

DESIGN OF AN ORAL NANO THERAPEUTIC DRUG DELIVERY SYSTEM FOR CONTROLLED RELEASE OF AN ANTIDEPRESSANT DRUG.

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A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the requirements for the degree of Master of Science in Medicine in the field of Pharmaceutical Affairs.

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Johannesburg, 2021

DECLARATION

I, Dimakatso Benigna Mmakola declare that this Dissertation is my own work. It is being submitted for the degree of Master of Science in Medicine in the field of Pharmaceutical Affairs in the Faculty of Health Sciences as a research component at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

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This09.....day of ...July 2021.....

ACKNOWLEDGEMENTS

First of all I would like to express my gratitude to the God of all creation, the Lord of my life, the one who has granted me the strength and wisdom to complete my studies, thank you my Lord Jesus Christ. My greatest appreciation to my supportive husband, Pastor Keletso Mmakola. Thank you for the constant counselling and encouraging words. Thank you for your love and support. To my babies, Tamar and Kgosi Mmakola, thank you for your understanding, life is not always about what is convenient but about the inconveniences that make us reach our greatest potential.,

To Doctor Jonathan Monwabisi Pantshwa, thank you for your guidance, patience and strict mentorship that made all of this possible. I would not have completed this research project without your direction and leadership. My gratitude to my brother in Christ, Dr Phil Ubinako. Thank you for your friendly face and always willing to help attitude. Thank you also for your scientific proof of concept in completion of this project. A great thank you to my supervisor, Professor Pierre Kondiah, your guidance, leadership and positive mentorship is highly appreciated. The greatest appreciation to my co-supervisors Professor Yahya E. Choonara and Professor Viness Pillay for what you have created at the WADDP, it has been a pleasure studying under your directive. My deepest condolences to the family of Professor Viness Pillay. Professor Pillay's legacy at the University of the Witwatersrand lives on.

I would like to acknowledge and thank the National Research Fund, Professor Kondiah's Thuthuka grant for funding this research project.

To my household supportive structure, Mmathabiso Alice Mahlo, thank you for taking care of my home when I was away. Thank you to my mother and father, Francina Lechalaba and Mpho Sethabela for your guidance, teachings, love and support, all of this made me the woman I am today. To my siblings, Nthabiseng Sethabela-Makoeng, thank you for being an excellent example to follow, and Teboho Sethabela thank you for your love and support. To Portfolio Laboratories (Pty) Ltd management, thank you for the financial assistance, and for your continued concern and care.

I would also like to extend gratitude to the staff at the Pharmacy and Pharmacology department, Ntate KB, Ntate Sello and Ntate Bafana, Mme Busi, thank you for all your assistance and support. To all my colleagues, thank you for making the journey even more pleasant. This dissertation is dedicated to my husband, my pillar of strength, my comfort and counsellor, my lifetime partner, Pastor Keletso Mmakola, and my beautiful children, Tamar and Kgosi Mmakola.

ABSTRACT

Depression is one of the most prevalent global mental illnesses affecting more than 264 million people of all ages worldwide. Presently, an excess of 4% of the global population is affected by mental depression. A significant number of bioactive agents (drugs) intended for treatment of depressive symptoms, enhance the availability of chemical neurotransmitters at the synapse by inhibiting the reabsorption of the neurotransmitter. Regardless, many antidepressant drugs have reduced efficacy due to their inability to reach the desired brain binding receptors, when administered via traditional routes of administration. Stability, solubility, absorption, bioavailability and site targeting are often challenges experienced by therapeutic agents orally administered. Duloxetine which is a first line treatment drug for depression, has low solubility resulting in low bioavailability when administered orally. The purpose of this study was to synthesize duloxetine (DLX) loaded eudragit-chitosan (Eud-CHT) nanoparticles, enclosed in gelatin capsules and to evaluate their potential for transport of Duloxetine to the CNS via oral delivery path for improved efficacy with reduced side effects. DLX loaded Eud-CHT nanoparticles preparation was by the solvent emulsion evaporation method and synthesis confirmed by physicochemical properties including Scanning Electron Microscopy (SEM), Fourier Transformation Infrared Spectroscopy (FTIR), zetasizer, Ultraviolet (UV) spectroscopy, Thermogravimetric Analysis (TGA) and Differential Scanning Calorimeter (DSC) for thermoanalysis. Scanning electron microscopy clarified the configuration of the surface and shape of the nanoparticles together with pore distribution of the nanoparticles. The synthesized nanoparticles were evaluated utilizing the zeta-sizer and the nanoparticles were found to be in the size range of 100 ± 73.41 nm, with polydispersity index (PDI) of 0.283 and with a zeta potential of 16 ± 2.79 mV. A drug entrapment efficacy (DEE) percentage of 72 was attained by the nanoparticulate formulation. DLX release studies showed a constant release over a 24-hour period. The synthesized DLX-Eud-CHT nanoparticles were dissolved in three pH environments simulating the stomach pH 1.2, intestinal pH 6.8 and physiological pH 7.4. At pH of 6.8, 40 % of DLX was released from the copolymeric nanoparticles, whereas only 20 % was released at pH 1.2 and 35 % was released at pH 7.4. This illustrates the shielding effects of the Eud-CHT nanoparticulate formulation on the DLX in an acidic environment of the stomach. FTIR results exhibited the formation of nanoparticulate structure with comparable peaks between the polymers, DLX and the DLX-Eud-CHT nanoparticles. DLX-HCL compared to DLX-Eud-CHT nanoparticles showed peaks at 1281 attributed to -C-O vibration, at 3328 attributed to -N-H stretching and -CH₂ bending at 1324. DSC and TGA thermal studies indicated that the Eud-CHT nanoparticulate structure increased the stability of DLX. In addition, HEK 293 neural cells were treated with DLX loaded Eud-CHT copolymeric nanoparticles and evaluated for cytotoxicity utilizing a 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide dye (MTT) assay and absorbance measured at 570 nm employing a Thermo Lab systems Multiskan MK3 microplate reader. From the MTT assay analysis conducted, the results indicated that the DLX-Eud-CHT nanoparticles were less toxic to the HEK cells compared to free DLX. The combined trials and results from the synthesis of DLX-Eud-CHT nanoparticles, showed evidence that these nanoparticles can be utilized as potential invaluable formulation for oral drug delivery of Duloxetine with improved neuro-availability.

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LIST OF ABBREVIATIONS

BBB	Blood Brain Barrier
BCSFB	Spinal Cord blood cerebrospinal Fluid Barrier
BCRP	Breast Cancer Resistance Protein
BS	Back Scattering
CSF	Cerebrospinal Fluid
CNS	Central Nervous System
DMSO	Dimethyl Sulfoxide
DSC	Differential Scanning Calorimetry
ECSC	European Commission Scientific Committee
EMA	European Medicine Agency
Eud-CHT-DLX	Eudragit-Chitosan-Duloxetine
FDA	Food and Drug Administration
FTIR	Fourier Transformation Infrared Spectroscopy
HCL	Hydrochloric Acid
HEK	Human Embryonic Kidney
LDLR	Low Density Lipoprotein Receptor
MDD	Major Depressive Disorder
MRP4	Multidrug Resistance Protein ABCC4
MTT	3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide dye
NP	Nanoparticle
PEG	Polyethylene Glycol
PDI	Poly dispersed index
PLGA	Poly (lactic-co-glycolic acid)
PVA	Polyvinyl Alcohol
SEM	Scanning Electron Microscopy

T	Transmission
TGA	Thermogravimetric Analysis
USP	U.S Pharmacopeia
UV	Ultra Violet
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Introduction

The World Health Organisation (WHO 2020) states that an excess of 264 million people worldwide are affected by depression. Depression causes persistent low mood, lack of interest in productive activities and often ideation of suicide (Sindhu et al., 2018). WHO 2020, declared suicide as the second leading cause of death among 15 to 29-year olds, with approximately around 800 000 people dying each year. Several therapeutic agents are designed for central nervous system activity but are significantly reduced by challenges in drug distribution to the Central Nervous System (CNS) (Sorets et al., 2020). The human body comprised of the brain, the spinal cord and the complex network of neurons that form the multifaceted unit called the CNS. The brain is safeguarded by the highly selective Blood Brain Barrier (BBB), which controls what enters and exits the central nervous system (Henrich-Noack et al., 2019). The BBB is formed by brain capillaries comprising of endothelial cells separating circulation of blood and brain tissue. The capillaries differ from peripheral capillaries in that they have tight junctions compared to peripheral capillaries which have gaps of up to 50nm in width (Mahringer et al., 2021). The endothelial cells are made up of three functional barriers, namely, the glycocalyx, the endothelial layer and the neurovascular unit. The glycocalyx is the first barrier which directly interacts with the blood. It is comprised of a thick carbohydrate layer with several other components that makes the BBB negatively charged. The endothelial layer which is 200nm thick with tight junctions, acts as a gate keeper controlling influx into the brain. Their pore size of 1.4 to 1.8nm is highly selective and inhibits diffusion of large molecules. The Neurovascular unit constituents are the vascular smooth muscles, microglia, pericytes, astrocytes, oligodendrites which all function together to control the BBB permeability (Reddy et al., 2021)

Although the BBB is an effective system against harmful chemicals and toxins, it also prevents useful therapeutic agents from entering the brain. Factors such as particle size, surface charge, chemical composition affects a molecule's ability to penetrate the BBB (Sharma et al., 2019). Nanotechnology provides a solution to the challenges associated with penetration across the BBB by therapeutic agents and facilitates the delivery to the CNS (Saeedi et al., 2019). The European Commission Scientific Committee (ECSC), 2006, defines nanotechnology as the science of designing, producing, and utilization of structural devices having one or more dimensions of about 100 millionth of a millimetre (100 nanometres) or less.

Orally administered drugs face several challenges following administration, including poor drug solubility, limited biodistribution, and lack of selectivity for targeted tissue absorption due to the BBB (Crawford et al., 2016 and Su et al., 2018). Although these drugs are actively received by patients, further challenges such as stability over a wide range of pH in the gastrointestinal tract, and enzymatic degradation by digestive enzymes are also encountered (Hu and Luo, 2018). Poor absorption through the GIT and extensive first pass metabolism ultimately affect the concentration of the drug at BBB and site of action. Duloxetine (DLX) was the model drug used in this study. DLX is a serotonin and norepinephrine reuptake inhibitor, and used as first line treatment in major depressive disorder (Sindhu et al., 2018). DLX is taken orally but has poor solubility, sensitive to low pH in the stomach, has 50% bioavailability after oral administration, and is highly metabolised in the liver after intestinal absorption (Sindhu et al., 2018 and Alam et al., 2012). Synthesised polymeric nanoparticles protect the drug from degradation, increasing bioavailability, facilitating target specificity and providing drug release that is controlled. The nano-range of the particles also aids in passage across the BBB. (Ding and Zhu, 2018 and Wilson, 2019).

Poly (lactic-co-glycolic acid) PLGA is one of the most widely used polymer because of its advantageous characteristics. PLGA is biodegradable and has been validated for usage by the Food and Drug Administration (FDA) and European Medicine Agency (EMA). This polymer can encapsulate a variety of drugs, e.g. amphiphilic drugs, and has surface modification properties, with possibilities of active cell targeting (Danhier et al., 2012). In a research conducted by Mir et al., 2017, PLGA nanoparticles loaded with loperamide showed evidence of crossing the BBB after surface functionalization with monoclonal antibodies, targeting transferrin receptors.

PLGA nanoparticles also have controlled or sustained release properties, depending on the molecular weight and lactide to glycolide copolymer ratio. The hydrophilicity of this polymer is due to the glycolide content, which leads to the shorter time it takes to biodegrade (Sharma et al., 2016). Chitosan coated PLGA nanoparticles have also showed modified drug release behaviour. Furthermore, positively charged chitosan nanoparticles attracts negatively charged cell membranes, improving the permeability of poorly permeable drugs. Chitosan has mucoadhesive properties utilized in sustained drug release (Badran et al., 2018). Chitosan surface modified PLGA or PLA nanoparticles have shown delivery of therapeutic agents targeted for CNS activity (Sarvaiya and Agravai, 2015).

In a study conducted by Zhu et al., 2018; PLGA coated with Chitosan improved the release properties of the model protein BSA from PLGA nanoparticles, by reducing the initial burst

release and extending sustained release (Ding and Zhu, 2018). Chitosan nanoparticles can be used for drug delivery intended for brain activity, and also widely used for oral delivery due to their mucoadhesive and cell permeability properties. It has been reported that orally delivered peptides are absorbed through the intestinal wall and through the BBB, when encapsulated in chitosan nanoparticles (Crawford et al., 2016).

In this study, Chitosan and Eudragit L100 constituent polymers were utilized in the synthesis of Chitosan-Eudragit L100 copolymeric nanoparticles formulated by solvent emulsion evaporation method. Eudragit L100 is a copolymer consisting of the methacrylic acid and methyl methacrylate polymers. This polymer is amongst the polymers that have approval from the FDA for incorporation in drug delivery systems (Dong et al., 2019). Eudragit is also non-toxic, biodegradable, mucoadhesive, and contains controlled drug release properties. Eudragit has a positive charge which provides advantageous interactions with the negatively charged cell surfaces of intestinal wall increasing cellular uptake. (Adibkia et al., 2011). It has been shown that positively charged molecules have an advantage of penetration through the intestinal wall due to the attraction to the negatively charged cells of the intestines thereby improving cellular uptake (Ren et al., 2018).

The focus of this study was on developing an oral nano therapeutic drug delivery system, loading an antidepressant drug, duloxetine, a serotonin and norepinephrine reuptake inhibitor, used as first line treatment for Major Depressive Disorders (MDD) (De Donatis et al., 2019). Chitosan coated Eudragit nanoparticles was prepared with the aim of shielding duloxetine from the harsh environment of pH extremities and enzymes in the stomach, increasing the concentration of duloxetine across intestinal mucosal membranes, thereby providing higher neuro-availability across the BBB.

1.2 Rational and motivation of this study

The most desired outcome from treating a patient is to have increased patient compliance, intended therapeutic results with decreased side effects. Orally delivered drugs are mostly preferred as they are easy to administer. However, orally administered drugs face several challenges after administration including unfavourable pH environment in the stomach, as well as the potential for enzymatic degradation by digestive enzymes. These therapeutic agents may also have dissolution, permeability and solubility challenges; decreasing their potential of reaching the desired target site. The BBB also poses another challenge for therapeutic agents, as it does not allow all agents to pass through or be taken up by transport mediums. The therapeutic agent must also be at the desired site of action, for a required amount of time and

at a certain concentration, for effective drug absorption. Therefore, controlled release properties of an oral nano delivery system will be beneficial for long-term antidepressant treatment. In a study conducted to develop and evaluate drug-loaded PLGA and chitosan nanoparticles for brain delivery, it was reported that these polymers have significant potential for the delivery of neuro-therapeutics, promoting improved pharmacokinetic profiles of the drug, compared to free orally administered non-nanoparticulate formulations (Tong et al., 2017).

This study aims to design biodegradable copolymeric eudragit-chitosan copolymeric nanoparticles, for sustained release of duloxetine to the brain. The polymeric eudragit-chitosan system, will protect the drug from chemical and enzymatic degradation, increasing its bioavailability. Eudragit has controlled and sustained release properties, which will have the potential of increasing the therapeutic efficacy of duloxetine (Sharma et al., 2016). Chitosan is biodegradable and biocompatible. It has sustained release properties and because of its positive charge, possesses easier cell permeability (Crawford et al., 2016 and Sharma et al., 2016). A formulation of duloxetine loaded eudragit-chitosan nanoparticles, will increase the potential of neuro-availability, providing higher therapeutic efficacy at the site of absorption.

The prospect for this delivery system, is for the encapsulated formulation of the nanoparticles to be orally administered, absorbed through the intestinal mucosa, distributed in the blood stream and para/intracellularly pass through BBB epithelial cells to the brain. Figure 1.1 illustrates the synthesis of Eud-CHT nanoparticles loaded with DLX and the proposed route of administration from oral intake to the BBB.

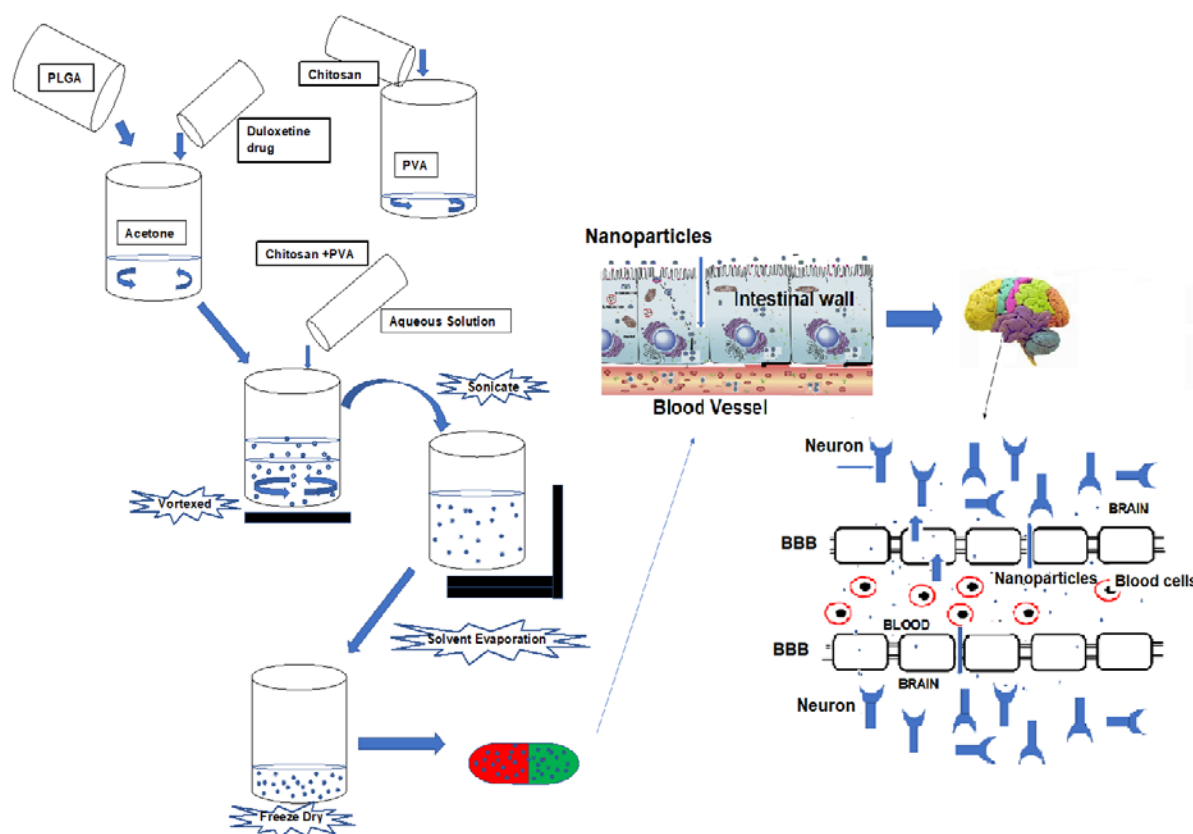


Figure 1.1: Stepwise representation of the synthesis of duloxetine-loaded copolymeric nanoparticles and route of drug absorption via intestinal to the BBB target sites.

Following synthesis of the DLX loaded Eud-CHT copolymeric nanoparticles, physicochemical properties and evaluation of various chemical bond shifts, responsible for particle solubility and drug entrapment efficacy were analysed using Fourier Transformation Infrared Spectroscopy (FTIR). The zeta potential and particle size of the polymeric nanoparticles was determined for surface charge receptor interactions, thereby designing an ionic equilibrated system, for high affinity receptor binding. The Eud-CHT-DLX nanoparticles were evaluated for cytotoxicity to the HEK 293 neural cells utilizing MTT assay. The designed polymeric nanoparticles will thus possess the potential of delivering duloxetine with a lower drug-loaded dose, releasing drug in a continuous manner, with higher proposed affinity drug uptake at the BBB site.

1.3 Aims and Objectives of this research

The aim of this study was to design and synthesize an oral copolymeric, biodegradable nanoparticulate drug delivery system, for the release of duloxetine to the CNS. The following objectives were undertaken and fulfilled:

- Objective 1: To design a biodegradable duloxetine-loaded, copolymeric nanoparticulate delivery system, using eudragit and chitosan, with particle sizes in the range of 80- 200nm.
- Objective 2: To evaluate the physicochemical properties of the copolymeric nanoparticles, employing Fourier Transformation Infrared spectroscopy determining molecular state composition of the polymer matrix.
- Objective 3: To evaluate particle size and charge distribution, determining the uniformity and surface charge distribution of the copolymeric nanoparticles.
- Objective 4: To determine drug entrapment efficiency and determine the drug release profile of duloxetine *in vitro*, employing a USP 2 apparatus.
- Objective 5: To undertake thermal and morphological evaluation, employing Differential Scanning Calorimetry and Scanning Electron Morphology, evaluating the particle morphological properties and the glass transition nature of the copolymeric nanoparticulate delivery system respectively.
- Objective 6: To determine cytotoxicity of Eud-CHT-DLX nanoparticles utilizing a HEK 293 cell line and undertaking a MTT assay.

1.4 Overview of this dissertation

Chapter 1: Introduces the rationale of the study profiling clinical depression, a debilitating disease affecting more than 4% of the global population. Therapeutic agents targeted at clinical efficacy face challenges from solubility; absorption; bioavailability decreasing their potential of reaching the desired target site. This chapter also discusses challenges to cross the BBB experienced by therapeutic agents, such as molecular size and composition, lipophilicity and ability to be taken up by transport systems. Nanotechnology using polymers is briefly described and presented as one of the mechanisms that can be used to overcome the BBB. This chapter summarises the results from the analyses conducted for proof of concept. The aims and objectives with motivation of the study is also explained.

Chapter 2: Presents a comprehensive literature review on nanotechnological strategies employed to enhance bioavailability of therapeutic agents targeted to the brain. It describes the challenges of the gastro-intestinal system before absorption and the restrictions at the BBB site before internalization into the brain. The CNS is the regulator of all bodily function, and although nutrients and oxygen have readily access to the brain, most molecules/therapeutic agents have limited access across the BBB. The main focus is on treatment of depression disorder and innovative mechanisms that have been employed to ensure therapeutic efficacy are outlined. Several strategies and nanotechnological designs

such as exosomes, dendrimers, nanogels are discussed including several transport mechanisms through the BBB.

Chapter 3: Describes the design, materials and methods utilized in the synthesis of the Eud-CHT-DLX nanoparticles. It illustrates the characterization of the nanoparticles, performed utilizing the FTIR, SEM, Zeta sizer, and Thermal analysis. Drug release profiles were determined by dissolution and UV Spectroscopy. Cell viability studies were also conducted utilizing MTT assay for proof of concept and confirmation of non-toxicity to physiological tissue. The nanoparticles were found to be less than 200 nm in size, with favourable poly-dispersity index. The morphological studies and the zeta sizer confirmed the synthesis of nanoparticles, with FTIR data exhibiting the formation of Eud-CHT nanoparticulate structure with incorporation of DLX.

Chapter 4: Provides a conclusion and future recommendations in which the study can be enhanced. Efficacious Eud-CHT nanoparticles loaded with DLX were formulated, with significantly positive results from the physicochemical characterization, dissolution analysis and ex-vivo studies. Cellular viability studies showed no toxicity to physiological tissue. Further studies should be conducted on the combination of these polymers, their behaviour in vivo and stability over time.

CHAPTER 2

LITERATURE REVIEW OF NANOTECHNOLOGICAL STRATEGIES AND BRAIN TARGETING

2.1. Introduction

Mental depression is a severe psychiatric condition, identified by low emotional state, hopelessness, distress, low self-confidence, trouble sleeping and frequently with thoughts of taking one's life. (WHO, 2020; Kircanski et al., 2012). A significant amount of bioactive agents (drugs) prescribed for treatment of depressive disorder enhances the accessibility of chemical neurotransmitters at the endogenous synapse by inhibiting their neuronal reabsorption. Nonetheless, the treatment success of antidepressant drugs is frequently reduced, as these molecules are impotent to reach the brain depression site by regular conventional pathways of administration. Stability, solubility, absorption, bioavailability and brain site targeting are often challenges experienced by therapeutic agents orally administered (Jacob et al., 2020; Lenox and Frazer, 2010).

The brain serves as the central regulator of bodily functions and forms part of the Central Nervous System (CNS) including spinal cord blood-cerebrospinal fluid barrier (BCSFB) and selective blood–brain barrier (BBB), which restrict access of most psychotropic drugs into the brain (Pamreddy, 2018; Mdanda et al., 2019). When damaged or dysfunctional, different areas are affected including memory, sensation, motor skills and personality (Opris et al., 2020; Lee et al., 2015). Blood supply to the brain is through tightly packed cell layers that form a physical barrier, commonly known as the BBB. Despite the fact, the brain is highly accessible for other substances like nutrients and oxygen, the BBB prevents undesirable material to be transported through and protects in several ways from potentially damaging toxins of the bloodstream. Consequently, the BBB keeps out 98 percent of small and 100% of large drug molecules greater than 500 dalton (Da) from entering the brain allowing very few drug molecules to penetrate through, based on their physico-chemical properties and molecular size (Andrade et al., 2018). Lipinski's rule described the characteristics required for molecules to gain excess through the BBB (Lipinski, 2004). Overcoming the hindrances of the BBB in delivering drug molecules to targeted regions of the brain in depression disorder is the most challenging aspect in medical treatment of neurological diseases. Diseases of the nervous system ranging from the paroxysmal disorders, namely epilepsy, migraine and the affective illnesses to the degenerative disorders including depression disorder, Alzheimer's disease, Parkinson's disease and Motor neuron disease therefore are tedious and complicated to treat (Saeedi et al., 2019).

For enhancement of targeted brain site delivery of medicinal products, several invasive and non-invasive strategies are employed. Some invasive strategies include convection, temporary disruption of the BBB, or local delivery of drugs by neurosurgery (Pehlivan, 2013). While these strategies are effective, the risk posed to patients by allowing access of toxic substances to the brain cannot be eliminated. Current neurotherapeutic studies are faced with the challenge of small particles delivery to targeted brain site and are focusing their attention on combating these challenges. (Opris et al., 2020). In this context, nanotechnology provides a solution to the challenges associated with penetration across the BBB by antidepressants and facilitates the transportation to the CNS (Saeedi et al., 2019).

Nanotherapeutics are the specific focus of this study and the developed nanotechnologies for transporting pharmaceutical agents past the blood-brain barrier for improved therapeutic efficacy to target depression disorder brain area and assist in decreasing drug related side-effects (Figure. 2.1). Several molecular nanodrug delivery platforms with exceptional increased effectiveness of therapeutic molecules targeted for brain activity have been developed (Khosa et al., 2018). The intended tailor-made molecular drug loaded nano systems are normally able to cross the BBB due to their small size less than 200nm, deliver specific therapeutics and molecular genetic materials to the brain tissue, leading to effective payload delivery to the desired brain area (Adebileje et al., 2017; Dendisová et al., 2018). The lipophilic nature and molecular mass range parameters enables these nanodrug molecules to cross the BBB via passive diffusion. These nano systems assist in maintaining the concentration of drug in the therapeutic range, and increase their duration in the body, enhance stability, improve solubility and permeability of brain targeted antidepressant drugs including amitriptyline, citalopram, clomipramine, dosulepin, Doxepin and duloxetine (Posadas et al., 2016). Advanced functionalization of nanostructures with ligands further enhances permeability across the BBB through transport media on the BBB to the brain (Kim et al., 2019).

This literature review's objective is to present recent advances in nanotechnological strategies as improved therapeutic intervention approaches for neurological diseases with particular focus on depression disorder. Major barriers to effective therapy are also highlighted. The re-engineering of the polymeric nanomaterial as a drug nano-carrier system used to cross BBB and trojan horse genomic constitution, ligand functionalization details, drug therapeutic applications and future prospects of the biomaterial are also profiled.

2.2. Diseases of the CNS and Strategies Employed

A complete range of common neurological disorders include meningitis, Parkinson disease, Alzheimer's disease, aneurysm, brain tumour, concussion, dementia, epilepsy, headaches and migraines, multiple sclerosis, psychiatric conditions (severe depression, obsessive-compulsive disorder), seizures and stroke (Zhang et al., 2017). Currently, approaches for delivery of brain targeted therapeutic agents, invasive delivery, the use of drug delivery systems e.g. nano therapeutics, invasive methods such as direct injections, and temporary disruption of the BBB are employed. Some methods of delivery are not always suited for the condition, e.g. direct injection can be used for some tumour treatment the local drug delivery, but are unsuited for neurodegenerative diseases such as Parkinson's disease or cancers that spread from original site, which require the entire brain to be covered by therapeutic agent. Methods such osmotic or chemical applications of reversable BBB opening, enable drugs in the brain, but can cause irreversible brain damage. One would conclude the systemic drug delivery approaches such as drug delivery systems, are a less invasive and safer method of drug delivery

Over the past decades, numerous strategies as shown in Table 2.1, were employed to deliver medicinal products into the brain (Dong et al., 2018). Several strategies employed including viral vectors known as the foremost effective means of gene transfer to modify particular cell type or tissue, and can be manipulated to express therapeutic genes (Warnock et al., 2011). Furthermore, several types of nanoparticles (NPs) encapsulate neurotherapeutics in polymer materials to surmount the BCSFB and BBB limiting characteristics. The formulated NPs slowly release the therapeutics into the CNS or the brain in a targeted manner with decreased peripheral toxicity (Figure. 2.1.)(adapted from Mumper et al., 2002).

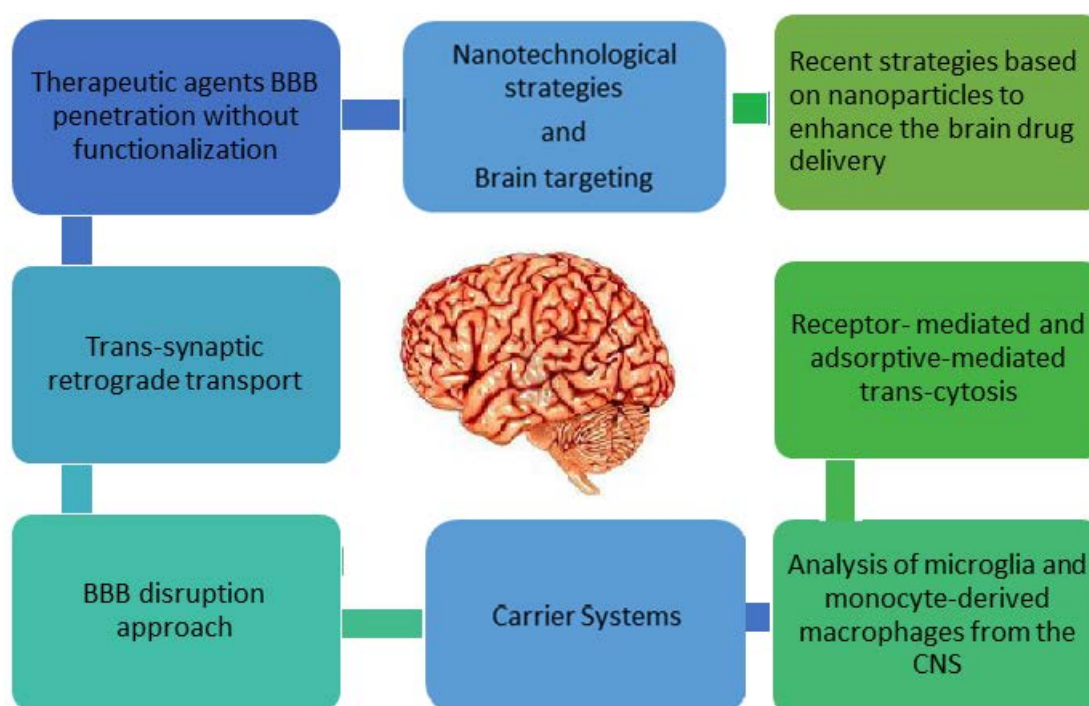


Figure 2.1: Illustration of the development of recent neurotherapeutic strategies employed for drug delivery with particular focus on Nanoparticle technology (modified and adapted from Masserini, 2013; Saeedi, Eslamifar et al., 2019).

Despite the fact that remarkable progress has been made in developing drug delivery systems for treating neurological disorders such as depression disorder, brain tumours, tuberculosis meningitis and HIV-neurocognitive dementia, there is still a growing need for more innovative, safe and effective strategies (Dong, 2018). These neurological disorders with therapeutic delivery strategies to combat the disorders are concisely articulated in this section and in Table 2.1. respectively.

Table 2.1: Therapeutic strategies for brain drug delivery (adapted fromDong, 2018).

Strategies	Advantages	Limitations	References
Viral vectors	High gene transfection efficiency	Safety concerns; brain direct injection; crossing the BBB; high dose by intravenous administration	(Warnock, Daigre et al., 2011)
Nanoparticles	Actively targeted delivery; brain targeting using specific physiological conditions	Crossing the BBB	(Lockman, Mumper et al., 2002)

Exosomes	Gene delivery to brain; potential ability to cross the BBB	Exosome donor cells; loading procedure; in-vivo toxicity and pharmacokinetics	(Zhang, Liu et al., 2019)
Delivery through active transporters in the BBB	Potential ability to cross the BBB by intravenous injection	Mainly for small molecules	(Barar, Rafi et al., 2016)
Brain permeability enhancer	Transiently open the BBB	Mismatch between findings in rodents and humans	(Zhang, Liu et al., 2017)
Delivery through the permeable BBB under disease conditions	Potential to cross the BBB	Limited knowledge about dynamic changes in the BBB and their mechanisms	(Zhang, Liu et al., 2017)
Non-invasive techniques to enhance brain drug uptake	Potential to open the BBB and decrease efflux transporters	Toxicity	(Zhang, Liu et al., 2017)
Alteration of administration routes	Bypass the BBB through nasal administration	Suitable for low dose	(Naidoo, Mdanda et al., 2019)
Nanoparticles for brain imaging/diagnostics	Enhance imaging; cross the BBB through the hyper-permeable BBB under disease conditions	Cross the BBB; understand dynamic changes in the BBB	(Meyers, Doane et al., 2013)

In depressive disorder patients present with low mood, lack of interest in previously enjoyed activities, decreased ability to think, worthless feeling and ideation of suicide (Sindhu et al., 2018). In depressive patients, the synaptic levels of neurotransmitters, Serotonin and Noradrenaline are significantly lowered, thus medication linked to treatment aims to increase levels of these neurotransmitters at the synapse to improve depressive symptoms (Khan et al., 2018 and Gudayol-Ferre et al., 2021). Therapeutic treatment includes the usage of Selective Serotonin Reuptake Inhibitors (SSRIs) such as Sertaline, Citalopram, Escitalopram, Paroxetine and Fluoxetine. Duloxetine, Venlafaxine and Desvenlafaxine have dual effect, inhibiting the uptake of both Serotonin and Noradrenaline (Gudayol-Ferre et al., 2021).

These currently available pharmaceutical treatments have adverse effects on patients resulting in negative effect on patient compliance with continued treatment and high probabilities of relapse (De Raedt et al., 2015). These symptoms include sexual function changes, dizziness, tiredness, excess weight gain, tremors, blurred vision, urinary disturbances, diarrhoea, insomnia, and headaches (Lader, 2007 and De Raedt et al., 2015).

Apart from adverse events, antidepressant drugs face several challenges to reach the brain site of action and several strategies have been investigated.

In a neuroimaging study by Khan et al., 2018, it was revealed that there are two neuro-circuits involved in depressive disorder, the dorsolateral prefrontal cortex and dorsal regions of the anterior cingulate cortex and the medial prefrontal cortex and ventral subcortical regions (amygdala). The first region is responsible for attention, cognitive control and the second region is sensitive to emotional stimulation. These regions have been found to have reduced activity during depressive episodes and are normal in patients who have responded to treatment (Khan et al., 2018). In a review performed by De Raedt et al., 2015, neurostimulation strategies to treatment are discussed as an alternative to pharmacotherapy. Electroconvulsive Therapy (ECT) which has been used for more than 20 years and has proven efficacy, is an introduction of electrically induced seizures by placing electrodes on the scalp of a patient. A major disadvantage in this therapy is that an anaesthetic must be injected multiple times, and use may cause degradation in memory and learning. A newer technique called transcranial alternating current stimulation was investigated by Haller et al., 2020. This technique involves non-invasive introduction of gamma (40hz) frequencies, stimulated for a period of 10-20 min sessions per day. Results showed safe and effective treatment with cognitive functional improvements, and an improved rating using depression rating scale and Beck Depression Inventory. Haller et al., 2020, however concluded that the technique necessitates further investigation as the sample size was only of 6 participants. An alternative treatment to ECT with decreased brain functioning has negative effects including Magnetic Seizure Therapy (MST) which is a form of electro-brain stimulation but has not been widely researched (De Raedt et al., 2015).

Transcranial Magnetic Stimulation (TMS), a non-invasive neurostimulation strategy which electrical stimuli is introduced by an electromagnetic coil, placed on the scalp and operated in such a way that it produces a magnetic field which have an effect on the firing of neurons (Mahoney et al., 2020). However, the treatment requires continuous therapy up to 25 repetitive sessions, with remission being a probability (De Raedt et al., 2015). There are also few studies conducted on the long-term effects of treatment using TMS, although it has fewer side effects and more tolerable than ECT (De Raedt et al., 2015). A clinical trials review performed by Loo et al., 2005, investigating the efficacy of TMS for treatment of depression found that with the extension of the treatment course efficacy has shown to be greater. The studies reviewed were limited in that they had small sample groups and the studies lasted to a maximum time of 2 weeks. The TMS treatment showed great effect over placebo but no significant clinical

effect (Loo et al., 2005). A more recent technique with more favourable side effects, easy to administer and more cost effective is called the Transcranial Direct Current Stimulation (tDCS) and differs to TMS in that it cannot directly activate the neurons but manipulates the membrane potential of the neurons (De Raedt et al., 2015).

Chronic Deep Brain Stimulation (DBS) is another technique discovered in the recent years; the technique is however invasive although has proven favourable results. The treatment requires electrodes to be implanted in the brain and is a chronic application. The electrodes function to regulate abnormal impulses. Vagus Nerve Stimulation (VNS) is another invasive technique, in which electrodes are surgically implanted into the brain (De Raedt et al., 2015). The techniques mentioned above although showing potential for treatment still requires intensive studies on effectiveness of treatment especially long term, Invasive therapies pose a risk of exposure to toxic substances to the brain, safety profiles on long term exposure to magnetic and electric stimulation is not thoroughly understood, and greater population size would need to be studied to determine efficacy. (De Raedt et al., 2015). Nanotechnology has become increasingly favourable as novel drug delivery strategy for treatment of Neurological disorders especially chronic disorders such as Major Depressive Disorder.

Tong et al, 2017, investigated the potential of PLGA-chitosan nanoparticles loaded with Desvenlafaxine which is used in the suppression of depressive symptoms, for improvement of pharmacokinetic and pharmacodynamic profile. PLGA-chitosan nanoparticles were administered orally and were compared to free Desvenlafaxine drug, orally administered. Results of the study showed significant reduction in symptoms of depression and increased level of monoamine in the brains of rodents treated with PLGA-chitosan-Desvenlafaxine loaded nanoparticles compared to the ones treated with free Desvenlafaxine (Tong et al, 2017). Nanoparticles have been used in maintaining the concentration of drug in the therapeutic range, and increase their duration in the body, enhance stability, improve solubility and permeability of drugs. These nano formulations also have the capacity to transport other functional entities if necessary, leading to theranostic molecules. The nanoformulations in Table 2.2 are applied in the delivery of diagnostic or remedial substances/molecules to the CNS. These nanoparticles are detailed in the nanotechnological strategies section 2.5.

Table 2.2: Neurotherapeutics and nano-technological strategies for brain delivery (adapted from Chowdhury et al., 2020).

Drug	Nano-technological strategies	Action	References
Doxorubicin	PEGylated nano-liposomes	Enhanced tumour growth inhibiting potential of doxorubicin in vivo	(Barenholz, 2012)
Efavirenz	Solid lipid nanoparticles	Improved bioavailability and brain targeting	(Gupta et al., 2017)
Curcumin	Polymeric nanoparticle-encapsulated curcumin	Bioavailability of curcumin and curcumin glucuronide in the central nervous system of mice after oral delivery of nano-curcumin	(Szymusiak et al., 2016)
L-dopamine	Cyclodextrin-nanosponges	Controlled drug delivery in Parkinson's diseases	(Trotta et al., 2016)
Elvitegravir	poloxamer-PLGA nanoformulation	Delivery of elvitegravir across BBB with the aim of halt HIV neurocognitive disorder	(Gong et al., 2020)
Cornell Dots	Silica nanoparticles with a fluorophore, PEG-coated	Malignant brain tumours imaging	(Juthani et al., 2020)
Dexmethylphenidate HCl	Nanocrystals	To halt attention-deficit/hyperactivity disorder	(Malamatari et al., 2018)

Daunorubicin	Liposomes	Paediatric brain tumours	(Lippens,1999; Ying et al., 2010)
L-DOPA	Gold nanoparticles	Parkinson's disease	(Gonzalez-Carter et al., 2019)
α -mangostin (α -M)	poly(ethylene glycol)–poly(l-lactide) (PEG–PLA)	Alzheimer's: A β clearance in brain	(Gu et al., 2016)
Desvenlafaxine	PLGA-chitosan nanoparticle	Management of depression	(Qin et al., 2017)
Pretomanid	oil-in-water nanoemulsion	Tuberculosis meningitis	(Pamreddy et al., 2018)

2.3. Factors that influence pharmacotherapy targeted to the brain depression site

Complication of delivering therapeutics into the targeted regions of the brain represents the most challenging aspect in neurological medical treatment (Ntshangase et al., 2019). The transportation of medicinal agents to the brain presents a challenge because of the limitations posed by the BBB in that it limits passage of molecules to the brain with the aim of protecting the brain from harmful toxins (Mahringer et al., 2021).

2.3.1 Gastric absorption of therapeutic agents and cell permeability

Oral administration of therapeutics remains the suitable delivery method of choice due to its patient compliance, painless and non-time consuming administration (Ghosh et al., 2019). However oral route, presents certain challenges to the bioavailability of therapeutic agents, since it is subjected to first-pass metabolism which occurs in the liver and the gut wall (Ghosh et al., 2019). Bioavailability is the quantity of drug substance that enters the bloodstream after absorption and hepatic metabolism. The gastric pH, which is 1.2, 5 - 7 in the small intestines and 6 – 7.5 in the colon affects drug stability. Moreover, the large surface area of the small intestines and its enzymes create another barrier of drug absorption. The bioavailability of drug substances depends on their physicochemical characteristics shown in Table 2.3. Low molecular weight hydrophobic drugs readily pass through the intestinal wall through transcellular pathways, whilst hydrophilic drugs require paracellular transport. For a drug to pass through the intestinal epithelium, aqueous solubility and cell permeability are essential. In addition, the intestinal membrane drug transporter proteins are observed as a determining factor for therapeutic disposition in the body and this factor has a direct impact on the movement of therapeutic agent from the intestines to the blood stream, pharmacokinetics of the drug, interactions with other drug molecules, and safety profiles (Yokooji et al., 2007). The

main efflux transporters of the ABC family are MDR1 (P-glycoprotein, P-gp, ABCB1), BCRP (ABCG2), and MRP2 (ABCC2). These transporters are all localized on membrane barrier tissues of the intestine and affect wide range of xenobiotics such as bupropion antidepressant, statins, macrolide antibiotics, angiotensin blockers, and chemotherapeutic agents, affecting exposure and clearance *in vivo* (Yokooji et al., 2007). After successful absorption through the intestinal track, therapeutic agents are transported via the circulatory system to their site of action.

2.3.2 Blood Brain Barrier

CNS is well protected from the entry of substances that may be harmful, however, this provides an ideal environment for neuro-disease to replicate independently whilst the therapeutics are circulating in plasma (Mdanda et al., 2020). CNS is protected by two biological barrier systems known as BBB and BCSFB. The Surface area of the BBB is 5000 times greater compared to BCSFB, and for therapeutics that are administered orally or intravenously the BBB is the most important route for absorption into the brain. (Pardridge, 1999). Tightly sealed joints of capillary endothelial cells form the BBB. Endocytic vesicles and fenestrae that are typically numerous in other organs are particularly fewer in brain capillaries. The tight joints in the BBB significantly reduces small molecules passage through passive diffusion into the CNS and less than 2% of therapeutic agents can penetrate the BBB (Sharma et al., 2019). The movement of bioactive substances through the BBB depend on size, configuration, surface charge, and chemical composition (Sharma et al., 2019). Only molecules smaller than 4 nm can passively penetrate the epithelial cells of the BBB (Kim et al., 2016). The restrictive transport mechanisms that control the entry of essential substances would be required for molecules not meeting the passive diffusion characteristics (Pardridge, 1999). For an effective passive penetration of nonfunctionalized therapeutic drugs across membrane, Lipinski's rule of physicochemical properties must be applied, Figure 2.2. (Sharma et al., 2019). The permeability of molecules through the BBB is highly impacted by their size and physicochemical properties, especially via passive diffusion. As Lipinski's "rule of five", five key physiochemical parameters (molecular weight, lipophilicity, polar surface area, hydrogen bonding, and charge) require optimization to improve BBB permeability by passive diffusion (Lipinski, 2004).

Lipinski's Rule of 5 mention that parameters listed below determines the permeation through biological membranes: (Lipinski, 2004)

- H-bond donors not more than 5 (expressed as the sum of OHs and NHs).
- H-bond acceptors not more than 10 (sum of nitrogen or oxygen atoms).
- Molecular weight less than 500 Daltons.

- An octanol-water partition coefficient log *P* greater than 5.
- Rotational bonds less than 10.

Table 2.3: Factors which contribute to the permeability of therapeutics across the BBB and BCSFB (adapted from Lu et al., 2014).

Therapeutic Agent	Molecular weight (Da)	Protein binding (%)	Lipid solubility	Log <i>P</i>	Efflux transporters	References
Doxorubicin	543.5	74-76%	low	-0.5	Pgp, MDR1, ABCB5	(Frank, Margaryan et al., 2005; Shen, Chu et al., 2008)
Duloxetine	333.9	-	high	4.2	P-gp	(Kumar et al., 2021; Islin et al., 2018)
Efavirenz	315.7	> 99%	high	4.6	BCRP	(Boffito, Back et al., 2003; Peroni, Di Gennaro et al., 2011)
Curcumin	368.4	-	high	3.29	MRP2, BCRP	(Ge, Yin et al., 2016; Her, Venier-Julienne et al., 2018)
Elvitegravir	447.9	97-98%	high	5.3	P-gp	(Custodio, Rhee et al., 2014; Gong, Chowdhury et al., 2017)

Dexamethylphenidate HCl	233.3	12 - 15%	moderate	2.25	-	(Robinson and Keating 2006)
Daunorubicin	527.5	50-60%	moderate	1.83	P-gp	(Spoelstra, Dekker et al., 1991)
α -mangostin (α -M)	410.5	-	high	7.7	ABCG2	(Wu, Hsiao et al., 2017)
Desvenlafaxine	263.4	30%	moderate	2.72	P-gp	(Kamath and Handratta 2008; Bachmeier, Levin et al., 2013)
Pretomanid	359.3	93%	moderate	2.75	-	(Rakesh, Bruhn et al., 2016)

The therapeutic benefits of drugs which are used for the treatment of several disorders of the CNS such as neurodegenerative diseases particularly depression is limited due to the selective nature of the BBB. Patients suffering from such disorders have limited therapeutic options and poor prognosis. There are various transport mediums across the BBB for the transportation of essential molecules, solutes, enzymes, proteins and nutrients for normal functioning of the brain (Figure. 2.2). Although small water-soluble molecules can move across tight junctions of the epithelial cells and hydrophobic molecules readily passing through the epithelial cells, many molecules such as glucose, proteins and amino acids cannot pass through the BBB and require transport mediums (Chen and Liu, 2012).

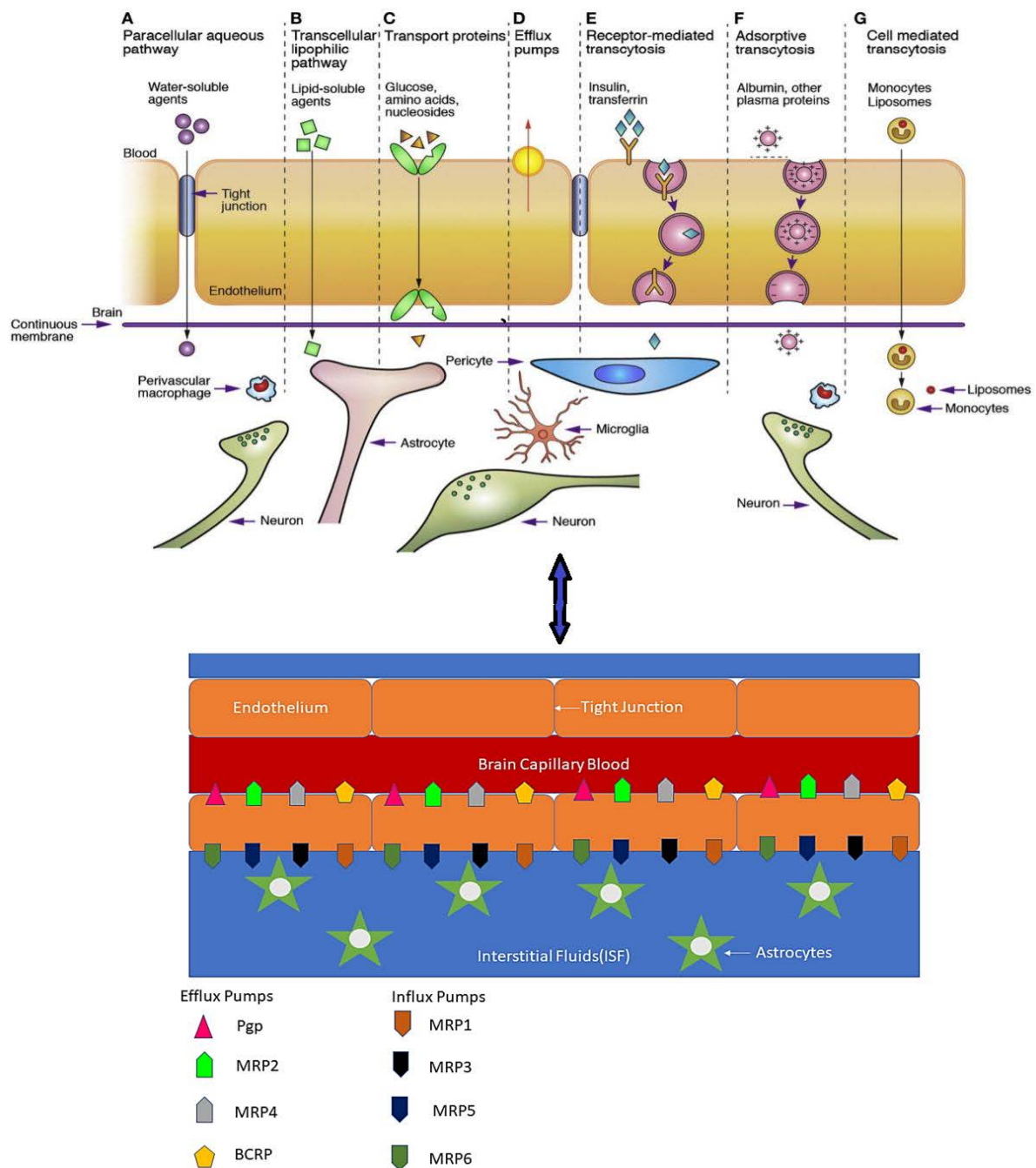


Figure 2.2: Transport pathways across blood brain barrier also illustrating BBB junctions and efflux pumps (adapted and modified from Vidu et al., 2014 and Sharma et al., 2019).

Receptor-mediated transport is usually for large molecules and Carrier Mediated transport for smaller molecules (Sharma et al., 2019). Various mediums of passage through the BBB are classified as paracellular transport, transcellular transport, transcytosis, and receptor-mediated transport (Saeedi et al., 2019). Nanotechnology takes advantage of these various transport mediums and provides a solution to the difficulty in penetration of the BBB by therapeutic agents and enhance CNS delivery (Saeedi et al., 2019).

2.4. Overcoming the Limitations

Nanostructures synthesized for brain delivery possess certain characteristics such as small size and high lipophilicity for permeability. These nanomolecules are limited by low solubility, rapid metabolism, and poor absorption. Traditionally, the main purpose of nanostrategies is to decrease normal tissue toxicity by improving drug specificity to target areas such as tumours (Manzoor et al., 2012). The effective non-invasive nanostrategies can enhance the permeability and retention of therapeutics allowing passive accumulation in targeted areas such as tumour interstitium (Manzoor et al., 2012). However, suboptimal delivery is accomplished with most nanoformulations due to heterogeneities of vascular permeability, which limits nanoformulations penetration. Scientists have developed several strategies to overcome such hindrances, however, some of these strategies employed to deliver drugs to the brain are classified under invasive and non-invasive strategies. Some examples of the non-invasive strategies include AuNPs aerosol sprays, Metallic particles, Liposomes, Dendrimers and polymeric biodegradable particles (Sajja et al., 2009; Raliya et al., 2017). Furthermore, some examples of invasive strategies for disruption of BBB are focused on ultrasound and the use of pharmacological agents for increasing the permeability of BBB (Kasinathan et al., 2015).

2.5. Nano-technological Strategies to combat the limitation of delivery of therapeutics to the brain

Numerous nanotherapeutic delivery strategies for brain targeting have been researched, these include dendrimers, liposomes, polymeric micelles, nanogels, nanocapsules and vesicles, nanoemulsion and carbon nanotubes as depicted in Figure. 2.3 & Table 2.2. These nanoformulations show a notable advancement in the impact of neurological therapy of various neurodegenerative diseases in particular depression disorder due to their nanosize, protection of drug molecule form degradation by encompassing it inside its interior core, improved *in vivo* drug release kinetics and prolonged drug half-life, as well as future expectations of greater scale production. Nano-technological innovations are however sometimes inadequate in terms of accurate delivery and sustained release capabilities. Employing surface functionalization of nanostructures with antibody-based ligands can significantly enhance the selective uptake at BBB cellular level.

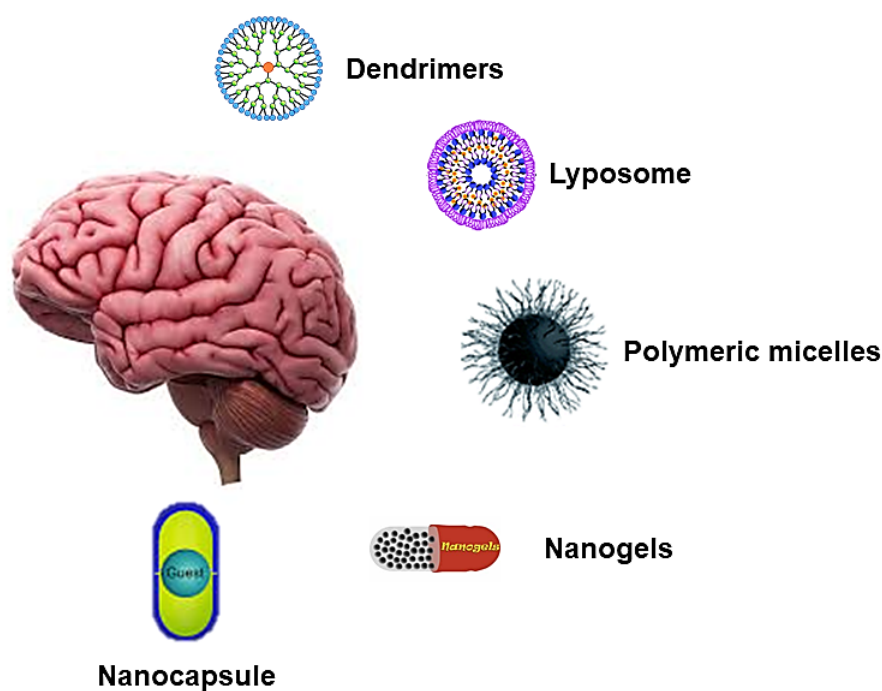


Figure 2.3: Nano-technological systems on basis of therapeutic delivery in brain targeting (adapted from Mukhtar et al., 2019).

2.5.1. Dendrimers

Dendrimers are three-dimensional molecules that consist of a core, numerous layers and repetitive branches. A lot of therapeutic agents consist of numerous restrictions namely low water solubility and a limited blood circulation time, as a result it was found necessary to develop new drug delivery systems which can deliver a drug efficiently to surmount BBB. (Kumar et al., 2014). Dendrimers are versatile and able to carry hydrophobic and hydrophilic drugs with a high drug loading capacity (Saeedi et al., 2019; Venditti, 2019). These nanostructures are useful in prolonging the half-life of therapeutic agents, aid in solubility, permeability, stability of drugs and easily cross biological barriers (Venditti, 2019). Dendronized polymers improve the loading of hydrophobic drugs (Venditti, 2019). Dendrimers consist of fascinating properties that are appreciated by the researchers in drug carrier developments and have been used in various therapeutic and biomedical applications. However, with all extensive applications of dendrimers, their application in biological systems may be limited due to toxicity issues associated with them (Madaan et al., 2014). The toxicity issues associated with this technological application in biological systems has remained a matter of concern till today. Dendrimers that are normally linear polymers such as polyethylene glycol (PEG), polyglutamic acid, polysaccharide, poly (allylamine hydrochloride) and N-(2-hydroxypropyl) methylacrylamide have been investigated as drug vehicles and accepted for clinical applications (Brannon-Peppas and Blanchette, 2004).

2.5.2. Nanoparticles

Polymeric Nanoparticles are synthesised from degradable and non-degradable polymers, and have small sizes, with sizes ranging from 10 – 1000nm with various structures such as spherical, rod, branched or shell like (Vendetti Iole, 2017). The small size of nanoparticles allows easy uptake into the cells (Vendetti Iole, 2017). Resveratrol, a polyphenol used in treatment of various neurological disorders including depression disorder was investigated by Fonseca-Santos et al, 2020, using nanoparticles to enhance delivery to the brain. Resveratrol has a poor solubility profile, hence decreasing its bioavailability and clinical effects. Three studies conducted incorporating Resveratrol in polymeric nanoparticulate structures showed excellent drug entrapment efficacy and results showed improvements in patient therapy (Fonseca-Santos et al., 2020).

2.5.3. Liposomes

Liposomes are lipid drug delivery systems that have superior advantages over polymeric nanoparticulate system due to their lipid nature, permeability through the BBB more efficient than their polymeric counterpart, but still require surface modification for enhanced permeation (Ghosh et al., 2019). Liposomes are a type of Lipid carriers that are spherical vesicles consisting of one or more phospholipid bilayers fabricated from cholesterol and other natural phospholipids enclosing an aqueous core (Khosa et al., 2018). Liposomes protect the drug from elimination, increase the time for circulation, decrease side effects and increases the effectiveness of therapeutic action of CNS drugs (Saeedi et al., 2019). Liposomal carriers increase the dissolution of hydrophobic drugs in the intestines, therefore, increasing absorption and transport through epithelial cells. Once taken up through the intestinal wall, liposomes are transported through the lymphatic system, thus resulting in decreased first-pass metabolism, increasing the concentration of therapeutic agents and enhanced bioavailability (Ghosh et al., 2019). There are several registered Liposome products on the market including Depocyt® for management of lymphomatous meningitis, Morphine Sulphate in Depodur® for management of Pain, and several preparations of Doxorubicin (Doxil®, Myocet® and Lipodex®) for treatment of cancer. These preparations are however limited because of the intravenous or intramuscular mode of administration. To overcome the physical stability of liposomes, niosomes are another type of Lipid carrier vesicles formed when mixing non-ionic surfactant and cholesterol.

2.5.4. Polymeric micelles

Polymeric micelles are composed of copolymers with an amphiphilic structure consisting of a hydrophilic copolymer block shell and hydrophobic core block. Micelles size range is from 10-100nm assist in drug delivery to surmount the BBB. The core can be loaded with a poorly soluble/hydrophobic drug thus improving the solubility, bioavailability and stability of drug molecules (Sezgin-bayindir et al., 2016). In a research study conducted to evaluate block polymers for the formation of micelles and brain delivery, it was concluded that PEG-PLA copolymeric micelles effectively passed through endothelial cells of the BBB and improved cellular uptake (Sezgin-bayindir et al., 2016). In another study, the bioavailability of dapoxetine, a drug used for the treatment of premature ejaculation was improved by formulating it with micelles. Dapoxetine micelles showed substantial improvement in bioavailability and efficacy (Abourehab et al., 2018).

2.5.5. Nanogels

Nanogels are defined as highly cross-linked nano-sized hydrogel systems that are either co-polymerized or monomers. They are arrangements of interconnected chains of polymers of nanosize of ionic or non-ionic nature formed from PEG, polyethylene amine and, or polyacrylic acid. The hydrogel Nanogels are favoured because of the advantageous characteristics of both hydrogels and nanogels. The major benefit of nanogels is their capacity to load the drug as high as 40-60% (Saeedi et al., 2019). Nanogels consist of great qualities that makes them better as a delivery system (Tan, Tan et al., 2010). These significant characteristics include thermodynamic stability, elevated capacity of solubilization, relatively low viscosity and capability of undergoing vigorous sterilization techniques (Tan et al., 2010).

2.5.6. Nanocapsules

Nanocapsules and vesicles are sphere-shaped hollow structures with a polymer membrane surrounding the cavity that holds the drug. Nanocapsules are useful in targeted drug delivery, for sustained and controlled release strategies and improve the stability and bioavailability of drugs (Venditti, 2019). Their sizes range from 50 – 400 nm. They are made out of a liquid/solid core, and the location of the drug would be in the cavity (Kothamasu et al., 2012). The surrounding membrane of the nanocapsule, consists of natural/synthetic polymers. These nanocapsules have attracted great interest to drug carrier developers, since they have a protective coating, which can be pyrophoric in most cases and it is easily oxidized and delay the release of active ingredients (Kanumur et al., 2012).

2.5.7. Nanoemulsions

Nanoemulsions are thermodynamically stable surfactant molecules produced by simple emulsification method such as titration and by mixing oil, water, surfactant and co-surfactant. Curcumin is a drug used in the treatment of toxoplasmosis and can be prepared in a nanoemulsion to tackle the challenges of its poor water solubility and low bioavailability. Tween 80&85 are used as surfactants. The findings by Azami et al., 2018 showed a statistically significant difference in levels of the parasites between rats treated with curcumin nanoemulsion and control groups. In this study, a preparation of nanoemulsion containing Atovaquone (used for the treatment of toxoplasmosis), was studied with the aim of improving bioavailability and efficacy of Atovaquone. Nanoemulsions were prepared using the spontaneous emulsification method with grape seed extracts. Compared with free atovaquone, nanoemulsion-containing atovaquone showed significant improvement in bioavailability after oral administration, mice survival time was also increased, and the number and size of brain cysts were reduced (Azami et al., 2018).

2.5.8. Carbon Nanotubes

Carbon nanotubes are carbon cylinders made up of one or more layers classified as single/multi wall nanotubes. This carbon based approach is one of recent strategies proposed in this current decade to be incorporated with nanotechnological principles, through the application of carbon nanotubes, which leads to the modulation of undesired effects and creating new conjugates with promising and improved pharmacological profiles (Aboofazeli, 2010). Carbon nanotubes were introduced and intensively investigated as multipurpose novel nanocarriers for therapeutic delivery and clinic applications (Aboofazeli, 2010). Carbon nanotubes consist of excellent physicochemical characteristics that allows covalent and non-covalent binding of several pharmaceutical entities and for rational design of novel candidate nanoscale structures in drug development (Aboofazeli, 2010). Carbon nanotubes are functionalized with different functional groups and carry simultaneously several moieties for targeting, imaging and therapy (Aboofazeli, 2010).

2.6. Surface modification of nanoparticles specific to CNS therapy of depression

Although several nanoparticles can cross the BBB, they are not all efficient when employed as drug delivery agents for brain targeting. Hence, surface-modification which involves the use of physiological and synthetic peptides to functionalize or coat nanoparticles is critical to enhance the delivery of pharmaceutical products to the CNS (Cox et al., 2019). Surface modification for targeting of certain receptors has been explored. Lactoferrin, transferrin and Low-density lipoprotein being amongst the researched receptors (Cox et al., 2019).

Surface functionalization of nanoparticles in mental depression is articulated in the subsequent paragraphs as models for enhanced neurotherapeutics delivery to the brain. Selective serotonin reuptake inhibitors are frequently used drugs for treatment of depression; nevertheless, long-term uptake of SSRIs may induce high toxicity negating its beneficial effects (Amare et al., 2019). Ongoing studies are focused in developing new antidepressants to improve Major Depressive Disorder. Previous studies showed that Cur/SLNs-HU-211 nanoparticles in the existence of CBR1 facilitated an efficient motor function improvement in CORT-induced MDD mice model. Cur/SLNs-HU-211 nanoparticles alleviated symptoms on CB1^{+/+} MDD mice and resulted in dopamine and norepinephrine recovery following CORT-induced neurotoxicity. This study confirmed that Cur/SLNs-HU-211 nanoparticles based CBR1 *in vivo* targeting would be a potentially feasible and safe way for future therapeutic strategies of Major Depressive Disorder (Wosko et al., 2018).

2.7. Physiological Transport Mechanisms for the delivery of neurotherapeutics into the brain

The delivery of drugs to the brain is achieved through BBB permeation by endogenous blood brain barrier transporters and these are broadly classified into three types namely transporter-mediated delivery, adsorptive mediated transport, receptor-mediated transcytosis (Figure. 2.2). The larger sum of nanosized formulations that can pass through the BBB utilize two main systems namely the receptor mediated transcytosis and adsorptive-mediated transcytosis for delivery across the BBB (Singh, 2013). The archetypical approach used to enable the carriage of nanoparticles into the CNS consist of incorporating a targeting molecule or binding to blood molecules that are recognized by receptors present at the brain endothelium, mediating endocytosis or transcytosis (Figure. 2.2).

2.7.1 Adsorptive mediated transport (AMT)

AMT depends on ligand's static charge for interactivity with the luminal exterior charges of endothelial cells forming the BBB. Endothelial cells of the BBB possess negatively charged vesicles that attract cationic molecules. Functionalisation of nanoparticles with cationic bovine serum albumin (CBSA), transactivating transcriptional peptides and MMP-2200 can be a strategic route to penetrate the BBB through adsorptive mediated transport (Sharma et al., 2019). CBSA-functionalized nanoparticles have been synthesised in previous studies (Gao, 2016; Lu, 2005). In an aim to enhance brain permeability Wei and colleagues, 2005 synthesised polyethylene glycol-poly lactic acid CBSA surface-modified nanoparticles and compared them with BSA-surface modified nanoparticles for brain capillary targeting. The rat brain capillary endothelial cells absorbed much more of the CBSA surface-modified nanoparticles than the BSA modified nanoparticles (Lu et al., 2005). In another study, Solid

lipid nanoparticles were conjugated with CBSA and loaded with doxorubicin for brain tumour targeting. The findings from the trans-endothelial studies exhibited incorporation of CBSA with improved cellular uptake by up to 6 times when compared to free doxorubicin (Agarwal et al., 2011).

TAT-functionalised nanoformulation of Paclitaxel-loaded liposomes functionalized with TAT were synthesized. TAT addition was to enhance cellular uptake of paclitaxel by the brain endothelial cells. Drug delivery efficacy was greatly improved in mice and the rate of tumour inhibition was up to 69% (Fu et al., 2015). In a research study by Liu et al., 2018 iron silver nanoparticles functionalized with TAT were developed for brain tumour targeting and drug delivery. Incorporating doxorubicin in the formulation showed a growth reduction of tumour cells by 29% (Liu et al., 2018). Proteases, and matrix metalloproteinases (MMPs) functionalized gold nanoparticles (AuNPs) have been employed in treatment and diagnosis of numerous different pathological conditions, such as cancer, AIDS, inflammation. A peptide with two cleavage sites for MMP-7 has been synthesized and immobilized gold nanoparticles (AuNPs) through a cysteine residue (Chen et al., 2013).

2.7.2 Receptor-mediated transcytosis

The biological characteristics of the BBB consist of various transporters including glucose carriers, transferrin receptors, Nicotinic acetylcholine receptors, insulin receptors, lipoprotein receptors, amino acid carriers LAT1, etc, by which several solutes including nutrients move across the BBB (Chen & Liu, 2012). Nanoparticles have ligands such as transferrin, nicotinic, integrin and use receptors for entry to the brain (Sharma et al., 2019).

Dual-functionalised transferrin-penetrating liposomes have been successfully synthesized for transport across the BBB targeting transferrin receptors for treatment of neurodegenerative disease employing gene therapy. Compared to plain liposomes, transferrin-penetrating liposomes transport across the barrier layer was significantly higher ($p < 0.001$) than that of plain liposomes (Rodrigues et al., 2018). Lakkadwaka & Singh, 2019 prepared doxorubicin liposomes functionalised with transferrin for transferrin receptor targeting for penetration of the BBB. The results of the study showed that the transferrin-functionalised liposomes targeted transferrin receptors and cell penetration was improved (Lakkadwala & Singh, 2019). Choline receptors with cationic water-soluble vitamins play an essential role in the synthesis of Lecithin. Choline hardly diffuses across biological membrane and has specialised choline receptors on the BBB. Choline receptor transportation is one of the widely explored transportation mediums across the BBB, and Choline modified doxorubicin being an example (Su et al., 2019). The brain requires glucose as a source of energy to function and is

transported via Glucose receptors highly expressed on the BBB. These receptors are also used to deliver pharmaceutical products to the brain (Su et al., 2019).

2.8. Nose to Brain Strategy specific to depression therapy

It is pertinent to understand the anatomy of the nasal cavity when discussing the intranasal administration, absorption and transportation of drugs to the brain and CNS. Figure. 2.4 shows the anatomy of the nasal cavity and delivery route of neurotherapeutics to the targeted brain site. The nasal cavity is located within the skull and spans from the base of the skull to the roof of the mouth. This cavity is a midline structure that is divided into two equal halves by the nasal septum (Pardeshi, 2013). The use of intranasal route has been explored in previous studies and established to be a promising route of transferring therapeutic substances directly to brain targeted site of action by employing the trigeminal and olfactory nerve pathways through the nasal mucosa in order to bypass the BBB and its associated limitations. Intranasal administration gives advantage to drug delivery because the drug is transported directly into the blood system and the brain, as a result the first-pass metabolism in the liver is avoided and bioavailability is significantly improved (Bourganis et al., 2018).

Intranasal administration of drugs is a conducive and suitable route for delivering drugs to the brain and CNS for the treatment of neurodegenerative diseases. Lipophilic compounds penetrate rapidly and effectively passing through the nasal membranes into the systemic circulation that shows the same pharmacokinetic behaviour to that of intravenous injection in terms of absorption with bioavailability of drugs up to a 100%. Because of this rapid action, lipophilic compounds hardly pass through the olfactory pathway in the nasal cavity. However, hydrophilic compounds and larger compounds have serious BBB challenges in passing across the nasal membrane into the blood circulation for systemic delivery. It is established that the bioavailability of less lipophilic molecules and large molecules is about 1-10% (Bourganis, 2018).

The trigeminal nerve and olfactory nerve paths are an inevitable route for the transportation of neurotherapeutic compounds to the targeted brain and CNS site of action via the nasal cavity. The intranasal route of administration is used to deliver a number of medicinal formulations to the CNS. Enzymes, proteins and insulin are also delivered via the nasal mucous membrane to the brain.

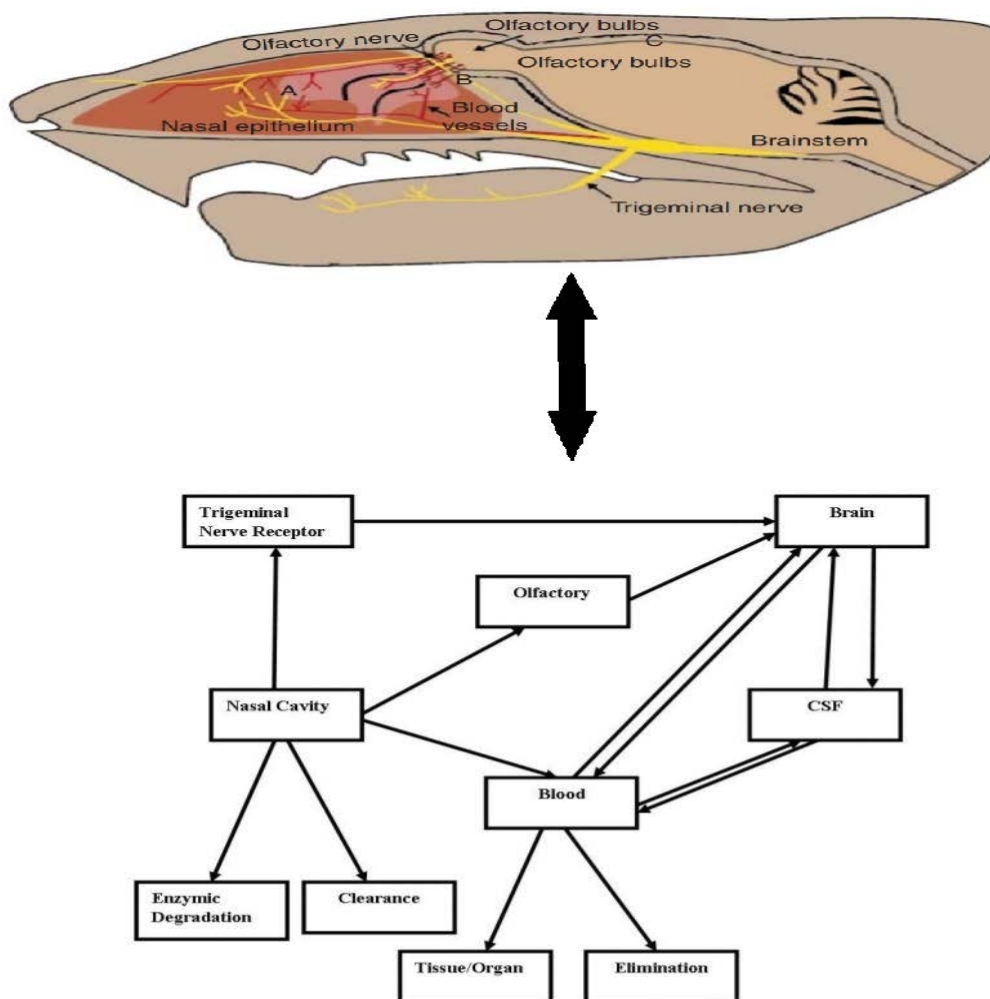


Figure 2.4: Possible drug pathways introduced into the nasal cavity and CNS, supplied with olfactory and trigeminal nerves (Adapted from Pardeshi, 2013).

Nose to brain drug delivery mediated along the olfactory and/or trigeminal nerve pathways are located at the roof of the nasal cavity as its neuroepithelium, which is the only part of the CNS that is directly exposed to the external environment. Thus, better targeting action can be achieved due to direct movement of drug from the submucosal space of the nose into the cerebrospinal fluid (CSF) compartment of the brain (Figure. 2.4). Limited research has been conducted on the olfactory or trigeminal route in humans because of the complications in quantitative measurements of drugs in brain tissues; however, several animal studies have been reported for CNS drugs namely, venlafaxine, oestradiol, zonisamide, risperidone, olanzapine, ropinirole, zolmitriptan, etc. Clusters of authors have reviewed diverse aspects relevant to nasal drug delivery systems but there are only a few reports highlighting the crucial aspects of nose to brain drug delivery and associated problems (Kozlovskaya et al., 2014).

This present review discusses advancement of collection of prominent facts that embody the potential of nasal mucosa in targeted and site specific delivery of neurotherapeutic agents, mechanisms of drug absorption across nasal mucosa, metabolism of drugs in the nasal cavity and in brain tissue, various formulation strategies for enhanced brain targeting via nasal route, physicochemical factors affecting brain uptake of neurotherapeutics, various drug delivery systems explored to date for brain targeting through the nasal mucosa route and future opportunities. Additionally, detailed emphasis is on discussion of olfactory and trigeminal nerve systems that play a major role in facilitating transportation of pharmaceutical agents via the nose to the brain. The advantages and limitations of drug delivery via the nose to brain route are presented in Table 2.4.

Table 2.4: Positives and limitations of drug delivery systems utilizing the nose to brain path (Adapted from Mistry, 2015 & Pardeshi, 2013).

Advantages	Limitations
It is a rapid, safe, non-invasive and convenient method of drug delivery	Rapid elimination of drug substances from nasal cavity due to mucocilliary clearance
It avoids drug degradation in gastrointestinal tract, particularly peptide drugs	Absorption enhancers used in formulation may create mucosal toxicity
It avoids hepatic first-pass metabolism and gut-wall metabolism of drugs, allowing enhanced bioavailability	There is a great deal of variability in the concentration attainable in different regions of brain and spinal cord
It bypasses BBB, thereby providing CNS targeted drug delivery, thereby reducing systemic exposure of drugs and associated systemic side effects	High molecular weight of drugs may result in decreased permeability across nasal mucosa
Therapeutic agent does not require any modification for being delivered via nose to brain drug delivery platform	Some therapeutic agents may cause irritation to nasal mucosa or may be susceptible to enzymatic degradation and metabolism in the nasal milieu at mucosal surface
Rapid drug absorption and quick onset of action can be achieved through highly vascularized and permeable structure of nasal mucosa	Nasal congestion due to cold or allergic condition may interfere with this technique of drug delivery
Better patient compliance, because self-medication is also possible with this route of drug administration	Frequent use of this route may cause mucosal damage like infection or anosmia
It serves as an alternative route for parenteral administration, especially for protein and peptide drugs or even for stem cells	Mechanical loss of the dosage form could occur due to improper technique of administration

Shows excellent bioavailability for low molecular weight drugs	Mechanisms of drug transport are still unclear
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2.8.1 Factors that influence nose to brain delivery

Nanoparticles with small size are transported via axons through the olfactory bulb into the olfactory cortex and to the caudal pole of the cerebral hemisphere and into the cerebrum and the cerebellum. Paths involving nerves joining the nasal passages to the brain and spinal cord are significant. Additionally, paths connecting the vasculature, CSF and lymphatic system are employed in carrying molecules from olfactory mucosa to the CNS. It is possible that a blend of these paths is responsible, however one of the paths may be the main contributor, depending on the qualities of neurotherapeutics, the attributes of preparations and the delivery tool utilized. Thus, the different modes of drug transport across the nasal olfactory epithelium include transcellular passive diffusion, paracellular passive diffusion, carrier-mediated transport, transcytosis and efflux transport (Illum, 2014). The permeability at the site where the formulation is deposited and the area of the nasal cavity exposed also affects nasal absorption of drugs. Absorption and permeability of drugs across nasal mucosa are influenced by various factors as illustrated in Figure. 2.5.

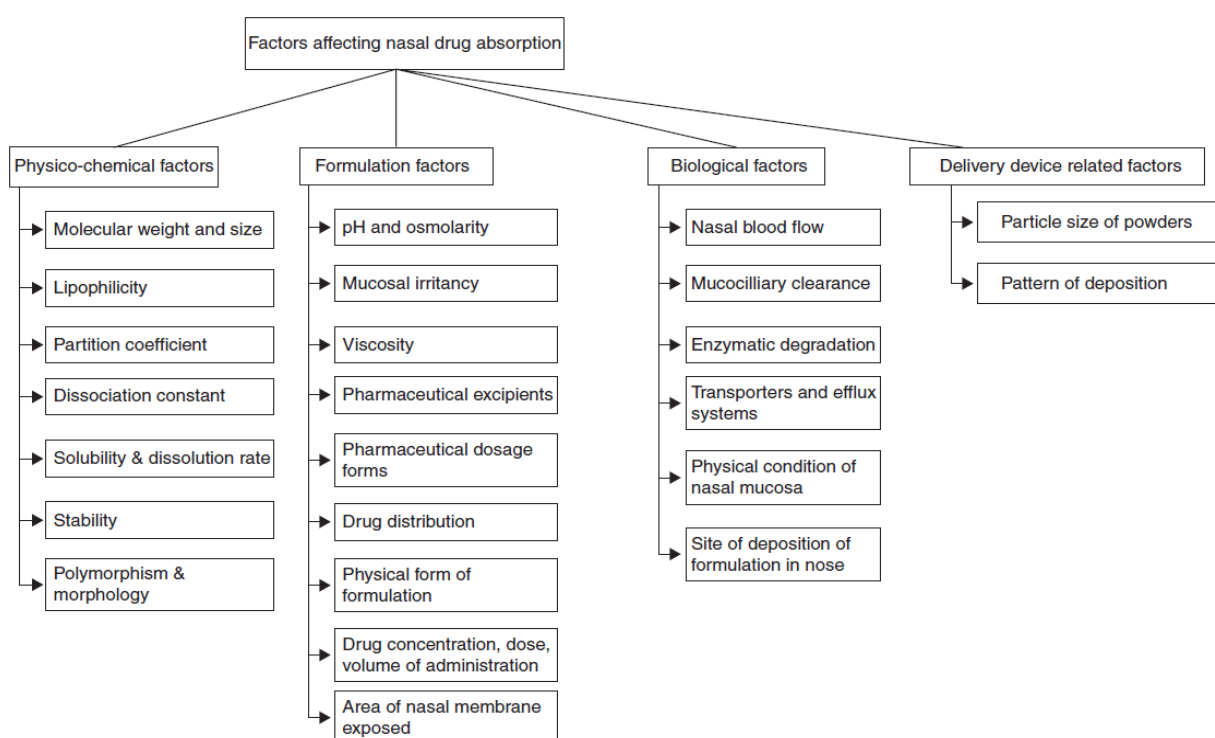


Figure 2.5: Factors that influence the absorption and permeability of drugs across nasal mucosa (Adapted from Pardeshi, 2013).

Multiparticulates, including micro- and nano-dimensional drug delivery vehicles have excellent and broad prospects in pharmaceutical field. Scientists from various disciplines are harnessed

by the superior outcomes obtained from such carrier systems viz. greater therapeutic efficacy with reduced dosing frequency. A variety of therapeutic agents loaded in various carrier systems are said to be utilizing the intranasal route of delivery for brain targeted delivery. A brief summary of drug molecules, proteins, peptides, hormone and/or biological cells including stem cells and respective drug carrier systems delivered by direct nose to brain delivery route are illustrated in Table 2.5.

2.8.2 Patents on drug delivery systems utilizing the nose to brain method

Despite the fact that many of the patents on nose to brain delivery have not reached the market, a good number of patents have been received on various drug molecules loaded in a variety of carriers to deliver pharmaceutical agents directly to the brain via nose to brain method (Table 2.5). Due to the challenges in large scale commercial production, only few patents have been transferred into commercial marketed products. Table 2.5. illustrates some nose to brain systems of drug delivery patents.

Table 2.5: Drug molecules and drug carrier systems delivered by direct nose to brain drug delivery route (Adapted from Pardeshi, 2013).

Drug	Carrier System	Problem	Outcome	Reference
Venlafaxine	Polymeric Nanoparticles	Slow onset of action, poor oral bioavailability	Quick onset of action, enhanced brain uptake	(S. Haque et al., 2012)
Oestradiol	Polymeric Nanoparticles	Nasal mucocilliary clearance, low permeability	Enhanced retention, high brain uptake	(X. Wang et al., 2008)
Zonisamide	Microemulsion	High hepatic first-pass metabolism, limited uptake across BBB	High bioavailability, high brain uptake	(A. Shahiwala et al., 2010)
Risperidone	Polymeric Nanoparticles	Poor oral bioavailability, non-targeted delivery	High bioavailability, high brain uptake	(M. Kumar et al., 2009)
Olanzapine	Nanoemulsion	High hepatic metabolism, high P-glycoprotein efflux	High bioavailability, high therapeutic efficiency	(M. Kumar et al., 2008)
Ropinirole	In situ gel	Low oral bioavailability, limited uptake across BBB	High brain uptake, high targeting efficiency	(S. Khan et al., 2010)
Zolmitriptan	Polymeric micelle	Slow onset, low oral bioavailability	Quick onset, high distribution in brain tissues, high bioavailability	(R. Jain et al., 2010)
GDF-5	Lipid microemulsion	Neuronal degeneration	Enhanced drug targeting	(L. R. Hanson et al., 2012)
Iduronidase	Solution	Inability to cross BBB	Treatment of lysosomal storage disease	(D. A. Wolf et al., 2012)
Melanocortin	Solution	Restricted BBB passage	Treatment of obesity	(M. Hallschmid et al., 2008)
HC-1	Solution	Limited BBB uptake	Brain tumour targeting	(S. V. Dhuria et al., 2009)
Insulin	Solution	Cognitive deficits	Improved memory	(C. Benedict et al., 2011)
Diazepam	Microemulsion	Poor bioavailability	High bioavailability	(L. Li et al., 2002)
MSCs	Solution	Neuronal degeneration	Neuronal regeneration	(L. Danielyan et al., 2011)

2.8.3 Limitations and Toxicity

There are several challenges facing direct nose-to-brain delivery (Table 2.4). It has been recorded that drugs arrive at the CNS and the brain following nasal delivery but the quantity of drugs recorded to have reached the brain is limited with evidence of preferential brain delivery documented limited. Current intranasal delivery systems available cannot exploit the merits of nasal drug delivery maximally due to large amounts of dose deposited at the anterior segment of the nose and thereby reducing drastically the number of drugs eventually reach the olfactory region resulting in low bioavailability. The drug remaining at the surface of the nasal cavity cause irritation and foul taste that result in patient compliance problems. Most nasal formulations are in liquid form, powder or gel. Liquid formulation is controlled by solubility, stability and dose volume. Powder and gel formulations may encounter challenges including stability, absorption across the mucosal epithelium cells, residence time and degradation. In general, nasal drug delivery systems may face problems of low bioavailability in the CNS and brain due to various factors.

2.9. Conclusion

The direction of Nanotechnology is headed towards advancement of delivery systems that provide safe and efficacious systems of delivery, that across BBB for the sufficient treatment of disorders of the CNS, in particular depression disorder. Bioavailability, minimization of adverse side effects and improved patient compliance are essentials components of drug delivery. Clinical depression management relies on an efficacious drug delivery, that will deliver therapeutic agents to the localized site of action, at required concentrations and intervals with sustained drug release profile. Nanoparticulate system of delivery is particularly advantageous, as it provides non-invasive method of delivery, enhancing treatment of CNS disease with a fewer side-effects. FDA validated polysorbate 80 that was coated with PBCA on the surface, accomplished desired action in the delivery of contrast agent that was previously unable to cross the BBB (Koffie et al., 2011). Examination of an APP/PSI mice amyloid plaques, previously injected with fluorescent agent (Alexa-488) loaded PBCA NPs, showed a presence of fluorescent signals, whereas free-Alexa-488 injected mice were found to have no fluorescent signals (Sharma et al., 2019). The success of nano technological approaches has beneficial potential in effectively decreasing the percentage of active ingredient in a formulation, and increasing drug circulatory retention time in the body. Receptor Mediated Transcytosis aid in transportation of nanostructures with specialized ligands such as Transferrin, Nicotine, and Integrin discussed under section 2.7.2, further enhance drug concentration in the CNS. Adsorptive Mediated transport which is dependent on surface charge is also responsible for successful neuroavailability of neuroactive drugs. TAT-functionalized liposomes loaded with Paclitaxel was found successful in tumour treatment of

mice (Fu et al., 2015). Nose to brain delivery also offers an innovative and alternative potential for successful delivery of pharmaceutical agents, at lower doses, avoiding the hepatic system and providing instant action on targeted brain site. It would seem that nanotechnology incorporation in drug delivery is yet in its infancy stage, but the research and studies conducted previously, present a worthwhile area of study, for safe, effective and efficient system that will revolutionize traditional medical therapy.

2.10. Future Perspectives

Enhanced mastery of processes of BBB cellular ingestion, and requirements in absorption of nanostructures will provide a foundation for further transformation and understanding of process design of nanoformulations to overcome the BBB. Toxicity profiles of nanotechnological entities should be incorporated in future studies when consideration is made for upscaling and commercial development. Additional studies into nano-formulation long-term effects on the brain is a critical consideration and should be included in future developments. The above mentioned will required diagnostic measures of safety and toxicity over a long period of time. While nanotechnological developments are yet at infancy, there is enough evidence to prove its advantages and useful benefits that investing in nanotechnology has untold potential enhancing the lives of many that are suffering from debilitating mental disorders.

CHAPTER 3

DESIGN AND SYNTHESIS OF A NANOPARTICULATE SYSTEM FOR ORAL DELIVERY OF DULOXETINE TO THE BRAIN

3.1 Introduction

Depression is a mental disorder in which patients experience chronic symptoms of low mood, decreased interest in previously enjoyed activities, loss of appetite, insomnia, exhaustion, and more severely suicidal thoughts (Denning et al., 2016 and Akechi et al., 2019). In depression disorder patients often require pharmacological treatment for at least 6-9 months (Akechi et al., 2019 and Sakurai et al., 2020). Duloxetine (DLX) is a novel antidepressant from the class of SSRIs used as first line treatment of depression disorder and it functions in the brain to inhibit reuptake of Noreadrenaline and Serotonin to alleviate symptoms of depression (Paulzen et al., 2016). DLX has an edge over other existing antidepressants including amitriptyline, citalopram, clomipramine, dosulepin and doxepin due to its favourable pharmacodynamic features such as dual inhibition, improved efficacy, tolerability, safety, faster recovery, fewer side effects, and low affinity for other neuronal receptors (Posadas et al., 2016). In this study DLX was used as the model antidepressant drug for the treatment of depression disorder.

However, because of the systemic use of the duloxetine, side effects such as liver damage, weight gain, drowsiness and fatigue, dry mouth, altered sexual function are experienced (Chahal et al., 2020). This often results in patient non-compliance, and possibility of relapse (Boisseau and Loubaton, 2011 and Akechi et al., 2019). DLX is susceptible to hydrolytic degradation which is more pronounced at low pH values. DLX formulations that are on the market, are largely metabolised in the liver leading to low bioavailability, ranging from 30 – 80% . A steady-state plasma concentration of drug is desired for effective treatment of any disorder. Three times daily dose of DLX may cause fluctuation in the plasma concentration with reduced patient compliance due to conventional multiple daily dosing (Akechi et al., 2019).

Approximately 90% of active pharmaceutical excipients are administered via the oral route. The oral route is preferred because of its advantageous attributes such as patient compliance, cost effective, does not have sterility restrictions, and most convenient especially for chronic treatment of disease (Patel et al, 2018). But the oral route of delivery is not always favourable, as some therapeutic agents encounter challenges of dissolution, permeability, and solubility

in the gastro-intestinal tract (Viswanathan et al, 2017). Other drugs are not stable in low pH conditions and experience degradation, even after absorption some drugs face substantial first pass metabolism in the liver. All these factors adversely affect the bioavailability and control the efficacy of the therapeutic agent. Nanomedicine provides an opportunity to improve the delivery efficiency of orally administered drugs.

Numerous nanotherapeutic delivery strategies for brain targeting have been developed including branched nanoemulsion, dendrimers, liposomes, polymeric micelles, nanogels, nanocapsules, vesicles, and carbon nanotubes. These nanoparticulate have proven to be very successful in transporting drug molecules across the blood-brain barrier due to their small size less than 200nm with improved solubility of drug, absorption and assist in decreasing drug related side-effects (Khosa et al., 2018). With this nanotechnology there is a great potential to improve drug delivery as it can facilitate targeted drug delivery, controlled released characteristics, improves stability of drugs.

The purpose of this study was to synthesize duloxetine (DLX) loaded eudragit-chitosan (Eud-CHT) nanoparticles, encapsulated in gelatin capsules and to evaluate their potential for transport of Duloxetine to the brain by oral delivery route for improved efficacy with reduced side effects. Nanoparticles have shown to be a promising drug delivery vehicle for therapeutic agents to the brain (Maksimenko et al., 2019). Moreover, polymeric nanoparticles have shown to improve bioavailability, form a protective barrier against drug degradation, facilitate sustained release of drug molecules and improve penetration at cellular level (Summerlin et al., 2015). CHT is a natural carbohydrate-based polymer acquired from a variety of living organism, such as insects, fungi, animals etc. CHT is non-toxic, biodegradable, has controlled release properties (Xu et al., 2018 and Patel et al., 2015). Eudragit RL100 is a copolymer consisting of methacrylic acid and methyl methacrylate polymers. This polymer is one of the polymers that are FDA approved for use in therapeutics. (Dong et al., 2019). Eudragit is also non-toxic, bio-adhesive, bio-degradable with controlled release properties. Eudragit has a positive charge which provides advantageous interactions with the negatively charged cell surfaces of targeted tissue increasing cellular uptake of nanoparticles. (Adiblela et al., 2011). Incorporation of duloxetine in the nanoparticulate formulation possesses a great potential for improving solubility and absorption of the duloxetine through the GIT wall, the nanoparticulate structure also protect duloxetine from acidic conditions in the stomach. DLX loaded Eud-CHT nanoparticles formulation was by solvent emulsion evaporation method and synthesis confirmed by physicochemical properties including Scanning Electron Microscopy (SEM), Fourier Transformation Infrared Spectroscopy (FTIR), zetasizer, Ultraviolet (UV) spectroscopy, Thermogravimetric Analysis (TGA) and Differential Scanning Calorimeter

(DSC) for thermoanalysis. The synthesized DLX-Eud-CHT nanoparticles were subjected into three pH environments simulating the stomach pH 1,2, intestinal pH 6,8 and physiological pH 7,4. Drug release was determined by dissolution and analysed by UV spectroscopy, and thermal analysis by Thermogravimetric analysis and Differential scanning calorimetry were conducted. In addition, HEK 293 neural cells were treated with DLX loaded Eud-CHT nanoformulation and evaluated for cytotoxicity utilising a 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide dye (MTT) assay and absorbance measured at 570 nm employing a Thermo Lab systems Multiskan MK3 microplate reader.

3.2 Materials and Methodologies

3.2.1 Materials

Eudragit RL-100 (Eud), polyvinyl alcohol (PVA) (Av. Mw 30 – 70 kDa), chitosan (CHT) (medium Mw; 450 kDa), HPLC grade acetone, acetonitrile, acetic acid, chloroform, hydrochloric acid, sodium phosphate, Dimethyl sulfoxide, Fetal bovine serum (FBS), 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide dye (MTT), 0.25% w/v trypsin and mannitol were purchased from Sigma (Sigma-Aldrich, Missouri, USA). Human Embryonic Kidney (HEK) 293 cells (CRL# 1573, passage 21, Batch # 203970) were purchased from the American Type Culture Collection (ATCC) (Rockville, MD, USA) and Duloxetine HCL was purchased from Sigma (Sigma-Aldrich, Missouri, USA). All reagents and chemicals used were of high analytical grade.

3.2.2 Synthesis of the Eud-CHT-DLX Nanoparticles

The Eud-CHT-DLX nanoparticles were prepared using the method of single emulsion solvent evaporation (Figure. 3.1) from Wang et al., 2013. 100mg of Eudragit was weighed and mixed with 2.5 mL of acetone until it dissolved, then mixed with 10mg of Duloxetine HCL and added to an aqueous phase of 0.5% acetic acid solution, which contained PVA (0.5 % w/v) and CHT (0.5% w/v). The resultant emulsion was then vortexed for 10min and then sonicated. The rotary evaporation and vacuum method was used to remove the acetone. Ultracentrifugation (was conducted to the mixture to obtain the nanoparticles using the Beckmann coulter, USA at 4 °C for 18min per 23,000g. The downstream processing procedure of centrifugation, washing, and lyophilization was followed as per the emulsification solvent evaporation protocol (Wang et al., 2016). The concentrated nanoparticles were freeze dried using mannitol as to protect from damage from freezing, (2.5% $\frac{w}{v}$) and to ensure stabilization (Szymusiak et al., 2016; Tong et al., 2017 and Chaves et al., 2018). The freeze-dried nanoparticles were subsequently lyophilized (FreeZone® 2.5, Labconco®, Kansas City, MS, USA) at -80 °C for 48 h to yield a powder.

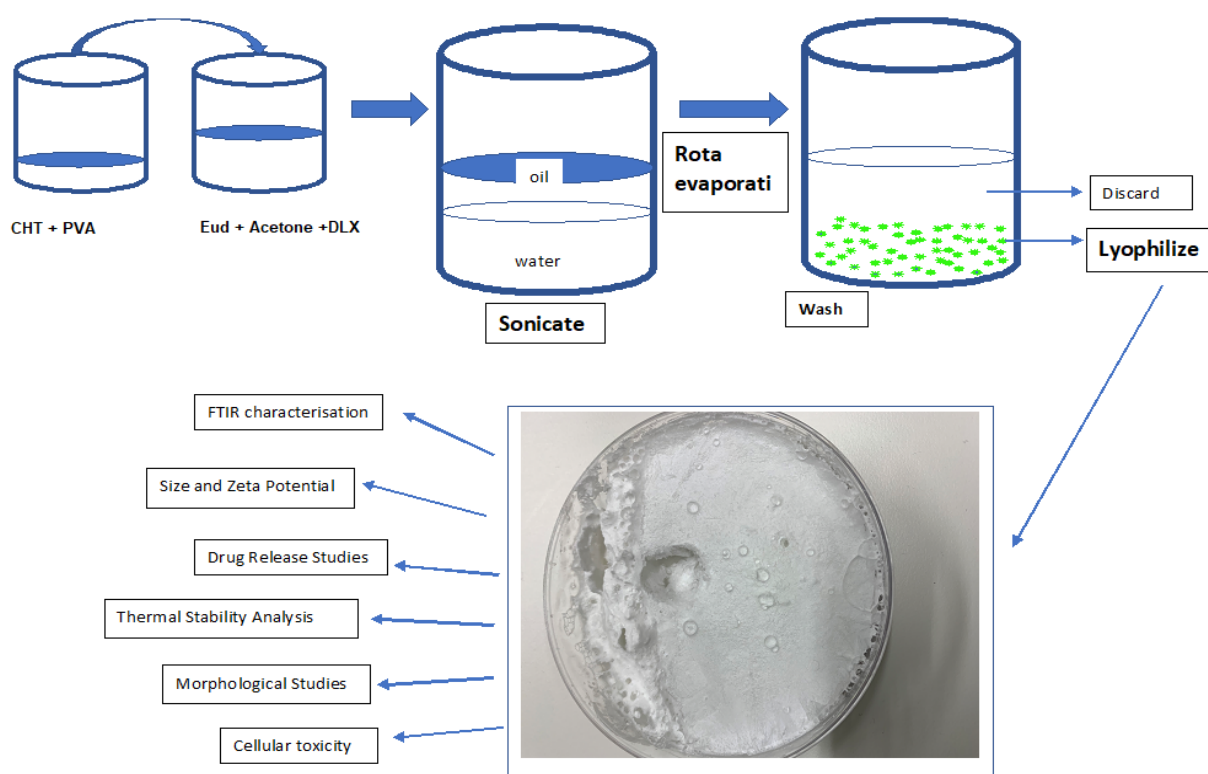


Figure 3.1: Schematic illustration of solvent emulsion evaporation synthesis and physicochemical characterization of duloxetine-loaded Eud-CHT nanoparticulate system.

3.2.3 Physicochemical analysis using Fourier Transformation Infrared (FTIR) spectroscopy

The native polymers and different nanoparticle formulations were analyzed using Attenuated Total Reflectance Fourier Transformation Infrared (ATR-FTIR) analysis to evaluate the chemical and stability of the nanoparticles. Characteristic peaks were compared to confirm the molecular vibrations of the polymers in the formulations. The Perkin Elmer Spectrum 2000 FTIR Spectrometer was employed, applying a single-reflection diamond MIRTGS detector (Perkin Elmer Spectrum 100, Llantrisant, Wales, UK). Samples were analysed at a resolution of 4 cm^{-1} using universal ATR polarization accessory. Samples of each polymer, duloxetine HCL and synthesized nanoparticles were placed on a diamond crystal and each ran at scan rate of 100 order to minimise signal to noise ratio, in the wavelength range of $4000 - 600\text{ cm}^{-1}$ at a constant pressure of 120 psi. (Kondiah et al., 2013).

3.2.4 Determining Particle size and zeta potential of copolymeric nanoparticles

Evaluation of zeta potential of nanoparticles and particle size distribution were conducted using a Zetasizer NanoZS analyser (Malvern Instruments Ltd. UK) that functions by dynamic light scattering. Light scattering angle of 90° and a temperature of 25°C was utilized. The nanoparticles were dissolved (1mg of each formulation in 1mL of Millipore water). Samples were diluted and sonicated before measurement, with the sample temperature maintained at 25 °C throughout the analyses. A 2 mL sample of the nanoparticle suspension was then filtered through 0.22 µm filter and placed in disposable cuvettes. Particle size, polydispersity index and zeta potential values were analyzed to screen formulations and select the most favorable preparation parameters such as the organic solvent, the concentration of stabilizer and sonication for the emulsification solvent evaporation approach.

3.2.5 SEM Morphological of DLX-Eud-CHT nanoparticles

The Eud-CHT-DLX nanoparticles were evaluated using the JEOL 840 SEM equipment (JEOL, Japan), under various magnifications at 20keV. The dry formulation, in lyophilized form, was loaded on studs and sputter-coated with gold-palladium shadowing. Micrographs of the formulation were then critically evaluated, determining the surface morphology and texture of the synthesised nanoparticles.

3.2.6 Thermal analysis using Differential Scanning Calorimetry and Thermogravimetric Analysis

The Eud-CHT-DLX nanoparticles were analysed by Differential Scanning Calorimetry (DSC), for evaluation of glass transition temperature and melting point of the copolymer. Data was collected using the Mettler Toledo DSC-1 STARE system, heated from 25-300°C, at a heating rate of 10°C per minute, resulting in thermograms of heat flow versus temperature. Thermogravimetric analysis (TGA) was conducted on the Eud-CHT duloxetine loaded nanoparticles, 10mg of sample was heated at 10 °C/min in an open aluminium pan, using nitrogen as a purge gas (flow rate of 25 mL/min). The percentage copolymer mass loss over temperature heating conditions was evaluated. (Nasef et al., 2017).

3.2.7 Optical Characterization and Stability of Eud-CHT-DLX nanoparticles utilizing the Turbiscan

Optical interactions and formulation stability of the formulated nanoparticle suspensions were investigated utilizing the Turbiscan™ LAB (Formulation, L'Union, France). A 20-mL aliquot of Eud-CHT-DLX nanoparticles and Duloxetine-free nanoparticle suspensions were

introduced into specialized sample holders. The Turbiscan measures stability based on turbidity with transmission light or back scattering light transmitted from a sample (Gagliardi et al., 2019 and Sumaila et al., 2019). The turbiscan can scan the entire length of a sample and scans at 40µm intervals. 10mg of Eud-CHT-DLX nanoparticles and Duloxetine free nanoparticles were dissolved in buffer solution in their respective beakers. Samples were analysed for 60min utilizing the Turbiscan TM LAB. Backscattering results were used as a measure of stability.

3.2.8 Establishing the loading efficiency of DLX inside the nanoparticulate formulation

A mass of 15 mg of Nanoparticulate formulation was weighed, and mixed in phosphate buffer saline (10mL) at pH of 7 and temperature of 37°C. The suspension was then placed for 60 min at speed of 10 000 rpm under centrifuge. The resultant solution was filtered through a 0.22 mm Millipore filter for removal of residue that may have not dissolved. (Tong et al, 2017). The Calibration curve (Figure. 3.2.) was used to determine drug entrapment efficiency (DEE), at 290 nm utilizing the calculated linear Equations (1 & 2) where $r^2 = 0.9962$. The specific absorptivity can be used on the determination of drug concentration of released using the constructed standard calibration curve relating absorbance to concentration by Beer Lamberts Law of linearity (Equation 1).

$$y = mx + C \quad (1)$$

Equation 2: Duloxetine Concentration

$$\text{Concentration of Duloxetine} = \frac{y}{m} - b \quad (2)$$

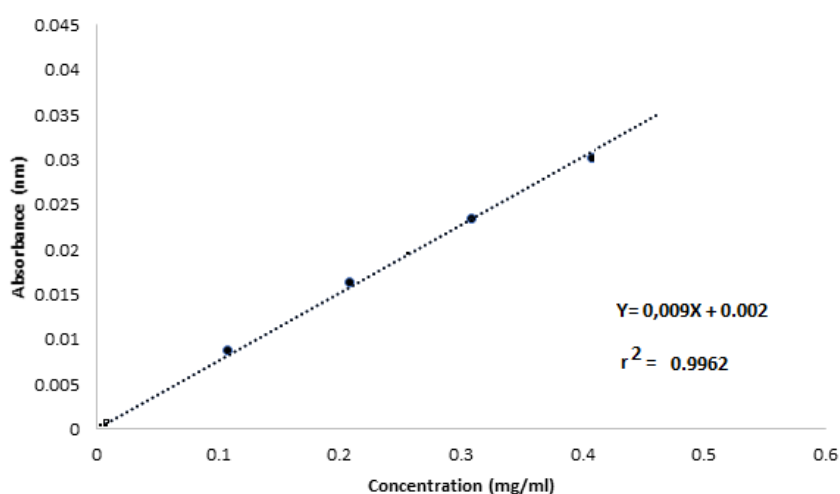


Figure 3.2: Calibration curve of Duloxetine Hydrochloride in 0,1 N HCL using Cecil 3021 scanning spectrophotometer at 303 nm (All instances: n = 3 and SD < 0.025).

The DLX-loading capacity (%^{w/w}) was calculated using Equation 4. All measurements were conducted in triplicate.

Equation 3: Drug Entrapment Efficiency

$$DEE(\%) = \frac{\text{Mass of DLX in nanoparticles}}{\text{Mass of Nanoparticles}} \times 100 \quad (3)$$

Equation 4: DLX loading Capacity

$$DLX \text{ Loading } (\% \frac{w}{v}) = \frac{\text{Mass of DLX in Nanoparticles}}{\text{Mass of Nanoparticles}} \times 100 \quad (4)$$

3.2.10 *In vitro* drug release analysis of the copolymeric nanoparticles containing Duloxetine HCL

In vitro release studies of duloxetine from the Eudragit-chitosan nanoparticles were determined by using a USP 2 apparatus (ERWEKA, USP Apparatus II, DT 126, UK) operated at a paddle revolution of 50 rpm over a 24-h period. 30 mg of Duloxetine-loaded Eudragit-chitosan nanoparticles was weighed and encapsulated into hard gelatine capsules. 300 mg of capsules were placed into vessels containing 900 mL PBS buffer of three pH mediums, pH 1.2 simulating stomach gastric fluid, pH 6.8 simulating the small intestinal environment and pH 7.4 for human physiological settings with temperature at 37°C. Subsequently, to exposure of samples to the dissolution media separately for 2 hrs. 5 mL of sample in triplicate was collected at predetermined intervals time 0 min, 5 min, 30 min, 60 min, 1hr, 2hrs, 4hrs, 8hrs until 24 hours. At each time interval of sampling, the sample quantity (5mL) in triplicate was pipetted out and replaced with the same quantity of buffer medium to maintain sink conditions. UV spectrophotometer was used to measure the absorbance at wavelength of 290 nm (Methuku et al., 2018). The DLX stock solution was prepared by dissolving duloxetine HCL (10 mg) in 20% Acetonitrile in 100 mL volumetric flask. Serial dilutions of 1- 5 mL were prepared from stock solution and made up to 50 mL with stock solution (Nasef et al., 2017; Methuku et al., 2018). Absorbance values were measured at the obtained maximum wavelength of 290 nm for duloxetine and the calibration curve obtained in Figure 3.2, with release curve in Figure 3.14 showing the concentration of Duloxetine at different time intervals, each point depicted as mean ± SD (n=3).

3.2.11 Kinetic modelling of drug release of Eud-CHT-DLX Nanoparticles *In vitro*

Drug release kinetics were determined using Zero order release model, first order release kinetics, Fick's law of diffusion, Korsmeyer-Peppas's equation, and Hixson-Crowell equation,

(Equations (5) - (9)). Statistics and Arithmetic were determined using Systat Sigma Plot software 12. The zero-order model (Equation (5)) represents a stable release process.

$$Y = a_0 + K_0t \quad (5)$$

Equation 6 (first-order release kinetics) defines the spread of the drug within the nanoparticulate structure with release rate of drug correlating with its concentration.

$$\text{Log } Y = \text{Log } a_0 + K_1t \quad (6)$$

Fick's law of diffusion (Equation (7)) gave way to the establishment of Higuchi's square root of time model, for determination of drug release from a complex network.

$$Y = a_0 + Kt^{1/2} \quad (7)$$

For authentication of the therapeutic release steps, the initial 35% of released therapeutic was substituted in Korsmeyer-Peppas model (Equation (8)).

$$F = \frac{Mt}{M^\infty} = Kt^n \quad (8)$$

where Mt/M^∞ represents fraction of released drug at time t, Mt represents the amount of released drug at time t, M^∞ represent the maximum amount that can be released, t represents the time period of release in hours, k represents the kinetic constant, and n represents the release exponent. There was an expectation of nanoparticle diameter increase due to DLX incorporation therefore the Hixson-Crowell law (Equation (9)) was used to describe the release of drug by dissolution, impacted by surface area and diameter of nanoparticles changes.

$$\sqrt[3]{Qa} - \sqrt[3]{Qa} = K_1t \quad (9)$$

The highest R^2 value determined from the kinetic model was chosen to represent the most appropriate model for authenticating the release of DLX from the nanoparticles. There are different types of release profiles namely Fick's diffusion, anomalous diffusion or erosion. The release exponent value (n) for Korsmeyer-Peppas law was using in determining the nature of release from the nanoparticles.

3.2.12 *In vitro* cytotoxicity studies of the Eud-CHT-DLX nanoparticles utilizing HEK 293 cells

Cellular toxicity studies were conducted utilizing HEK 293 cells and MTT assay method (Jafari et al., 2016). HEK 293 cells were subcultured in 96-plate wells, at a seeding density of 695000 cells/mL in trypsin medium. The cells (HEK 293) remained at 37°C and CO₂ of 5 % in a controlled incubator. Treated of the cells was performed with three variant concentrations of Duloxetine HCL, Eud-CHT-DLX nanoparticles and blank nanoparticles. The treatment period was 48 hours, and after which there was a removal of the culture medium, then substituted with fresh medium and MTT reagent, further incubated for 4 hours. Before crystallized all medium was aspirated, and Dimethyl Sulfoxide (DMSO) was added to solubilize the formazan crystals. Absorbance was then measured at 570nm utilizing the Thermo Lab systems Multiskan MK3 microplate reader (Pantshwa et al., 2017). Percentage cell viability was calculated using quantity of cells counted over the count of quadrants, shown in Equation 10&11. DF represents dilution factor that was used and 10⁴ is the constant.

Equation 10: Calculation of viable cell number

$$\text{Total Cell Number} - \text{Dead Cell Number} = \text{Viable Cell Number} \quad (10)$$

Equation 11: Calculation of percentage cell viability (11)

$$\% \text{ Viable cells} = \frac{\text{Number of cells counted}}{\text{Number of Quadrants counted}} \times DF \times 10^4 \text{ cells/ml}$$

3.2.12 Statistical Analysis

Data for the preparation and characterization of nanoparticles and drug release studies were processed and analyzed by Origin software (version 8.5.0 SR1, Origin Lab Corporation, Northampton, MA, USA). All results were represented by the mean ± standard error of three experiments.

3.3 Results and Discussions

3.3.1 Characterization of the synthesised nanoparticulate formulation utilizing FTIR spectroscopy

The molecular composition and characteristics of the Duloxetine-Eudragit-Chitosan (DLX-Eud-CHT) nanoparticles were evaluated for their chemical characteristics. FTIR spectra of starting material eudragit, chitosan and combinational blank Eud-CHT nanoparticles are illustrated in Figure. 3.3. Comparable peak in Eud and Eud-chitosan of methylene stretching due to ester bonds is also observed at 2952 cm⁻¹. Figure. 3.4 illustrates the FTIR spectra of

the blank nanoparticle, plain DLX and DLX-Eud-CHT nanoparticles. FTIR Spectral evaluation of Duloxetine HCL and Duloxetine loaded nanoparticles shows comparable peaks at 1281 cm^{-1} and 1724 cm^{-1} . The first peak can be attributed to C-O vibration, while the second peak⁻¹ is attributed to -CO stretch. A peak at 3328 cm^{-1} is observed and attributed to N-H stretching of Duloxetine HCL. CH_2 bending can be observed at peak 1324 and 1321 cm^{-1} . The results observed were found to be in correlation with those concluded in studies by Sindhu et al., 2018 and Mani et al., 2014. Comparison of Eud-CHT-DLX nanoparticles and Chitosan (Figure. 3.3) depicts similarities in peaks at 1377 and 1374 cm^{-1} for C-H bending, 1147 and 1144 cm^{-1} for C-O-C stretching band and 888 and 892 cm^{-1} for C-N vibration of Chitosan (Menezes et al., 2019). Comparing Eudragit FTIR spectra, depicted in Figure. 3.3, and the FTIR spectra for nanoparticles (Figure. 3.4.), they thus displayed distinct peaks noted at 2936 and 2952 cm^{-1} attributed to the C-C chains stretching vibration, 1451 cm^{-1} and 1448 cm^{-1} for the OR-C=O group, and 1147 and 1150 cm^{-1} for stretching of the ester from the eudragit. (Guo et al., 2017 and Mohammadi et al., 2017). The results of the FITR characterisation thus, indicate the synthesis of the complexed nanoformulation.

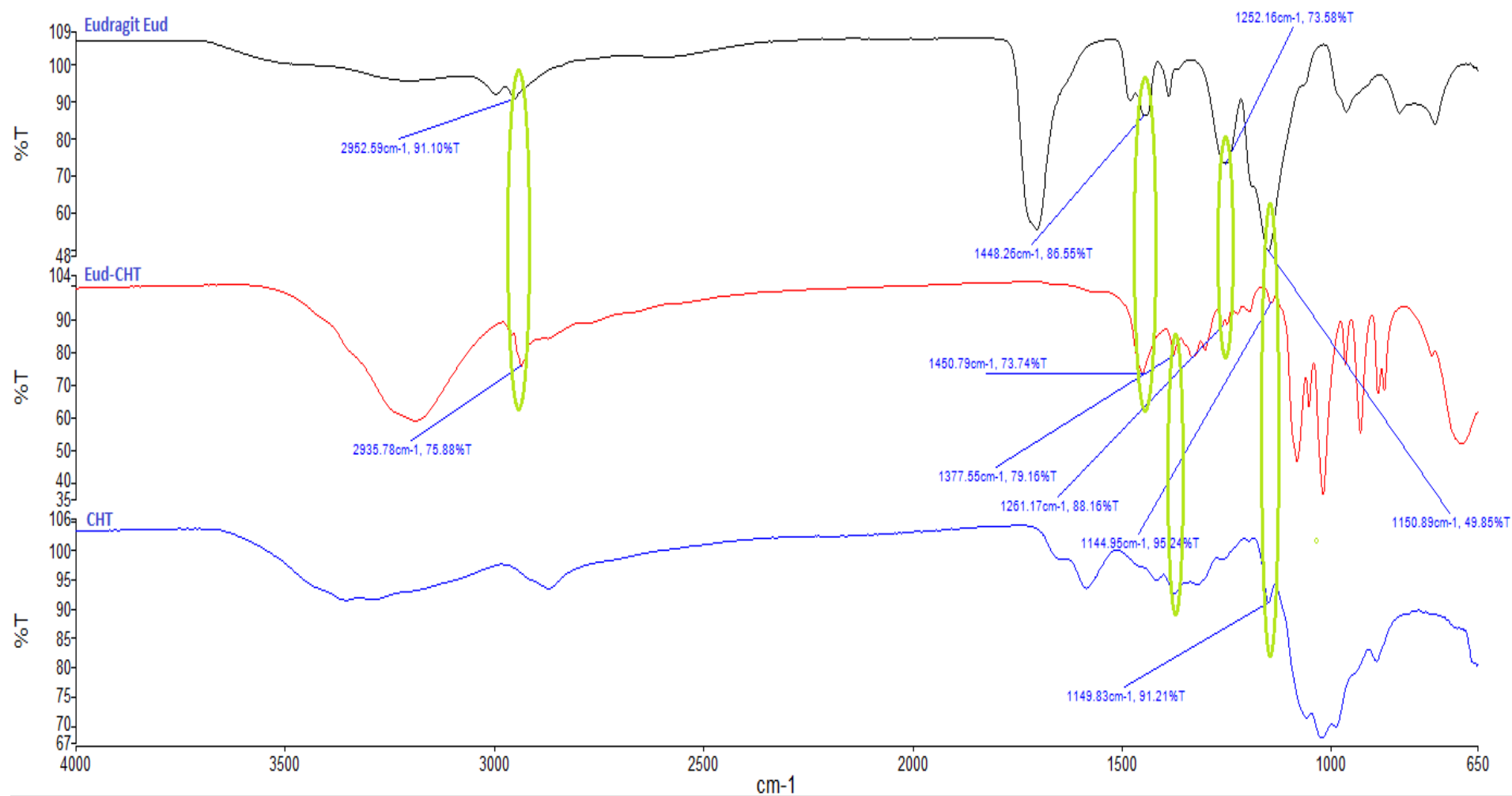


Figure 3.3: Fourier Transformation Infrared Spectra of eudragit RL-100 (Eud), chitosan (CHT) and blank Eud-CHT nanoparticles.

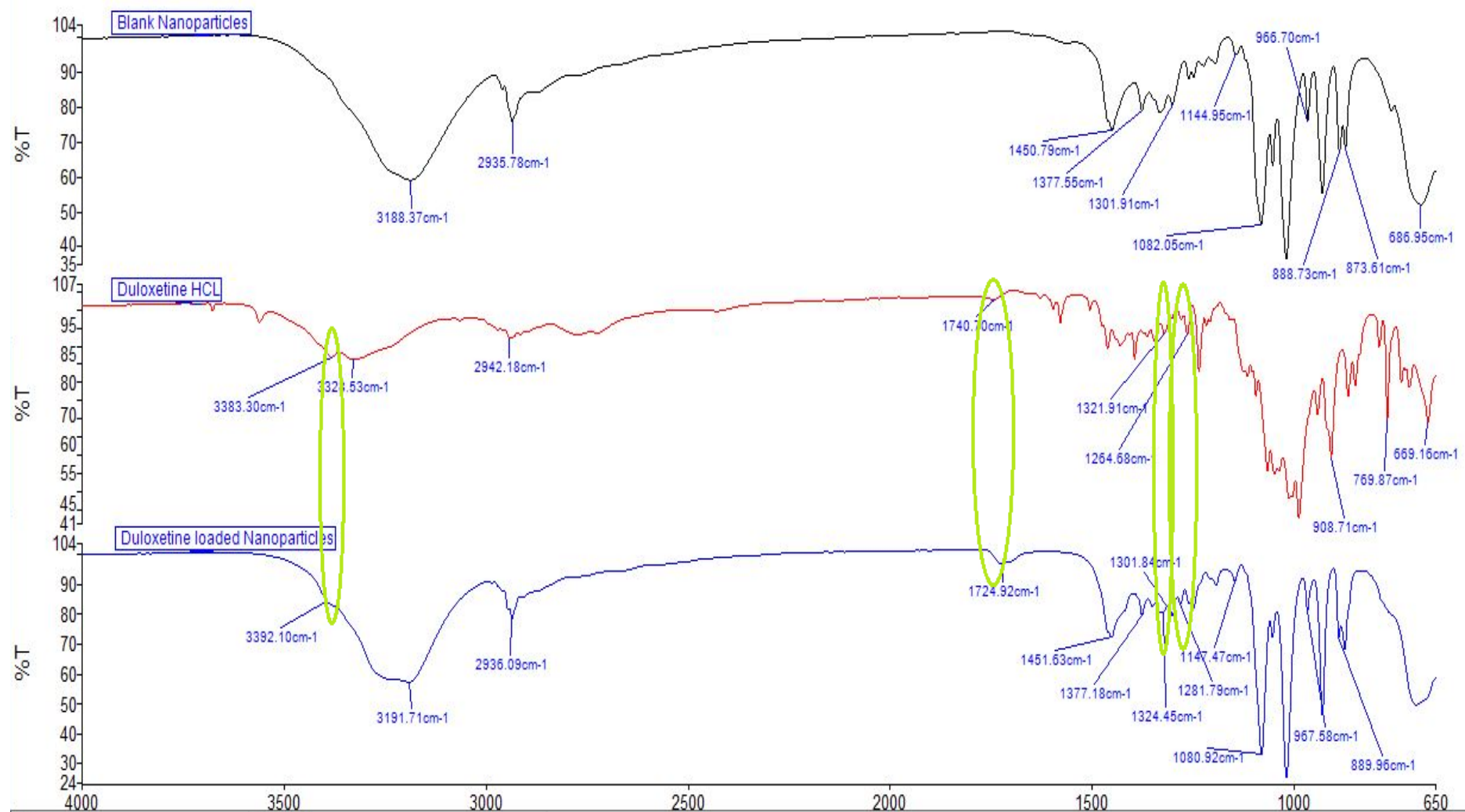


Figure 3.4: Fourier Transformation Infrared Spectra of synthesised blank nanoparticles, Duloxetine HCL and Eud-CHT-DLX Nanoparticles.

3.3.2 Eud-CHT-DLX nanoparticles size, zeta potential and SEM morphological evaluation

The nanoparticles both Placebo/blank and Duloxetine loaded were evaluated for size of nanoparticle and zeta potential employing the Zetasizer NanoZS analyser (Malvern Instruments Ltd. UK). Figure. 3.5(a) illustrates the particle size and distribution of the Placebo nanoparticles, with particle size of 100 nm with polydispersity index (PDI) of 0.445. PDI less than 0.5 indicates good and narrow size distribution that is, perfectly uniform/homogenous sample dispersion and stability with respect to the particle size. The observed particle size for Duloxetine loaded nanoparticles, in Figure. 3.5(b), is 106 nm with PDI of 0.283. The average particle size for drug loaded nanoparticles was comparable to placebo nanoparticles, regardless of Duloxetine HCL loading. Particle size of < 200nm is favourable for BBB crossing (Zaman et al., 2018). Surface Morphology was investigated and characterized utilizing SEM. The particle size compared with SEM images (Figure. 3.7), shows spherical shaped particles with a narrow size distribution and particle size less than 100nm. This observation shows a corresponding result compared to Zetasizer. The nanoparticles also show various agglomerated particles, this indicates good interaction between the nanoparticles. The observed zeta potential for placebo and drug loaded nanoparticles (Figure. 3.6 a(ii) & b(ii)) was $-10 \pm 3.26\text{mV}$ and $-11.5 \pm 2.79\text{mV}$ indicating zeta potential standard less than -25 mV with high degree of stability and decreased potential for flocculation (Sindhu et al., 2018 and Tong et al., 2017).

Concentrations of the polymers used also affected level of stability due to interference of cohesion (Keck and Muller, 2006). Although aggregation of nanoparticles was also observed to be dependent on the concentration of nanoparticles used during characterization and application (McNally et al., 2003), at high concentration nanoparticle aggregated whilst small concentrations were evenly dispersed as shown with SEM image (Figure. 3.7). The chitosan coating provided a positive charge which favors increased interaction with negative cell membranes. The degree of nanoparticle adhesion corresponds to the amount of chitosan present in the formulation and it has been demonstrated that higher ratios of chitosan: Eud leads to increased GIT wall uptake of the nanoparticles which in turn results improved neuro-availability(Keck and Muller, 2006) .

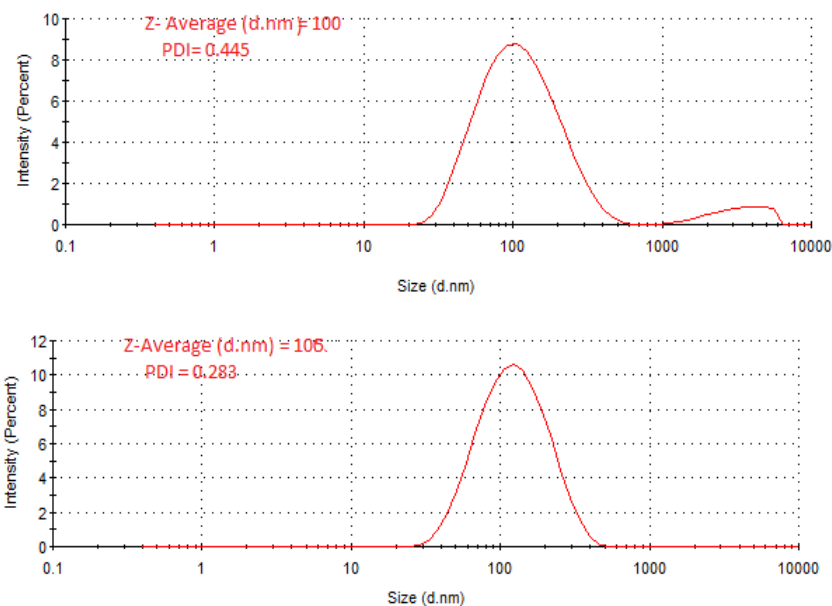


Figure 3.5: Particle size distribution graph of a(i) Placebo nanoparticles and b(i) Duloxetine loaded nanoparticles

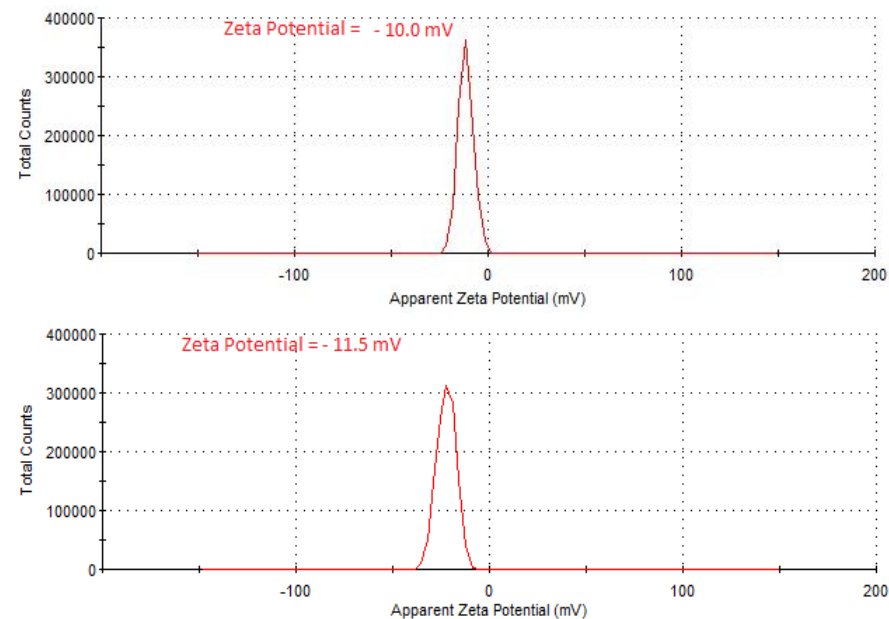


Figure 3.6: Zeta potential graph of a(ii) Placebo nanoparticles and b(ii) Duloxetine loaded nanoparticles.

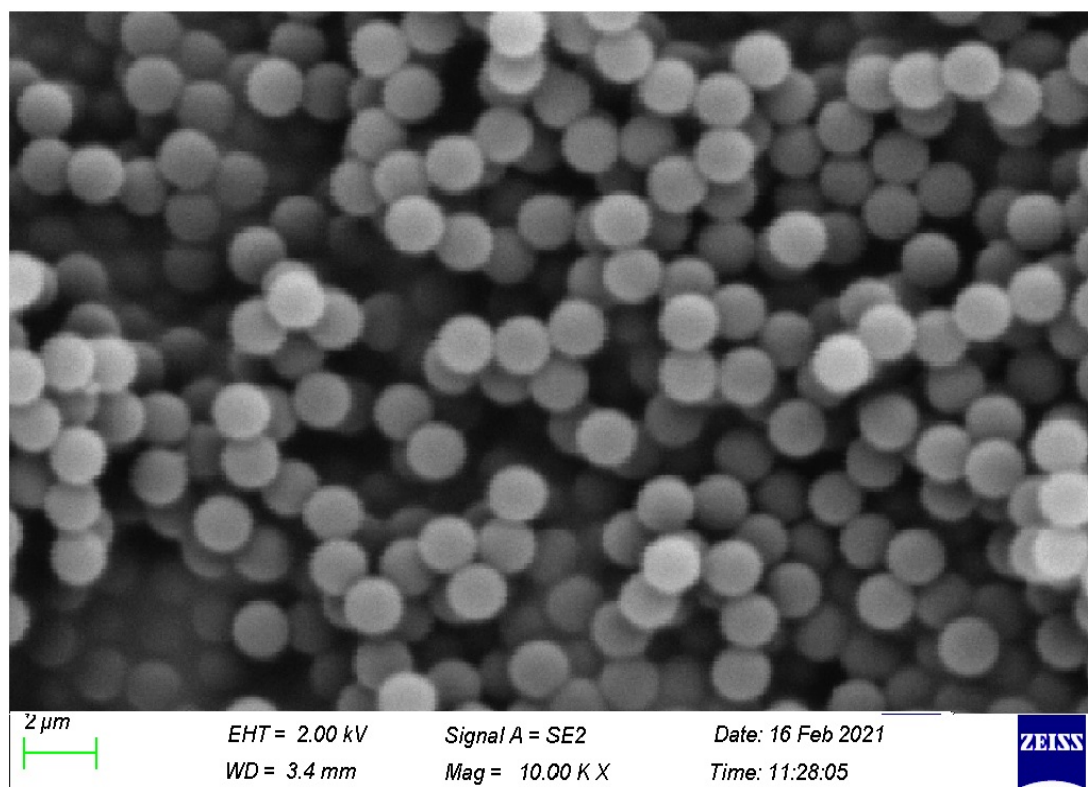


Figure 3.7: Illustration of scanning electron micrograph (SEM) image of self-assembled Eud-CHT-DLX nanoparticulate formulation in dry state.

3.3.3 Thermogravimetric analysis

Thermal stability studies were conducted as evaluation of integrity and changes in the mass of the polymeric nano-formulation during application of thermal stress (Jothimani et al., 2017). Duloxetine is known to be thermally stable; analysis was conducted to examine the magnitude of change in thermal stability properties of duloxetine incorporated in the nano-formulation. Utilizing the TGA, the thermal degradation of Duloxetine-loaded Eudragit-Chitosan (DLX-Eud-CHT) nano-formulation, generated the thermogram showed in Figure 3.8 a (red solid). The Eud-CHT-DLX nano-formulation TGA thermogram illustrated a one-step degradation with weight loss of 62% on heating from 36.2-907.5°C. However, its related derivative TGA (DTGA, blue) demonstrates a degradation in two distinctive steps shown by DTGA peaks at 68.1°C and 310°C, respectively. These points correspond to the points of inflection on the nano-formulation TGA thermogram.

The thermograph for model drug Duloxetine HCL shows a two-step weight loss, beginning at 40 to about 910°C, showing in the DTGA graph at peaks 300°C and 410.70°C, the first and second step, which can be concluded that it may have been the reason for the first and second nano-formulation degradation steps (Figures 4.9a and 4.9b). Degradation of Duloxetine started at the extrapolated onset of 300°C then the second degradation step began before the first degradation step ended. The extrapolated end of the degradation of Duloxetine was at 410°C and the total Duloxetine weight loss on heating from 40-910°C was 77.935%. Blank nanoparticles show a two-step weight loss and degradation, the onset at 190-210 °C with total loss of 85% of weight, which is attributed to the copolymeric structure degradation, and next between 210-250°C. Comparison of DLX loaded and blank nano-formulation show a difference of a one-step degradation at 330 – 450°C, which can be attributed to the inclusion of the drug in the formulation. A similar result of a two-step degradation of Duloxetine, between 200 and 400°C was found during a study conducted by Mani et al, 2014. The initial loss is associated with the melting point of Duloxetine HCL at 118 - 162°C (Chahal et al., 2020). The inclusion of Duloxetine into the nanoparticulate structure showed enhancement in the thermal stability of Duloxetine (Akhtar et al., 2020).

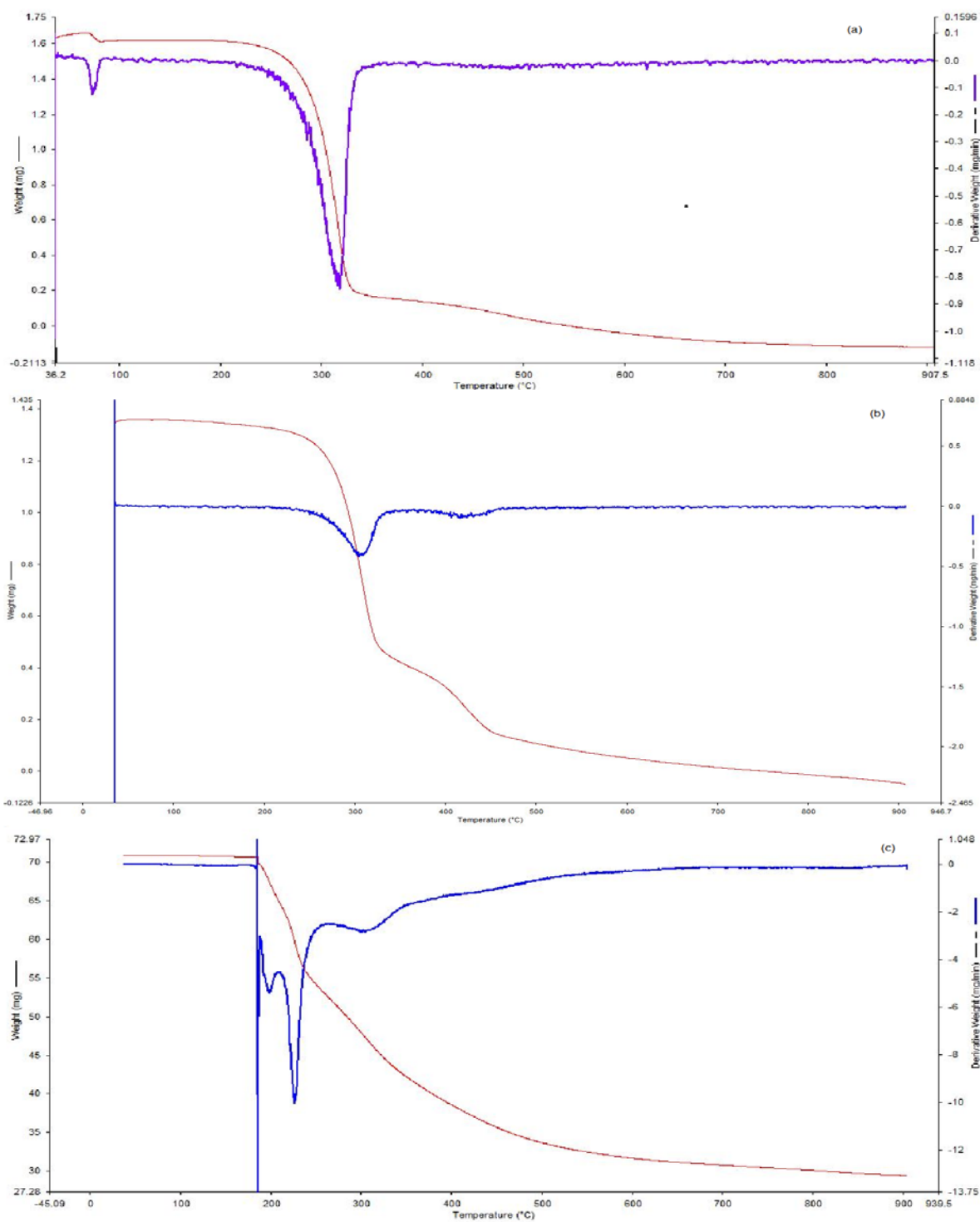


Figure 3.8: Thermogravimetric curves (TGA in red) and derivatives (DTGA- blue) depicting the stability and thermal degradation of (a) Eud-CHT-DLX nanoparticles, (b) Plain Duloxetine drug (c) Blank nanoparticles.

3.3.4 Thermal analysis by Differential Scanning Calorimetry (DSC)

DSC analysis allowed the interpretation of transitions in melting points and thermal degradation in response to DLX-Eud-CHT nanoparticulate formation and DLX encapsulation. Condensed differential scanning calorimetry thermographs of blank nanoparticles, DLX and DLX loaded Eud-CHT nanoparticles are illustrated in Figure 3:10. Glass transition of Eudragit RL is observed at 62°C similarly demonstrated by Jana and colleagues. (Jana et al., 2014). An Endothermic peak was observed between 195°C - 235°C (Figure 3.9a). Figure. 3.9b shows a thermograph for Chitosan and endothermic peak observed at 60°C - 150°C due to chitosan's dehydration (Chatzitaki et al, 2020). Figure. 3.10 which shows thermograph for Eudragit/Chitosan blank nanoparticles, demonstrates a distinct endothermic peak between 145°C - 160°C. The endothermic peak for Eudragit was reduced by 50°C and the endothermic peak for Chitosan was increased by 80°C respectively. This can be attributed to the formation of the solid polymeric nanoparticles. DLX loaded nanoparticles shows a slight decrease in temperature of start of degradation, 5°C, as compared to DLX free nanoparticles. The Duloxetine melting point at 130°C attributed to melting point of Duloxetine is not observed in the DLX loaded nanoparticles thermogram (Mani et al., 2014). The thermogram indicates a protection of Duloxetine from degradation by the nanoparticulate complex.

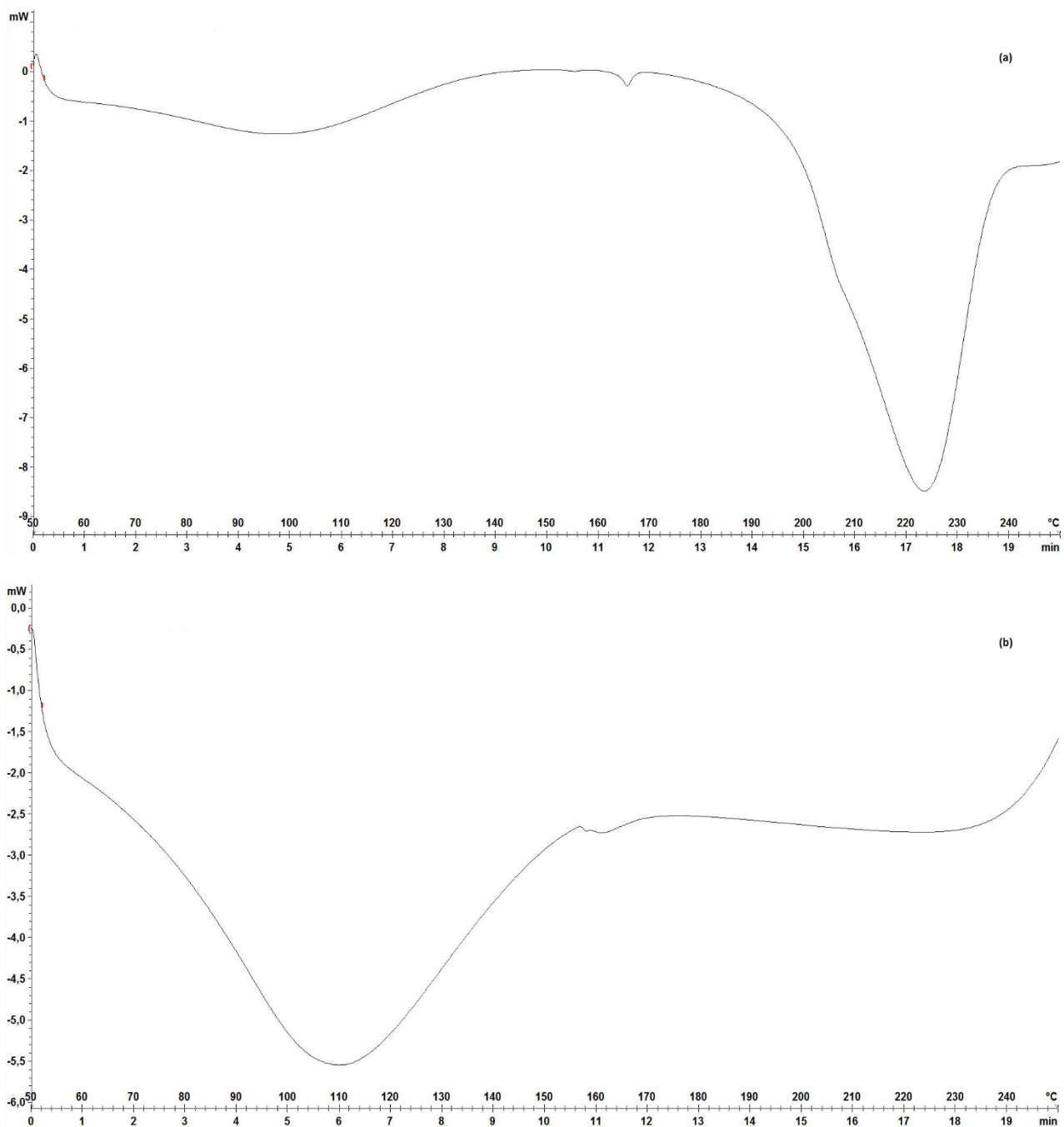


Figure 3.9: Differential Scanning Calorimetry thermograph graph for (a) Eudragit RL 100 and (b) Chitosan Polymer.

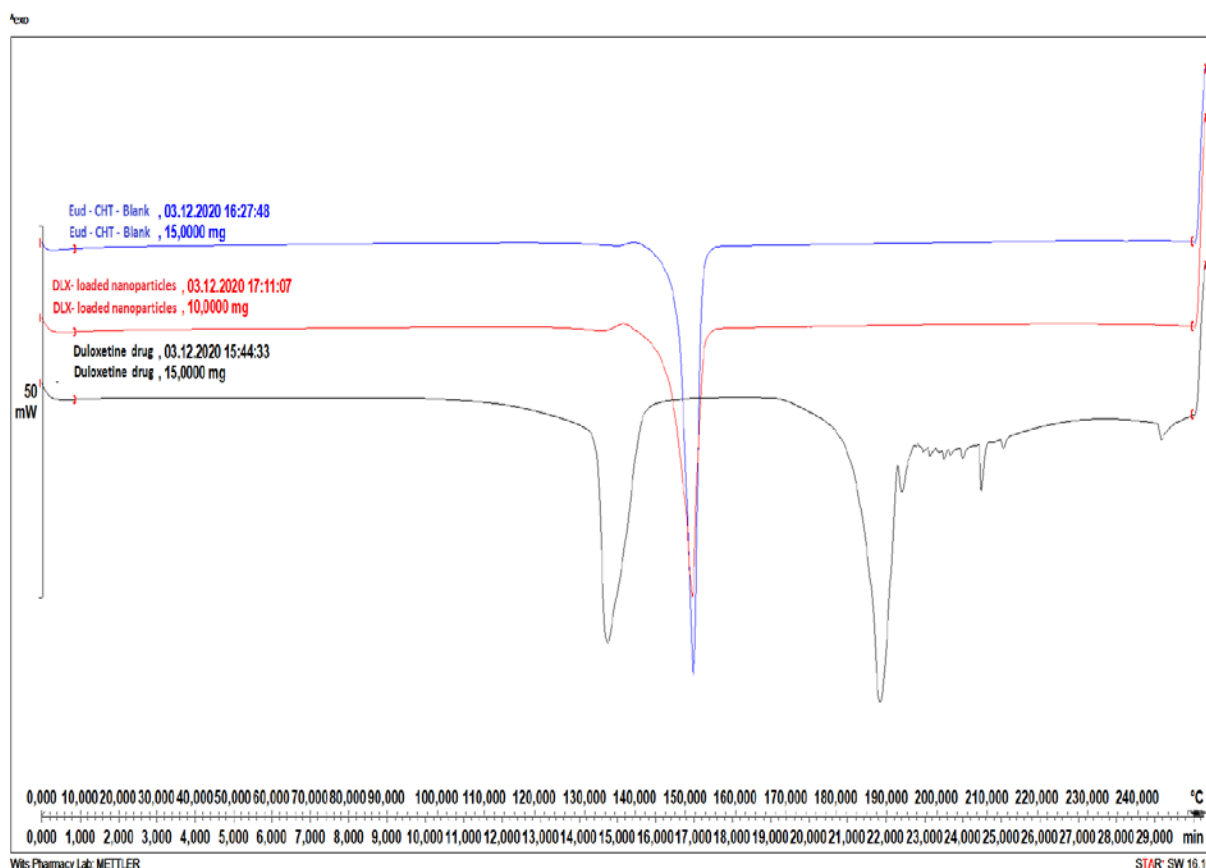


Figure 3.10: Condensed differential scanning calorimetry thermograph for blank Nanoparticles, DLX loaded nanoparticles and plain Duloxetine.

3.3.5 Investigation of stability of nanoparticles utilizing the Turbiscan™ LAB

The EUD-CHT-DLX nanoparticle suspensions were analysed for stability using light scattering analysis. The light scattering analysis is based on the principle of particle dispersion within a solution. It analyses flocculation and coalescence at different heights of the sample. Relationship between Back Scattering (ΔBS) vs Transmission (T) determines the level of creaming at the top of the sample, aggregation in the middle of the sample and sedimentation at the bottom of the sample (Sumaila et al, 2019). Figure. 3.11 and 3.12 represents the Light Transmission graph for Eud-CHT-DLX Nanoparticles and Duloxetine HCL plain drug respectively. In both graphs there is a form of destabilization. The two graphs show a similar trend, a destabilization at the bottom of the sample, a decrease in ΔBS and T at the middle of sample. The plain drug and Eud-CHT-DLX nanoparticles exhibited changes in ΔBS from bottom to middle of sample of 20% and 30% respectively. This may be due to sedimentation kinetics of nanoparticles and heterogenous particle size in the nanoparticulate formulation as noted in the Zeta size analysis, size range of $100.6 \pm 73\text{nm}$. The plain drug exhibits an increase in ΔBS in the middle of sample, this demonstrated coalescence in the plain drug solution, no destabilization is noted for nanoparticles here, but at the top of the sample where a change in

Δ BS of 10% is observed. Nanoparticles have eudragit hydrophobic core and chitosan hydrophilic shell which can explain the creaming kinetics. The two formulations of nanoparticles and plain drug both exhibit destabilization at different levels, and this technique alone is not sufficient determinant of stability.

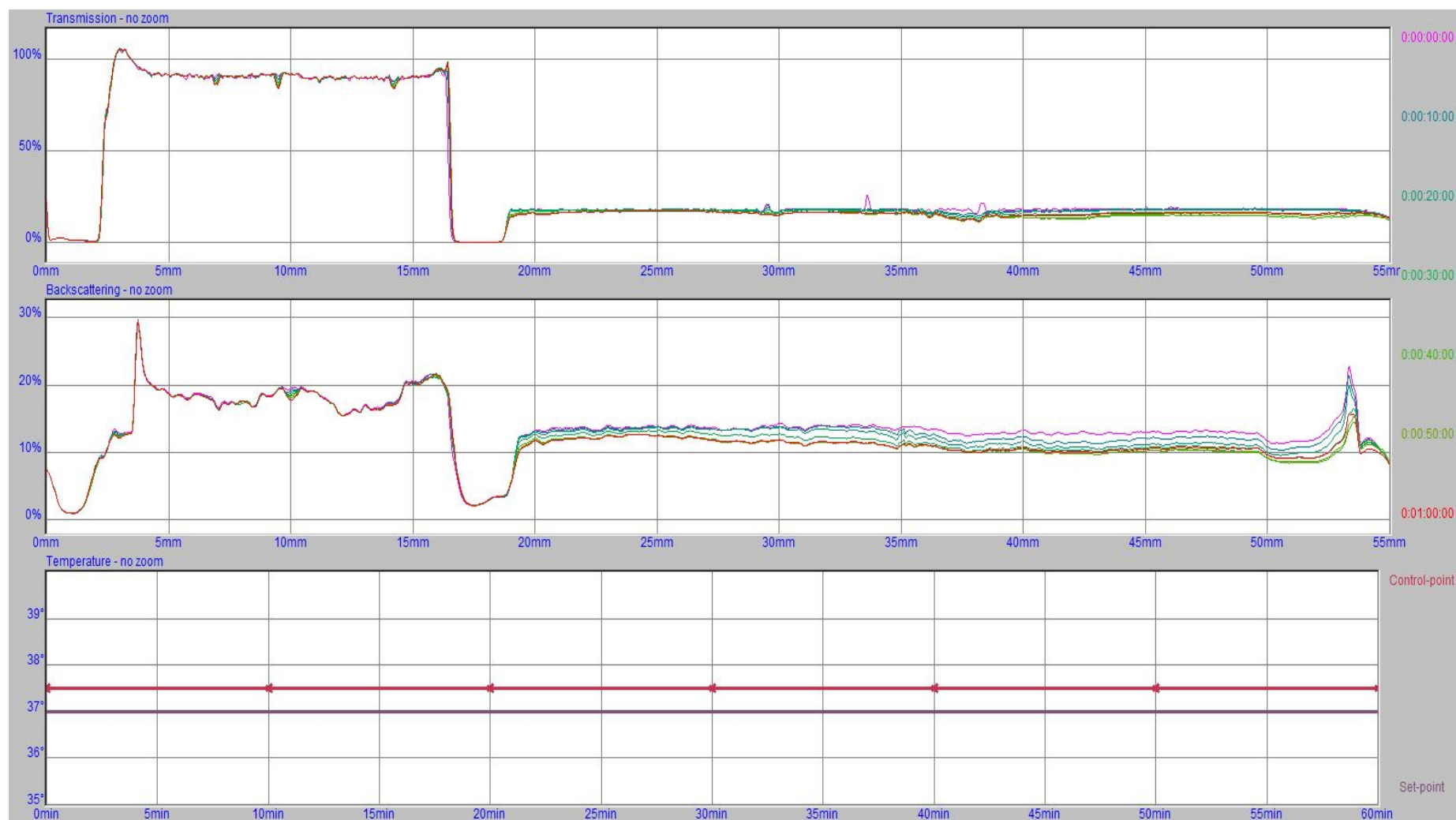


Figure 3.11: Representation of destabilization kinetics of Eud-CHT-DLX nanoparticles, indicating Δ BS (Back Scattering) and T (Transmission) measured at 37°C for 1hour.

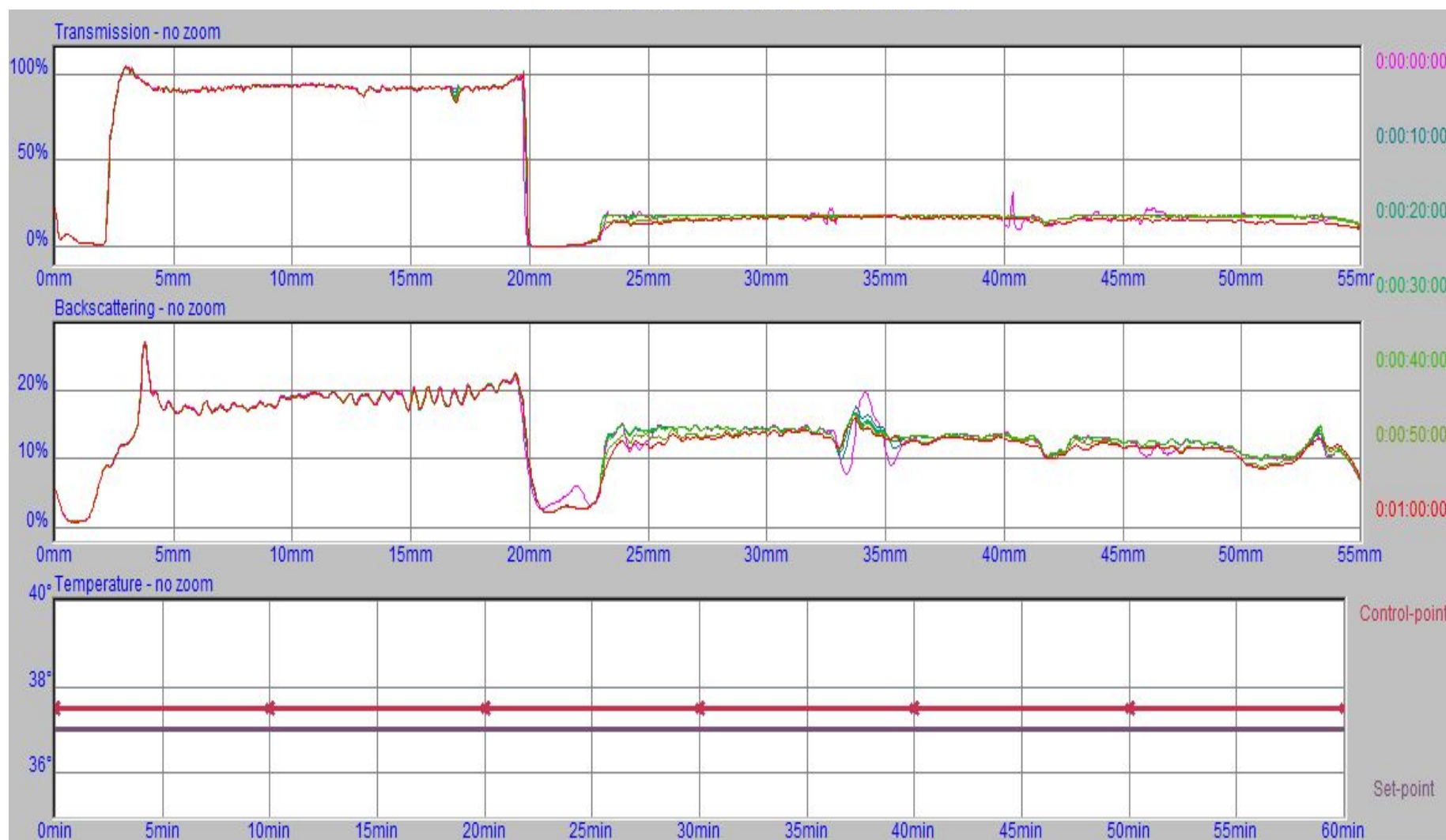


Figure 3.12: Representation of destabilization kinetics of Duloxetine plain drug, indicating Δ BS (Back Scattering) and T (Transmission) measured at 37°C for 1 hour.

3.3.6 Eud-CHT-DLX Nanoparticles Drug Release Studies

The invitro release of Duloxetine from Eud-CHT-DLX nanoparticles is presented in Figure. 3.13. The nano-formulation was dissolved in mediums simulating gastric, intestinal and normal physiological pH, at 1,2, 6,8, and 7,4 respectively. The study was conducted over a 24-hour period observing the release at hourly intervals. Each point represents mean $\pm$ SD (n=3).

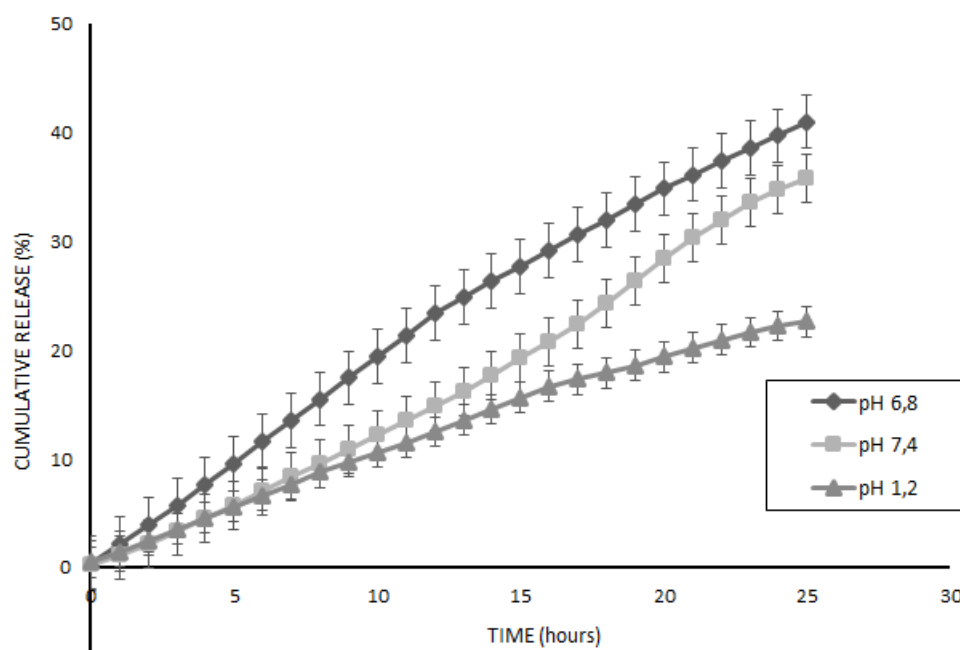


Figure 3.13: Duloxetine release from Eud-CHT-DLX nanoparticles at simulated conditions of the stomach pH, intestinal pH, and normal physiological pH (1,2; 6,8 & 7,4). Each individual point represents: mean $\pm$ SD (n=3).

The purpose of design and synthesis of Eudragit-Chitosan nanoparticles was to protect the antidepressant drug (Duloxetine HCL) from degradation in the stomach acid, improve bioavailability and potentially create a controlled release drug delivery system. Percentage drug release was analysed up to 25 hours at different pH mediums, representing the stomach environment, intestinal environment, and neutral environment. Figure. 3.13 shows release of drug up to 20% after 24hours for nanoparticles placed at a medium of pH1.2. It can be observed that the nanoparticulate system has protected Duloxetine from the harsh pH environment. At pH 6.8, percentage drug release was up to 40% after 24hours, and at pH 7.4, the percentage of drug release slightly differed from 6.8 pH at 35%. Nanoparticulate formulation released drug faster at elevated alkaline pH, less drug was released at an acid pH. In a study conducted by Rana et al, 2020, solid lipid nanoparticles (SNL) loaded with Duloxetine to optimize delivery of Duloxetine to the brain, it was found that plain duloxetine

drug is released at pH 7,4, with 94% of drug released after 8hrs. In comparison to the SLN formulation which released 20,8% of the drug and after 24hrs, 52,9% of the drug was released. (Rana et al, 2020). A comparable drug release profile was observed in this study and at 24hrs, pH 7,4, 35% of Duloxetine was released from the nano-formulation. The nanoparticulate formulation also demonstrated a prolonged release over a 24hr period, and 41% of the drug was released over 24 hours. Eud-CHT-DLX nanoparticles thus, have the potential of increasing the bioavailability of Duloxetine and sustained release.

3.3.7 Kinetic modelling of drug release of Eud-CHT-DLX Nanoparticles In vitro

Drug release kinetics were determined using Zero order release model, first order release kinetics, Fick's law of diffusion, Korsmeyer-Peppas's equation, and Hixson-Crowell equations. The obtained data from drug release studies at pH 6,8; 7,4 and 1,2 at 37°C was used in the above equations to determine the values in Table 3.1. The release profile of Eud-CN-DLX nanoparticles in 24 hours was controlled in nature, attributed to the dissolving of the Eud-Cn copolymer structure. Higuchi's model best describes the release of DLX from the nanostructure. DLX was released through the disintegrated outer hydrophobic Eudragit layer at 37°C, dependent on the square root of time according to Fick's law of diffusion. At 6,8; 1,2 and 7,4 Korsmeyer-Peppas's model also clearly defined the release kinetics.

Table 3.1: Drug release kinetics results for several models of Duloxetine-loaded nanoparticles for targeted drug delivery.

NM in vitro release conditions	Zero-order	First-order	Higuchi	Korsmeyer-Peppas	Hixson-Crowell n-value	n-value
37°C, pH 6,8	0,592	0.711	0.812	0.781	0.671	0.482
37°C, pH 7,4	0,652	0.750	0.845	0.830	0.712	0.461
37°C, pH 1,2	0,891	0.901	0.971	0.961	0.894	0.531

Abbreviation: NM-nanoparticles

According to Korsmeyer-Peppas model, if the release power coefficient (n value) is greater than 0.45 and smaller than 0.89, it implies that the therapeutic release is due to anomalous transport behavior (Non-Fickian diffusion), while $n < 0.45$ implies that the drug release was due to Fickian diffusion. For release from the Eud-CHT nanoparticles, for drug release at 37 °C, the n value was close to 0.45 at both pH values, implying that the drug release was mostly due to Fickian diffusion. DLX was released from the inner core by means of the disintegrated Eudragit whose disintegration is temperature controlled, which is consistent with Higuchi's square root law. There was no distinguished effect of pH on the release behavior kinetics. This

suggests that DLX-loaded Eud-CHT nanoparticles provide favorable release of hydrophobic drugs through the GIT wall with improved neuro-availability.

3.3.8 Evaluation of Cytotoxicity of Eud-CHT-DLX nanoparticles utilizing HEK 293 cells

A cytotoxicity assay was conducted to evaluate the impact of Eud-CHT-DLX nanoparticles on the viability of HEK-293 cells. After 48 hours of treatment with duloxetine HCL, Eud-CHT-DLX and blank nanoparticles at concentrations of 1.5, 3, and 6 $\mu\text{g/mL}$, brightfield microscopic images were captured (at 10X) and an MTT assay was conducted as described in Section 3.2.11. Figure. 3.15. Depicts the graph for MTT assays for plain duloxetine HCL, Eud-CHT-DLX nanoparticles and blank nanoparticles. Figure. 3.14. Displays light microscopic images of the HEK 293 cells treated with a) Untreated cells, b) 1.5 $\mu\text{g/mL}$ Eud-CHT-DLX Nanoparticles c) 3% DMSO d) Plain duloxetine drug e) 1.5 $\mu\text{g/mL}$ blank nanoparticles. All treated and untreated cells show comparable pattern of cell viability, the positive control cells treated with 3% DMSO display a high cytotoxicity as expected. On the graph, the plain duloxetine HCL drug shows a dose-dependent effect, in that at 1.5 $\mu\text{g/mL}$ and 3 $\mu\text{g/mL}$ cell viability was high, the assay was 131 % and 125 % compared with untreated cells (100%), and at 6 $\mu\text{g/mL}$ duloxetine concentration, the assay shows a decline at 98%. The Eud-CHT-DLX nanoparticles showed increased cell viability at 140, 119 and 155 for the 1.5, 3 and 6 $\mu\text{g/mL}$ concentrations. The cell viability of 119 was probably due to experimental error. The blank nanoparticle treatment slightly inhibited growth compared with untreated cells. Both Eud-CHT-DLX and blank nanoparticles at 3% concentration show less cell viability than at lower (1.5 $\mu\text{g/mL}$) and higher (3 $\mu\text{g/mL}$) dose. Cells treated with 3% DMSO were used as a positive control and showed a low viability of 2%. The Eud-CHT-DLX nanoparticles depicted an increase in cell viability when compared with plain duloxetine. This suggested that the Eud-CHT-DLX nanoparticles are less cytotoxic than the pure drug as reported previously (Jafari et al., 2016). Eud-CHT-DLX showed no growth inhibition of the HEK 293 cells and are biocompatible. The data suggest that Eud-CHT-DLX nanoparticles can be an effective and safe carrier for the delivery of duloxetine to the brain. Moreover, due to a lower cytotoxic profile observed with less side effects with the nanoparticles as compared with the plain duloxetine HCL (Jafari et al., 2016).

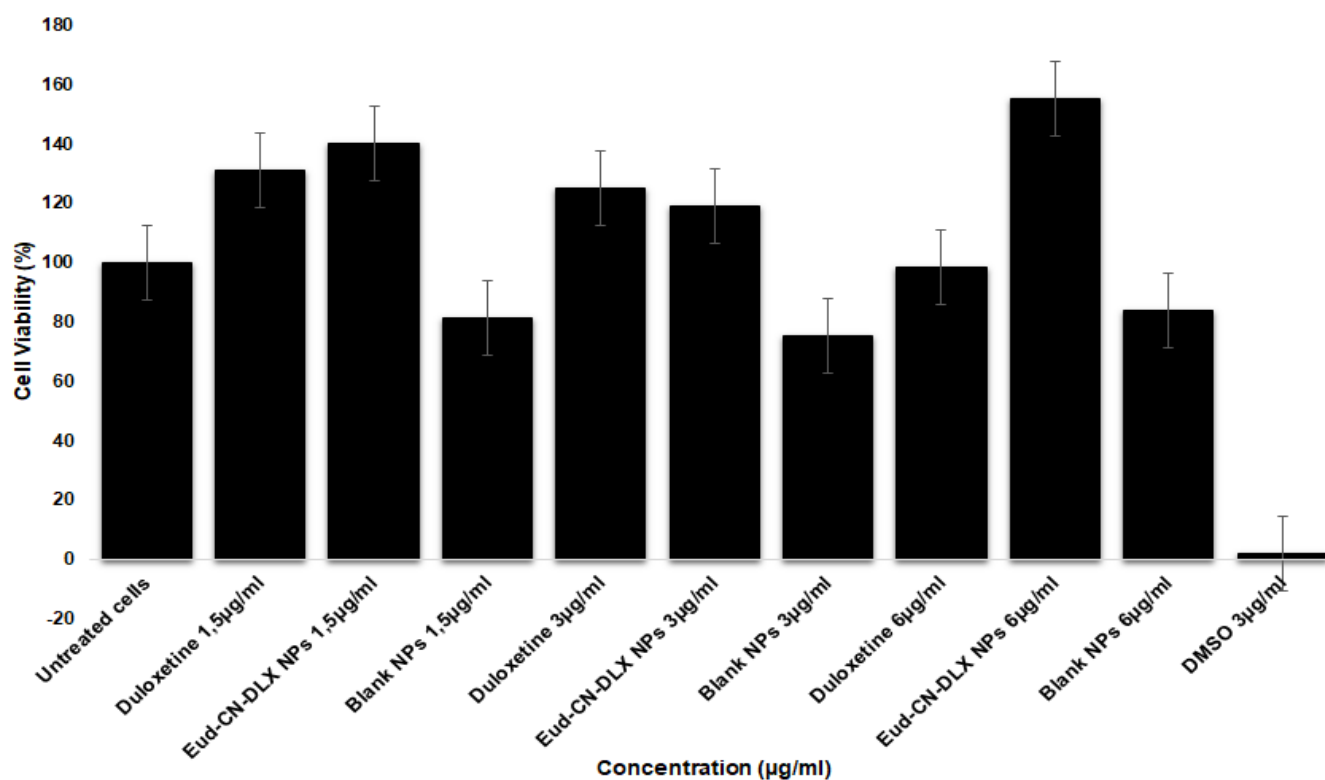


Figure 3.14: Tetrazolium salt MTT assay for evaluation of the outcome of Duloxetine loaded nanoparticles (Eud-CNT-DLX), blank nanoparticles and Duloxetine plain drug on percentage viability of HEK 293 cells. The cells treated with various formulations were incubated for 48h, prior to evaluation of cell viability. Each percentage cell viability point represents average $\pm$ SD (n=3).

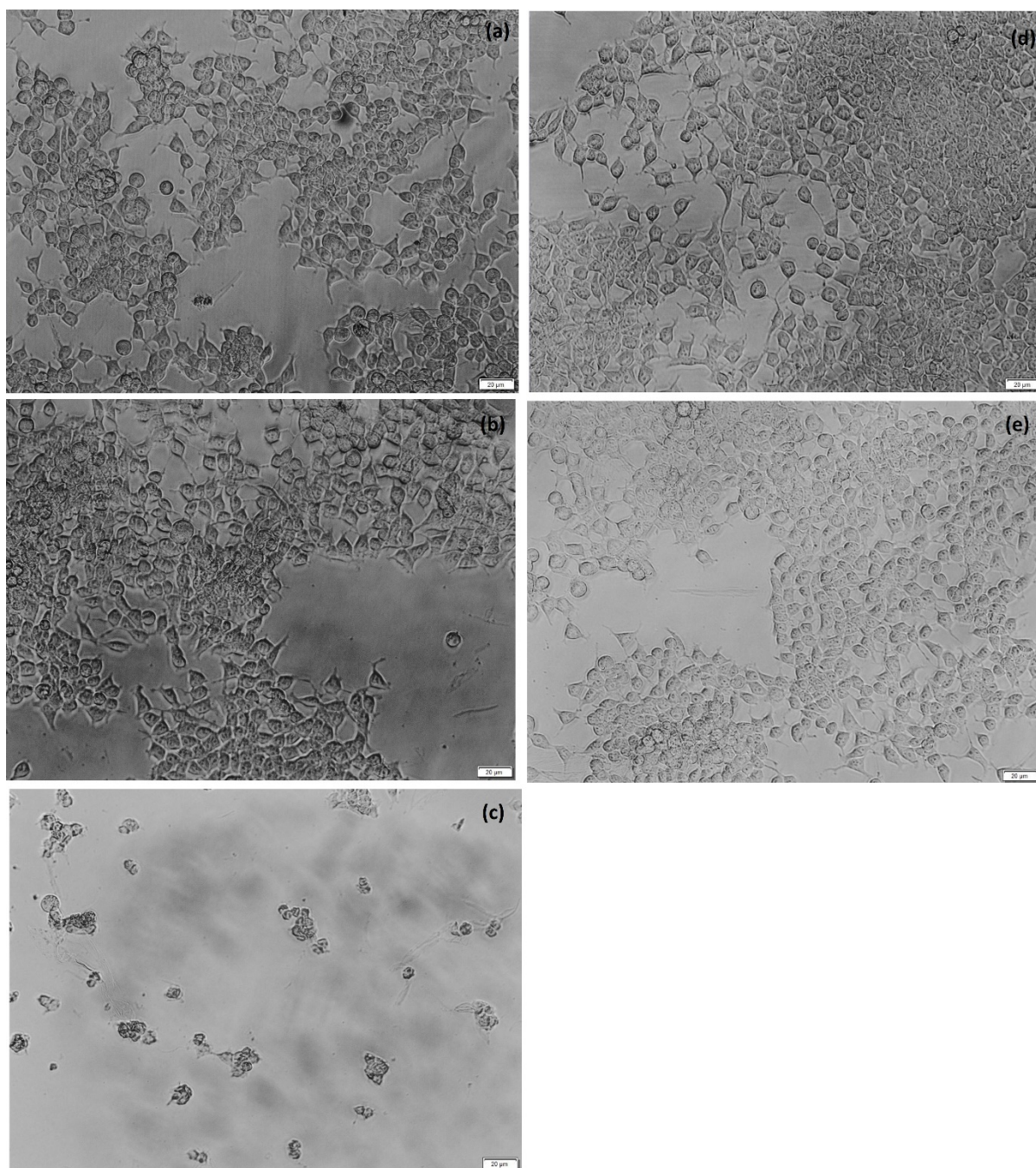


Figure 3.15: Olympus Inverted Microscope Images (scale 20 µm) of HEK cell growth after treatment with a) Untreated cells, b) 1.5% Eud-CHT-DLX Nanoparticles c) DMSO 3% d) Plain Duloxetine drug e) 1.5% Blank Nanoparticles.

CHAPTER 4

CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusions

The need for the development of new strategies to enhance site-specific targeted, non-toxic, drug delivery systems is vital in improving drug efficacy of anti-depressive agents. Nanotechnological strategies are of great interest in surmounting the limitations associated with conventional pharmaceutical drugs to the brain. This study was aimed at developing a polymeric nanoparticulate vehicle for the transportation of DLX to the brain, with the objective of increasing bioavailability, reducing drug degradation by stomach acid, and improving absorption through the intestinal mucosa. Increase of bioavailability would ultimately improve availability of required drug molecule at brain site of action. Eud-CHT-DLX nanoparticles were successfully synthesised utilizing the single emulsion solvent evaporation method. The SEM images showed the required spherical shaped particles, with narrow size distribution corresponding with Zetasizer results of favourable distribution of less than 0.5. The nanoparticulate systems for brain delivery are less than 200nm size to overcome the blood brain barrier (BBB), and in this study, the size of formulated duloxetine-loaded nanoparticles was 100.6 ± 73.41 nm, with drug entrapment efficacy (DEE) value of 72% indicative of potential to improve neuroavailability. FTIR characterization confirmed the integration of Duloxetine, Eudragit and Chitosan with assembly of nanoparticles.

Eud-CHT-DLX nanoparticles presented a sustained release of duloxetine in a time-frame of 24 hours as compared to free conventional duloxetine which has mean plasma half-life of 12 hours, and these findings can be useful for the treatment of depression. Nanoparticulate formulation protected duloxetine from low pH environment simulating the stomach acid during the drug release studies, with only 20% of the drug released after 24hrs. This would mean that less of the drug molecule is degraded by the harsh acidic environment and more of the DLX is available for absorption at small intestinal site. Thermal studies of Eud-CHT-DLX nanoparticles showed an improved stability profile of Duloxetine, compared to plain drug. Duloxetine is characterised as a drug that is low in solubility and absorption through the intestinal wall, it is unstable in gastric pH, highly metabolised in the liver, and binds to plasma protein. Pharmacokinetics profile of Duloxetine will be effectively improved by the polymeric nanoparticulate structure as DLX will be protected from acidic degradation, absorption will be enhanced by the polymeric profile, the stability of DLX will be enhanced, and bioavailability will be improved. From the MTT assay analysis conducted, the results indicated that the DLX-Eud-CHT nanoparticles were less toxic to the HEK cells compared to free DLX. Lower toxicity of the nano-formulation would suggest decreased side effects of Duloxetine. DLX-Eud-CHT

nanoparticles can be considered as a biocompatible vehicle for transport of DLX. The combined experiments and results from the synthesis of DLX-Eud-CHT nanoparticles, strongly suggested that these nanoparticles can be utilized as potential invaluable formulation for oral drug delivery of Duloxetine with improved neuro-availability.

4.2 Recommendations and future outlook

Brain diseases are highly challenging to treat due to the Blood-Brain Barrier (BBB). The current therapeutic interventions used in managing these diseases are faced with low bioavailability in the brain. Different strategies have been investigated for brain delivery including intranasal drug delivery, intracerebral administration, intracerebroventricular administration and physical/chemical disruption of the BBB. Although these methods have proven successful, they are invasive and expensive, often pose risk of infection and toxicity, and thus have less patient compliance (Teixera et al., 2020). Nanotechnology has become an invaluable tool for targeted delivery because of its advantages including small size facilitating entry into the brain, the ability to load hydrophilic and hydrophobic drugs, improvement of stability profile, enhancement of bioavailability and potential of decreased side effects.

Lipid based formulations including nanoparticles, liposomes, micro-emulsions, nano emulsions have become of significant interest in brain delivery, as they naturally pass through the endothelial cells of the BBB. These lipid formulations are biocompatible, biodegradable, and have a low cytotoxic nature. Surface modification further enhance their capability for interaction with transport mediums and cell receptors at the BBB surface (Teixera et al., 2020). In this study, duloxetine (DLX) loaded eudragit-chitosan (Eud-CHT) nanoparticulate system was formulated, and encapsulated in gelatin capsules, an effective depression disorder therapeutic, characterized employing techniques such as DSC, FTIR, SEM, zetasizer, Ultraviolet (UV) spectroscopy, TGA and MTT assay. This oral nanoparticulate system has high drug loading capacity, and the *in vitro* duloxetine release profile was concluded to be appropriate. Cytotoxicity results of Eud-CHT-DLX nanoparticles exhibited increased cell viability when compared with plain duloxetine. This suggested that the Eud-CHT-DLX nanoparticles were less cytotoxic than the pure drug. In addition, this oral nanoparticulate system is non-invasive and may therefore be a promising replacement of the highly invasive conventional system currently used in the treatment of depression disorder. Further studies should be conducted to optimize drug loading to provide uniform dosing and evaluate *in-vivo* behavior of the Eud-CHT-DLX nanoformulation. In addition, nanoparticles interaction with the immune system must be researched as there is an unclear health impact. Toxicity of the

nanoparticles to the brain also should be further studied to determine safety levels of the biocompatible and biodegradable nanomaterials. This study can also be advanced by inducing depression disorder in the animal model and the oral duloxetine loaded Eud-CHT nanoparticulate system applied to further elucidate the efficacy of the delivery system. Preceding animal studies validation of techniques to optimize the nano formulation for industrial manufacturing can be recommended, however challenges with large scale expensive manufacturing equipment and testing instruments such as SEM/TEM and the specialist skills required to handle such instruments could be a hindrance.

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