FORMULATION AND EVALUATION OFLEVODOPA AND GLUTATHIONE LOADED LIPID-POLYMER HYBRID NANOPARTICLES AS BRAIN TARGETED DRUG DELIVERY FOR TREATMENT OF PARKINSON'S DISEASE.

A thesis submitted to

ASSAM SCIENCE AND TECHNOLOGY UNIVERSITY, ASSAM

for the partial fulfillment of the requirement in the award of degree of

Master of Pharmacy in Pharmaceutics

Under the supervision of

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DECLARATION BY THE CANDIDATE

I hereby declare that the matter embodied in the dissertation entitled "**Formulation and Evaluation of Levodopa and Glutathione Loaded Lipid-Polymer Hybrid Nanoparticles as Brain Targeted Drug Delivery for Treatment of Parkinson's Disease"** is a bonafide and genuine research work carried out by me under the supervision of Dr. Pulak Deb, HOD department of Pharmaceutics, Girijananda Chowdhury Institute of Pharmaceutical Science (GIPS), Hatkhowapara, Azara, Guwahati. The work embodied in this thesis is original and has not been submitted the basis for the award of any degree, diploma or fellowship in any other university or institution.

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Contents:

Chapter 6: Result and Discussion

1. Introduction

1. Introduction

Parkinson's disease (PD) also known as **paralysis agitans**,is one of the most common neurodegenerative disorders which is caused by a loss of dopamine (DA) producing neurons in the basal ganglia in the brain. Patients of PD suffer from motor symptoms such as tremor, bradykinesia and rigidity and treatment with levodopa (LD) has positive effects on these symptoms. Several factors affect the availability of orally given LD. The etiology still remains unknown but age, genetics and environmental factors has a positive influence. [1]

The treatment of PD with conventional drugs carries serious adverse reactions and cannot fix the root cause of PD i.e the degeneration of dopaminergic neurons, which limits conventional drug usage in clinical practice. PD is characterized by resting tremor, bradykinesia, myotonia and gait disorders with or without non-motor symptoms such as depression, constipation and sleep disorders. [2]

There are two main aspects about the pathogenesis of Parkinson's disease, one is the α-synuclein, the other is the oxidative stress. [1] α -synuclein is mostly soluble in normal brain while the α -synuclein inclusions are detergent insoluble in PD. α -synuclein may contribute to PD pathogenesis in a number of ways, but it is generally thought that its deviant soluble oligomeric conformations termed as protofibrils are the toxic species that mediate disruption of cellular homeostasis and neuronal death, through effects on various intracellular targets, such as synaptic function.[1]The brain is particularly susceptible to the risk of oxidative damage due to its high level of polyunsaturated fatty acids and relativelylow antioxidant activity, so oxidative stress plays an important role in the degeneration of dopaminergic neurons in Parkinson's disease (PD).[2]

Over half a century ago and counting, replacement of the neurotransmitter dopamine with the precursor levodopa (L-DOPA) is the backbone therapeutic for standard PD therapy. However, L-DOPA only treats the motor symptoms of the disease; it does not alter the degeneration of the affected neurons as the disease progresses, and it is associated with significant adverse effects in advanced disease.[1]

Therefore, it is imperative that novel disease-modifying therapies should be developed that have the potential to halt the neurodegenerative process itself, and to stimulate the protection, repair, and regeneration of dopaminergic neurons. PD has certain features that

give firm rationale for the development of targeted therapies,but challenges include the unknown etiology, patient-topatient variability in Parkinsonian pathology and symptoms, and the length of time over which the therapy needs to remain active to be effective.

The blood–brain barrier (BBB) is a challenging obstacle for the delivery of drugs into brain. Drugs exceeding 500 Da in molecular weight with low lipid solubility rarely cross the BBB. The blood-brain barrier (BBB) which protects the Central Nervous System, consequently, hampers the success of current therapies by blocking drugs access to brain. Thus, the need to develop innovative CNS treatments and improving the ability to cross BBB is urgent [3].

The barriers at three interfaces separate the blood from the Brain Interstitial Fluid:the **blood–brain barrier (BBB)**, which is formed by the endothelial cells lining the microvessels; the **blood–CSF barrier**, formed by the choroid plexus epithelium, which secretes cerebrospinal fluid (CSF); and the **arachnoid epithelium**, which forms part of the meningeal covering. At each of these layers, cell to cell tight junctions forms a physical barrier. Specific transport proteins or transcytosis mechanisms mediate uptake and efflux forming a transport barrier and enzymes forms a metabolic barrier. Together, these mechanisms regulates the molecular traffic between blood and brain, both inward and outward. Gaseous molecules such as O_2 and CO_2, and small lipophilic agents, including many central nervous system (CNS) drugs, can diffuse passively through the lumen-facing (apical) and brain-facing endothelial cell membranes.[3]

Nanotechnology has abundant of applications in the 'real world' [4]. It encompasses the production and application of physical, chemical and biological system or devices at submicron level as well as the integration of resulting nanostructures into larger systems. [5]

TheBBBis composed ofseveralkindsofcells, which includes braincapillary endothelial cells(BCECs),pericytes,astrocytesandneuronalcells[6]. BCECs are the main component of BBB. BCECspossess of thecontinuoustightjunctionsbetweenthe cells which prevent paracellular transport of compounds from blood to brain. The tight junctions also result in extremely high trans endothelial electrical resistance(TEER) between the blood and brain and thus the passive diffusion of compounds is considerably restricted [5]

Several approaches like BBB disruption (osmotic and biochemical), drug manipulation (prodrug, lipophilic analogs, chemical drug delivery, carrier mediated drug delivery, and receptor or vector mediated drug delivery) and alteration in the route of administration, including intracerebroventricular, intrathecal, and olfactory pathways (intranasal route) are used for the targeting of drugs to the brain. [7]

Lipid-Polymer Hybrid Nano- particles (LPHNs) are solid, submicron particles composed of at least two components: the polymer and the lipid. Lipid-polymer hybrid nanoparticles are a Nano medicine formulation platform that can be used for targeting brain in different diseases. The anatomy of a hybrid nanoparticle consists of a hydrophobic polymer core, a lipid monolayer surrounding the core, and a lipid-PEG which is distributed within the lipid monolayer to form a polyethylene glycol (PEG) corona. The polymeric core affects drug encapsulation and release. Drug release from the nanoparticles begins with diffusion processes, followed by erosion, then swelling of the matrix. The polymer degrades due to hydrolysis and the degradation rate depends on the polymer composition and molecular weight. The lipid shell serves the purpose as a biocompatible shield, a template for surface modifications, and a barrier for preventing water-soluble drugs from leaking out of the core. [8]

Nano particulate carriers such as lipid emulsions, polymeric nanoparticles, liposomes and micelles within the size range of 10–400 nm, allow easy access across BBB by efficiently encapsulating drug molecules and increasing their diffusion through biological membranes. [9, 10]

The success of polymeric nanoparticles and liposomes has motivated the development of lipid-polymer hybrid nanoparticles (LPHNs), which integrates the unique advantages of both polymeric nanoparticle and liposomal systems while overcoming some of their limitations. Lipid-coated polymeric nanoparticles comprising a PLGA core, a PEG shell, and a lipid monolayer at the interface were recently described and characterized. The PLGA core is capable of carrying poorly water-soluble drugs, while the PEG shell helps to decrease biofouling and increase circulation half-life. The lipid monolayer that resides at the interface between PLGA core and PEG shell acts as a molecular fence, promoting drug retention and sustained release from the polymeric core. When compared to PLGA and PLGA–PEG nanoparticles, this lipid-coated PLGA nanoparticle allows for higher

drug encapsulation, tunable and sustained drug release over a longer period of time, and excellent serum stability [9]

LPHNs combine the mechanical advantages of biodegradable polymeric nanoparticles and biomimetic advantages of liposomes, have emerged as a robust and promising delivery platform. LPHNs systems can be described as a polymeric core coated with single or multiple layers of lipids that constitute the shell. [12]

This review makes one such effort to combine the positive attributes of both liposomes and polymeric nanoparticles into a single delivery system, called lipid-polymer hybrid nanoparticles.[13] Since the nanoparticles are targeted in the brain so the BBB permeability is of utmost importance. This type of nanoparticles is typically comprised of three distinct and organised functional components: (i) a polymeric core where drugs are incorporated; (ii) a lipid layer surrounding the core that acts as a highly biocompatible shell and as a molecular fence to promote drug retention inside the polymeric core; and (iii) a lipid coat outside the lipid shell to enhance nanoparticles BBB permeation. Biodegradable polymeric nanoparticles have shown great therapeutic potential as a drug delivery vehicle. Biodegradable polymers such as poly(D, L-lactic-co-glycolic acid) (PLGA), poly(caprolactone) (PCL), chitosan have been used in several approved therapeutic products. Their co-polymers with PEG are commonly used to form core-shell structured nanoparticles to encapsulate a variety of therapeutic agents. [14, 15, 16]

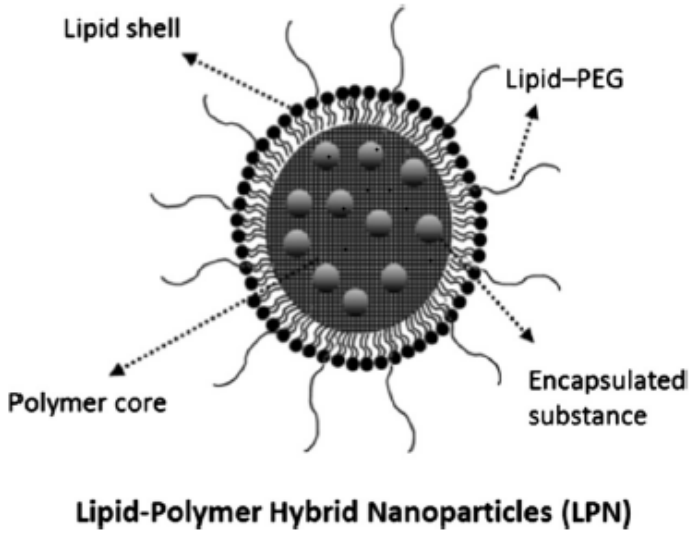

Fig 1.1: Structure of a lipid- polymer hybrid nano particles

1.1 Synthesis of Lipid-Polymer Hybrid Nanoparticles

Lipid-polymer hybrid nanoparticles can be synthesized through two distinct approaches:

- one approach involves a two-step process in which the polymer core and lipid shell are prepared separately and then merged together to form the hybrid nanoparticle;
- the other approach involves a single-step process, in which the hybrid nanoparticles are prepared through a one-step nano precipitation and self-assembly method.[17]

1.1.1 Two-step synthesis approach:

In this method the polymeric core and lipid shell are prepared separately using two independent processes; then the two components are combined by direct hydration, sonication, or extrusion to obtain the desired lipid shell–polymer core structure [30]. It is typically used to prepare lipid-polymer hybrid nanoparticles which consist of a lipid bilayer or multilayer shell. In this approach, the polymer core is formed through an emulsion method, high-pressure homogenization method, or nano precipitation method.
Liposomes are prepared by using a sonication method or extrusion method. Then the previously prepared polymer cores are mixed with the preformed liposomes at desirable molar ratios to prepare lipid-polymer hybrid nanoparticles by needle extrusion, high-pressure homogenization, or simply by vortexing. [18] This mixture is then heated to a temperature above phase transition temperature (Tm) of the lipid to facilitate the reorganization of the lipid onto the particle surface. [31] If the payload is miscible with the polymer in an organic solvent or the payload is covalently conjugated to the polymer chain, a single emulsion method is usually applied to prepare the payload-encapsulated polymer core. [19] First, the payloads and the polymers are dissolved in a water-immiscible organic solvent such as chloroform and then, the emulsion will be formed by adding the polymer solution into an aqueous solution that contains a proper emulsifier, followed by high-speed homogenization. If the payloads are hydrophilic and cannot be dissolved in the organic solvent, a water-in-oil-in-water (w/o/w) double emulsion method is needed to prepare the polymer core. [20] Briefly, a water-in-oil (w/o) emulsion is

formed by adding a payload containing aqueous solution into a polymer-containing organic solvent. The resulting w/o emulsion is subsequently added to a second aqueous solution to form w/o/w double emulsion. The double emulsion is then hardened in an aqueous solution by evaporating the organic solvent. [21]

High-pressure homogenization represents another method to prepare polymer core by using very high pressure to break polymer solutions or melted polymers into small droplets as they pass through a very narrow nozzle. The obtained sub-micron-sized droplets are subsequently hardened by spray drying or simply cooling down to room temperature. This method is simple and easy to control and the resulting polymer particles are typically hundreds of nm in diameter. [21]

Nano precipitation method is also widely used to prepare sub-100 nm polymer particles. This method involves the use of two miscible solvents; one is a good solvent of the polymer and the other one is a poor solvent. The polymer is first dissolved in the good solvent and then added to the poorer one. As the good solvent diffuses into the poor solvent, the polymer will spontaneously precipitate out to form small particles. The mixing of the two solvents can be done through dropwise addition, stirring or sonication. Polymer concentration, the volume ratio of the two solvents, and mixing rate can be molded to control the size and polydispersity of the particles. Once the polymer particles are prepared, can be mixed with preformed lipid films to form lipid-polymer hybrid nanoparticles. The lipid components are usually dissolved in an organic solvent such as chloroform. A thin lipid film is formed by evaporating the organic solvent, to that, an aqueous solution is added to rehydrate the lipids. The polymer particles can be added together with the aqueous solution to rehydrate the lipid films or mixed with liposomes after they are prepared. Nonetheless, upon mixing through high-pressure homogenization, high-speed vortexing, or extrusion, the lipid films or liposomes fuse on the surface of polymer core resulting in the formation of lipid-polymer hybrid nanoparticles. When the concentrations of both lipids and polymer core are properly controlled, a lipid bilayer will form on the surface of the polymer core due to non-covalent interactions between them. [26]

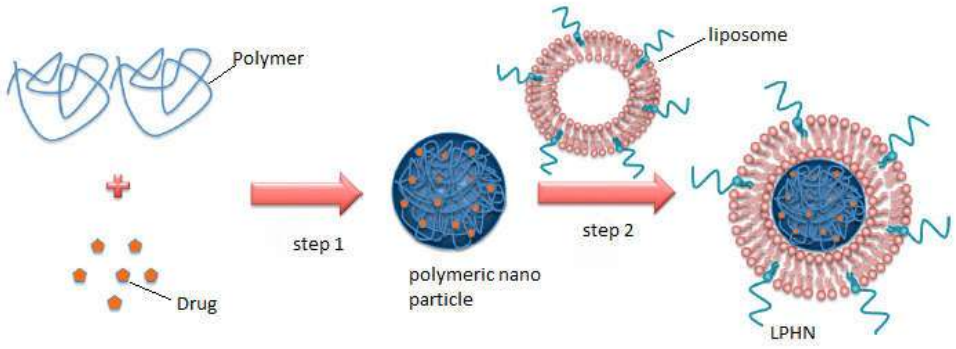

Fig 1. 2: Formation of lipid polymer hybrid nanoparticle (LPHN) by two step synthesis approach. **Step 1** shows the addition of polymer and drug to form polymeric core and **Step 2** shows the addition of processed liposomes and polymeric nano particles to form LPHN.

1.1.2 One-step synthesis approach

It is a relatively simple approach that combines the dual steps of the two-step method into a single step. [30] This one-step synthesis approach is typically used to prepare lipid-polymer hybrid nanoparticles with a lipid monolayer shell. Here free polymers and hydrophobic drugs are dissolved in a water-miscible organic solvent such as acetonitrile, while lipids and lipid-PEG conjugates are dissolved in an aqueous solution. The polymer solution is then added to the lipid-aqueous solution in dropwise manner. The organic solvent diffuses into the aqueous solution quickly, leaving polymer to precipitate into nanoparticles. The lipids and lipid-PEG will self-assemble on to the surface of polymer nanoparticles through hydrophobic interactions to reduce the system's free energy. As the self-assembled lipid monolayer forms due to hydrophobic interactions, a hydrophobic polymer such as PLGA and PCL should be used. [17] One of the critical factors influencing the successful preparation of CSLPHNs using this method is the quantity of lipid needed for uniform lipid coating of polymeric core particles.

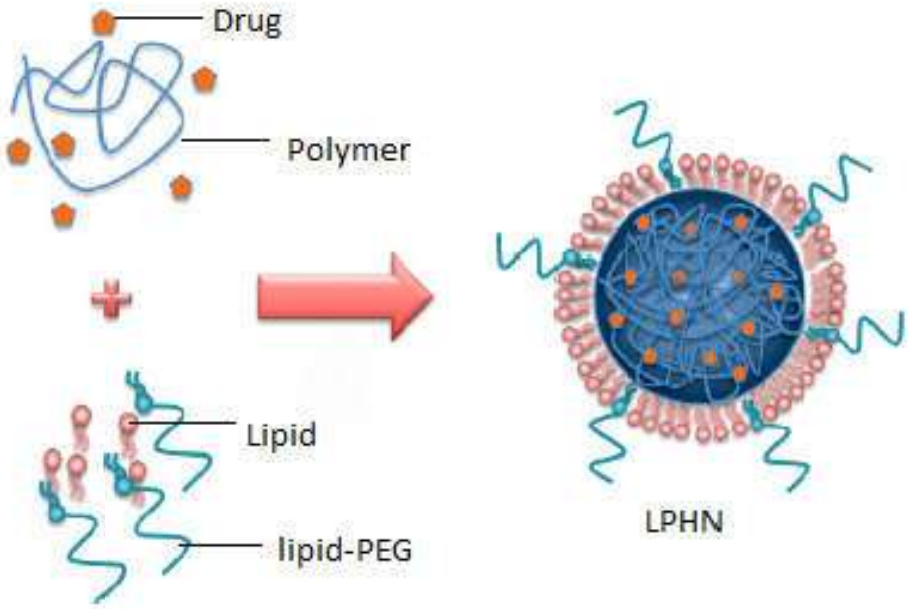

Fig 1.3: Formation of lipid polymer hybrid nanoparticle (LPHN) by one-step synthesis approach.

Variations of the single-step method include modified solvent extraction/evaporation and nanoprecipitation methods.

1.1.2.1 Modified solvent extraction/evaporation method

This method is a modification of the emulsion/solvent evaporation method. Here the polymer and drug are dissolved in a water-immiscible organic solvent. A predetermined amount of lipid is then dispersed in water by mechanical stirring, bath sonication, or sometimes by heating. The organic solution is mixed with the aqueous phase, and the resulting dispersion is sonicated using a probe sonicatorand ice bath. The organic dispersed phase is broken into tiny nanodroplets, which are solidified to form nanospheres coated with a lipid layer. The organic solvent is then usually removed by evaporation in a rotary evaporator under reduced pressure or by stirring overnight. The particle suspension is purified by centrifugation followed by controlled washing. The washed particles are then freeze-dried to obtain a dry powder. [30]

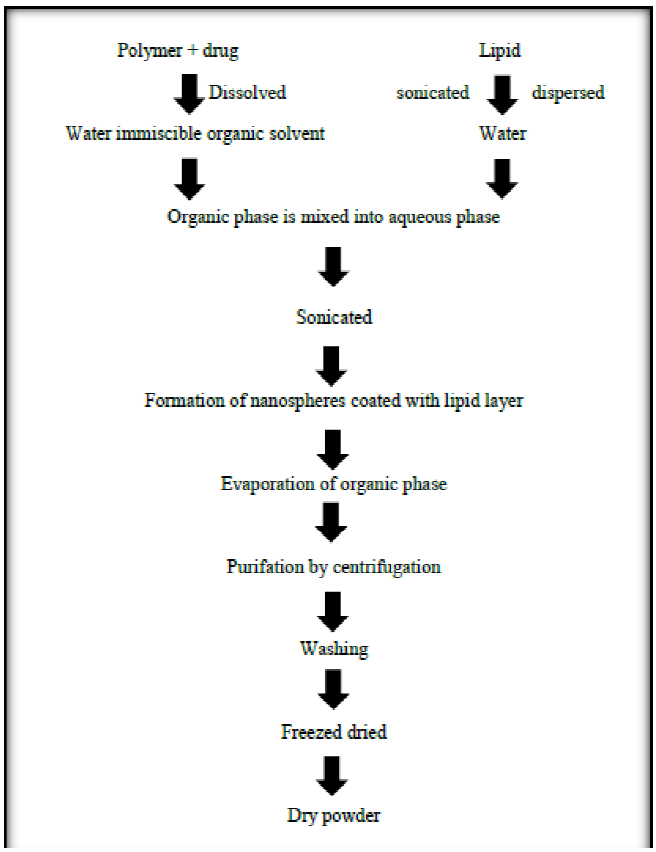

Fig 1. 4: flow chart of Modified solvent extraction/evaporation method

1.1.2.2 Modified nanoprecipitation method

In this method, polymer(s) and hydrophobic drug(s) are dissolved in a water-miscible organic solvent. The organic solution is then added, dropwise, to the aqueous dispersion containing lipid and/or lipid-PEG conjugate. The mixture is then vortexed and subsequently homogenized or ultrasonicated to reduce the particle size within nanometer range. To ensure proper dispersion of lipid and lipid-PEG conjugate, it is necessary to heat the aqueous dispersion (generally ~65 °C) before addition of the organic solution. To uniformly coat the polymeric core with a lipid shell and also to evaporate the organic solvent, the dispersion was stirred for several hours with a magnetic or mechanical stirrer. CSLPHNs formed were then purified by ultracentrifugation, centrifugal ultrafiltration, or dialysis.

The critical factors that have to be optimized for particle size, polydispersity, and surface charge include the type of the lipid, lipid/polymer ratio, phase/volume ratio of organic to the aqueous phase, and viscosity of the polymer. [30]

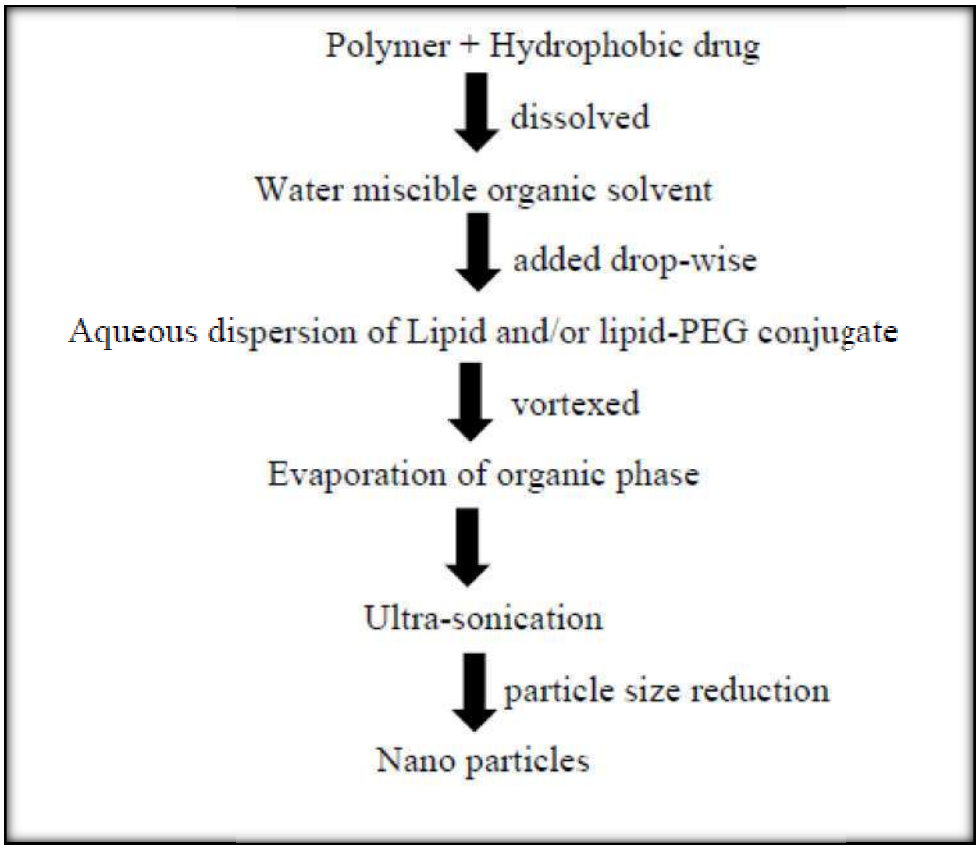

Fig 1.5: Flow chart of Modified Nano precipitation method

1.1.2.3 Self-assembled Nanostructures

Self-assembly has a special place in nanoscience and it comes into the role because of the limitations of both the methods. The top-downmethod provides the ability to build patterns, but are capital intensive, two dimensional, and cannot provide materials in quantity. Bottom-up methods can make large quantities of nanostructures, but without a regular pattern in their arrangement. Self-assembly bridges the two process i.e it provides a strategy that makes possible the patterning (in a broad sense) of nanostructures made by bottom-up synthesis; it also uses patterns to guide the ordering of nanostructures made by

bottom up methods. The novelty of self-assembly is in the focus on the formation of matter which are structured rationally at scales less than 100 nm. [22]

1.2 Designing of Nanoparticles

Factors that affect the clearance and biodistribution of nanoparticles, such as particle physicochemical properties and targeting ligand functionalization, should be carefully considered for the optimal design of therapeutic nanoparticles. [23]

1.2.1 Size

Size is an important parameter on the basis of physiological parameters such as hepatic filtration, tissue extravasation/ diffusion, and kidney excretion. Nanoparticles smaller than 10 nm can be rapidly cleared by the kidneys or through extravasation, while larger nanoparticles may have higher tendency to be cleared by cells of the mononuclear phagocyte system (also referred as reticuloendothelial system, RES) [24] To capitalize on the enhanced permeability and retention effect and to efficiently escape from the physiological barriers, many studies show that the optimal nanoparticle size range of approximately 10–250 nm. [25]

1.2.2 Surface Charge

It has been established that the surface charge of nanoparticles also can affect their uptake by the mononuclear phagocyte system (MPS) cells. Neutrally charged particles have demonstrated much lower opsonization rates than charged particles [26]. It was found that positively charged nanoparticles generate a higher immune response compared to that of neutral or negatively charged nanoparticle formulations [27]. Davis *et al*. in a study has proposed that the optimal range of nanoparticle surface charge should be between 10 and +10 mV for reduced phagocytosis and minimized nonspecific interactions of nanoparticles [28].

1.2.3 PEGylation

Surface modification of nanoparticles with PEG, with favorable intrinsic physicochemical properties, was found to reduce nanoparticle accumulation in off-target organs such as liver and spleen [31]. A PEG shell on the nanoparticle surface shields hydrophobic or charged particles from being attached to blood proteins, leading to

prolonged circulation half-life. Based on the PEG density there is two type of configurations 'mushroom' and 'brush'. It has been stated in studies that the brush configuration would create more effective blocking or repulsion of opsonins than the mushroom one. [29]

1.2.4 Ligand Functionalization

The conjugation of targeting ligands to the surface of PEGylated nanoparticles has shown to affect the biodistribution. Although targeting ligands could improve the cell or tissue-specific delivery of nanoparticles, they may compromise particle's surface properties by masking the PEG layer and adversely affecting the nanoparticles' anti-biofouling properties in vivo. The successful development of targeted nanoparticle technology for efficient drug delivery depends on striking a balance between cellular targeting and immune evasion. [29]

1.2.5 Targeting Ligands

An essential aspect for the successful development of targeted nanoparticles depends on the choice of targeting ligands. Several variables that could be considered include ligand biocompatibility, cell specificity, binding affinity, and purity of the ligand [32]. Other important factors that have to be considered are the size and charge of the ligand molecule and their ease of modification and conjugation to the nanoparticles. The five different classes of targeting ligands, includes antibodies and antibody fragments, aptamers, peptides, sugars, and small molecules.

1.2.6 Physicochemical characteristics

1.2.6.1 Interaction and mechanism of hybrid particle formation

Different mechanisms of lipid-polymer hybrid particle formation can be distinguished based on the method of preparation. In the single step method, polymer particle formation involves the precipitation of the polymer from an organic solution and the diffusion of the organic solvent in an aqueous medium. Then, the lipid molecules self-assemble spontaneously by hydrophobic interaction on the polymeric particle surface to form a monolayer. [33]

The mechanism of hybrid particle formation in the two-step method the process occurs in two steps. First, after forming a bilayer of phospholipid in aqueous solution it attaches to the polystyrene particle surface by adsorption to form homo dispersed and stable phospholipid vesicle-covered particles. Second, after bilayer attachment, hydrophobic attractions between the polystyrene surface and hydrocarbon chain of the phospholipid bilayer results in the collapse of the bilayer structure and leave a monolayer covering the polymer particle. In the process, the lipid and polymer contact is favored by forces like electrostatic interactions, hydrophobic attractions, or van der Waals forces. The input of external energy such as heating, sonication, or agitation aids to rearrange lipids onto the polymer particles. Surface charges play a major role in forming the lipid layer onto polymer particles. Stable particles are formed by electrostatic interactions between a negatively charged polymer and a cationic lipid. Moreover, the affinity of the phospholipid for polymer particle depends on the hydrophilicity of the polystyrene surface. Surface hydration of the polystyrene particles can shield the attractive forces and decrease affinity for the lipid monolayer coverage. [34]

1.2.6.2 Structure

Polymeric particle's morphology, two-dimensional fluidity, lipid shell permeability, and distribution of lipids have been assessed using confocal laser scanning microscopy and cryo-transmission electron microscopy (Cryo-TEM).[29] lipid composition and its concentration play a significant role in the formation of various structures of hybrid nanoparticles. Thus, the presence of excess lipid during the preparation leads to the formation of multilamellar lipid coatings on the particle or may form free liposomal vesicles. [32]

1.2.6.3 Stability

The phospholipids that constitute the shell of the hybrid nanoparticles may act as surfactants to stabilize the hybrid nanoparticle.[30] Four major factors that affect the colloidal stability of lipoparticles have been so far identified, pH and ionic strength of the aqueous medium, temperature, curvature of radius of lipoparticles, and vesicle-to-particle ratio.

Lipoparticles usually tends to become unstable with an increase in ionic strength of the continuous phase. The adsorption of lipid onto polymer particle is affected by its incubation temperature. When incubation occurs at a temperature (T) below glass transition temperature (Tg), the entire vesicle adheres onto particles without rupturing. However, when T is greater than Tg, lipid reorganization onto the polymeric particle is accelerated. Small vesicles having a higher curvature radius tend to coat the smaller polymer particles. [33] One approach to improving the colloidal stability is by creating steric repulsions between particles after incorporating a lipid-PEG conjugate into the formulation. Another approach to improve the colloidal stability is to incorporate suitable amounts of additional surfactants along with the phospholipids. [30]

1.2.6.4 Drug loading and entrapment efficiency

A reason for poor drug loading (DL) and entrapment efficiency (EE) in hybrid nanoparticles is the presence of excess lipids that can form vesicles by entrapment or adsorption of the drugeither by hydrophobic interactions and/or by hydrogen bonding.[34] Moreover, during purification, these vesicles are washed away, leading to drug loss.

Various techniques exist for loading the drug into hybrid nanoparticles (HNs). The drug can be loaded into both the polymeric core and the lipid shell, thereby increasing the total drug payload, or two different drugs can be loaded into the core and the shell.[35] The most commonly used strategy is to incorporate the drug into core production or lipid film formation. Another option is by adsorbing or absorbing the drug in the cores and lipid vesicles separately before combining to form HNs. However, the DL is generally expected to be better in the incorporation approach than the adsorption approach. The macromolecules or proteins show highest loading efficiency near their isoelectric point when they have minimum solubility and maximum adsorption.For smaller molecules, using ionic interactions between the drug and polymer can be an effective way to increase drug loading. [36]

Factors that influence the DL and EE are aqueous solubilities of the drug, affinity, and miscibility of the drug in both polymer and lipid phases,amount of lipid,drug-lipid charge interactions,aqueous phase pH,and methods of preparation. [34]

A spectrophotometric method was developed and validated to quantify drug content in the loaded HNPs.

1.3 Characterization of Hybrid Nanoparticles

1.3.1 Particle Size and Polydispersity Index (PDI)

Particle size measurements were performed by using dynamic light scattering (DLS) technique (Malvern Zetasizer, ZEN 3600). Here the samples were suitably diluted with purified water before measurement. [37]

1.3.2 Zeta Potential

Zeta potential measurements were taken using the Malvern Zetasizer (ZEN 3600) in which the electrophoretic mobility on the surface of the nanostructures was measured.[37]

1.3.3 Scanning Electron Microscopic (SEM) Analysis

Scanning electron microscopy is the technique used to look at morphology and surface structure of the materials.The nanoparticles are typically dried or fixed on a silicon wafer substrate for scanning electron microscopy (SEM) imaging, from which an actual physical size and size distribution can be obtained. With high resolution SEM, surface morphology of hybrid nanoparticles may also be observed. [37]

1.3.4 Transmission Electron Microscopic (TEM) Analysis

Transmission electron microscopy is the technique used to look at the internal structure of the materials. The internal core-shell structure is typically measured by transmission electron microscopy (TEM), in which negative stains are often used to increase electron contrast in order to highlight specific particle components. [38]

1.3.5 Fourier-transform infrared (FTIR) spectroscopy

FTIR analysis was used to characterize any chemical interaction that occurred in the nanoparticulate system among the drug, polymer and lipids. The FT-IR spectra of the sample illustrates characteristic bands due to different functional groups present corresponding to the bending and stretching vibrations. [37]

1.3.6 Differential scanning calorimetry (DSC)

It is a suitable thermal analysis technique for determining the purity, the polymorphic forms and the melting point of a sample. Here the difference in the amount of heat required to increase the temperature of a sample and reference are measured as a function of temperature. Both the sample and reference are maintained at nearly the same temperature throughout the experiment. [37] The powered samples are analyzed by using Perkin Lemer's DSC instrument (model: DSC 4000)

1.3.7 Drug release study

Drug release study is usually performed through a dialysis method. Briefly, a dialysis cassette containing drug-loaded nanoparticles is placed in a large volume release medium maintaining a temperature at 37 C with moderate agitation. The drug molecules will continuously diffuse out of the nanoparticles and leach into the release medium. The released drugs or the drugs remained inside the nanoparticles are collected at a series of time points for quantification using analytical tools such as high performance liquid chromatography (HPLC) and mass spectrometer. [39]

1.4 Application of Hybrid Nano-particles

1.4.1 Therapeutics delivery

Lipid-polymer hybrid nanoparticles can be formulated to efficiently encapsulate and deliver a wide variety of therapeutic agents. These drugs can be loaded inside the nanoparticles alone or in a combination with two or more different types of drugs. Hydrophobic drugs can be directly and physically entrapped in the polymer core during the nano precipitation process and lipophilic drugs can be incorporated into the lipid shell. To further control the release kinetics of the drugs, they can be covalently linked to the polymer chains. (17)

1.4.2 Imaging agent delivery

Besides delivering therapeutic agents, the lipid-polymer hybrid nanoparticle can also be used to deliver a variety of imaging agents such as iron oxide, fluorescent dyes, and quantum dots (QDs) by encapsulating them inside the polymer core. (17)

1.4.3 Vaccine adjuvants

NPs are promising adjuvant delivery systems for enhancing and directing the adaptive immune response of vaccine antigens. The surface display of antigen onto lipid-based NPs has been shown to induce robust antibody responses by mimicking the structure and surface chemistry of microbial pathogens.For example, high IgG titers ($>10^6$) were observed with sustained levels over 100 days after immunization with nanograms of ovalbumin antigen conjugated onto the surface of CSHLPNs along with monophosphoryl lipid A or α- galactosylceramide as molecular danger signals [39].

1.5 Drug Profile

1.5.1Drug 1: Levodopa (core drug)[40]

1.5.1.1 Structure-

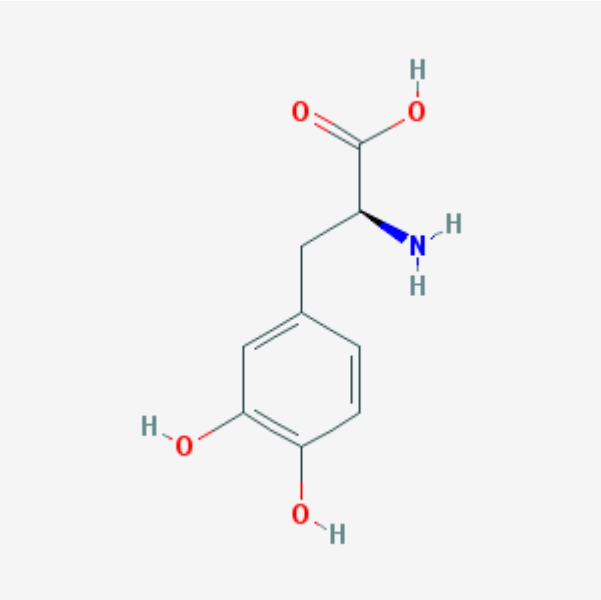

Fig 1.6: structural representation of Levodopa

1.5.1.2 IUPAC Name- (2S)-2-amino-3-(3,4-dihydroxyphenyl)propanoic acid

1.5.1.3 Molecular formula- $C_9H_{11}NO_4$

1.5.1.4 Physical and Chemical Properties-

Property Name	Property Value
Molecular Weight	197.19 g/mol
Hydrogen Bond Donor Count	4
Hydrogen Bond Acceptor Count	5
Rotatable Bond Count	3

Property Name	Property Value
Exact Mass	197.069 g/mol
XLogP3	-2.7
Heavy Atom Count	14
Isotope Atom Count	0
Covalently-Bonded Unit Count	1

1.5.1.5 Physical Description-

State	Solid
Color	Colorless to white crystals or crystalline powder
Odor	Odorless
Taste	Tasteless
Melting Point	284-285 C
Solubility	**Water solubility:** 5000 mg/L (at 20 °C)
	Readily soluble in dil hydrochloric and formic acids; practically insoluble in ethanol, benzene, chloroform, ethyl acetate.
Stability	In presence of moisture rapidly oxidized by atmospheric oxygen & darkens.
Decomposition	When heated to decomposition it emits toxic fumes of nitrogen oxides.
Dissociation constant	pKa = 2.32

1.5.1.6 Pharmacology-

Levodopa (L-dopa) is used to replace dopamine lost in Parkinson's disease because dopamine itself cannot cross the blood-brain barrier where its precursor can. However, L-DOPA is converted to dopamine in the periphery as well as in the CNS, so it is administered with a peripheral DDC (dopamine decarboxylase) inhibitor such as carbidopa, without which 90% is metabolised in the gut wall, and with a COMT inhibitor if possible; this prevents about a 5% loss. The form given therapeutically is therefore a prodrug which avoids decarboxylation in the stomach and periphery, can cross the blood-brain barrier, and once in the brain is converted to the neurotransmitter dopamine by the enzyme aromatic-L-amino-acid decarboxylase.

Levodopa is an amino acid precursor of dopamine with antiparkinsonian properties. Levodopa is a prodrug that is converted to dopamine by DOPA decarboxylase and can cross the blood-brain barrier. When in the brain, levodopa is decarboxylated to dopamineand stimulates the dopaminergic receptors, thereby compensating for the depleted supply of endogenous dopamine seen in Parkinson's disease. To assure that adequate concentrations of levodopa reach the central nervous system, it is administered with carbidopa, a decarboxylase inhibitor that does not cross the blood-brain barrier, thereby diminishing the decarboxylation and inactivation of levodopa in peripheral tissues and increasing the delivery of dopamine to the CNS.

1.5.1.7 Pharmacological Classes-

Established Pharmacologic Class [EPC]	Aromatic Amino Acid Decarboxylation Inhibitor
Mechanisms of Action [MoA]	DOPA Decarboxylase Inhibitors
Established Pharmacologic Class [EPC]	Aromatic Amino Acid
Chemical/Ingredient structural concept [Chemical/Ingredient]	Amino Acids, Aromatic

1.5.1.8 Mechanism of Action

Levodopa increases the level of dopamine & thus activation of dopamine receptors in extra-pyramidal centers in the brain (primarily in caudate nucleus &substantianigra).

1.5.1.9 Pharmacokinetics of the Drug

1.5.1.9.1 Absorption

Levodopa is rapidly absorbed from the proximal small intestine by the large neutral amino acid (LNAA) transport carrier system.

1.5.1.9.2 Metabolism

95% of an administered oral dose of levodopa is pre-systemically decarboxylated to dopamine by the L-aromatic amino acid decarboxylase (AAAD) enzyme in the stomach, lumen of the intestine, kidney, and liver. Levodopa also may be methoxylated by the hepatic catechol-O-methyltransferase (COMT) enzyme system to 3-O-methyldopa (3-OMD), which cannot be converted to central dopamine.

After IP injection into mice, biotransformation of 60% of radioactively labelled DL-DOPA takes place within 10 min, & peak dopamine levels are reached 20 min after administration.

1.5.1.9.3 Distribution

More than 95% of levodopa is decarboxylated in periphery by widely distributed extracerebral aromatic l-amino acid decarboxylase. Little unchanged drug reaches cerebral circulation & probably less than 1% penetrates into CNS.

1.5.1.9.4 Excretion

Most part converted to dopamine metabolites are rapidly excreted in urine, about 80% of radioactively labeled dose being recovered within 24 hr. these metabolites 3,4-dihydroxyphenylacetic acid & 3-methoxy-4-hydroxyphenylacetic acid, as well as small amt of levodopa & dopamine, also appear in cerebrospinal fluid.

After administration of l-dopa to rats by mouth, 18 metabolites were excreted in urine. Excretion of l-dopa was less than 1% of dose.

1.5.1.10 Biological Half-lives: 50 to 90 minutes.

1.5.1.11 Identification-

Ultraviolet absorption spectrophotometry with comparison to standards.

1.5.1.12 Drug Interactions-

acebutolol, acetaminophen / aluminum hydroxide / aspirin / caffeine / magnesium hydroxide, acetaminophen / diphenhydramine, acetazolamide, alprazolam, aluminum hydroxide, amiloride / hydrochlorothiazide, amiodarone, amitriptyline, belladonna / butabarbital, benzthiazide, calcium / vitamin D,disulfiram, docetaxel, entacapone, esmolol, ethambutol, ethanol.

1.5.1.13 Dosage-

Initial: 250 to 500 mg orally twice a day with meals.

Maintenance: 3000 to 6000 mg/day in 3 or more divided doses.

1.5.2 Drug 2: Glutathione [42]

Glutathione (**GSH**) is an important antioxidant in plants, animals, fungi, and some bacteria and archaea. Glutathione is capable of preventing damage to important cellular components caused by reactive oxygen species such as free radicals, peroxides, lipid peroxides, and heavy metals. It is a tripeptide with a gamma peptide linkage between the carboxyl group of the glutamate side chain and the amine group of cysteine, and the carboxyl group of cysteine is attached by normal peptide linkage to a glycine.

1.5.2.1 Structure-

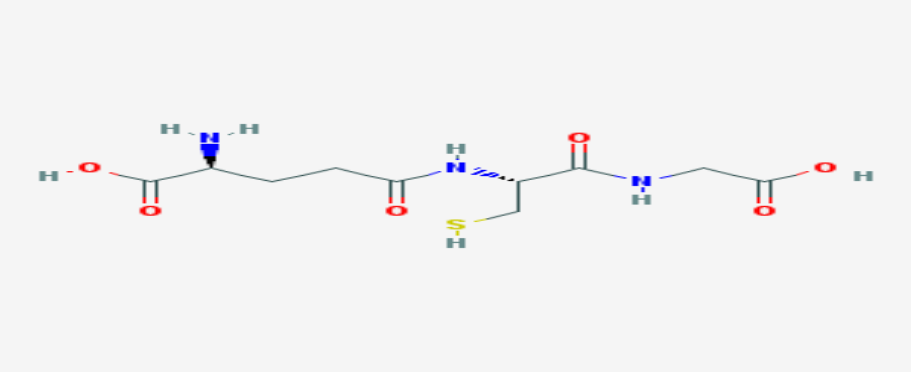

Fig 1.7: structural representation of Glutathione

1.5.2.2 IUPAC Name- (2S)-2-amino-5-[[(2R)-1-(carboxymethylamino)-1-oxo-3-sulfanylpropan-2-yl]amino]-5-oxopentanoic acid.

1.5.2.3 Molecular formula- $C_{10}H_{17}N_3O_6S$

1.5.2.4 Physical and Chemical properties-

Property Name	Property Value
Molecular Weight	307.321 g/mol
Hydrogen Bond Donor Count	6
Hydrogen Bond Acceptor Count	8
Rotatable Bond Count	9
Exact Mass	307.084 g/mol
Heavy Atom Count	20
Isotope Atom Count	0
Covalently-Bonded Unit Count	1

1.5.2.5 Physical Description-

State	Solid
Color	Colorless to white crystals or crystalline powder
Odor	Odorless
Taste	Tasteless
Melting Point	195 °C
Solubility	freely soluble in water, and insoluble in methanol, di ethyl ether.

1.5.2.6 Pharmacology:

Glutathione is a tripeptide comprised of three amino acids (cysteine, glutamic acid, and glycine) present in most mammalian tissue. Glutathione acts as an antioxidant, a free radical scavenger and a detoxifying agent. Glutathione is also important as a cofactor for the enzyme glutathione peroxidase, in the uptake of amino acids, and in the synthesis of leukotrienes. As a substrate for glutathione S-transferase, this agent reacts with a number of harmful chemical species, such as halides, epoxides and free radicals, to form harmless inactive products. In erythrocytes, these reactions prevent oxidative damage through the reduction of methemoglobin and peroxides. Glutathione is also involved in the formation and maintenance of disulfide bonds in proteins and in the transport of amino acids across cell membranes.

1.5.2.7 Mechanism of Action-

Glutathione (GSH) participates in leukotriene synthesis and is a cofactor for the enzyme glutathione peroxidase. It is also important as a hydrophilic molecule that is added to lipophilic toxins and waste in the liver during biotransformation before they can become part of the bile. Glutathione is also needed for the detoxification of methylglyoxal, a toxin produced as a by-product of metabolism. This detoxification reaction is carried out by the glyoxalase system. Glyoxalase I catalyzes the conversion of methylglyoxal and reduced glutathione to S-D-Lactoyl-glutathione. Glyoxalase II catalyzes the conversion of S-D-Lactoyl Glutathione to Reduced Glutathione and D-lactate. GSH is known as a cofactor in both conjugation reactions and reduction reactions, catalyzed by glutathione S-transferase enzymes in cytosol, microsomes, and mitochondria. However, it is capable of participating in non-enzymatic conjugation with some chemicals, as it is hypothesized to do to a significant extent with n-acetyl-p-benzoquinone imine (NAPQI), the reactive cytochrome P450 reactive metabolite formed by toxic overdose of acetaminophen. Glutathione in this capacity binds to NAPQI as a suicide substrate and in the process detoxifies it, taking the place of cellular protein sulfhydryl groups which would otherwise be toxically adducted. The preferred medical treatment to an overdose of this nature, whose efficacy has been consistently supported in

literature, is the administration (usually in atomized form) of N-acetylcysteine, which is used by cells to replace spent GSSG and allow a usable GSH pool.

1.5.2.8 Pharmacokinetics of the Drug-

1.5.2.8.1Absorption

Glutathione is not orally bioactive, and that very little of oral glutathione tablets or capsules is actually absorbed by the body.

1.5.2.8.2 Distribution

Glutathione is the predominant low-molecular-weight thiol (0.5–10 mmol/L) in animal cells. Most of the cellular GSH (85–90%) is present in the cytosol, with the remainder in many organelles (including the mitochondria, nuclear matrix, and peroxisomes) (8). With the exception of bile acid, which may contain up to 10 mmol/L GSH, extracellular concentrations of GSH are relatively low (e.g., 2–20 μmol/L in plasma).

1.5.2.8.3 Metabolism

In healthy adult humans, the endogenous disappearance rate (utilization rate) of GSH is 25 mol/(kg h), which accounts for 65% of whole body cysteine flux [38.3 mol/(kg h)].

1.5.2.9 Drug Interactions-

Glutathione has no known severe or serious interactions with other drugs.

1.5.2 10 Dosages of Glutathione:

- Intravenously

  Dosage Considerations – Should be Given as Follows:

  Chemotherapy Adjunct

  - 600 mg/day intramuscularly days 2-5 of chemotherapy
  - 1.5 g/m2 intravenously before chemotherapy

  Male Infertility

  - 600 mg intramuscularly every other day for two months
- Oral
  - 250 mg orally once daily
  - Dose range: 50-600 mg/day

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2. LITERATURE REVIEW

Literature Review:

- **For drug delivery vehicle**

1. **Avhad P.S., Patil P.B., et. al.,** in their study on **A Review on Different Techniques for Brain Targeting** reported techniques that can easily cross the Blood Brain Barrier (BBB). Number of drugs unable to cross BBB easily, so that by using these techniques drug can easily reach to targeted sites. Techniques such as Pro drug, Liposome's, Nanotechnology, Microspheres, Polymeric Micelles and Micro emulsions and Dendrimers are helpful to transfer drug at brain targeted site. [1]
2. **Li Zhang and Liangfang Zhang**, in their study on **Lipid-Polymer Hybrid Nanoparticles: Synthesis, Characterization and Applications** found that lipid-polymer hybrid nanoparticles offer numerous advantages as a drug delivery platform including simple fabrication process, tunable size and surface charge, high loading capacity of poorly water-soluble drugs, sustained and controllable release profile of the drugs, high in vitro stability, and excellent in vivo properties which make the lipid-polymer hybrid nanoparticles an ideal drug delivery platform. also highlighted few unmet challenges in further developing this new nanoparticle platform such as first, optimizing the targeting ligand density on the nanoparticle surface is critical to achieve optimal therapeutic efficacy, secondly, precise control of multiple drugs with different hydrophobicity inside the same hybrid nanoparticles remains challenging, lastly, large-scale fabrication of these hybrid nanoparticles. [2]
3. **BivashMandal, George C.Wood, et. al**.,in their study on **Development and in vitro evaluation of core–shell type lipid–polymer hybrid nanoparticles for the delivery of erlotinib in non-small cell lung cancer** reported the development of CSLPHNPs composed of the lipid monolayer shell and the biodegradable polymeric core for the delivery of drug. Single-step sonication method was employed to prepare and optimize CSLPHNPs at 8–16%$W_{Lipid}/W_{Polymer}$ and 16%$W_{Lipid\text{-}PEG}/W_{Polymer}$. The mean size of erlotinib loaded CSLPHNPs ranged from 150 to 180 nm with PDI less than 0.2 and zeta potential between 15 and 30

mV. They optimized different formulation variables including initial drug input, molecular weight of PCL, pH of the external aqueous medium before erlotinib loaded CSLPHNPs preparation, and type of phospholipids were studied. The drug entrapment efficiency and drug loading in erlotinib loaded CSLPHNPs ranged 18–66% w/w and 1.6–2.1% w/w. [3]

4. **Kathy Stavropoulos** in her work on **Synthesis and Characterization of Lipid-Polymer Hybrid Nanoparticles for Combinatorial Drug Delivery** provides an approach to address the aforementioned concerns. By combining multipledrugs into a single delivery system, which can be functionalized to target specific tissues and cells, the drugs could have the opportunity to reach the targeted sites with the correct mass ratios and the possibility to induce therapeutic synergism. [4]
5. **BivashMandal, Laura A. Thoma, et. al**., in their study on **Core–shell-type lipid–**polymer **hybrid nanoparticles as a drug delivery platform** reported current research trends on CSLPHNs including classification, advantages, methods of preparation, physicochemical characteristics, surface modifications, and immunocompatibility and several applications for cancer chemotherapy, vaccines, and gene therapeutics. [5]
6. **J-F. Le Meins, C. Schatz, et. al.,**in their study on **Hybrid polymer/lipid vesicles: state of the art and future perspectives** reported a comprehensive overview of the fundamental aspects related to these structures, and discuss emerging developments and future applications in this field of research. They further concluded that the design of hybrid polymer/lipid vesicles with good control over the molar composition and the membrane structure at the micro- and nano-scales remains highly challenging. [6]
7. **Liangfang Zhang, Juliana M. Chan, et. al.,** in their study on**Self-Assembled Lipid-Polymer Hybrid Nanoparticles: A Robust Drug Delivery Platform** reported the engineering of a novel lipid-polymer hybrid nanoparticle (NP) as a robust drug delivery platform, with high drug encapsulation yield, tunable and sustained drug release profile, excellent serum stability, and potential for differential targeting of cells or tissues. The NP is prepared by self-assembly

through a single-step nanoprecipitation method in a reproducible and predictable manner, making it potentially suitable for scale-up. It has been demonstrated that the hybrid NP has tunable size and surface charge, high drug loading yield, sustained drug release profile, favorable stability in serum, good cellular targeting ability. [7]

8. **Aditya Grover, Anjali Hiraniet.al.,**in their study on **Nanoparticle-Based Brain Targeted Delivery Systems** described the blood-brain barrier and the current state of nanoparticle therapeutics that aim to cross the blood-brain barrier to improve drug delivery to this highly sensitive region. They reported several nanoparticle-based strategies for brain-targeted deliveries. Of thementioned brain delivery systems, the glutathione- and thiamine- coatedmethods seem the most promising. The unique properties of each nanoparticle carrier, along with the drug it encapsulate and its surface modification with PEG to induce BBB permeability. [8]
9. **Salatin S, Jaleh B, Mohammad BJ et al**., in their study **on Development of a nanoprecipitation method for the entrapment of a very water soluble drug into Eudragit RL nanoparticles** reported the preparation and evaluation of Eudragit RL 100 nanoparticles as a model scaffold for providing a sustained release profile for water soluble drug [9].

- **For drug**

10. **Faria Afroz and Christian Bach** in their study on **Improvement in Drug Delivery System for Parkinson's Disease** reported a method on chitosan based nano particles for delivery of drug in the brain and observed an improvement in DA transport across the BBB. In vitro studies confirmed that free-drug is more cytotoxic than DA-Cs-NP. After 3 hr there was an increase in transport of DA across the cells and oxygen reactive species reduction was observed. Because of its high loading and good delivery capacity for hydrophilic molecules chitosan was chosen as the polymer. The developed nanoparticles are solid matrix like colloidal particles which are composed of polymers or lipids and are mostly given

by the intravenous route and are developed for the targeted delivery of therapeutic agent. [10]

11. **Hirotaka Iwaki, Noriko Nishikawa, et. al.**, in their study on **Pharmacokinetics of levodopa/benserazide versus levodopa/ carbidopa in healthy subjects and patients with Parkinson's disease** studied the pharmacokinetics of levodopa in the two kinds of levodopa/ decarboxylase inhibitor formulations and reported the pharmacokinetics of the drug in healthy volunteers and in patients with parkinsonism. [11]
12. **HongjiaZhang ,Rongsheng Tong, et. al**., in their study **on Emerging targets and new small molecule therapies in Parkinson's disease treatment** analyzed and summarized the several novel targets in PD and their small molecule targeted pharmacologically active agents based on their mechanisms of action and pharmacodynamic profiles. Targets like Angiotensin-converting enzymes inhibitors, Microtubule-stabilizing agents, Phospholipase A2 (PLA2) inhibitors, Erythropoietin (EPO) receptor agonists, Leucine-rich repeat kinase 2 (LRRK2) inhibitors, D3 receptor agonists, Monoamine oxidase (MAO) inhibitors, Triple monoamine neurotransmitters reuptake inhibitors, L-type calcium channel blockers, Phosphodiesterase inhibitors, Catechol-O-methyltransferase (COMT) inhibitors, Adrenergic receptor antagonists, PD treatment with natural medicinal ingredients has been studied. [12]

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12. Jianyou Shi. Emerging targets and new small molecule therapies in Parkinson's disease treatment. Bioorganic & Medicinal Chemistry. 2016; 24: 1419–1430.

3. Aim and Objective

Aim and Objective

3.1 Aim:

Formulation and Evaluation of levodopa and glutathione loaded Lipid-Polymer Hybrid Nano particles for the treatment of Parkinson's Disease.

3.2 Objective:

- Preformulation study of drug and polymer
- Drug- excipient compatibility studies using DSC and FT-IR techniques.
- Formulation and evaluation of levodopa and glutathione loaded lipid-polymer hybrid nanoparticles.

4. Plan of work

Plan of Work

4.1 Pre-formulation study

4.1.1 Organoleptic Property

4.1.2 Solubility Studies

4.1.3 Drug – excipient compatibility study by DSC and FT-IR.

4.2 Formulation of Drug loaded lipid- polymer hybrid Nanoparticles

4.3 Evaluation of the drug loaded lipid- polymer hybrid Nano particles

4.3.1 Particle size Distribution

4.3.2 Surface morphology

4.3.3 Drug loading

4.3.4 Drug entrapment efficiency

4.3.5 In-vitro drug release study

4.4 Results and discussion

4.5 Conclusion

5. Methodology

5.1 Materials

Table 5.1: Materials and its suppliers are mentioned .

Sl no.	Materials	Source
1.	Levodopa	Yarrow Chem Product ; Mumbai
2.	Glutathione	Yarrow Chem Product ; Mumbai
3.	Eudragit RL 100	BS Trading; Kolkata
4.	Cholesterol	BS Trading ; Kolkata
5.	Ethanol	Infinity solutions; Guwahati
6.	Methanol	Infinity solutions; Guwahati
7.	Tween 80	Krishna Enterprise; Guwahati
8.	Potassium di hydrogen phosphate	Krishna Enterprise; Guwahati
9.	Di sodium hydrogen phosphate	Krishna Enterprise; Guwahati
10.	Sodium chloride	Infinity solutions; Guwahati

Table 5.2: Instruments used for the preparation and evaluation of formulation.

Sl. No.	Instrument	Company
1.	U.V visible spectrophotometer (Model No. UV 1800 240 V)	Shimadzu ; Japan
2.	FT-IR spectrophotometer (Model No. 10059756)	Bruker; Germany

3.	Differential Scanning Calorimetry (model No. DSC- 4000)	PerkinElmer 2000, USA
4	Zeta-sizer (Model No. Nanosight NS 500)	Malvern; UK
5	High speed homogenizer (Model No. IKA T25)	Digital ultra turrax R; Germany
6	Analytical Balance Cs-09-05-11/2	Citizen, Scale (l) Pvt. Ltd. , Parwanoo; India
7	Sonicator	
8	Magnetic Stirrer with hot plate	Rolex; India

5.2 Methods

5.2.1 Pre-formulation study

5.2.1.1 Organoleptic Properties

The organoleptic properties like color, odor and taste of drud and the polymer were examined.

5.2.1.2 Solubility

The solubility of the drug and the polymer in different solvent such as ethanol, water, phosphate buffer 7.4 was studied.

5.2.1.3 Melting Point

Melting point of the drug was determined by using Melting Point Apparatus. A small amount of drug was taken in a capillary tube which was sealed on one end. The capillary was placed in the sample chamber and the temperature at which the drug melts is noted. This was performed thrice and the average value was calculated.

5.2.1.4 Differential Scanning Calorimetry Study

The DSC thermograms of Levodopa, Eudragit RL 100, Glutathione, cholesterol, physical mixture of levodopa and eudragit RL 100, and mixture of glutathione and cholesterol were recorded by Differential Scanning Calorimetry (PerkinElmer 2000, USA). Samples weighing between 5-10 mg were sealed into standard aluminium pan and heated from 20 C to 500 C at a rate of 10 C per min.

5.2.1.5 FT-IR Spectroscopy study

FT-IR study was performed by Bruker's FT-IR Spectrometer for both the drugs and polymers used. Small amount of sample was taken and triturated in a morter. The sample was kept onto a sample holder and scanned from 400 cm^{-1} to 4000 cm^{-1} . Samples were compared with official standard and interpreted for the functional group peaks. [1]

5.2.2 Preparation of Levodopa and Glutathione loaded lipid-polymer hybrid Nano-particles

Lipid-polymer hybrid nanoparticles were prepared in two steps:

Step 1*:* Preparation of the polymeric core

The polymeric core was prepared by using Eudragit RL 100 containing Levodopa through a nanoprecipitation method using different drug to polymer ratios (1:4, 1:7, and 1:10). 40 mg of Levodopa was dissolved in 5ml of distilled water. Simultaneously, different amount of Eudragit RL 100 160 mg, 280 mg, and 400 mg was dissolved in 10 ml of acetone [1].

Table 5.3: levodopa loaded polymeric NPs prepared by nanoprecipitation method

Formulations	Drug: polymer	Water (ml)	Levodopa (mg)	Eudragit RL 100 (mg)	Acetone (ml)	Poloxamer (mg)
A1	1:4	5	40	160	10	200
A2	1:7	5	40	280	10	200
A3	1:10	5	40	400	10	200

The mixture was formed by injecting the aqueous solution of Levodopa dropwise into Eudragit RL 100 organic solution and was homogenized for 30 mins at 15000 rpm. The mixture was then added into 20 ml of external aqueous solution containing 2% of poloxamer 407 as a stabilizer. The mixture was then magnetically stirred at room temperature at 500 rpm for 2 hours to evaporate the organic phase.

The solution was then centrifuged at 5000 rpm for 1 hour and washed via resuspending the nanoparticles in distilled water, followed by centrifugation. Nanoparticles were then lyophilized and stored under the desicator for further studies [1].

Step 2: Preparation of the lipid film by film hydration method

When preparing liposomes with mixed lipid composition, the lipids must first be dissolved and mixed in an organic solvent to assure a homogeneous mixture of lipids.

Cholesterol is dissolved in a solution of chloroform or mixture of chloroform and methanol. Once the lipids are thoroughly mixed in the organic solvent, the solvent is removed to yield a lipid film. The organic solvent should be removed by rotary evaporation yielding a thin lipid film on the sides of a round bottom flask. The lipid film is thoroughly dried to remove residual organic solvent by placing the vial or flask on a vacuum pump overnight. To the dried film previously prepared aqueous polymeric solution was added and agitated [2, 3, 4]. The temperature of the hydrating medium should be above the gel liquid crystal transition temperature (Tc or Tm) of the lipid. After addition of the hydrating medium, the lipid suspension should be maintained above the Tc during the hydration period [5, 6, 7].

Table 5.4: glutathione liposomes prepared by film hydration method

Formulations	Glutathione (mg)	Cholesterol (mg)	Methanol: Chloroform	Tween 80 (%)
B1	100	100	1:1	2
B2	100	150	1:1	2
B3	100	200	1:1	2

5.2.3 Physicochemical characterization of Nano-particles

5.2.3.1. Particle size analysis

A dynamic light scattering particle size analyzer (Malvern nano sizer, UK) was used for measuring the mean particle size and polydispersity index of the prepared nanoparticles. The PDI offers the indication of the breadth of variation in the nanoparticle size distribution pattern.

5.2.3.2. Morphological analysis

The morphology of nanoparticles was examined using high- resolution scanning electron microscopy (SEM). The samples were mounted on a metal stub using double sized carbon adhesive tape and coated with platinum/palladium alloy under vacuum.

5.2.3.3. Encapsulation efficiency and drug loading

The drug Encapsulation Efficiency and drug loading of the prepared hybrid nanoparticles were determined by dissolving a known amount of lyophilized nanoparticles in 5 ml of ethanol and centrifuged at 4000 rpm for 10 mins [1]. The amount of incorporated drug in the precipitate of centrifuged nanosuspension was measured using UV-visible spectrophotometer (UV 1800 240 V, Shimadzu; Japan) at wavelength of 237.8 nm and 280 nm. Then, by substituting the data in the previously obtained standard curve the encapsulation efficiency and drug loading were determined by using following equations:

$$\textit{Entrapment efficiency (EE \%)} = \frac{actual\ drug\ content\ in\ nanoparticles}{total\ drug\ used\ in\ formulation} \text{x } 100$$

$$\textit{Drug loading (\%)} = \frac{(total\ amount\ of\ drug - unincorporated\ drug\ amount)}{amount\ of\ nanoparticles\ recovered} \text{ x } 100$$

5.2.3.4. In vitro release study

The in-vitro drug release from the hybrid Nanoparticles was performed by using dialysis bag diffusion technique. A known amount of lyophilized nanoparticles was placed into the dialysis bag, which was then immersed in 200ml of phosphate buffer, pH 7.4 dissolution medium in USP dissolution apparatus type II with a speed of 100 rpm at 37±0.5 C. Aliquots of 5 ml of the dissolution medium were withdrawn at specific time intervals and replaced with fresh medium to maintain the sink condition [1]. The concentration of drug present in the sample was determined by absorption ratio method spectrophotometrically at 237.80 nm (isobestic point) and at 280 nm.

6. Result and Discussion

6.1. Preformulation studies

Table 6.1: Organoleptic properties

Sample	Color	Odor	Taste
Levodopa	White	Odorless	Tasteless
Glutathione	White	Odorless	Characteristics
Eudragit RL 100	White crystalline powder	Characteristics	Tasteless
Cholesterol	White crystalline powder	Odorless	Tasteless

Table 6.2: Solubility data

Sample	Solubility		
	Souble	**Slightly soluble**	**Insoluble**
Levodopa	Dil. HCl	Water	Ethanol, chloroform
Glutathione	Dil. Alcohols , water	Ethanol	Methanol
Eudragit RL 100	Ethanol	-	water
Cholesterol	Ethanol, Chloroform	Water	-

Table 6.3: Melting point

Sample	Melting point (C)
Levodopa	289
Glutathione	330

6.2. FT-IR spectroscopy study

Levodopa

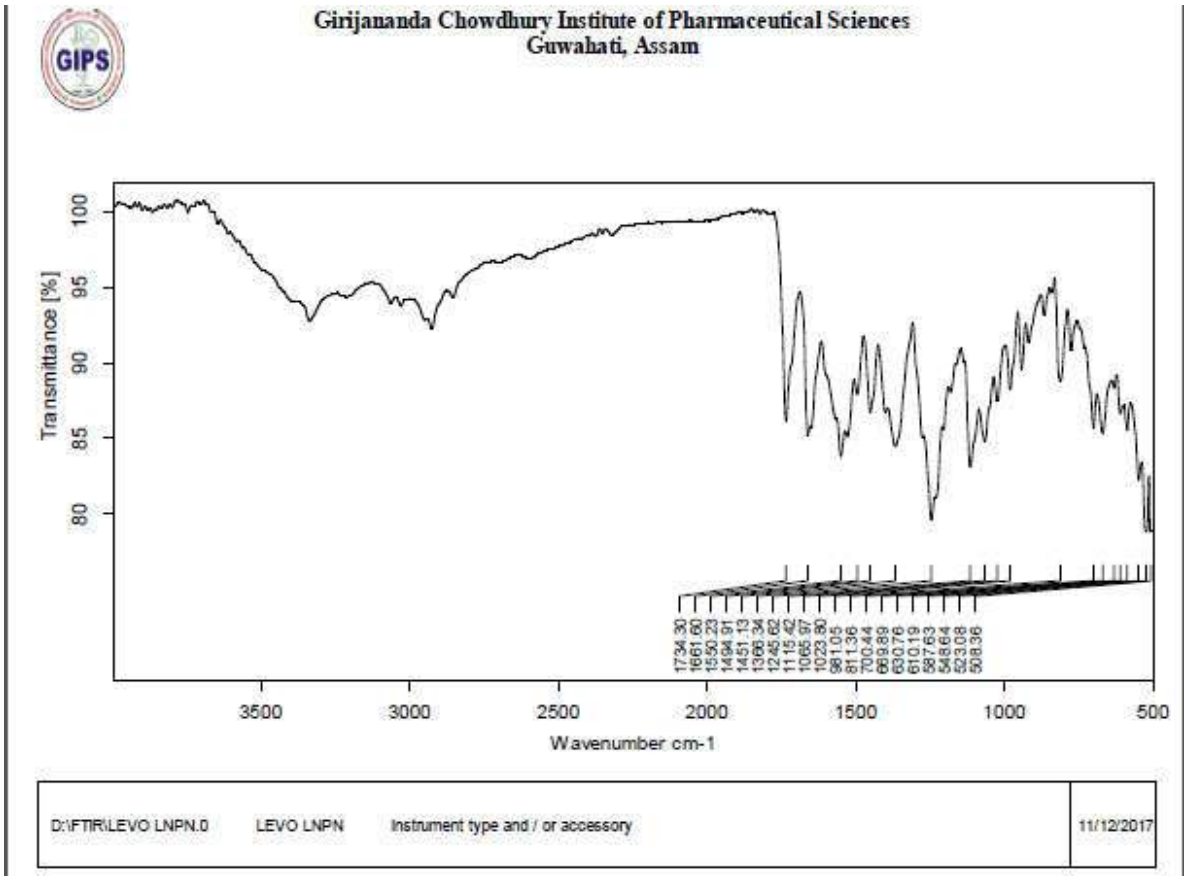


Table 6.4 Structural Assignment of Levodopa

Wave number (cm^{-1})	Structural assignment
3745.36	O-H str.
3335.94	N-H str
2925.46	C-H str
1734.30	C=O
1661.60	N-H bnd.
1366.34	C-H bnd
1115.42	C-N
1065.97	C=C

Glutathione

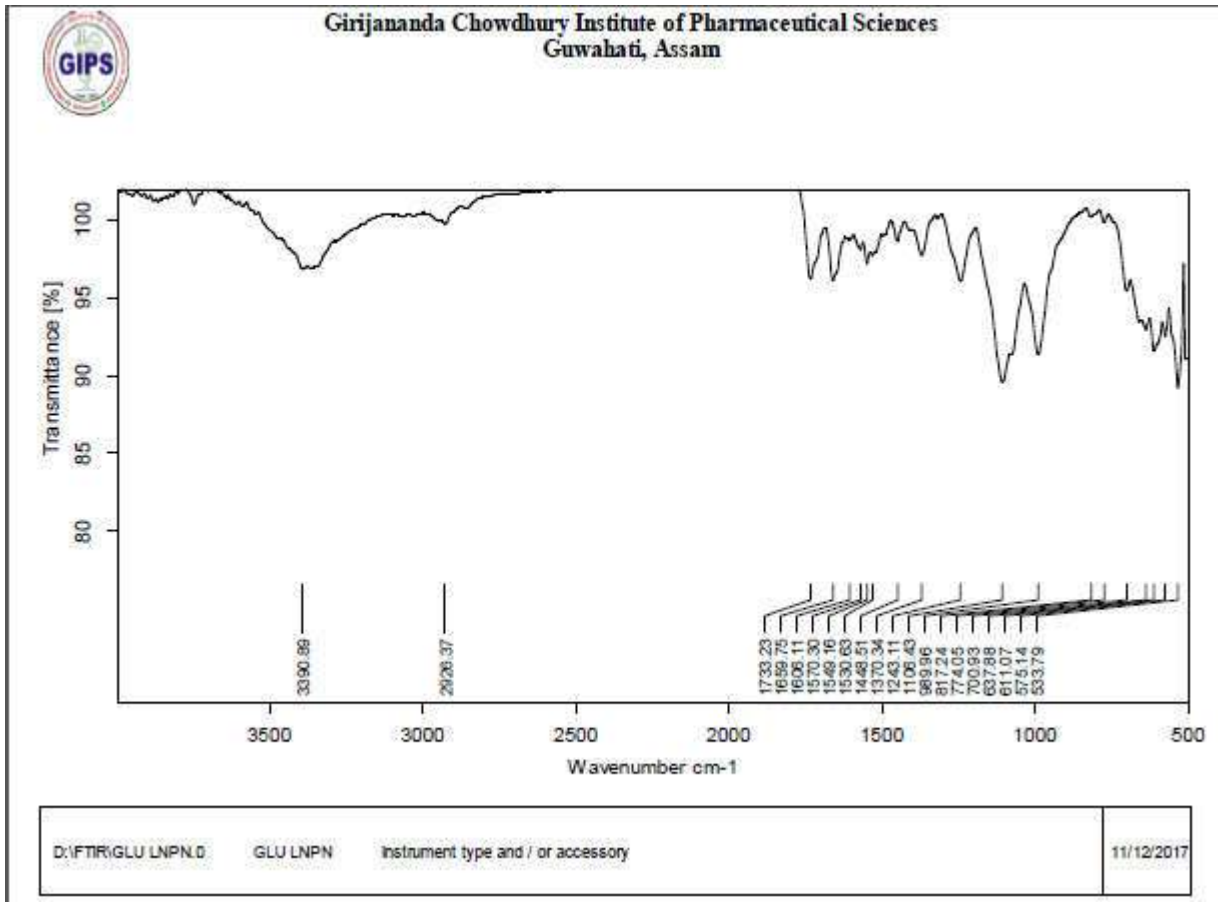


Table 6.5: Structural assignment of Glutathione

Wave number (cm^{-1})	Structural assignment
3588.00	N-H str
2926.37	S-H str.
1783.46	C=O
1448.51	$C\text{-}H_2$
1243.11	O-H
1106.43	C-N

Eudragit RL 100

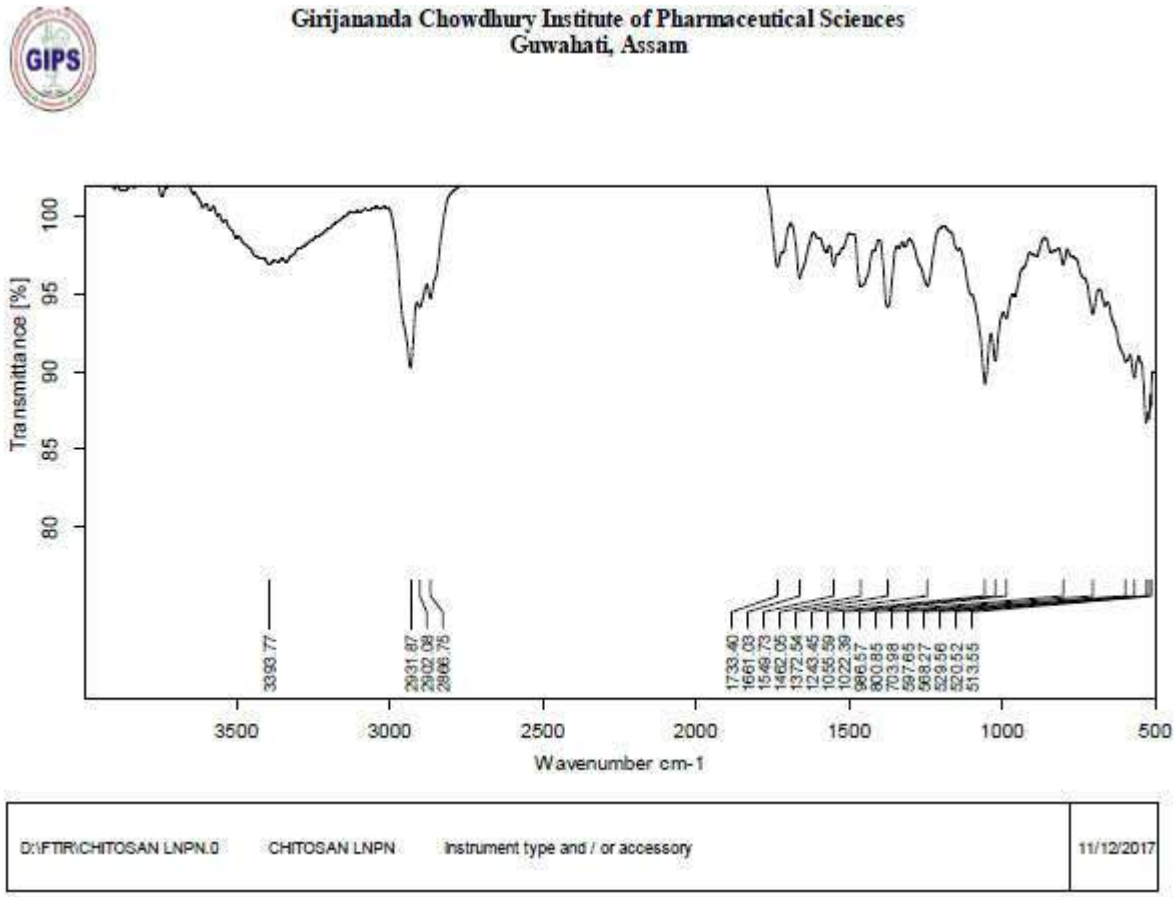


Table 6.6: Structural assignment of Eudragit RL 100

Wave number (cm^{-1})	Structural assignment
3587.72	N-H (1)
3393.77	N-H (2)
1733.40	C-O-C
1316.74	O-H

Cholesterol

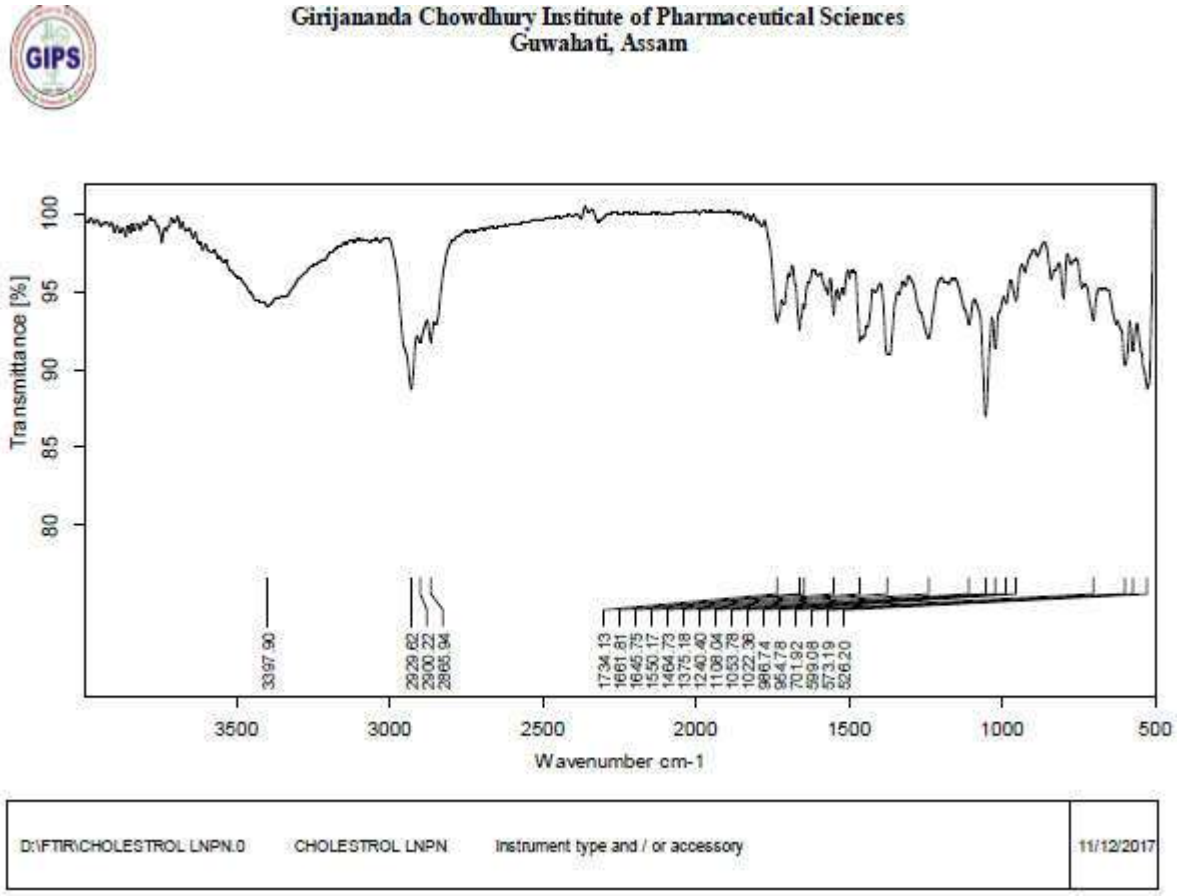
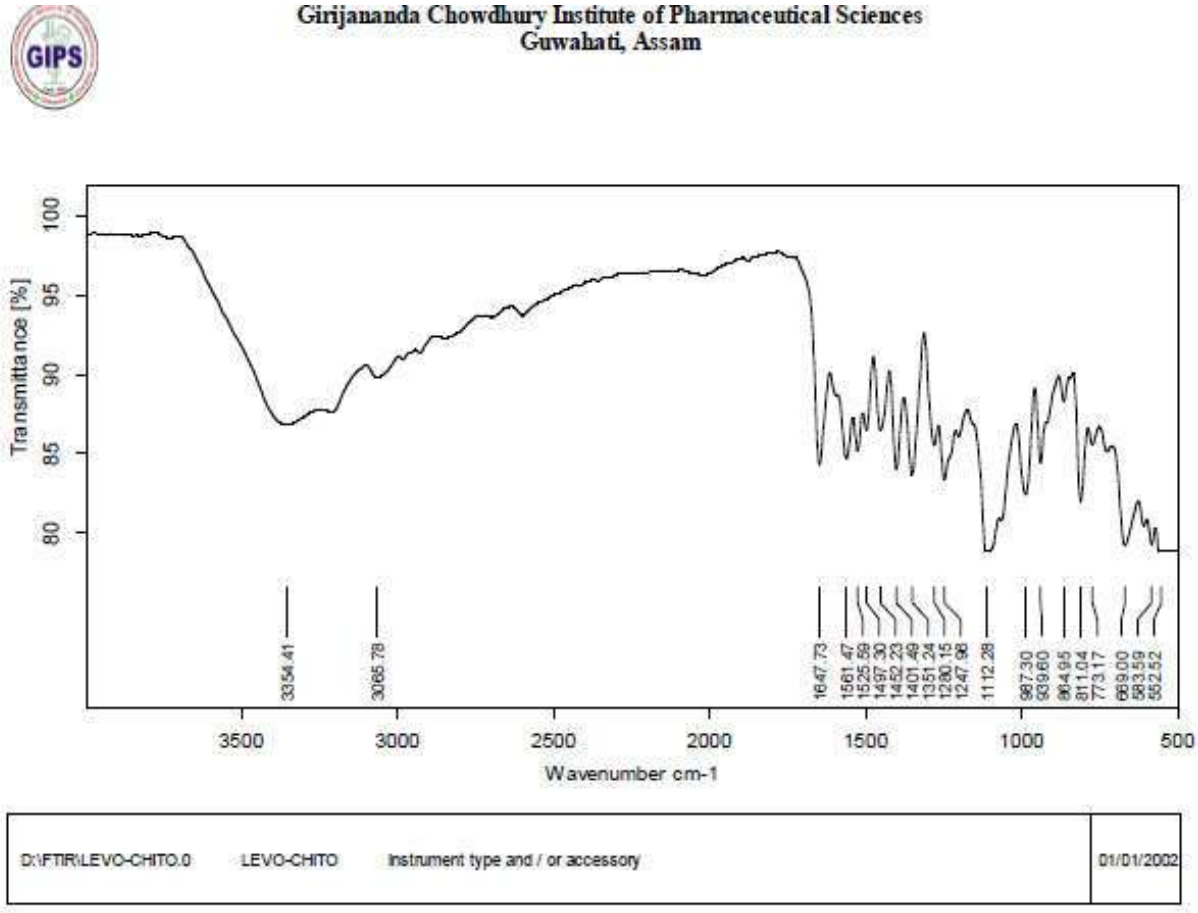


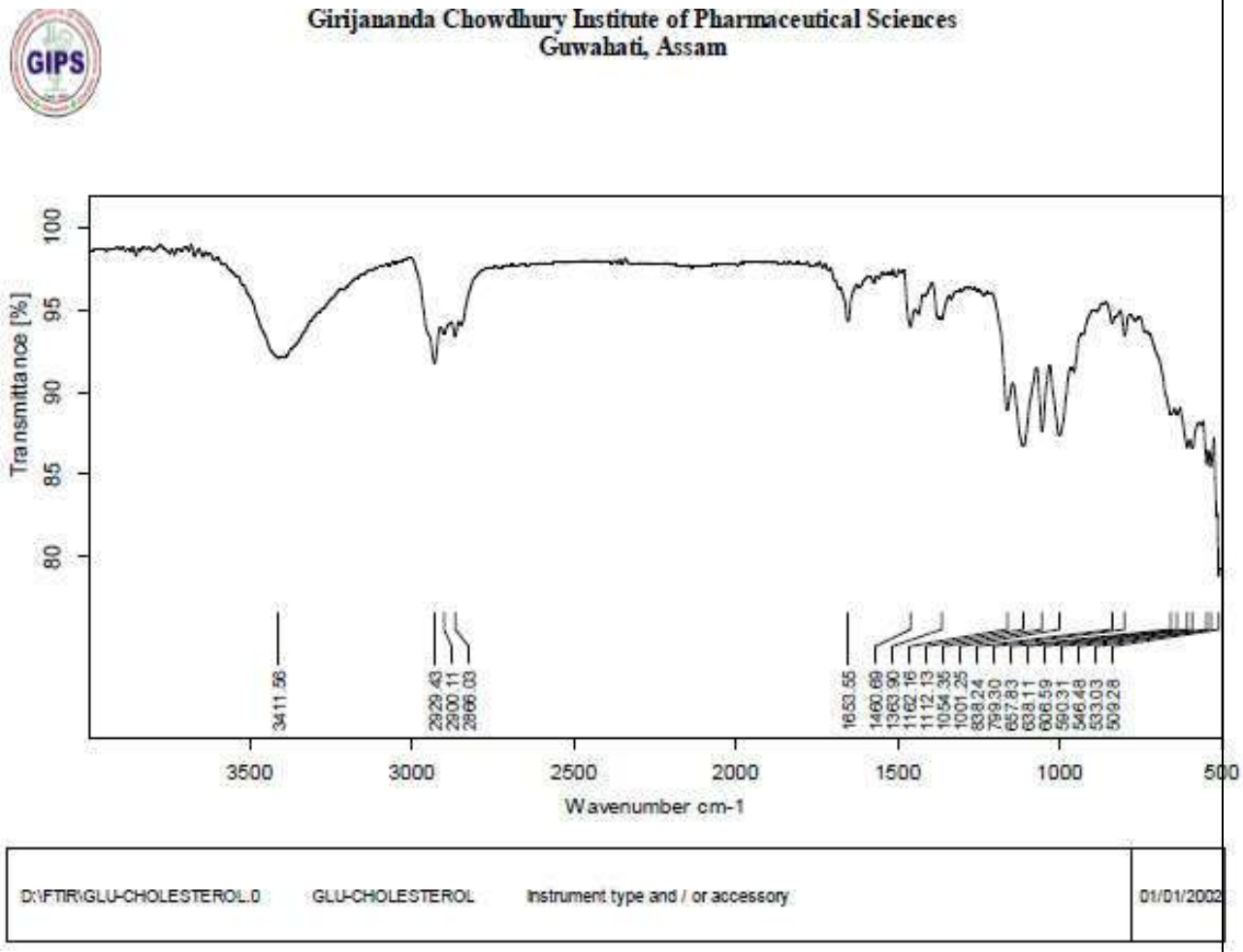
Table 6.7: Structural assignment of Cholesterol

Wave number (cm^{-1})	Structural assignment
2929.62	C-H str
1645.75	C=C str
1375.18	C-H bnd.
799.97	C-C

Levodopa + Eudragit RL 100



Glutathione + cholesterol



Interpretation of result:

FTIR analysis of samples was performed to investigate the interaction between the drug and polymer. Fig. 3 indicates the FTIR peaks of lipid-polymer hybrid nanoparticles. The pure Levodopa showed the OH str at 3745.36 cm^{-1}, NH str at 3335.94 cm^{-1}, CH stretching vibrations in the region of 2925 cm^{-1}. The emergence of a strong band in the IR spectra around 1734.30 cm displays the presence of the carbonyl group in the molecule, which is due to the C=O stretching. C-N peak at 1115.42 cm^{-1} and C=O peak at 1065.95 cm^{-1} were also seen.

The spectra produced by the FTIR for the Eudragit RL100 shows strong bands in the region between 1150–1190 cm^{-1} and 1240–1270 cm^{-1} are due to the stretching of carbonyl (ester) groups present in the Eudragit RL 100. There are also stretching bands of C(=O) ester vibration at 1734.01 cm^{-1} .

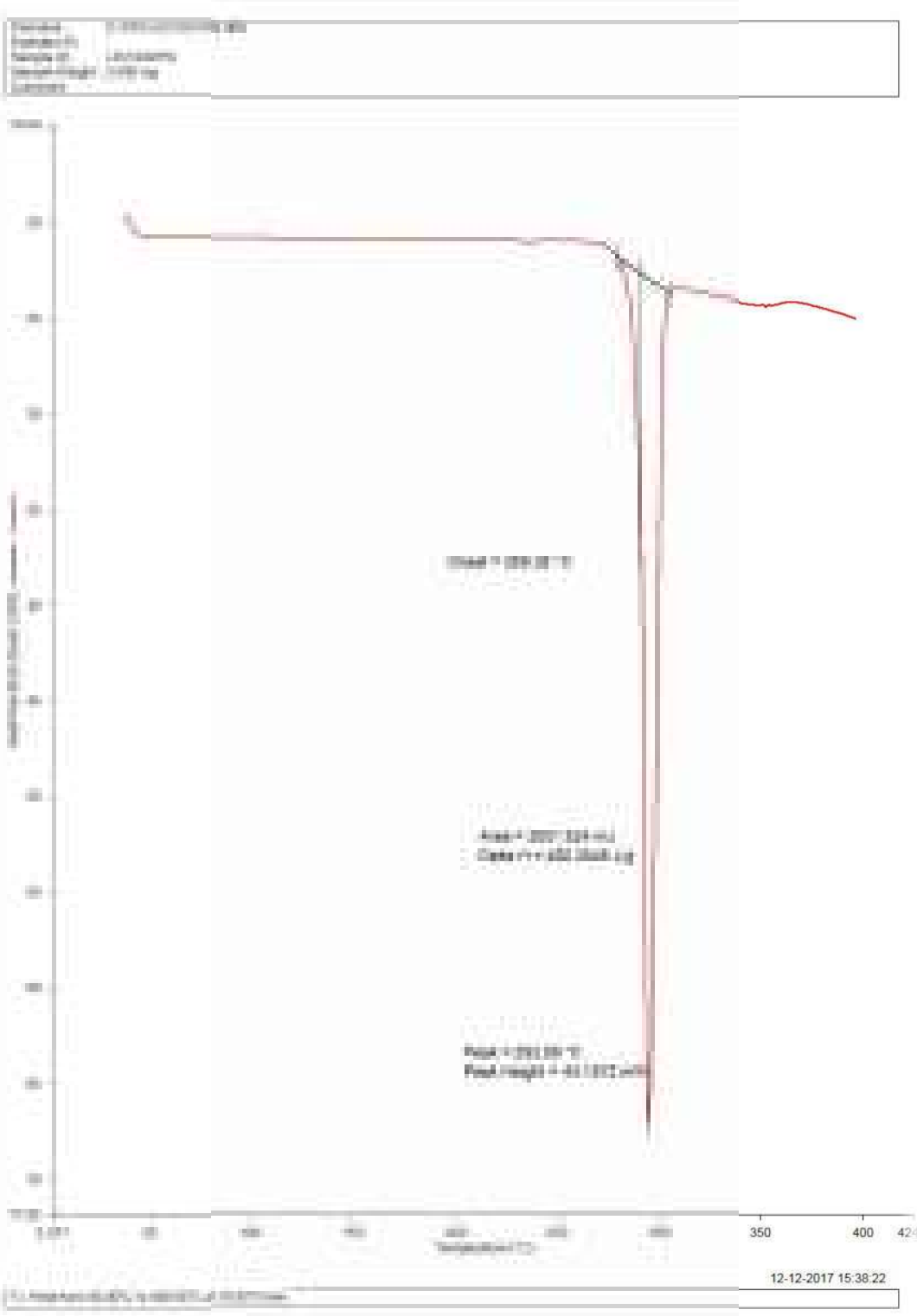
Cholesterol shows C-H peak at 2929.62 cm^{-1}, C=C peak at 1645.75cm^{-1}, C-H peak at 1375.18 cm^{-1} and C-C peak at 799.97 cm^{-1}.

The spectra produced by the FTIR for the Glutathione shows N-H peak at 3588.00 cm, S-H peak at 2926.37 cm^{-1}, C=O peak at 1783.46 cm^{-1}, C-H_2 peak at 1448.51cm^{-1}, O-H peak at 1243.11cm^{-1} and C-N peak at 1106.43cm^{-1}.

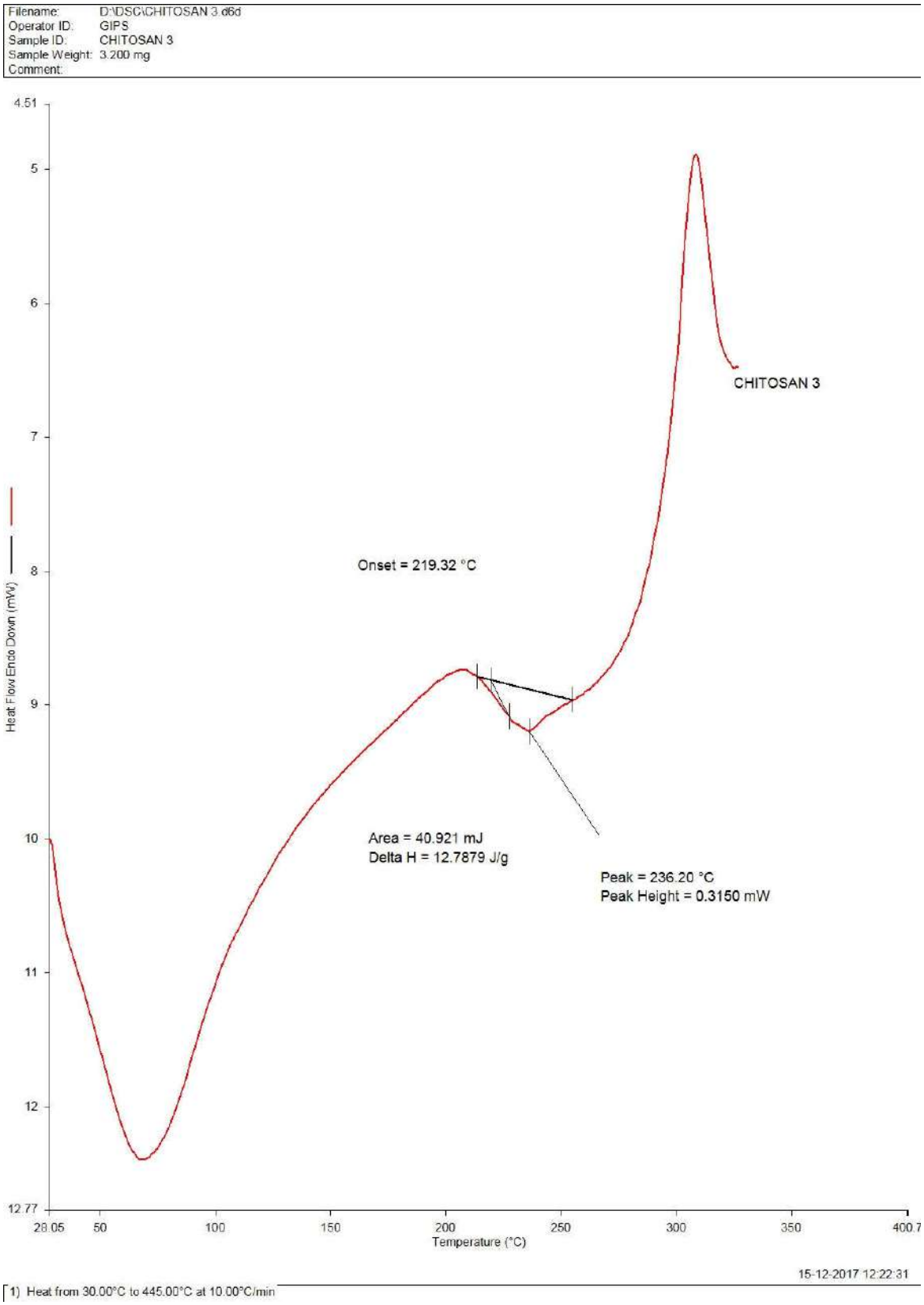
The FTIR spectra of the prepared LPHNs shows retention of major peak for levodopa and glutathione. It can be concluded that no strong drug polymer interaction occurred inside the nanoparticles.

6.3. Differential Scanning Spectroscopy

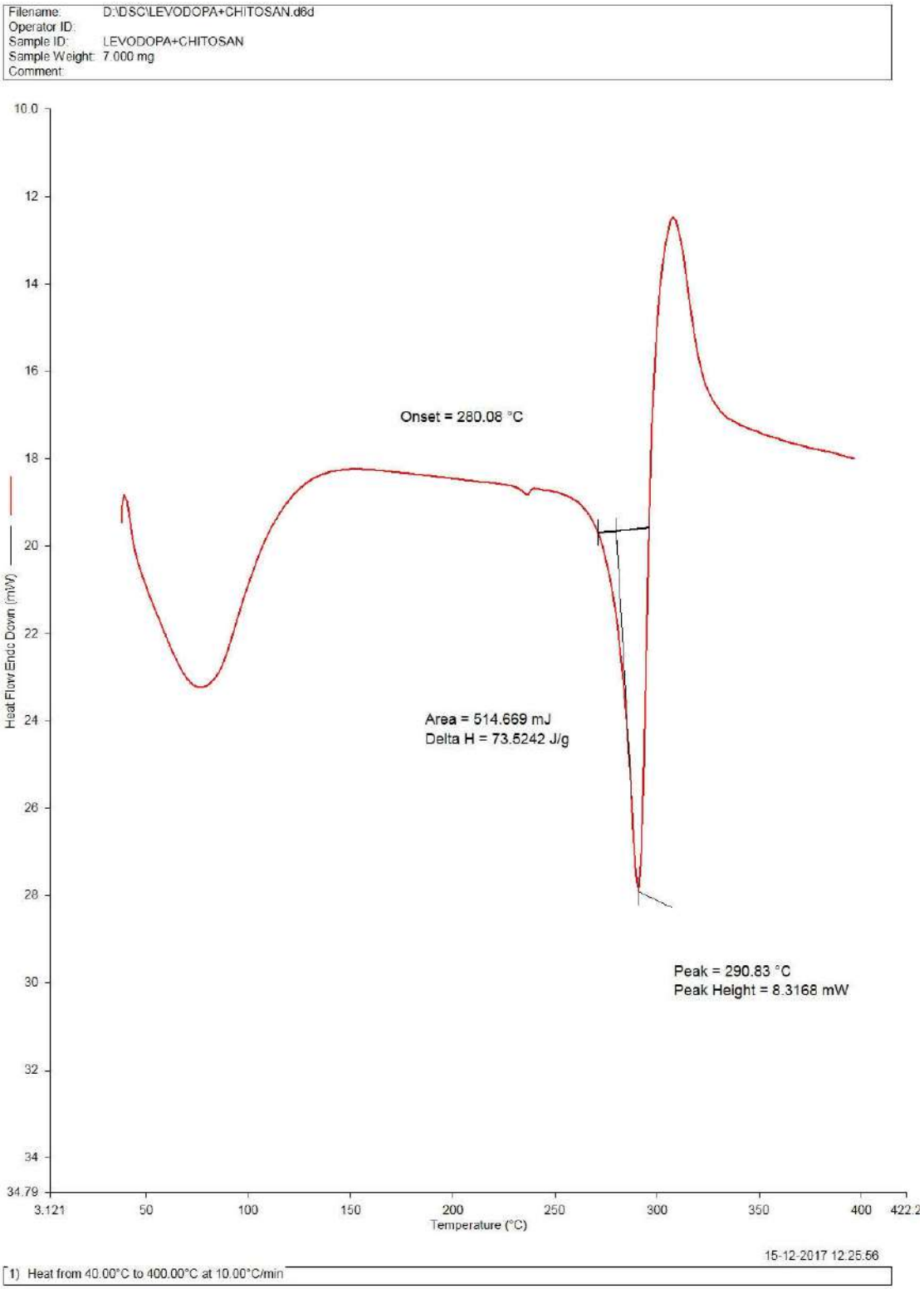
Levodopa



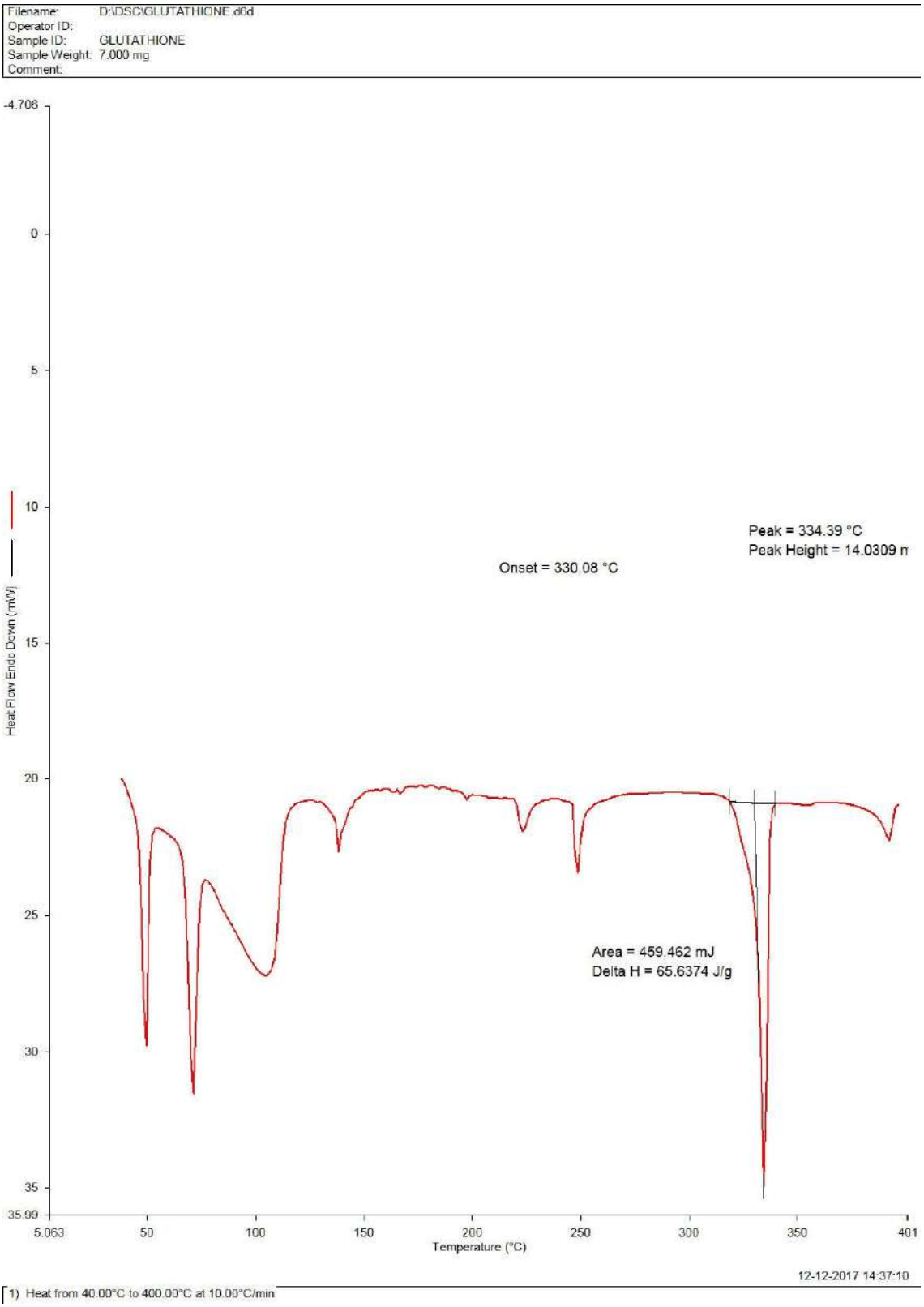
Eudragit RL


Filename: D:\DSC\CHITOSAN 3.d6d
Operator ID: GIPS
Sample ID: CHITOSAN 3
Sample Weight: 3.200 mg
Comment:
CHITOSAN 3
Onset = 219.32 °C
Area = 40.921 mJ
Delta H = 12.7879 J/g
Peak = 236.20 °C
Peak Height = 0.3150 mW
Heat Flow Endo Down (mW)
Temperature (°C)
4.51
5
6
7
8
9
10
11
12
12.77
28.05
50
100
150
200
250
300
350
400.7
15-12-2017 12:22:31
1) Heat from 30.00°C to 445.00°C at 10.00°C/min

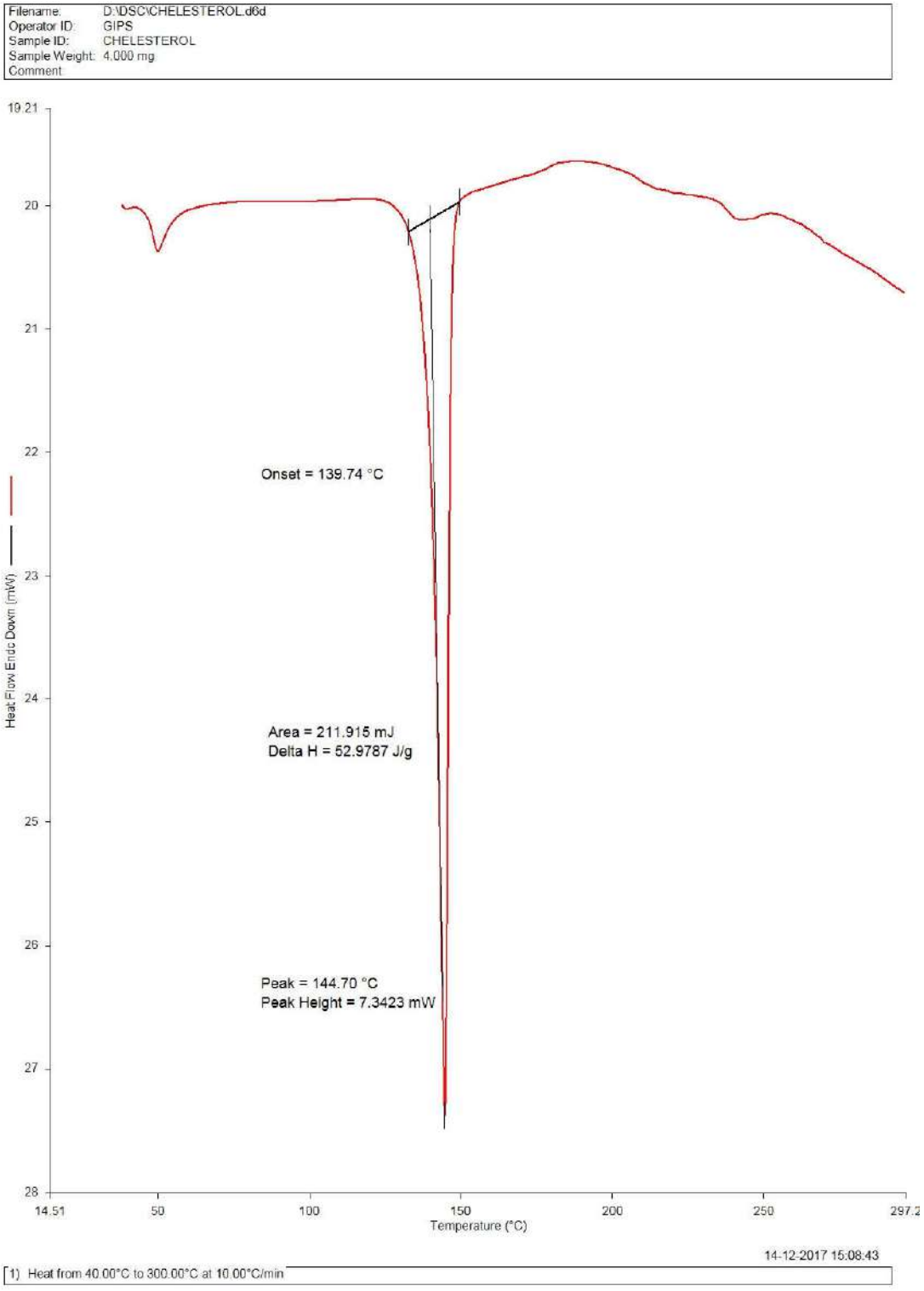
Levodopa + Eudragit



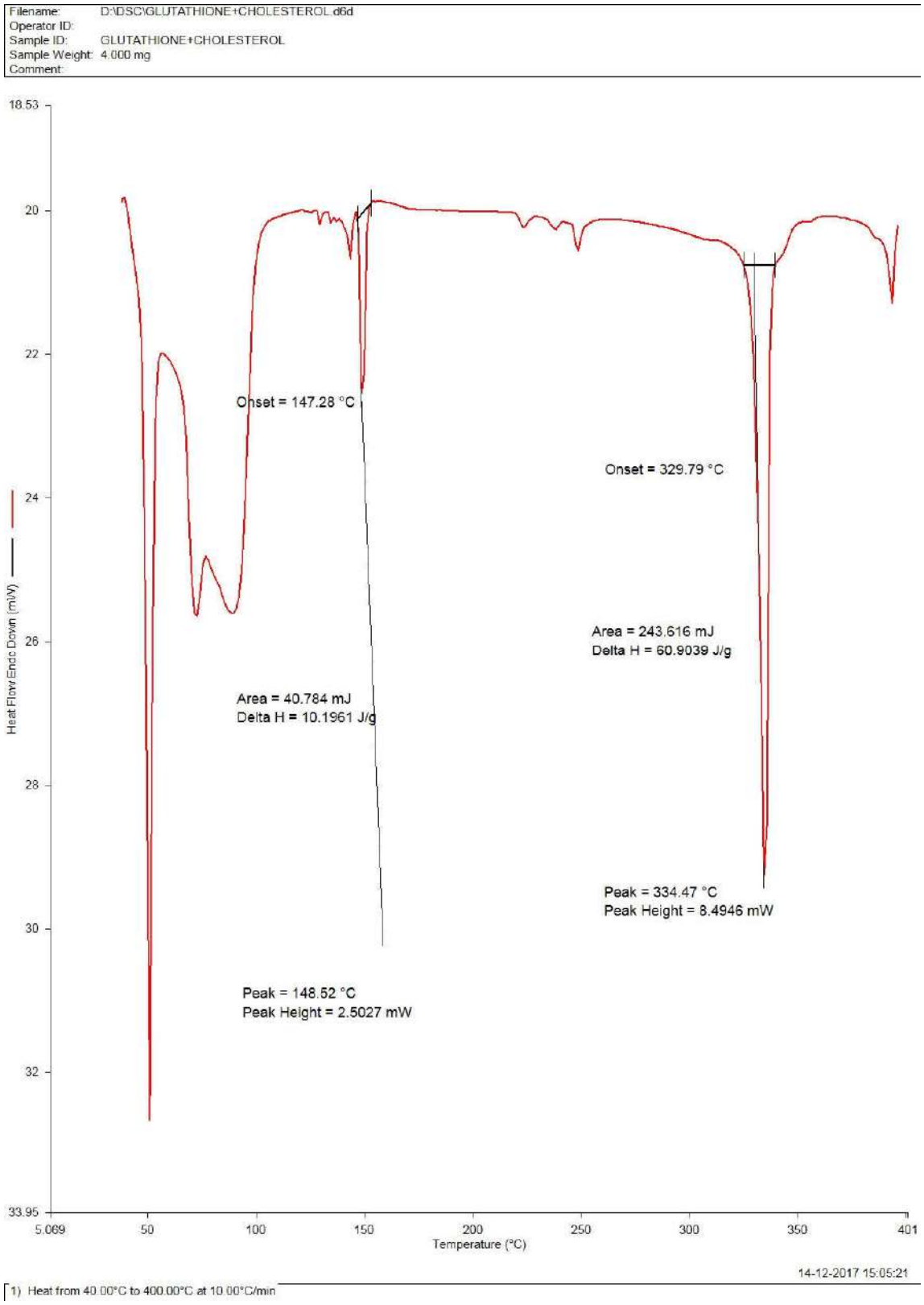
Glutathione



Cholesterol



Glutathione + Cholesterol



Interpretation of result:

DSC studies were carried out in order to study the crystalline or amorphous nature of formulations and to evaluate the interactions between the drug, polymer, and other materials. According to the results, pure Eudragit RL100 exhibited amorphous nature of polymer. The pure Levodopa demonstrated a sharp peak at 293.99°C which can be related to its melting point. In physical mixture, the levodopa peak (290.83 °C) shifted to a slight lower temperature probably due to drug dilution (fig 3). Cholesterol exhibits crystalline nature and shows sharp peak at 144.70 C and pure glutathione shows sharp peak at 334.39 C (fig 4). In physical mixture the glutathione peak is at 334.47 C which suggest no physical interaction.

6.4. Prepared Nanoparticle characterization

To synthesize LPHN we have combined nanoprecipitation method forming polymer core and the thin film hydration forming a lipid shell. The solid polymeric core acts as a scaffold to encapsulate levodopa. The lipid shell contains glutathione envelopes the core preventing internal drug leakage.

LPHN were designed to fit the 100–200 nm size range for enhanced delivery, permeability and retention. Scanning electron microscopy shows that LPHNs were dispersed, with a well-defined spherical shape (Fig.1).Particle sizes, polydispersity index (PDI), entrapment and loading of LPHNs are shown in table 3. The size of different LPHNs formulation ranges from 184.4 nm to 223.5 nm. Entrapment efficiency ranges from 53.98% to 86.76% and drug loading increases from 30% to 54% as the concentration of polymer and lipid increases.

Table 6.8: Physiocochemical parameters of LPHNs

Formulation	Polymeric NP/ Liposome ratio	Encapsulation efficiency (%)	Loading capacity (%)	Mean particle size (nm)	PDI
F1	A3:B1	53.98	30.78	184.4	0.378
F2	A3:B2	69.75	42.95	203.7	0.452
F3	A3:B3	86.76	54.72	223.5	0.517

6.5. Scanning Electron Microscopy (SEM)

Scanning electron microscopy of the prepared hybrid Nanoparticle (figure) revealed that the nanoparticle possess almost a smooth surface and spherical shape while the presence of clumps on some part were also seen. The SEM image typically revealed the presence of uniform coating of the Nanoparticles.

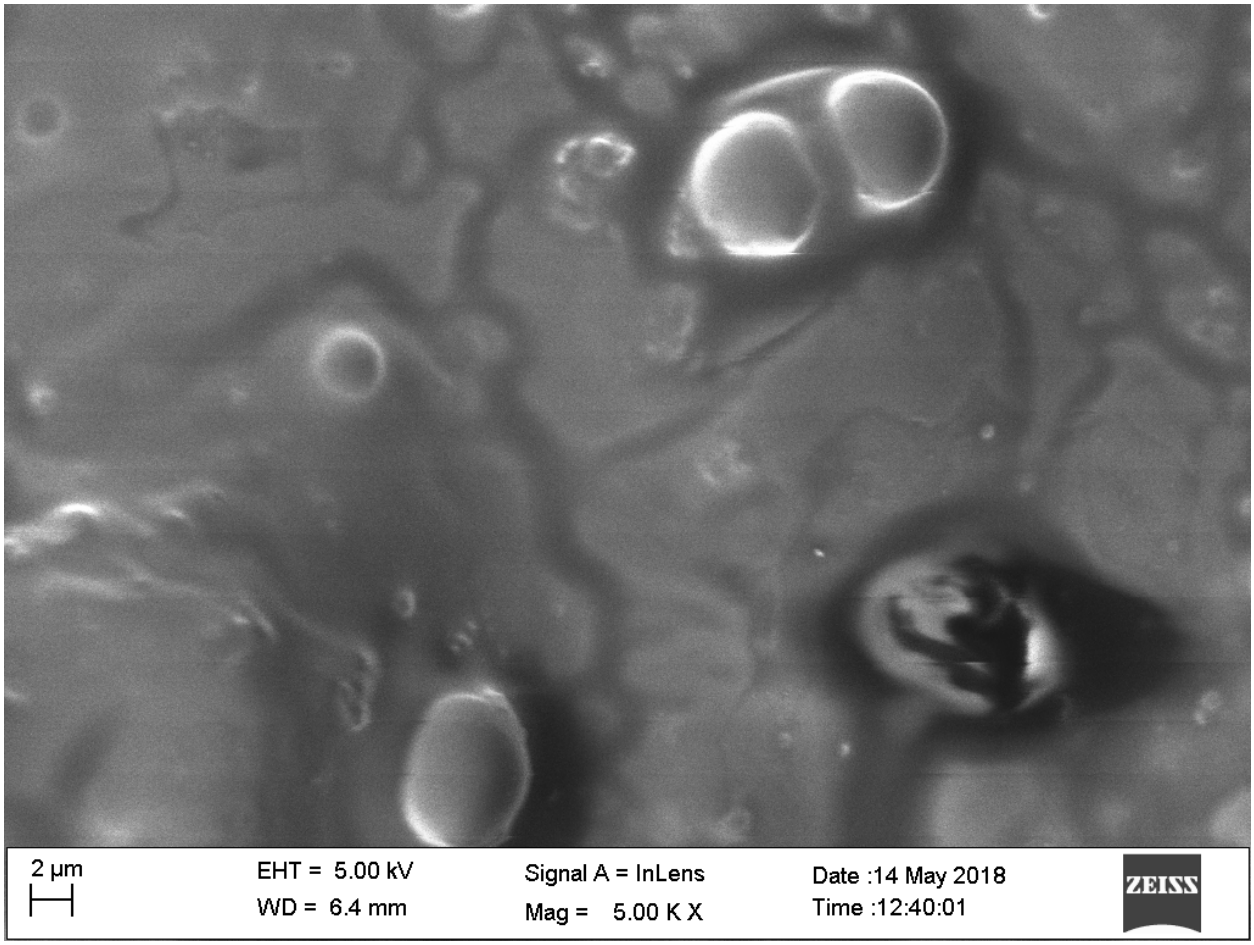


Figure 6.1: SEM imaging of prepared nanoparticles

6.6. In-vitro dissolution study

The formulations F1, F2 and F3 shows 67.71 %, 74.51% and 80.11% release respectively in 24 hour. The release profiles of the formulations suggest an initial fast release followed by a sustain release behavior. The sustaining behavior was more dominant as the polymer to lipid ratio was increased (F3).

Table 6.9: In-vitro release study

Formulation	[a]$Rel_{0.5}$ (%)	[b]Rel_{24} (%)
F1	41.64	67.71
F2	37.24	74.51
F3	34.41	80.11

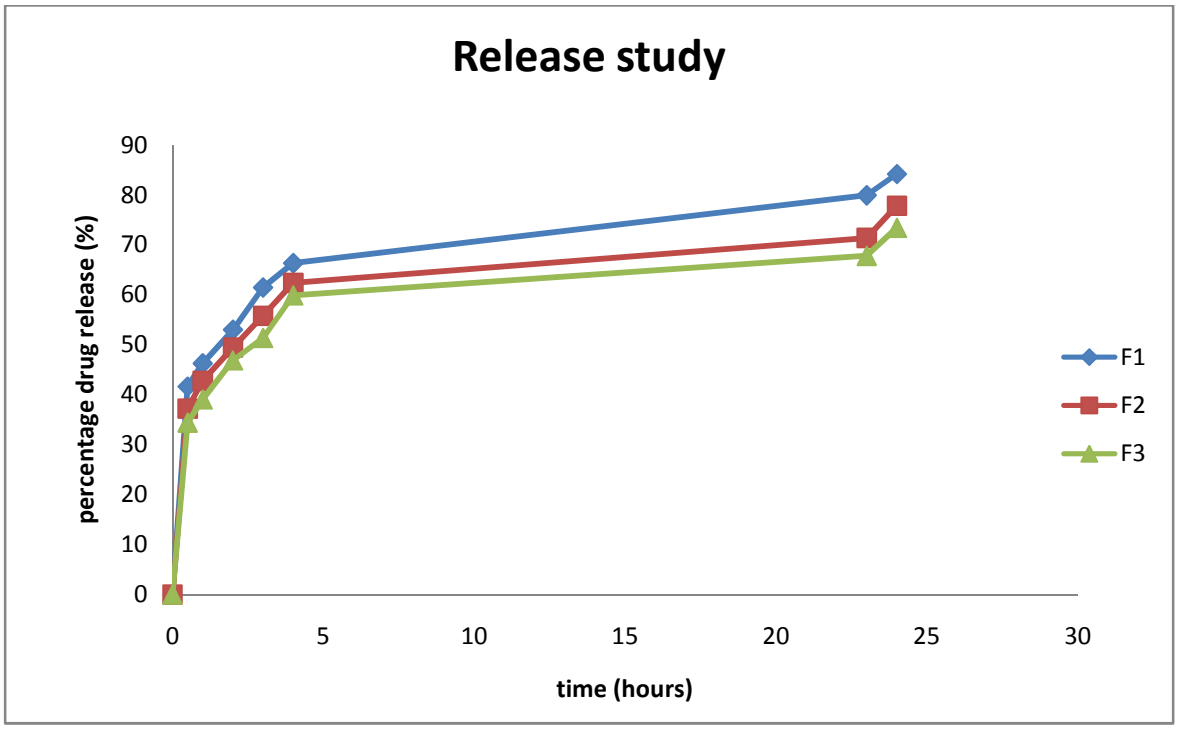


Figure 6.2: In-vitro dissolution data

Dissolution Data Modelling

Table 6.10: Parameter: K value

Formulations	Zero order	First order	Higuchi	Korsmeyer-Peppas/ n value		Hixson Crowell
F1	3.948	0.392	20.620	48.639	0.170	0.058
F2	3.601	0.333	18.860	44.804	0.167	0.055
F3	3.404	0.294	17.792	41.703	0.173	0.054
Standard Deviation	0.275	0.049	1.428	3.474	0.003	0.002

Table 6.11: Parameter: R^2 value

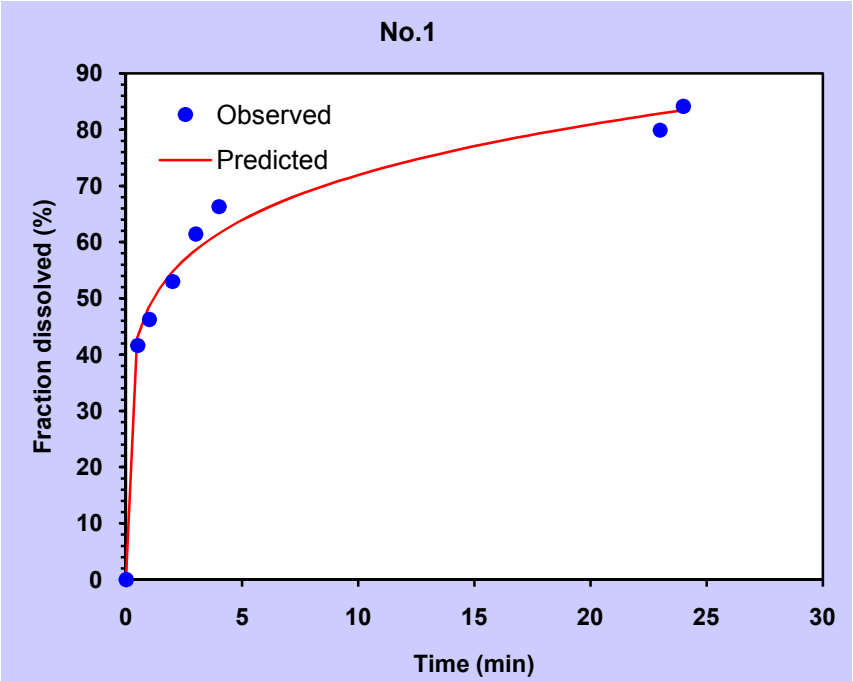
Formulations	Zero order	First order	Higuchi	Korsmeyer-Peppas	Hixson Crowell
F1	-1.1637	-0.0388	0.2085	0.9896	0.1918
F2	-1.2030	-0.6430	0.1821	0.9826	0.0676
F3	-1.1261	-0.9745	0.2228	0.9789	0.0017

Interpretation:

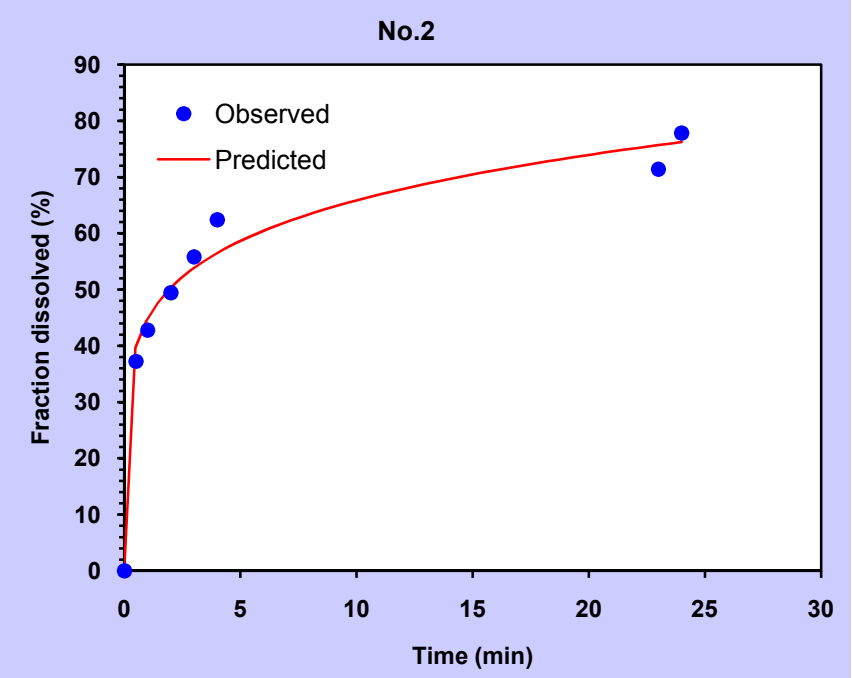
From the above mentioned data it is found that the best fit value for the dissolution data is Korsmeyer- Peppas model. Here Release exponent (n) shows a result less than 0.43(sphere) which indicates the drug transport mechanism follows fickian diffusion in case of matrix system, whereas in reservoir- type system release rate remains relatively constant and is not affected by concentration gradient, but most likely is related to the thickness and permeability of polymeric membrane.

Graphical representation of data

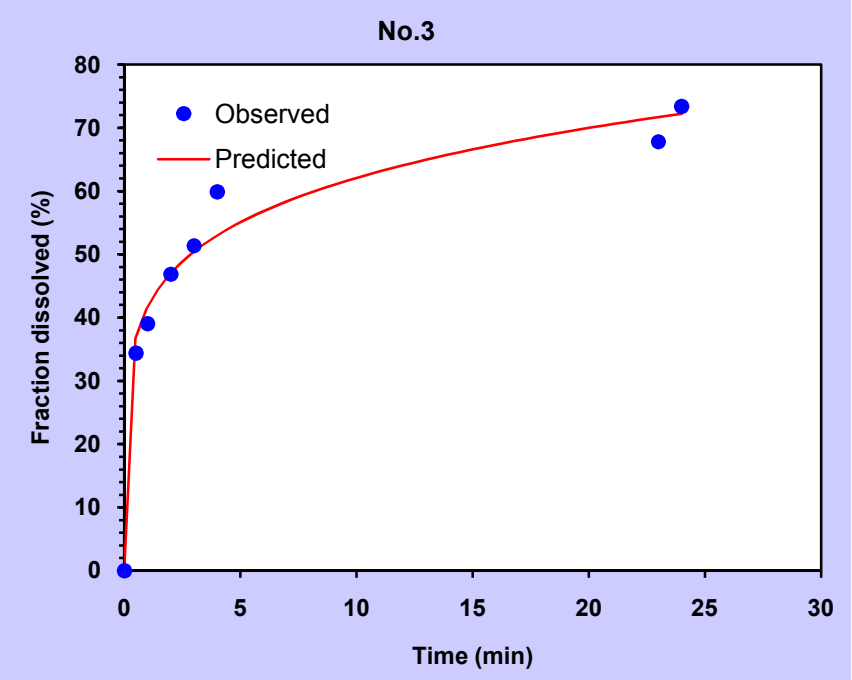
Formulation F1



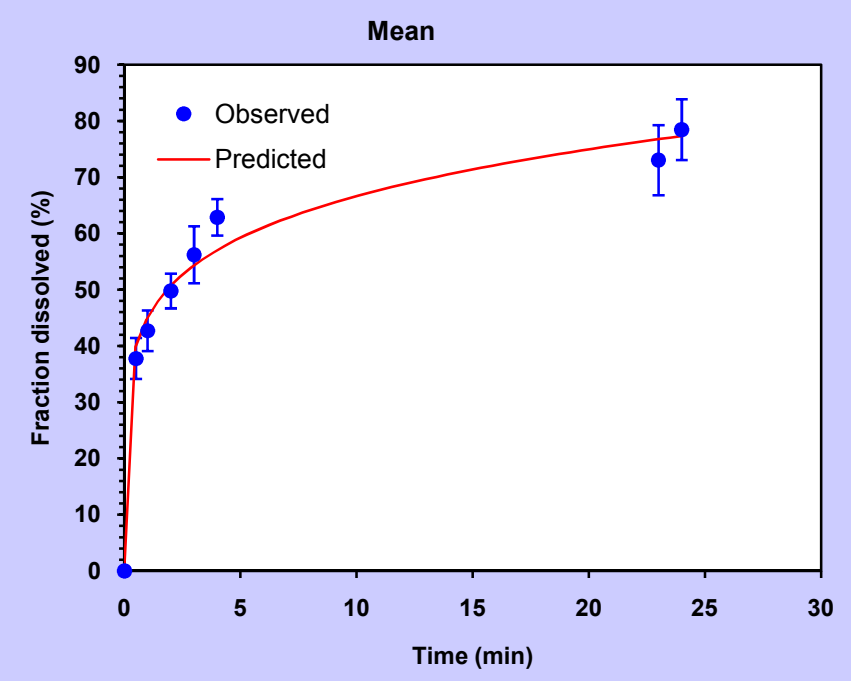
Formulation F2



Formulation F3



Mean



Conclusion

The lipid polymer hybrid nanoparticle was prepared by two steps method. The polymeric core was prepared by nano-precipitation method and the lipidic coat was formed by using thin layer hydration method. The formulation F3 was found to show maximum drug entrapment efficiency of 86.76%, loading of 54.72% having particle size of 223.5 nm having a polydispersity index of 0.517. The release pattern of the formulation shows a sustained behavior. These results demonstrated that Eudragit RL 100 and cholesterol nanoparticles are potentially promising systems for the efficient delivery of Levodopa and Glutathione in the treatment of Parkinsonism.

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5. Chang, WK., Tai, YJ., Chiang, CH., et al., The comparasion of protein-entrapped liposomes and lipoparticles: preparation, characterization, and efficacy of cellular uptake. Int J Nanomedicines. 2011; 6: 2403-2417.
6. Troutier, A., Delair, T., Pichot, C. Physicochemical and interfacial investigation of lipid/polymer particle assemblies. Langmuir. 2005; 21:1305-13.
7. Troutier, AL., Delair, T., Pichot, C. New insights into self-organization of a model lipid mixture and quantification of its adsorption on spherical polymer particles. Langmuir. 2005; 21: 1305-13.

Thank You...

List of Publications

1. **Poster Presentation:**

TITLE: Formulation and Evaluation of levodopa and Glutathione Loaded Lipid-Polymer Hybrid Nanoparticles as Brain Targeted Drug Delivery for Treatment Of Parkinson's Disease.

Presented on **National Conference on Recent Advances in Science and Technology (NCRAST), April 2018.**

2. **Review Article:**

Title: A REVIEW OF LIPID-POLYMER HYBRID NANOPARTICLES AS A DRUG DELIVERY SYSTEM

Published in **World Journal of Pharmaceutical Research, Volume 7, Issue 1, 372-388**.

3. **Research Article:**

Title: "LIPID-POLYMER HYBRID NANOPARTICLES AS BRAIN TARGETED DRUG DELIVERY FOR THE TREATMENT OF PARKINSON'S DISEASE"

The paper has been accepted for publishing in the coming issue of **International Journal in Current Research and Life Science.**

Lipid-Polymer Hybrid Nanoparticles as Brain Targeted Drug Delivery System for the treatment of Parkinson's Disease

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ABSTRACT

Lipid-polymer hybrid nanoparticle is a drug delivery platform that consists of a hydrophobic polymeric core, a lipid shell surrounding the polymeric core, and a hydrophilic polymer stealth layer outside the lipid shell. It combines the positive attributes of both liposomes and polymeric nanoparticles designed to load two drugs of different polarity into a single device and bypass the BBB efficiently. Levodopa has always been the drug of choice for the treatment of Parkinsonism. However, the oral bioavailability is very less due to its peripheral degradation and glutathione is used as a supplement to balance the depleted glutathione level which accounts for oxidative stress. The main aim of the study was to prepare Levodopa and Glutathione loaded hybrid nanoparticle as a brain targeted drug delivery model for providing a sustained release profile, thereby treating the motor symptoms and neurodegeneration. The formulations were optimized and evaluated in terms of particle size, zeta potential, surface morphology, Fourier transform infrared spectroscopy (FTIR), and differential scanning calorimetry (DSC). Drug entrapment and in-vitro release of the lyophilized particles were also examined and results were found to be satisfactory. The study revealed that the formulated nanoparticles were able to release the drug for a prolonged period of time with a reduced dose than that of the conventional formulations.

INTRODUCTION

- Parkinson's disease (PD) also known as **paralysis agitans**, is one of the most common neurodegenerative disorders which is caused by a loss of dopamine (DA) producing neurons in the basal ganglia in the brain.
- Lipid-Polymer Hybrid Nano- particles (LPHNs) are solid, submicron particles.
- LPHNs systems can be described as a polymeric core coated with single or multiple layers of lipids that constitute the shell.
- It is composed of at least two components: the polymer and the lipid.
- The anatomy of a hybrid nanoparticle consists of :
  1. a hydrophobic polymer core,
  2. a lipid monolayer surrounding the core.
- Drug release from the nanoparticles begins with diffusion processes, followed by erosion, then swelling of the matrix.
- The polymer degrades due to hydrolysis and the degradation rate depends on the polymer composition and molecular weight.
- LPHN integrates the unique advantages of both polymeric nanoparticle and liposomal systems while overcoming some of their limitations.
- LPHNs combine the mechanical advantages of biodegradable polymeric nanoparticles and biomimetic advantages of liposomes, have emerged as a robust and promising delivery platform.

METHODOLOGY

1.Preformulation Study

- Organoleptic study
- Melting Point study
- Solubility Studies
- Drug-Polymer compatibility studies by DSC

2. Preparation of Levodopa and Glutathione loaded lipid-polymer hybrid Nano- particles

Hybrid nanoparticles are prepared using two-step synthesis process:

A. Preparation of Polymeric Nanoparticles

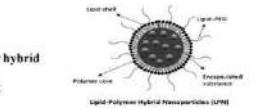
B. Preparation of Lipid Shells

C. Both polymeric nanoparticles and liposomes are mixed and incubated

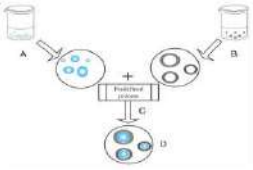
D. Final Hybrid Nanoparticle Formation.

3. Evaluation of the Prepared Hybrid Nano particles

- Particle size analysis
- Morphological analysis
- FTIR spectroscopy
- DSC analysis
- Encapsulation efficiency and drug loading
- In vitro release study



Lipid-Polymer Hybrid Nanoparticles (LPHN)



RESULTS

Formulation Table

A. For the preparation of Polymeric Nanoparticles

Formulations	Drug: polymer	Water (ml)	Levodopa (mg)	Eudragit RL 100 (mg)	Acetone (ml)	Polaxamer (mg)
N1	1:4	5	40	160	10	200
N2	1:7	5	40	280	10	200
N3	1:10	5	40	400	10	200

B. For the preparation of Liposomes

Formulations	Drug: polymer	Glutathione (mg)	Cholesterol(mg)	Chloroform (mL)	Methanol (ml)	Tween 80 (ml)
L1	1:1	100	100	5	5	0.2
L2	1:2	100	200	5	5	0.2
L3	1:4	100	400	5	5	0.2

Organoleptic Properties

Sample	Color	Odor	Taste
Levodopa	White	Odorless	Tasteless
Glutathione	White	Odorless	Characteristics
Eudragit RL 100	White crystalline powder	Characteristics	Tasteless
Cholesterol	White crystalline powder	Odorless	Tasteless

Solubility Study Data

Sample	Solubility		
	Soible	Slightly soluble	Insoluble
Levodopa	Dil. HCl	Water	Ethanol, chloroform
Glutathione	Dil. Alcohols , water	Ethanol	Methanol
Eudragit RL 100	Ethanol	-	water
Cholesterol	Ethanol, Chloroform	-	Water

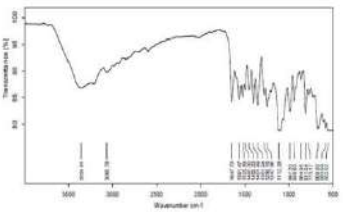
Physicochemical Properties

Formulation	Encapsulation efficiency (%)	Loading capacity (%)	Mean particle size (nm)	PDI
F1	39.92	14.78	231.2	0.351
F2	49.58	18.35	264.1	0.403
F3	61.37	22.72	278.5	0.517
B1(blank)	-	-	313.3	0.368

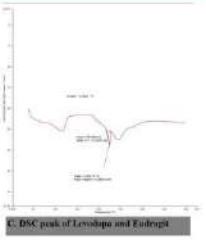
Release Study

Formulation	$^{a}Rel_{6\,h}$(%)	$^{b}Rel_{24}$(%)
F1	48.91	82.32
F2	41.34	78.52
F3	23.92	71.67

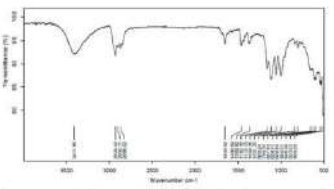
DSC and FTIR data



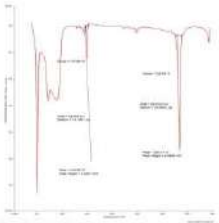
A. FTIR peak of Levodopa and Eudragit RL 100



C. DSC peak of Levodopa and Eudragit



B.FTIR peak of Glutathione and Cholesterol



D. DSC peak of Glutathione and Cholesterol

Conclusion

Various Levodopa–Glutathione loaded nanoparticles were prepared using the two-step approach. The formulation F3 was found to show the maximum drug entrapment efficiency (61.37%) with a mean particle size of 278 nm. The polymeric core nanoparticles were found to provide a biphasic release pattern; an initial burst release followed by a sustained release manner whereas the liposomes shows an immediate release behavior. These results demonstrated that prepared nanoparticles are potentially promising systems for the efficient delivery of Levodopa and glutathione and could be used as novel drug delivery systems for brain-targeting delivery of the levodopa.

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A REVIEW OF LIPID-POLYMER HYBRID NANOPARTICLES AS A DRUG DELIVERY SYSTEM

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ABSTRACT

Nano technologies have the potential to revolutionize the drug development process and change the landscape of the pharmaceutical industry. It is one of the most extensively explored area in the medical science in the past few decades to develop a functional nanostructures to facilitate the delivery of various therapeutic and imaging agents. Liposomes and polymeric nanoparticles represent two primary delivery vehicles that are being studied. While there are several advantages of these two particle platforms, some intrinsic limitations remain to limit their applications at a certain extent. Recently, a newer drug delivery platform, named lipid-polymer hybrid nanoparticle, has been developed that combines the positive attributes of both liposomes and polymeric nanoparticles while excluding most of their shortages. The nanoparticle consists of a hydrophobic polymeric core, a lipid shell surrounding the polymeric core, and a hydrophilic polymer stealth layer outside the lipid shell. In this review, we have discussed about the different method of synthesis of the lipid-polymer hybrid nanoparticle, followed by a review of factors and typical characterization of the particles. We then summarize the current and potential medical applications of the nanoparticle as a delivery vehicle.

KEYWORDS: Hybrid nanoparticle, liposome, polymeric nanoparticle, drug delivery vehicle, therapeutic agents.

INTRODUCTION

Lipid-Polymer Hybrid Nano- particles (LPHNs) are solid, submicron particles composed of at least two components: the polymer and the lipid. Lipid-polymer hybrid nanoparticles are a

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Dear Sir/Madam

Congratulation!!! We are pleased to inform you that, according to my record, your manuscript, entitled **"LIPID-POLYMER HYBRID NANOPARTICLES AS BRAIN TARGETED DRUG DELIVERY FOR THE TREATMENT OF PARKINSON'S DISEASE"** has been accepted for publication in the International Journal of Current Research in Life Sciences. We cover the costs partially through article processing fees. Our expenses are split among editorial costs, electronic composition and production, journal information system, manuscript management system, electronic archiving, overhead expenses, and administrative costs. Moreover, we are providing research paper publishing in minimum available cost.

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