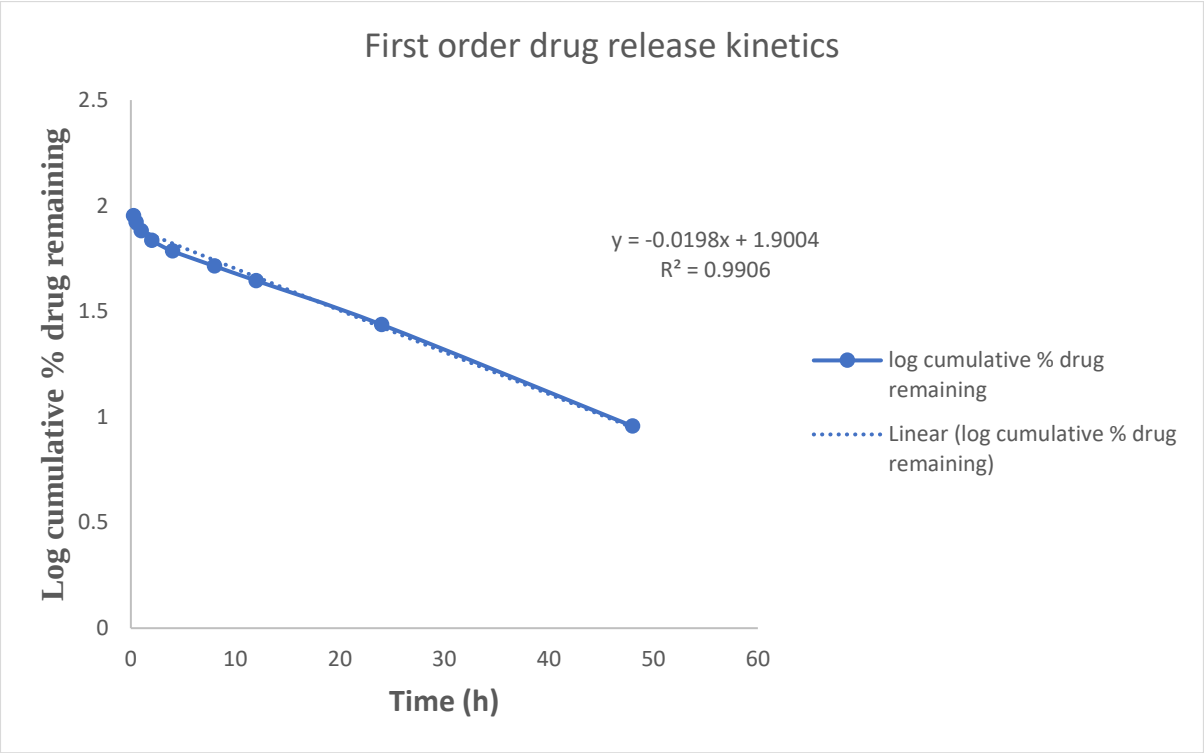

FIGURE 26: First-order drug release kinetic model

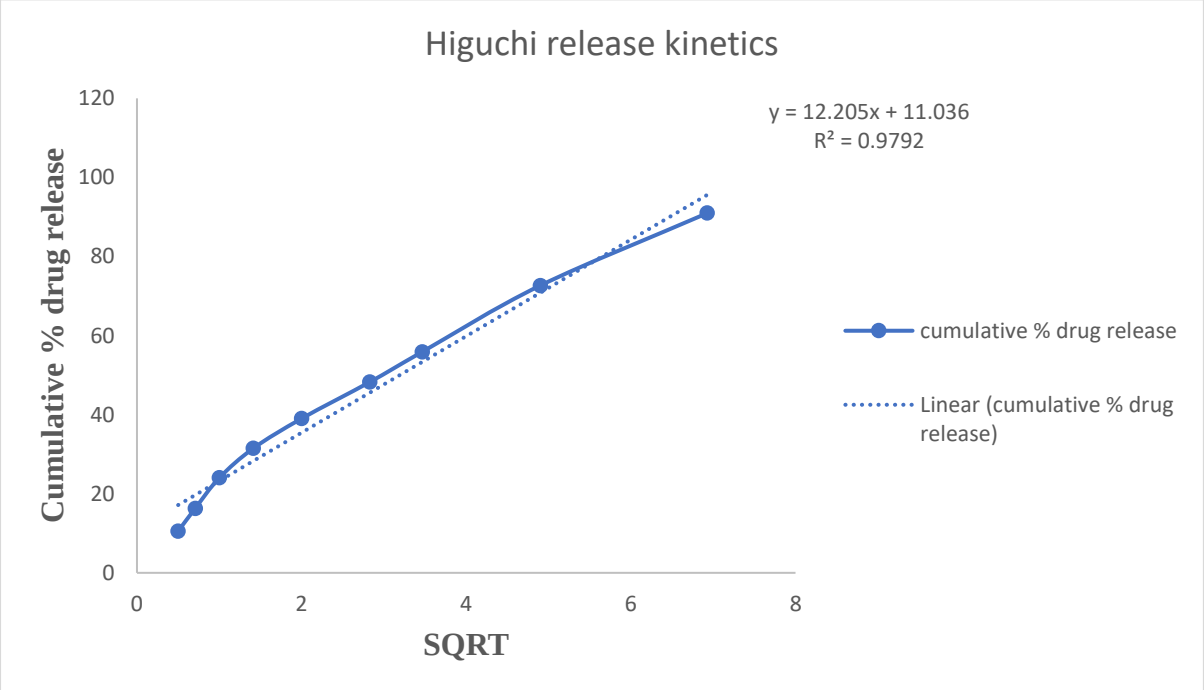

FIGURE 27: Higuchi drug release kinetic model

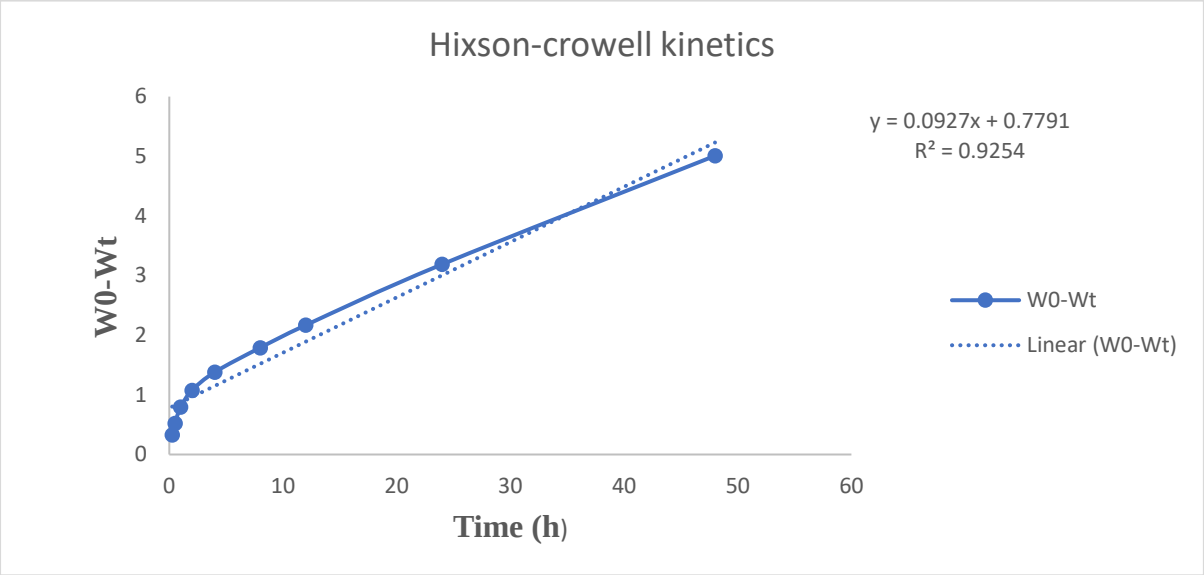

FIGURE 28: Hixson-Crowell drug release kinetic model

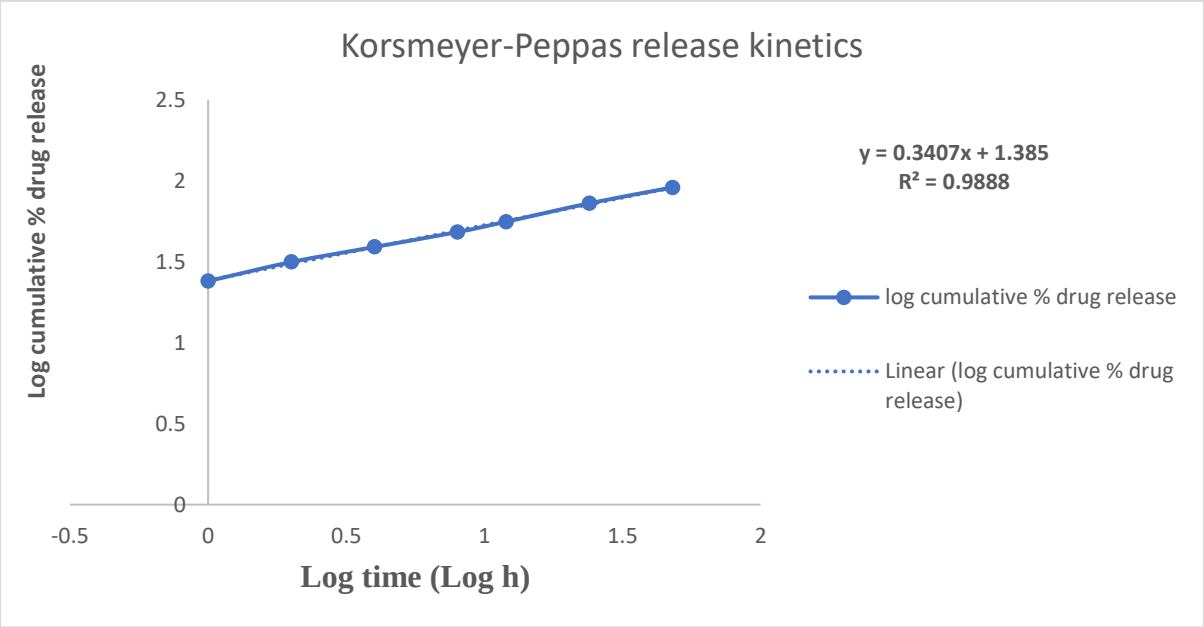

FIGURE 29 - Korsmeyer-Peppas drug release kinetic model

The drug release data suggest that drug release mainly followed **first-order kinetics** .

CHAPTER -7

DISCUSSION

DISCUSSION

- **The UV absorption spectrum of lamivudine in different solvent systems**

UV scanning of lamivudine in water and PBS pH7.2 yielded lambda max values at 269nm and 270nm respectively which was very close to the reported peak of lamivudine .This confirms the authenticity and purity of the product being used and yielded the reference wavelength for spectrophotometric calculation in further studies

The calibration curve of lamivudine in water and PBS 7.2

Two different calibration curves were prepared – One in water & one in pbs 7.2 to study in-vitro drug release and liposome entrapment efficiency. Each reading was made in triplicate, and the average values were taken, and standard deviations were measured. The correlation coefficient (R^2) values were 0.9837 in water and 0.9992 in PBS. The values favor the accuracy of the calibration curves used for further analysis. The low standard deviation in the graphs signified an accurate way of serial dilution to prepare stock and standard solutions.

- **Drug loading and entrapment efficiency**

The theoretical drug loading was 4.23(%), whereas the practical drug loading was found to be 1.85%. The entrapment efficiency was found to be 43.73.The drug, lamivudine is water soluble in nature , and thus it will be entrapped into the hydrophilic bilayer.

- **Study of morphology by SEM-**

The surface morphology as assessed by SEM has shown that the liposome vesicles were spherical in shape, their size was in the nanoscale range, and they had a smooth surface and has no leakage. SEM images of liposome revealed the 3 Dimensional networks of the formulation prepared at different magnification like-10X,30X,50X

- **Particle Size distribution Study-**

Particle size and size distribution of the liposomal formulation was carried out by dynamic light spectroscopy. 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. Determination of dose intensity fluctuation yields the value of Brownian motion of particle due to thermally induced collision between the particles which are converted into particle-size by

using DTS software. From the result it was observed that the particle size of the formulation is in nano range .

- **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 formulation. Zeta potential value of liposomal formulation of lamivudine is - **61.45**

- **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 may be greater or less than one. Generally for the nanoliposome sample the value is 0.05 and the values greater than 0.7 indicate that the sample has a very broad size distribution and is probably not suitable for the dynamic light scattering technique .PDI of the lamivudine-loaded liposomal formulation is **0.4151**

- **Drug –excipients interaction study-**

Drug –excipient interactions were investigted using FTIR spectroscopy. The dug –excipient interaction is an important preformulaion study which is a critical factor considered during liposome formulation.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. FTIR spectra assess the drug-excipient interaction at the level of functional group by determining their vibrational patterns. Here the spectra of drug ,the individual excipients (cholesterol and soya lecithin), liposome without drug and liposome with drug have been depicted

FTIR studies indicates four bands present in the lamivudine spectrum, namely; N-H, O-H, C=O, C-N linkages respectively. NH stretching observed at 3142.04cm^{-1}, O-H stretching observed at 3299.90 cm^{-1}.C=0 stretching observed at 1649.76 cm^{-1} . The liposome without drug showed the peaks of liposome at wave numbers 2926.01 cm^{-1}.(lecithin),1467 cm^{-1} (cholesterol).In case of drug loaded liposome shows no drug peak, indicating complete drug encapsulation within liposomal vesicle. Furthermore minor shifts in some of the peaks in formulation corresponding to cholesterol and soya lecithin could be due to physical interaction between the functional groups of the excipients and drug, most likely due to formation of Vander Waal's force of attraction of dipole-dipole interaction or weak hydrogen bond formation.

In –Vitro drug release study-

In vitro drug release study data were collected over 168 hours, and the cumulative release percentage of drug from the formulation was plotted against thime to determine the drug release pattern in various media. From this study it was seen almost 85%of drug content were released from the formulation in PBS media .The graph clearly showed the sustained release of the drug from the formulation.

The drug released pattern was also correlated with the established models of release kinetics, namely first order, Higuchi, Hixon-Crowell and Korsmeyer–Peppas. Considering the R^2values (0.9906,0.9792,0.9254,0.9880) .In all the above –mentioned kinetics model in PBS media.

CHAPTER -8

CONCLUSION

CONCLUSION

Upon successful completion of the project, in the present study, a novel delivery formulation for AIDS treatment, a liposome containing lamivudine was developed. The optimized formulation showed a substantial amount of drug loading and a 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 the crossing of the formulation across BBB is to be checked .How the drug crossed through BBB and becomes useful for treating AIDS is to be done.

CHAPTER 9

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REFERENCE-

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