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A SUBLINGUAL DRUG DELIVERY SYSTEM FOR THE TREATMENT OF DEPRESSION

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



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Johannesburg 2024

DECLARATION

I Mmakganya Le Roux declare that this Research Report is my own work. It is being submitted for the Degree Master of Science in Medicine: Pharmaceutical Affairs at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



_____

(Signature of candidate)

____ 31nd _____day of_____July_2024_____ in___Parktown_____

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And lastly, to my best friend Keamogetwe Sehapela. Thank you for always believing and encouraging me.

ABSTRACT

Depression is a common mental illness that has affected many individuals globally. Several antidepressant drugs have been developed over the years, and the primary method of drug administration is the oral route. The challenge with orally administered antidepressants is the bioavailability of the drug in the systemic circulation or its site of action. Only a fraction of the drug is available at the target site due to first-pass metabolism in the liver and digestive system. Another limitation that affects the drug delivery of an antidepressant is the presence of the blood-brain barrier (BBB). The ability of these drugs to achieve optimal therapeutic levels in the central nervous system (CNS) lies in their ability to effectively cross the BBB. To overcome these limitations, various innovative drug delivery systems have been developed. Nanoparticles have been explored as potential drug delivery systems (DDS) having shown the ability to encapsulate drugs and enhance drug delivery to the brain. Different types of nanoparticles, such as polymeric or liposome nanoparticles, can be designed to have particular drug release profiles, improving the efficacy of the drug and reducing side effects of therapy. The current study will assess the potential of poly (lactic-co-glycolic acid) as a drug delivery system for the sublingual delivery of amitriptyline for the treatment of depression. It will cover the statistics and impact of depression along with the currently available treatment options and their limitations such as low bioavailability and first-pass metabolism, to name a few. Furthermore, drug delivery methods that have been considered in previous studies, the use of alternative routes, drug delivery systems used to improve antidepressant therapy, the challenges with conventional drug therapy, the role of the blood-brain barrier, and alternative drug delivery methods currently available in an effort to improve conventional drug delivery systems for depression were discussed. The primary goal of the study was to synthesize a Amitriptyline loaded poly (lactic-co-glycolic acid) (PLGA) nanosuspension for sublingual delivery to the brain. Based on literature, PLGA enhances drug delivery to specific cells or tissues and improves the efficacy of the drug. A Amitriptyline loaded PLGA nanosuspension were formulated by nanoprecipitation. Fourier-transform infrared spectroscopy (FTIR), Zetasizer, and Differential Scanning Calorimetry (DSC) were used to determine the physiochemical properties of the nanosuspension. The synthesised nanoparticles were 137.7nm in size, with polydispersity index (PDI) of 0,064 and a zeta potential of -8.98. High Performance Liquid Chromatography (HPLC) was used to determine the drug release content of the nanosuspension at differing time intervals. The drug release profile of amitriptyline exhibited a burst release of approximately 60% in the first hour, followed by a gradual release up to approximately 68% within 8 hours. The high drug release profile of the nanosuspension and the

ability of the biomaterials to cross the BBB makes this drug delivery system a desirable system compared to the conventional tablet-based delivery systems.

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ABBREVIATIONS

ABC	ATP binding cassette
AMT	Amitriptyline
AdMT	Adsorptive-mediated transport
BBB	Blood Brain Barrier
CAT1	Cationic amino acid transporter
ChT	Choline transporter
CNS	Central Nervous System
CMT	Carrier-mediated transport
DDS	Drug delivery system
DSC	Differential Scanning Calorimetry
EC	Endothelial cells
EE	Entrapment efficiency
FTIR	Fourier-transform infrared spectroscopy
GIT	Gastrointestinal tract
GLUT1	Glucose transporter
HCl	Hydrochloride
HPLC	High Performance Liquid Chromatography
IV	Intravenous
LAT1	l-type amino acid transporter
MAOIs	Monoamine oxidase inhibitors
MCT1	Monocarboxylate lactate transporter
MRP	Multidrug resistant protein

NCs Nanocarriers

NPs Nanoparticles

NVU Neurovascular unit

P-gp P-glycoprotein

PBS Phosphate buffer saline

PDI Poly dispersed index

PLGA Poly (lactic-co-glycolic acid)

PTS-6 Peptide transport system-6

RMT Receptor-mediated transport

SGLTs Sodium-coupled glucose transporters

SLN Solid lipid nanoparticles

SNRIs Serotonin and norepinephrine reuptake inhibitors

SSRIs selective serotonin reuptake inhibitors

TCAs Tricyclic antidepressant

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CHAPTER 1

INTRODUCTION

1.1 Introduction on depression and treatment

Depression is the most prevalent and profoundly impactful mental health condition that affects more than 300 million people worldwide, and this figure is constantly climbing (Vitorino *et al*., 2019). This mental condition is characterized by depressive mood, loss of interest or pleasure, low energy, feelings of guilt or inferiority complex, insomnia, loss of appetite, difficulty concentrating and decision-making, and in more severe cases, recurrent thoughts of suicide or death (Mossie *et al*., 2016).

Depression is brought on by a number of variables, including genomic factors, the environment, health issues, life events and physiological factors (Thakkar *et al*., 2021). The primary variable found to be the cause of depression is abnormalities in the monoamine neurotransmitters like dopamine, serotonin, and norepinephrine, which are responsible for the brain's message-transmission systems (Kaltonboeck and Harmer., 2018). As a result of insufficient supplies of neurotransmitters, depression symptoms may arise (Alam *et al*., 2014).

For many years, depression has been treated with a variety of antidepressant types, including tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), serotonin and norepinephrine reuptake inhibitors (SNRIs) and selective serotonin reuptake inhibitors (SSRIs) (Thakkar *et al*., 2021). The route of administration of a drug affects its bioavailability, its concentration in the plasma and duration of effect (Tijani et al., 2021). Several antidepressants are predominantly delivered via the oral route of administration. Before reaching the target site, orally administered drugs are metabolised in the liver after passing through the gastrointestinal tract (GIT). Therefore, a lesser amount of the drug reaches systemic circulation due to enzymatic breakdown in the GIT and liver metabolism (Sharma and Dang., 2020).

Amitriptyline is a well-known TCA often prescribed for the treatment of depression (Elsharkawy and Elhazzazi., 2016). It is a highly lipophilic drug which undergoes extensive pre-systemic metabolism in the liver and its systemic bioavailability after oral administration is low as a result (Rico-Villademoros *et al*., 2015). The presence of the blood-brain barrier (BBB) is another limitation that prevents effective drug concentrations from reaching the brain (Sharma and Dang., 2020). If the drug cannot pass through the BBB to enter the CNS, the desired pharmacological effect is not achieved (Thakkar *et al*., 2021).

A study by Sharma and Dang, investigated intranasal drug delivery as an alternative route of delivery for brain targeted therapy (Sharma and Dang, 2020). Intranasal drug delivery is increasingly considered as a viable method for drug delivery due to its capacity to carry a drug to the systemic circulation and the CNS. Although nasal mucosa improves drug bioavailability and fast onset of action, the major drawback of nasal drug administration is the rapid mucocilliary clearance, which causes drug particles to be eliminated from the nasal cavity before sufficient absorption through the nasal mucosa (Chonkar *et al*., 2015). Drugs administered in the nasal cavity may get entrapped in the viscous gel layer which decreases the drug's effectiveness (FDA, 2021). Furthermore, the nasal cavity consists of several enzymes in the intranasal epithelium and lumen which may reduce the bioavailability of a drug (Lam *et al*., 2020).

In view of the above, this study will consider sublingual drug delivery as an alternative route of delivery. Sublingual drug delivery entails the delivery of a drug (Tekade *et al*., 2017) to the sublingual zone of the oral fissure and allowing it to enter the systemic circulation through the mucosal membrane. The mucosal lining is made up of three layers (Saha *et al*., 2017). The epithelial membrane which serves as a protective barrier, is the outermost layer (Saha *et al*., 2017). The basement membrane is the epithelial membrane's deepest layer, which refills the epithelium. The lamina propria is found underneath the epithelium, followed by the submucosa (Saha *et al*., 2017). The lamina propria is a connective tissue layer that is less thick and hydrated (Saha *et al*., 2017).

Blood vessels abound in the submucosa of the mouth (Saha *et al*., 2017). The drug enters the systemic circulation immediately after being absorbed via the mucosal membrane in the sublingual area. The drug is immediately available in the systemic circulation as a result of direct drainage into the systemic circulation (Saha *et al*., 2017). Drugs administered via the surface of mucosal tissue circumvent the hepatic first-pass metabolism and prevent enzymatic breakdown in the GIT, making it a straightforward and painless mode of administration (Chen & Wen.,2018). This delivery method, however, has some drawbacks. One disadvantage is the small accessible surface area of the administration site, and the possibility of saliva washing off the drug is a major concern in terms of drug component retention (Chen & Wen., 2018). Sublingual delivery is also hampered by physical and biochemical barriers. The saliva and mucus layer, mucosal epithelium, and the basement membrane are examples of physical barriers, while biochemical obstacles allow drug candidates to be destroyed by sublingual metabolising enzymes (Chen and Wen., 2018).

To overcome these limitations, efforts have been undertaken to utilise alternate delivery routes and develop innovative drug delivery systems (Macedo et al., 2020). Scientists and researchers have been working on new antidepressant treatment formulations and drug delivery systems for the brain (Han and Jian, 2020). The use of nanoparticles is one method of delivering drugs to the brain that has taken considerable interest. Nanoparticles are regarded as one of the most favourable drug delivery systems that can penetrate inaccessible areas like the brain and protect therapeutic molecules while delivering them efficiently (Saraiva *et al*., 2016). Another significant benefit of employing nanoparticles for drug delivery is that they enable drug delivery to the brain by masking the adverse physicochemical features of the drug without having to modify it (Wohlfart et al., 2012). Thus, the aim of this study is to investigate the sublingual drug delivery of amitriptyline and the potential of Poly (lactic-co-glycolic acid) (PLGA) nanosuspension as a drug delivery system to enhance the systemic absorption of amitriptyline following sublingual administration.

1.2 Rationale and motivation of the study

The capacity of antidepressants to pass the BBB is one of the defining variables used to assess their efficacy (Vitorino *et al*., 2019), and remain at the target site for a longer time period (Alam *et al*., 2014). Despite great breakthroughs in prior research that focused on the treatment of brain diseases, there has been little progress in developing therapeutic molecules for brain disorders (Kim *et al*., 2019). This is mostly due to the BBB. The BBB regulates the uptake and expulsion of tiny molecules, proteins, and even cells in the brain to protect it from the unanticipated movement of substances from the systemic circulation (Pollak *et al*., 2017). It is an essential component in maintaining homeostasis in the brain. The BBB's barrier function is primarily provided by the very restrictive impermeable junctions between the brain capillary endothelial cells that are responsible for the barrier qualities and hinder the transfer of most drugs across the BBB (Wohlfart *et al*., 2012).

Molecules with a molecular mass of less than 500 Da and those that are lipophilic can generally cross effortlessly through the BBB (Soni et al., 2019). Unfortunately, many antidepressant drugs lack certain physiochemical properties, such as, small molecular size, positive charge, and high lipid solubility are therefore ineffective in treating brain diseases because they have a low BBB penetration efficiency making it difficult to attain therapeutic drug levels in the brain (He *et al*., 2019 and Han and Jian, 2020). The pharmacological efficacy of these antidepressants is reliant on their sustained presence at the targeted site in the brain (Tong *et al*., 2017). Traditional oral and parenteral therapies are restricted by their lack of ability to efficiently enter the brain from

systemic circulation (Tong *et al*., 2017). In view of the foregoing, there is an urgent need for novel effective drug delivery systems that deliver the drug in the brain in a controlled manner to achieve therapeutic effect (Vitorino *et al*., 2019; Tong *et al*., 2017).

Extensive research has been done on the use of polymeric nanoparticles in the pharmaceutical and biomedical field (Monge *et al*., 2020; Chen and Wen., 2018) and there are significant benefits in the use of nanoparticles as drug delivery systems. Nanoparticles have previously proven to be effective drug delivery systems. The following are some of the benefits of using nanoparticles as drug carriers for sublingual delivery: ability to contain both hydrophobic and hydrophilic drugs; provision for the controlled release of drugs; high carrier capacity ; prevention of enzymatic degradation; functionality of the nanoparticles and their availability in a wide range of modifiable structures which aid in targeting specific sites of action; and the generation of good stability, resulting in a longer shelf-life (Chen and Wen., 2018).

1.3 Aim and Objectives of the study.

The aim of the study was to develop a nanoformulation for sublingual delivery of Amitriptyline to treat depression. To achieve this aim, the following objectives will be fulfilled:

1. To formulate amitriptyline loaded PLGA nanosuspension for the treatment of depression.
2. To measure physicochemical properties including polydispersity index, particle size and the zeta potential of the nanosuspension employing a Zetasizer.
3. To determine the chemical composition, structure, and functional groups characteristics of the nanosuspension using FTIR.
4. To determine the thermophysical properties of the starting materials and the nanosuspension using DSC.
5. To determine the amitriptyline release from the nanosuspension employing HPLC.

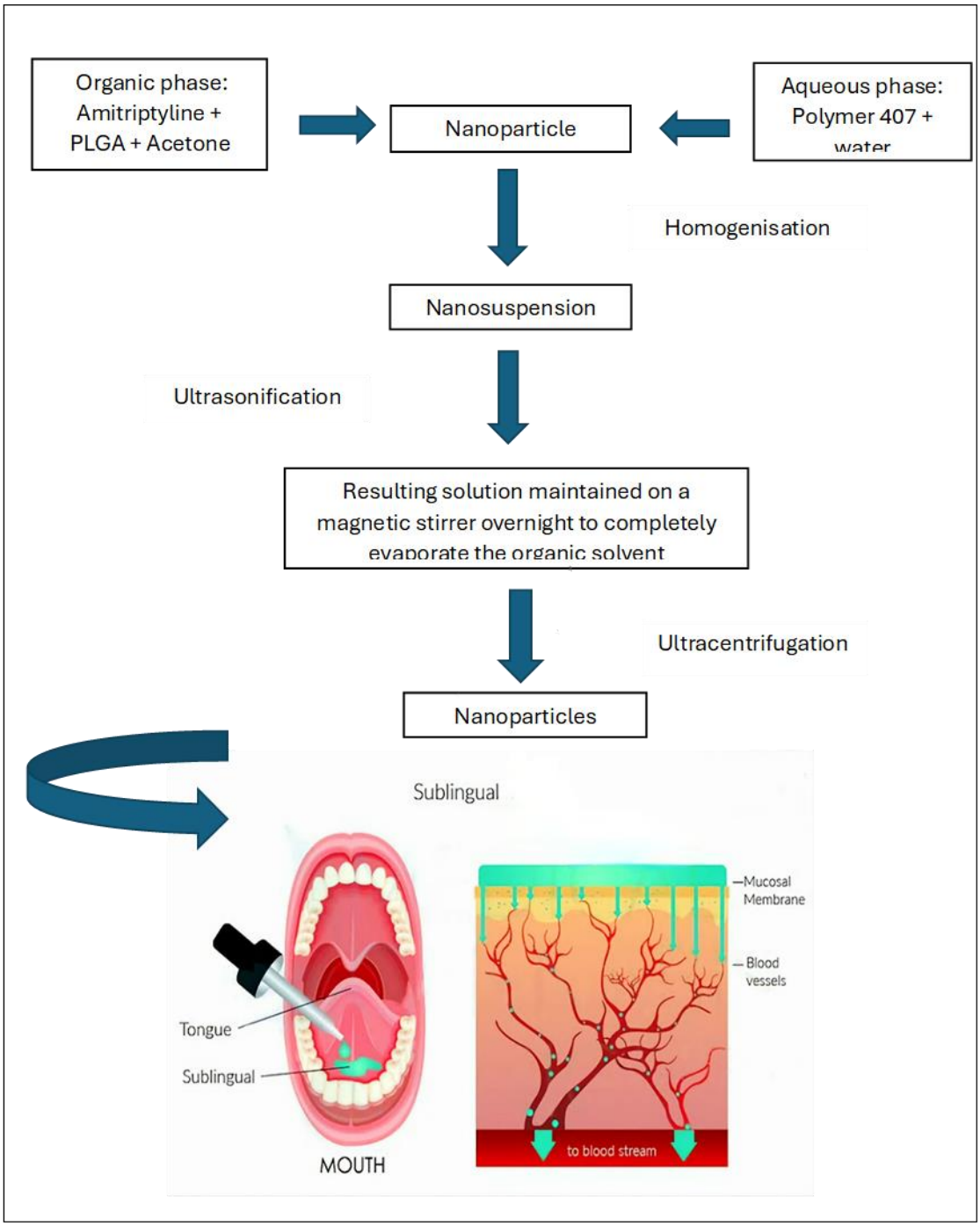


Figure 1.1 A schematic representation of a nanoparticulate drug delivery system used for the sublingual delivery of an antidepressant.

1.4 Overview of this dissertation

Chapter 1: This chapter describes the challenges in the treatment of depression due to the low systemic bioavailability of existing antidepressants and the inability of these drugs to cross the BBB and enhance therapeutic effect. It also highlights the use of nanoparticles as drug carriers for targeted drug delivery. The rationale and motivation along with aim and objectives are explained.

Chapter 2: This chapter provides a literature review on the advancements of nanoparticle drug delivery systems for the treatment of depression.

Chapter 3: This chapter describes the material and formulation of amitriptyline loaded nanosuspension. Characterisation of materials using Zetasizer, FTIR and DSC. Determining the drug release profile of the nanosuspension drug delivery system using the HPLC.

Chapter 4: This chapter provides conclusion and recommendation.

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CHAPTER 2

ADVANCEMENT OF NANOPARTICLE DRUG DELIVERY SYSTEMS FOR THE TREATMENT OF DEPRESSION

2.1 Introduction

The effectiveness of current treatments for depression is not dependant on the potency of the drug (Raghav *et al*., 2023), it is dependent on the drug concentrations in the brain. As a result, conventional oral therapies are limited because they necessitate the drug to cross the blood-brain barrier (BBB) (Vitorino *et al.,* 2019). The brain is an extremely sensitive organ that is expertly shielded by nature. The presence of the BBB controls the exchange of substances between the bloodstream and the CNS, and thereby maintains brain homeostasis. It protects the brain by restricting the passage of pathogens and harmful substances into the brain (Daneman and Prat, 2015). Only specific molecules are allowed to flow through it unhindered. Sadly, the very defences against invasive molecules can also work against therapeutic interventions. Certain therapeutic compounds, both large and small, are unable to pass through the BBB due to this regulation of brain homeostasis. It is estimated that nearly all large molecular weight drugs (primarily peptides and proteins) and over 98% of small molecular weight drugs developed for CNS pathologies are unable to cross the BBB. Therefore, finding new ways to effectively deliver drugs to the CNS is crucial for treating CNS disorders (Shrikant *et al*., 2015).

These challenges have spurred researchers to create more effective methods for novel drug delivery systems that are more effective in treating CNS disorders. Nanoparticles (NPs) have been researched as drug delivery vehicles for CNS therapeutics in an effort to get around the problem of BBB transport as well as other physicochemical constraints of conventional drug delivery systems (Kaushik *et al*., 2018). These drug delivery systems can non-invasively bypass the BBB and reach neurons through various routes (Saeedi *et al*., 2019). A number of NPs have been employed for penetrating the BBB. NPs allow drugs to cross the BBB by penetrating the tight junctions between the endothelial cells. Drug transfer across the endothelial cell layer can also be facilitated by NPs' endocytosis and transcytosis (Herda *et al.,* 2014). Lipid NPs possess lipophilic characteristics that allow them to penetrate the BBB and enter the brain via various transport pathways, such as transcellular, paracellular, transcytosis, and receptor-mediated endocytosis (Gilmore *et al*., 2016). Furthermore, they are ideal for treating CNS diseases because their structure can be change easily to encapsulate the desired medication (Vashist *et al*., 2018). Nanotechnology has created new avenues for sustained, controlled, and targeted delivery,

resulting in higher effective concentrations at the targeted site and fewer side effects by using various NCs such as nanoparticles, liposomes, dendrimers and micelles (Amirmahani *et al*., 2017). The creation these NCs could be a useful tool in the treatment of brain disorders (Leite *et al.,* 2015).

Sublingual drug delivery holds promise for the treatment of depression. The mucosa of the oral cavity has multiple administration sites, including the floor of the mouth, palate, gingiva, lips, and cheeks. Of these, the sublingual site is the most appealing because of its dense vascularization and thin epithelium. Due to its anatomic accessibility, low enzymatic activity, physiological pH range, and ability to avoid the first-pass effect, the oral cavity mucosa is regarded as an effective administration site for drug delivery. Furthermore, because it is a highly vascularized tissue, it has quick access to blood capillaries and can deliver compounds either locally (mucosal) or systemically (transmucosal) (Paris *et al*, 2021). This review will discuss the challenges of treating CNS disorders due to the current treatment not being able to penetrate the BBB. It'll provide an overview of nanoparticle drug delivery systems designed to overcome the limitations of conventional drug delivery to the CNS. Various strategies employed by nanoparticle formulations to enhance drug delivery to the brain.

2.2 Conventional antidepressant therapy

Improvements in drug formulations and alternative delivery methods have the potential to enhance patient medication therapy through various mechanisms. Drug formulations can provide targeted therapy, while alternative routes can improve patient, adherence, convenience, and comfort (Kaminsky *et al.,* 2015).

2.2.1 Oral therapy

Oral administration describes the process of drug delivery via the mouth. The oral route is the most frequently used route to deliver therapeutics owing to its non-invasive nature. It is the most common technique of delivery because of its benefits, which include ease of drug administration, non-invasiveness, and patient compliance (Alqahtani *et al*., 2021). Because of its convenience, it is the best method of drug administration for long-term or daily use. In some developing nations where there are limited options for drug administration, this technique is especially crucial. (Dilnawaz, 2017). However, gastrointestinal (GI) tract epithelium can act as a physical and biochemical barrier which can make it difficult for the drug to achieve therapeutic levels due to the poor solubility, stability, and bioavailability which affects the efficacy of the drug (Dilnawaz, 2017 and Renukuntla *et al,* 2023). In order to overcome these restrictions, formulations based on

nanoparticles have emerged as a glimmer of hope for oral drug delivery. They have made great strides toward resolving a number of solubility-related issues and are crucial to clinical applications (Dilnawaz, 2017).

2.2.2 Parenteral therapy

Intravenous (IV) injections are utilized to administer drugs directly into the bloodstream for rapid absorption (Jin *et al.,* 2015). It allows drugs to bypass first pass metabolism in the GIT and the liver and be directly absorbed into the systemic circulation, resulting in a quick onset of action and high bioavailability. One of the most significant disadvantages of IV drug delivery is the difficulty in delivering drugs to specific tissues and cells. Drugs are distributed uniformly throughout the body before reaching their sites of action, but they may be degraded or become inactive, leaving only trace amounts at the targeted sites. When the final effective concentrations at the site of action are lowered, medications may lose some of their effectiveness (Sanjay *et al.,* 2018). Furthermore, IV drug delivery systems release drugs in a burst rather than a sustained manner, which reduces the effectiveness of drugs. Higher concentrations of drugs may be required, ultimately increasing cytotoxicity and adverse effects. Finally, the drug's non-specificity, low solubility and undesirable release profiles further constrain this drug delivery method. (Cheung and Das, 2014). The goal of controlled drug delivery is to deliver drugs to the intended site of action at the desired rates and times in a sustained manner, improving the drug's bioavailability, pharmacokinetics, and efficacy while limiting adverse effects (Sanjay *et al,* 2018).

2.3 Oral transmucosal drug delivery

Sublingual delivery refers to the administration of drugs to the systemic circulation via the mucosal membranes lining the floor of the oral cavity. Owing to its distinct anatomical and physiological characteristics, the oral mucosa presents numerous prospects for systemic drug delivery. Any drug that diffuses across the oral mucosa membranes will bypass the hepatic metabolism and have direct access to the systemic circulation through capillaries and venous drainage due to the mucosa's high vascularization (Patel *et al.,* 2011). When a drug is absorbed through the sublingual mucosa it enters the reticulate vein, located underneath the oral mucosa. They are then drained into the facial veins, internal jugular vein, and brachiocephalic vein before entering the systemic circulation (Pawar *et al*., 2018). The main way that a drug enters the oral mucosa is by passive diffusion, which occurs through a lipoidal membrane. When taking the medication sublingually, absorption is three to ten times higher than when taking it orally (Mathur *et al.,* 2019). Increasing the duration of contact between the formulation and the mucosa to guarantee the best possible bioavailability of the delivered molecule is one of the primary challenges in sublingual

delivery. Therefore, to maximize this delivery route, mucoadhesive systems that retain the compound at the mucosa surface for an extended period of time are needed (Paris *et al*., 2021). A system like this guarantees that the absorbing membrane is in close contact with it, which optimizes the drug concentration gradient across the biological membrane and minimizes the differential pathway. The oral mucosa could be a suitable location for long-term or controlled medication administration (Nibha and Pancholi., 2012).

2.4 The blood-brain barrier

The blood brain barrier is an intricate multicellular structure that isolates the brain from the rest of the body by restricting the entry of some compounds into the brain from the bloodstream (Ding *et al*., 2020). This barrier protects the brain from harmful substances and pathogens by acting as a shield. Only a small number of molecules can pass through this barrier because of its highly selective structure (Mishra *et al*., 2023). The BBB is primarily composed of endothelial cells (ECs) which make up a significant portion of the brain, pericytes, astrocytes and microglia (Fig. 2.1) (Power *et al*., 2022). Together, these components make up the neurovascular unit (NVU) and form an uninterrupted impermeable barrier that controls the transcellular transport of nutrients, ions, and potentially neurotoxic substances while preventing the paracellular movement of these substances (Sweeney *et al*., 2019). The ongoing interaction among the components of the NVU ensures the functionality of the BBB and maintaining homeostasis in the brain. The ECs are sealed off from external substances by the BBB's tight and adherent junctions, which essentially stop substances from penetrating the brain (Shi *et al.,* 2023).

2.5 Drug delivery to the brain

The BBB restricts the entry of drug compounds from entering the CNS, which leads to poor treatment outcomes for CNS diseases (Nguyen *et al.,* 2021). This is caused by a few limiting factors that are connected to the physicochemical characteristics of drugs in addition to the existence of the BBB's protective mechanism. It has been suggested that the BBB is the main barrier preventing drugs from entering the brain through blood circulation (Bahadur and Jha., 2022). Therefore, to treat these conditions targeted transport therapies to cross the BBB are needed (Nguyen *et al.,* 2021).

Research on developing drugs for the central nervous system focuses primarily on two approaches, invasive and non-invasive methods (Markowicz-Piasecka *et al.,* 2022). Invasive drug delivery techniques modify BBB permeability by direct entry into the CNS, temporarily disruption the BBB. Invasive techniques can be aggressive aiming to deliver as much therapeutic to the

target tissue as possible with the least amount of exposure to nearby healthy tissue (Stockwell *et al.,* 2014). Unfortunately, the mechanical nature of invasive approaches may result in various injuries to the brain (Tam *et al.,* 2016). Therefore, although highly invasive techniques are appropriate for well-defined tumors, they should not be used for less localised illnesses like CNS disorders (Barbu *et al.,* 2009).

Non-invasive techniques pertain to the painless delivery of drugs across the BBB, typically without the need for direct instrument insertion (Ali *et al*., 2023). Developing drug carriers, such as liposomes and nanoparticles (NPs), that may be able to cross the BBB by non-disruptive pathways is one creative method to get around the BBB resistance to drug delivery (Rabanel *et al.,* 2020).

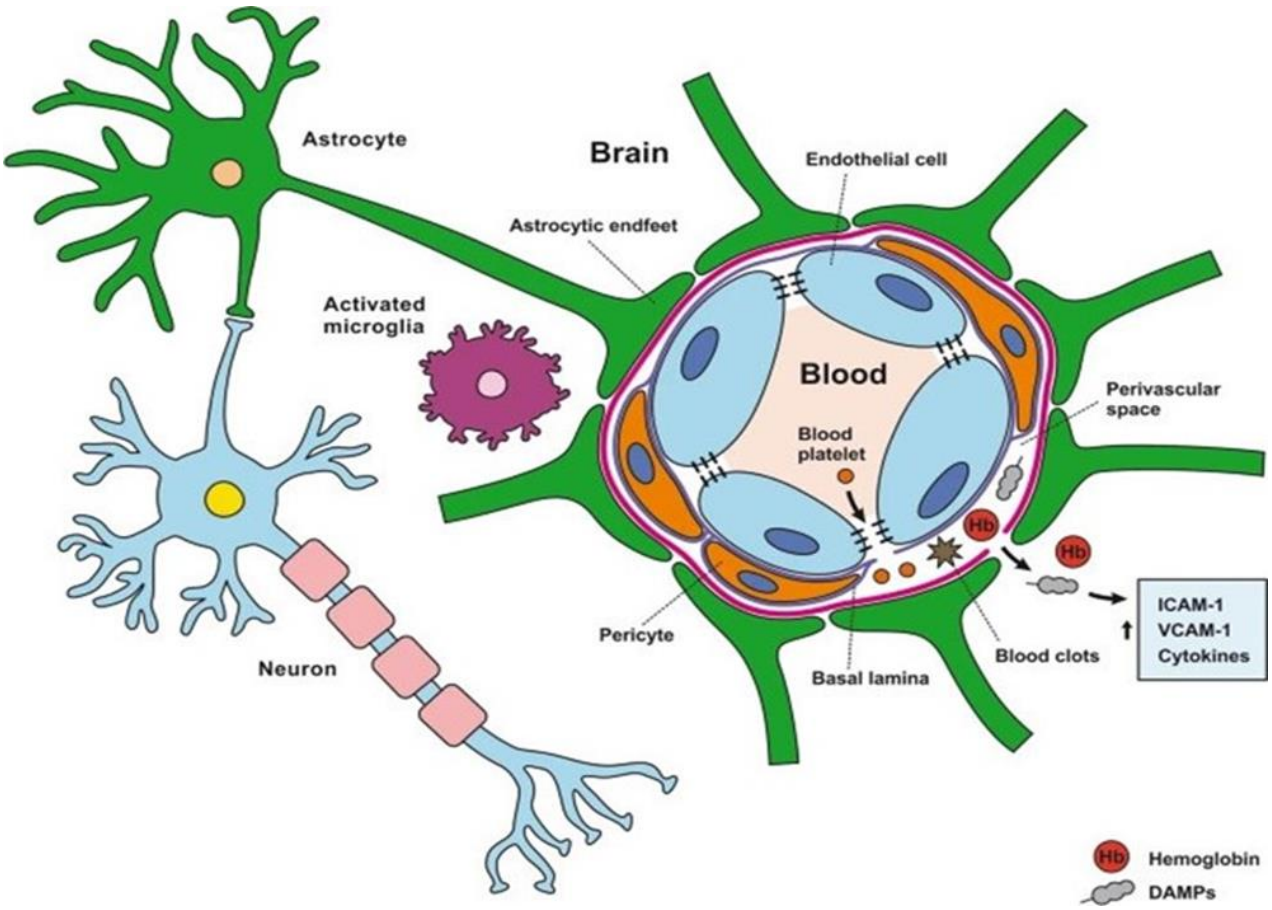


Figure 2.1 Schematic representation of the blood-brain barrier and the components of the neurovascular unit. Image obtained from Solár et al., 2022.

2.6 Transport across the blood-brain barrier

Despite the BBB acting as a barrier to some molecules being transported from the vasculature system to the brain, it can also facilitate the entry of appropriate materials through several mechanistic pathways to maintain brain homeostasis (Song *et al.,* 2023 and Ding *et al., 2020*). These pathways include, transcellular diffusion, paracellular diffusion, active efflux transport, carrier-mediated transport and transcytosis which are represented in Fig. 2.2 (Bellettato & Scarpa., 2018 & Deli. M., 2011).

2.6.1 Passive diffusion (transcellular & paracellular diffusion)

Most molecules travel across the BBB by passive diffusion, which is the most popular transport pathway for many medicinal substances (Mishra *et al*., 2023). In most cases, only positively charged, lipid soluble (lipophilic) molecules with molecular weights between 400 and 600 Da can passively diffuse through the BBB in the direction of the concentration gradient without requiring additional energy (Fong., 2015).

2.6.2 Active reflux transport

The blood-brain barrier's active efflux transport systems facilitate the removal of foreign substances from the brain, acting as a detoxification system within the brain (Kusuhara and Sugiyama., 2005). To achieve a clinically significant concentration in the brain, drugs must overcome these efflux mechanisms that operate in the brain. This efflux transport in the brain capillaries is mediated by a number of transporters with the primary efflux mechanism of these agents being the ATP binding cassette (ABC) transporter P-gp and multidrug resistant protein (MRP) (Kusuhara and Sugiyama., 2005 and Chen and Liu., 2012).

2.6.3 Carrier-mediated transport

Carrier-mediated transport (CMT) is an energy-dependent pathway typically used to transport low molecular weight nutrients from the bloodstream to the brain (Liu and Xu., 2022). Target molecules are recognized by particular receptors on the membrane of carriers, which then move them throughout the cell. This mechanism facilitates the transportation of peptides, amino acids, and monosaccharides which cannot diffuse through the cell membrane (Mukherjee *et al.,* 2017). Different specific transporters exist, each of which may be unique to one or more molecules. On one side of the membrane, a protein transporter is capable of binding to both glucose and amino acids. Compounds can cross the membrane in the direction of the concentration gradient by inducing a conformational change in the membrane (Wong *et al*., 2019). At the BBB, there is wide range of transporters, including influx transporters like sodium-coupled glucose transporters

(SGLTs), monocarboxylate lactate transporter (MCT1), l-type amino acid transporter (LAT1), glucose transporter (GLUT1), choline transporter (ChT), and cationic amino acid transporter (CAT1). P-glycoprotein (P-gp), and peptide transport system-6 (PTS-6) are a few examples of efflux transporters (Zeiadeh *et al*., 2018). Influx transporters promote the absorption of substances by the BBB, whereas efflux transporters impede it. Additionally, transporters can be energy independent (like GLUT1) or energy dependent (like P-gp). Either efflux inhibition or influx substrates can be used to improve drug delivery to the CNS through transporter-mediated uptake (Zeiadeh *et al*., 2018). The drug molecule should be changed so that it resembles pseudo-nutrient to enable transportation across the BBB via the specific transporters (Pardridge, 2005).

2.6.4 transcytosis

The primary pathway for large molecular weight compounds like proteins and peptides to enter the brain undamaged is through the transcytosis of macromolecules across the BBB using endocytotic mechanisms. The BBB and tight junctions physically block the passage of most large blood-borne molecules into the brain, but there are specific and some non-specific transcytotic mechanisms that can move a wide range of large molecules and complexes across the BBB (Abbott *et al.,* 2010).

Receptor-mediated transcytosis (RMT) is a vesicular transcellular pathway which facilitate the passage of different macromolecules via specific receptors across the BBB, including transferrin, lipoproteins and insulin (Zhang *et al.,* 2020). This pathway requires the binding of macromolecular ligands to specific transmembrane receptors to form receptor-ligand complexes. The process of binding triggers endocytosis, and after membrane penetration, the complexes are pinched off in a vesicle. Simultaneous exocytosis takes place as the complexes are transferred through the endothelial barrier. Numerous receptors, such as transferrin receptors, insulin-like growth factors, and lactoferrin receptors, were involved in this pathway (Wong *et al.,* 2019).

Adsorptive-mediated transport (AdMT) is a pathway that facilitates the transport of positively charged macromolecules across the endothelium and does not necessitate binding to a receptor. The surface layer of glycans and glycoproteins that coats the body's cell membranes has a negative charge, which mediates this non-specific process. The unspecific binding of cationic molecules is made possible by this anionic layer, known as the glycocalyx. These molecules are subsequently endocytosed and transported across the endothelial cells (Gosselet *et al.,* 2021). Both RMT and AdMT are commonly used to facilitate transport of nanomaterials into the brain. For nanomaterials consisting of cationic elements or positively charged surfaces, the primary

route of transport is AdMT, whereas nanoparticles functionalized to engage with cellular receptors use RMT as a transport mechanism (Kim *et al., 2019* and Sahay *et al.,* 2010).

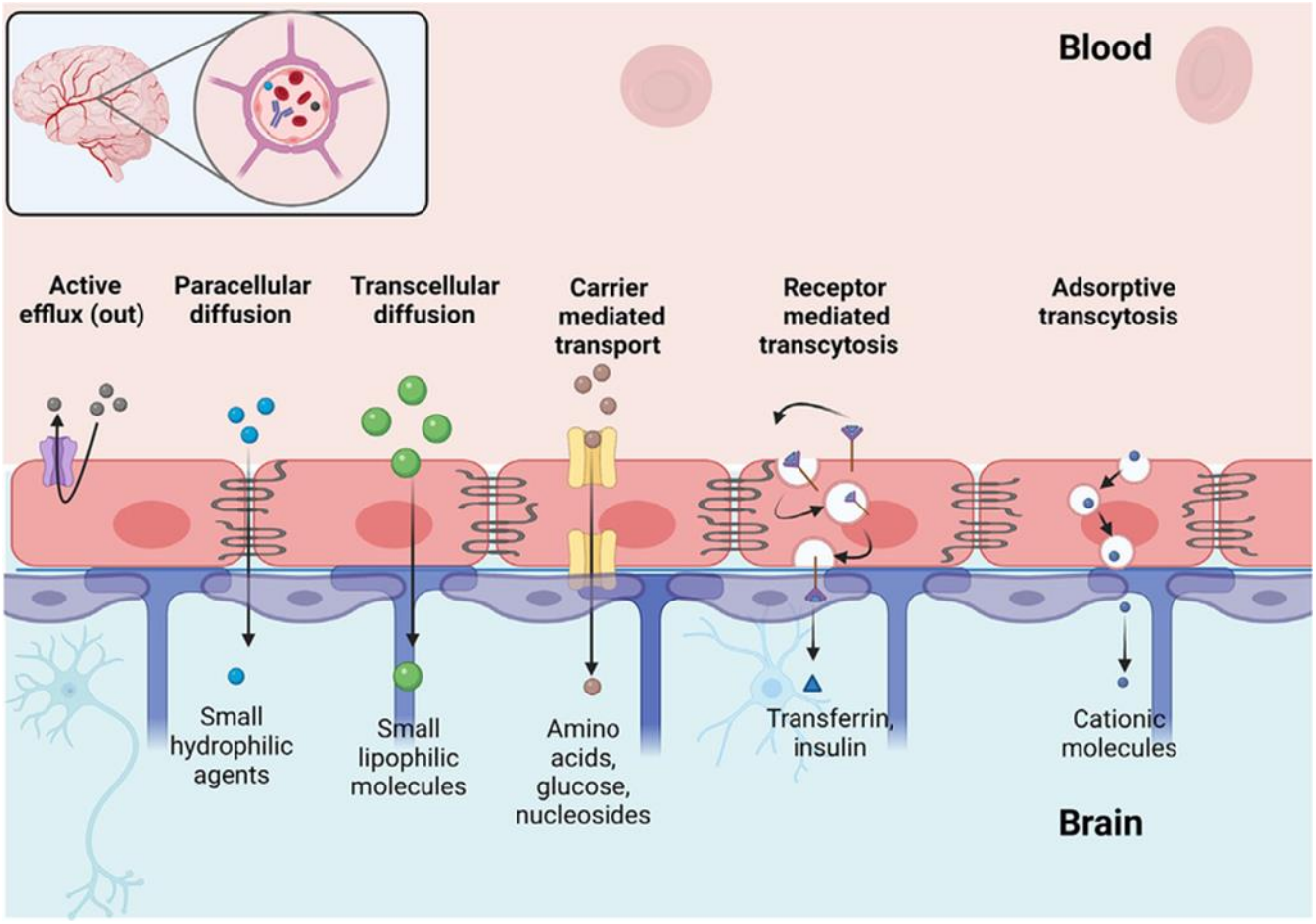


Figure 2.2 Transport pathways at the blood-brain barrier including receptor-mediated transcytosis, adsorptive transcytosis, carrier-mediated transport, and passive paracellular and transcellular diffusion (Rathi et al., 2022).

2.7 Nanoparticle based systems designed to cross the BBB.

Nanoparticles have demonstrated great promise in delivering therapeutic agents to the brain for the treatment of CNS diseases because of their unique physicochemical properties. Some of the primary features of nanoparticles are enhancing drug solubility and stability, facilitating transport across biological membranes, delaying the rate of chemical and enzymatic drug degradation, and increasing the therapeutic index and decreasing adverse effects by enabling targeted and controlled drug release. (Elumalai *et al.,* 2024 and Liu and Xu., 2022 and Bahadur and Jha.,

2022). The field of nanomedicine has advanced to a new level, revolutionising drug delivery and offering enormous promise for better patient outcomes. Healthcare practitioners can now precisely control the dosage and timing of medication release by utilizing drug delivery systems based on nanoparticles, resulting in more individualized and successful treatment plans (PourGashtasbi., 2015). Various types of nanoparticles like liposomes, polymeric nanoparticles, dendrimers, and micelles are considered as potential candidates for targeted drug delivery to the brain. Table 2 shows a summary of these nanoparticles.

Table 2.1 Different nanoparticles used in drug delivery.

Nanoparticle	Drug molecule	Size (nm)	Advantages	Disadvantages	references
Liposomes	Doxorubicin-loaded stealth PoP liposomes	100-400	Biocompatibility, reduced toxicity and degradation of drugs, targeted delivery, increased drug therapeutic index.	Poor stability, low encapsulation of hydrophilic drugs, cytotoxicity	Gbian and Omri, 2022; Luo *et al.,* 2016.
Polymeric nanoparticles	Paroxetine-loaded PLGA	1-100	Stability, non-toxicity, long clearance time, biodegradability, biocompatibility, and simple manufacturing processes	Self-aggregation	Hersh *et al.,* 2022; Sur *et al*., 2019; Li *et al*., 2023
Dendrimers	Cisplatin-loaded dendrimer	2-10	Water soluble, controlled delivery, multiple drug entrapment, controlled delivery.	Toxicity	Ambekar *et al*., 2020; Sherje e*t al.,* 2018, Chauhun, 2018; Jain *et al.,* 2010;
Micelles		10-100	Biodegradable, biocompatible, prolonged circulation time,	Inadequate drug loading capacity, poor stability in blood, and insufficient binding and uptake by cells.	Majumder *et al.,* 2020; Owen, *et al.,* 2012.

2.7.1 Liposomes

Liposomes are spherical vesicles, measuring between 100 and 400 nm, with a water-soluble core surrounded by a phospholipid bilayer (Fig 2.3) that improves lipophilicity, allowing macromolecules to pass through the blood-brain barrier more easily (Kurawattimath *et al.,* 2023). The main ingredients used to create liposomes are cholesterol and a variety of phospholipids, the latter whose chemical makeup ultimately determines the liposomal characteristics. Phosphatidylcholines, phosphatidylethanolamine, phosphatidylserines, phosphatidylglycerol, and sphingomyelin are the most widely used phospholipids (Trucillo *et al.,* 2020).

The structure of liposomes allows for the encapsulation of hydrophilic and hydrophobic compounds within the hydrophilic lumen and hydrophobic bilayer membrane respectively, (Kansız and Elçin., 2023) and the bilayer interface incorporates amphiphilic compounds (Ong *et al.,* 2016). Owing to their phospholipid composition, liposomes are thought to be non-toxic and biocompatible. They can also increase the therapeutic efficacy of incorporated medications by shielding them from enzymatic degradation (Md *et al.,* 2018). Liposomes are a promising option for use as targeted carriers in the treatment of a variety of illnesses because of their high loading, low toxicity, increased stability, and ability to control drug release. The primary benefit of their lipophilicity is their ability to cross the blood-brain barrier through an endocytotic process without surface functionalization, which makes them a viable option for drug delivery to the brain (Emad *et al.,* 2021).

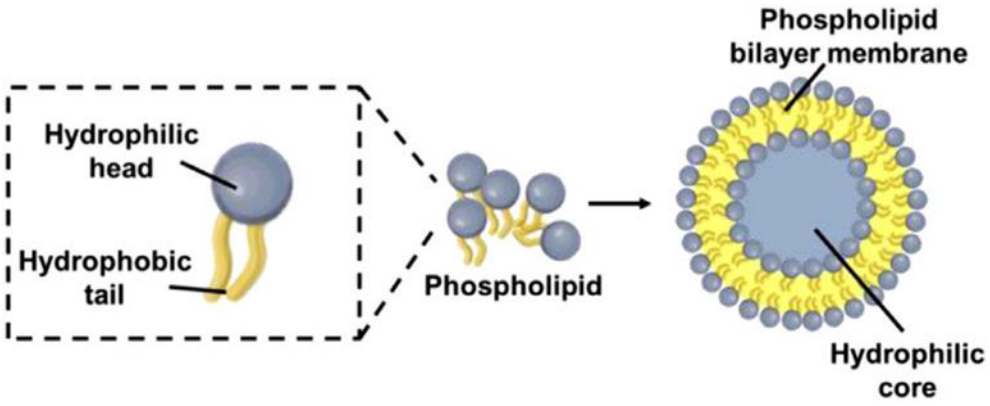


Figure 2.3 showing structure of liposome (Magar et al., 2021).

2.7.2 Polymeric nanoparticles

Polymeric nanoparticles are small particles ranging from 1 to 200 nanometers in size (Hickey *et al*., 2015). They are made from biodegradable or non-biodegradable polymers, including chitosan, albumin, gelatin, collagen, polylactic acid, polylactide co-glycolic acid (PLGA), polylactic acid (PLA), polyethylene glycol (PEG), polycaprolactone (PCL), respectively (Bhardwaj and Jangde, 2023). The hydrophilic corona of polymeric nanoparticles provides steric stability, while the dense polymer matrix core is designed to encapsulate lipophilic drugs. The drug molecules to be delivered can adhere to the surface of the nanoparticles by chemical conjugation, adsorption, or encapsulation (Parveen *et al*., 2012). Different forms, sizes, and architectural configurations can be achieved when producing these nanoparticles (Song *et al*., 2023). They can also be altered to carry neutral, negative, or positive surface charges. Desired properties of polymeric nanoparticles have been incorporated into them thanks to the ease with which different molecules can modify their surfaces to achieve targeted delivery and increased lifetime in circulatory systems. Another benefit is the potential to increase colloidal stability and lengthen their shelf life. Because of this, these nanoparticles have been widely used in bioimaging, brain targeting, vaccine development, cancer therapy, and many other fields (Song *et al*., 2023).

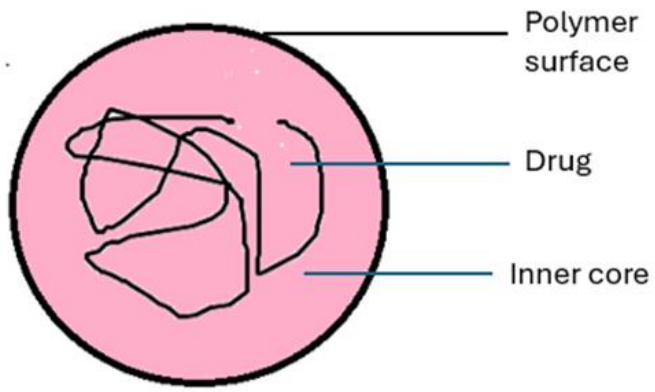


Figure 2.4 showing structure of polymeric nanoparticle.

2.7.3 Dendrimers

Dendrimers are uniformly sized and shaped polymeric macromolecules that are highly branched. They are made up of three structural components which determine their physiochemical and

biological properties: a central core, repeating branching units known as generations, and terminal groups that offer surface functionalities that can be changed (Mendes *et al.,* 2017 and Zhu *et al.,* 2019). The number of branch points between the dendron's core and periphery determines its generation. Higher-generation dendrimers are larger, more branched, and have more end groups on their periphery than lower-generation dendrimers (Lee *et al*., 2005).

Since their creation, dendrimers have been considered as promising drug delivery vehicles due to their abundance of internal cavities and surface functionalities. The use of dendrimers as drug delivery systems has several benefits, including increased stability of the active component, longer drug residence time in the circulatory system, protection of the drug from the environment, and tissue targeting (Markowicz-Piasecka *et al.,* 2022). Drug molecules can be physically encapsulated into the internal cavities of dendrimers or chemically conjugated to the terminal functional groups, depending on their structures and properties (Huang and Wu., 2018). Several dendrimers, including polyamidoamine (PAMAM), polyhydroxylamine, poly-L-lysine (PLL), and polypropylene amine (PPI), have been employed in the delivery of drugs and contrast agents (Saeedi *et al*., 2019). Both invasive and non-invasive approaches can be used to target specific areas of the brain with dendrimer-based drug delivery systems. Carrier-mediated transport (CMT) seems to be the most promising of these (Markowicz-Piasecka *et al*., 2022). Despite their widespread use in drug delivery, dendrimers' application in biological systems is restricted because of their toxicity concerns and intricate synthetic procedures (Naqvi *et al.,* 2020).

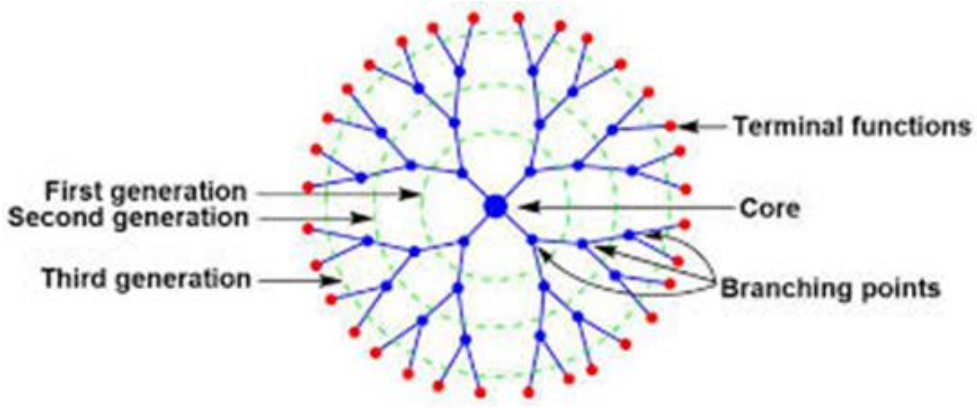


Figure 2.5 showing the main structural features of a dendrimer obtained from Caminade, 2022.

2.7.4 Micelles

Micelles are self-assembled, nanoscale (5-100nm), colloidal structures composed of amphiphilic molecules, typically surfactants or block copolymers that spontaneously form in aqueous solutions

(Fig. 6) (Movassaghian *et al*., 2015). These polymers exist as monomers below a certain concentration, called critical micellar concentration (CMC). Above the CMC, however, they self-assemble into micelles with an exterior hydrophilic shell and an interior lipophilic core (Ghezzi *et al.,* 2021). Its hydrophilic shell facilitates the stabilisation of the particle in an aqueous solution, while the hydrophobic core controls the sustained release of the hydrophobic drug embedded within (Bastakoti *et al*., 2013). Micelles have garnered significant interest as drug delivery systems due to their ability to enhance the solubility of hydrophobic drugs, encapsulate and protect the drug from enzymatic degradation, improve sustained release profile of the drug, and potentially target specific tissues (Wakaskar., 2017).

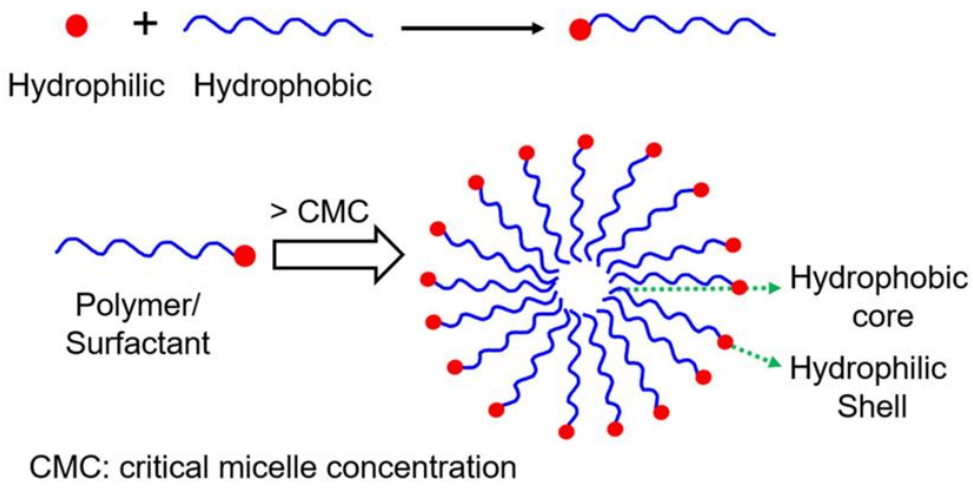


Figure 2.6 Polymeric micelles prepared by self-assembly of block copolymer and surfactants. Image obtained from Perumal et al., 2022.

2.8 Conclusion

Conventional (oral and parenteral administration) antidepressant drugs have been widely used over the years and have helped in the management of depression for many years. However, not all patients respond adequately to this treatment. These formulations, although effective have frequent drawbacks such as variable absorption, first-pass metabolism, inactivation, or degradation before reaching the targeted sites, drugs are not released in sustained manner, increased adverse effects, low solubility of drug and non-specificity to name a few. The research into alternative drug delivery methods for antidepressants represents an important field of research focused on resolving the shortcomings of existing treatment modalities and enhancing outcomes for depressed individuals. Furthermore, the BBB, which is responsible for the selective transport of molecules to the brain, is one of the major barriers to the efficacy of brain-targeted

drug delivery systems. 98% of small-molecule medications and 100% of large-molecule neurotherapeutics are unable to passively diffuse through the BBB due to microscopic tight junctions (Mishra *et al.,* 2023). Targeted drug delivery to the brain offers a promising method for treating neurological disorders. It can be facilitated by using strategies like liposomes, nanoparticles, dendrimers, and micelles, which have the potential to improve drug solubility, stability, and bioavailability, as well as facilitate targeted drug delivery to the brain. Over the past few years, researchers have been interested in the design, development, and implementation of these drug delivery system.

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CHAPTER 3

SYNTHESIS AND CHARACTERISATION OF AMITRIPTYLINE LOADED PLGA NANOSUSPENSION

3.1 Introduction

Depression is the most severe and incapacitating mental illness, having a detrimental impact on both mental and physical health (Abd-Elal *et al*., 2023). Depression has a negative impact on a person's quality of life because it affects their mood, behaviour, sleep and appetite. One's capacity to function normally may be compromised by the emotional and physical difficulties (Tawfik *et al*., 2020). It is primarily caused by disturbances in monoaminergic (norepinephrine, serotonin, and dopamine) transmission in the brain as a result of the complex interaction of several social, psychological, and biological factors. Therefore, most antidepressants used to treat depression increase the availability of monoamines at the synapse by blocking alpha-2 auto- and hetero-receptors on monoaminergic neurons, preventing their uptake by neurons, preventing their intraneuronal metabolism, or both. Nonetheless, the therapeutic efficacy of these antidepressants depends on their continuous and prolonged presence at the site of action in the brain (Tong *et al*., 2017 and Haque *et al.,* 2014).

Pharmacological treatments for depression include first-generation tricyclic antidepressants (TCAs), second-generation selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors (MAOIs), and serotonin and norepinephrine reuptake inhibitors (SNRIs) (Khatoon *et al*., 2019). Amitriptyline is a commonly used TCA for the treatment of depression. It acts by inhibiting the membrane pump mechanism that allows norepinephrine and serotonin to be absorbed by adrenergic and serotonergic neurons (Lv *et al.,* 2013). When used in a clinical setting, it is taken orally, reaching peak plasma concentrations in 4 to 8 hours. It undergoes significant first-pass hepatic metabolism and has a systemic bioavailability that ranges from 33 to 62% (Morineau *et al*., 2018 and Khurshid *et al.,* 2017).

Traditional formulations (such as tablets and capsules) for the treatment of depression have several drawbacks including, side effects, compromised patient compliance, prolonged dosing frequency, low bioavailability, toxicity, and limited drug penetration. Another major obstacle encountered during drug transportation to the CNS is the passage of drugs through the BBB (Song *et al.,* 2023). The effective use of readily available conventional therapies for depression is restricted by all these problems. Consequently, it continues to be a significant challenge for

global health organizations and pharmaceutical companies to evaluate an alternative delivery system that could safely and potentially manage depression without compromising patient compliance. (Laffleur and Keckeis, 2020 and Patel *et al*., 2022).

To overcome these issued novel drug delivery systems have been developed for targeted drug delivery, these include but are not limited to polymers (PLGA, micelles or dendrimers), liposomes, nanoemulsions, proteins (albumin, transferrin), nanostructured lipid carriers, solid lipid nanoparticles (SLN) etc (Wu *et al*., 2023 and Khan and Irchhaiya, 2016). Among the different nanoparticles used for drug delivery, this study will focus on PLGA nanoparticles. PLGA is an FDA approved biodegradable copolymer of lactic acid and glycolic acid (Kim *et al*., 2019; Sharma and Dang., 2020). It has been reported to display properties of low toxicity, high biocompatibility, and highly controlled drug release (Ding *et al*., 2020). PLGA nanoparticles have received substantial interest and are widely employed as polymer-based carriers for drug delivery to the brain. They are reported to improve drug absorption through the mucosa resulting in greater neuro-availability (Pandey *et al*., 2016).

This study will aim to synthesize amitriptyline loaded PLGA nanosuspension in order to minimise adverse effects by promoting targeted drug delivery and ensuring that the drug is only active at the intended location (Patra *et al*., 2018) and increasing therapeutic efficacy upon sublingual administration. After the AMT-PLGA nanoparticles were synthesized, their physicochemical characteristics were assessed using Fourier Transformation Infrared Spectroscopy (FTIR) to assess the solubility of the copolymer. Zeta potential and particle size and distribution were measured for stability, colloidal dispersion and surface charge. Differential scanning calorimetry (DSC) was used to the evaluate the thermal behaviour of the materials.

3.2 Materials and method

3.2.1 Materials

Poly (lactic-co-glycolic acid) (PLGA 50:50; Resomer RG504), poloxamer 407, amitriptyline (AMT) Hydrochloride (HCl), phosphate buffer saline (PBS), acetone, HPLC grade acetonitrile were obtained from Sigma-Aldrich (St. Louis, MO, USA).

3.2.2 Synthesis of AMT-PLGA nanosuspension

The modified nanoprecipitation method used for the preparation of AMT-PLGA nanosuspension was adopted from Sharma and Dang (2020). The method used was as follows: 5mg/mL of AMT HCl and 12.5mg/mL of PLGA (50:50) were weighed out and dissolved in 10ml of acetone to form

the organic phase. In preparation of the aqueous phase 200mg of poloxamer 407 was dissolve in 20ml distilled water. The organic phase was slowly added to the aqueous phase using a syringe while keeping the solution on a magnetic stirrer at a speed of 800rpm. The formulated was then homogenized using a high-speed homogenizer at 15 000rpm for 10 minutes to ensure that the nanoparticles are uniformly distributed. The nanosuspension was sonicated in an ice bath by a probe ultra-sonicator for 5 minutes at 50% amplitude to evenly disperse the nanoparticles. The resulting solution was maintained on a magnetic stirrer overnight at s speed of 400rpm to completely evaporate the organic solvent. The nanoparticles were obtained after centrifugation at 12 000rpm, 4ºC for 15 minutes and the supernatant liquid was discarded. The nanoparticles we gently washed three times with distilled water to remove unentrapped drug molecules. The nanoparticles obtained were resuspended in distilled water and placed in an ice bath in an ultra-sonicator for 30 minutes at 60% amplitude to agitate and redisperse them.

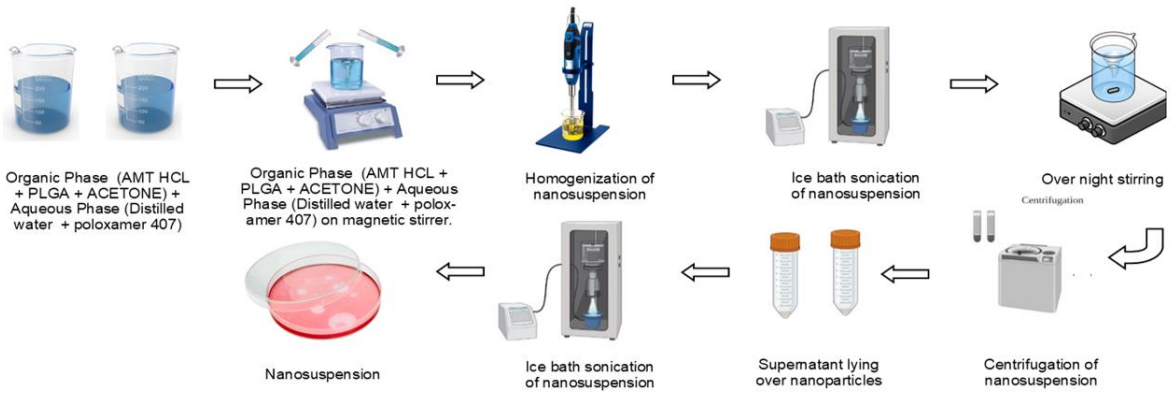


Figure 3.1 Schematic representation of preparation of AMT-PLGA nanosuspension using nanoprecipitation.

3.2.3 Characterization of the nanosuspension's particle Size Distribution and Zeta Potential

The evaluation of zeta potential, particle size and distribution of the AMT-PLGA nanosuspension were measured using the dynamic light scattering. All measurements were carried out by triplicate by a Zetasizer NanoZS analyser (Malvern Instruments Ltd. UK). The nanosuspension was sonicated by a probe ultra-sonicator to for 3 minutes to break the larger particles into smaller particles and prevent aggregation, then filtered using a 0.22µm syringe filter and loaded into a

cuvette (for size measurement) and a capillary cell (for zeta potential) for analysis. The results reported are average values from triplicate runs.

3.2.4 Analysing the chemical properties starting materials and the nanosuspension using FTIR

An Attenuated Total Reflectance Transformation Infrared (ATR-FTIR) spectra was used in the current study to evaluate the chemical interaction between PLGA and amitriptyline during nanosuspension development. The following samples were analysed; amitriptyline powder, PLGA powder, and the PLGA-amitriptyline nanosuspension. The spectra of AMT-PLGA, PLGA and amitriptyline HCl samples were scanned 20 times at a wavelength of 650-4000cm^{-1} using a PerkinElmer Spectrum 2000 ATR-FTIR (PerkinElmer 100, Llantrisant, Wales, UK) spectrometer fitted with a single-reflection diamond MIRTGS detector. The FTIR spectrum of all the samples will have the intensity in the y-axis and wavenumber on the x-axis. The FTIR spectrum will allow for analysis of the peaks for each starting material and also peak shift due to the chemical interaction taking place during formulation of the nanosuspension.

3.2.5 Thermal analysis of biomaterials using DSC

The AMT-PLGA nanosuspension was analysed by Differential Scanning Calorimetry to obtain information relating to the thermal characteristics of amitriptyline powder, PLGA powder and the PLGA-amitriptyline powder. PLGA-amitriptyline nanosuspension was lyophilized to allow for characterisation of DSC. Data was collected using the Mettler Toledo DSC-1 STARe system (Swchwerzenback, ZH, Switzerland). About 3 mg of each sample (PLGA, amitriptyline and amitriptyline-loaded PLGA) were put into a sealed, perforated aluminium pan, loaded into the DSC, and heated in a nitrogen atmosphere at a heating rate of 10º C/min over a temperature between of 25-300ºC. The resulting thermogram plotted heat flow(mW) versus temperature (ºC).

3.2.6 Calibration curve of amitriptyline hydrochloride in PBS and drug release

The calibration curve was obtained using the High-Performance Liquid Chromatography. The method used to obtain calibration curve was adopted from Farag and Ahmed, 2011. A stock solution of 250µg/ml was prepared in PBS pH 7.4. The stock solution was diluted to achieve a series of standard concentrations (0, 20, 40, 60, 80, 100, and 120µg/ml). Waters empower 3 HPLC (Microsep, Johannesburg, South Africa) was used to obtain the standard curve and quantified the amitriptyline. The mobile phase consisted of HPLC grade acetonitrile and distilled water adjusted to pH 4 using phosphoric acid. The ratio of acetonitrile to water was 50:50 and the

flow rate was 1ml/min. The absorbance was then measured at a wavelength of 254nm. The standard curve for amitriptyline was plotted on the HPLC empower 2 software, with concentration on the X-axis and absorbance on the Y-axis.

Drug release studies were performed using snakeskin dialysis tubing (size 3.5KDa MW). Briefly, nanosuspension was prepared into a snakeskin dialysis tubing, followed by immersion the dialysis tubing into a 100ml PBS solution. This was done in triplicate. A volume of 3ml was sampled out of at 1, 2, 4, 6, 8 hours and replaced with fresh PBS to maintain sink condition. Withdrawn samples were analysed using waters HPLC in the conditions used for the standard curve above. A concentration versus time graph was constructed from the amount of amitriptyline release after each time point.

3.3 Results and discussion

3.3.1 Characterization of the nanosuspension's particle size and PDI

Nanoparticles that are smaller in size are more favourable for active transport across the blood-brain barrier (Zaman *et al.,* 2018). The results of the investigations into the zeta potential and size distribution revealed a mean nanoparticle size of 137.7nm and a particle size distribution of 0.064. Particle size and BBB permeation have been examined in numerous of studies, and it has been noted that a particle size of <200nm is suitable for drug delivery to the brain (Jo *et al.,* 2015). Particle size distribution indicates the uniformity of the nanoparticles. A well-defined peak, as seen in the image below, suggests homogeneity in the particle sizes of the nanoparticles which can be beneficial in applications used to for targeted brain delivery.

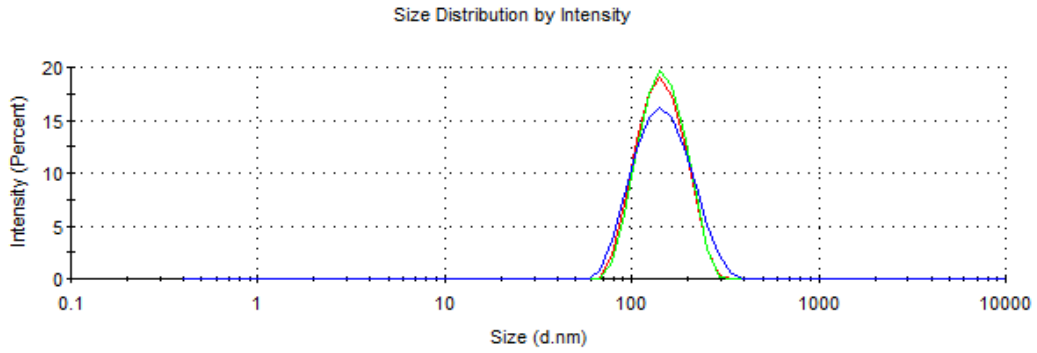


Figure 3.2 Particle size for AMT-PLGA nanosuspension.

3.3.2 Zeta Potential

Zeta potential is used to investigate the surface charge characteristics of the nanosuspensions. The Zeta potential observed for AMT-PLGA nanoparticles was mean of -8.98mV which suggests

repulsion between the nanoparticles, good dispersion stability and decreased potential for aggregation. Additionally, it ensures the development of a high-energy barrier to prevent the scattered particles from merging (Sindhu *et al.,* 2018) (Figure 3.3).

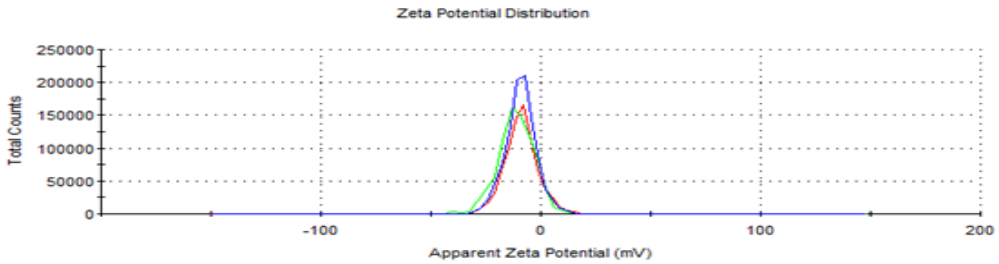


Figure 3.3 Zeta potential for AMT-PLGA nanosuspension.

3.3.3 Characterisation of starting materials and the nanosuspension using FTIR spectrometry

The chemical composition and characteristic of AMT-PLGA, PLGA and AMT were evaluated (Fig. 3.4). FTIR spectra for AMT, PLGA and AMT-PLGA were recorded (Fig. 3.5). The spectrum of AMT-PLGA nanosuspension was analysed in a liquid form and showed distinctive peaks at 2882cm^{-1} which correspond to C─H stretching, 1759cm^{-1} which corresponds to C═O stretching, 1342cm^{-1} which corresponds to C─N stretching, 1098cm^{-1} which corresponds to C─O stretching, 962.33cm^{-1} and 841.77cm^{-1} which corresponds to C═C bending and C─C stretching. The spectrum of AMT showed distinctive peaks at 3063cm^{-1} and 3017 cm^{-1} which corresponds vibration of C─H, 2925cm^{-1} and 2898cm^{-1} which corresponding to ─C─H stretching, 1485cm^{-1} and 1441cm^{-1} which corresponding to CH_2 and CH_3 bending, 966cm^{-1} which corresponding to C─H, and 743cm^{-1} which corresponding to C─C stretching. The spectrum of PLGA displayed characteristic peaks at 2950cm^{-1} which correspond to C─H stretching, 1384.36cm^{-1} which corresponds to CH_3 bending, 1165.36cm^{-1} which corresponds to C═O stretching, and 1086cm^{-1} which corresponds to C─O stretching. The drug's successful incorporation into the PLGA is indicated by the disappearance of the drug's characteristic peaks in the case of AMT- PLGA (Eissa *et al.,* 2023).

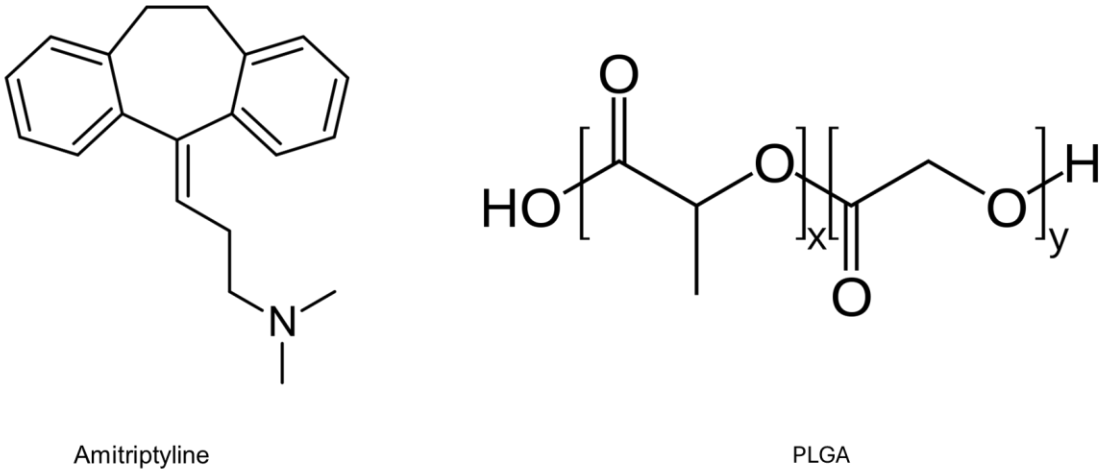


Figure 3.4 Schematic of the starting materials' chemical structures.

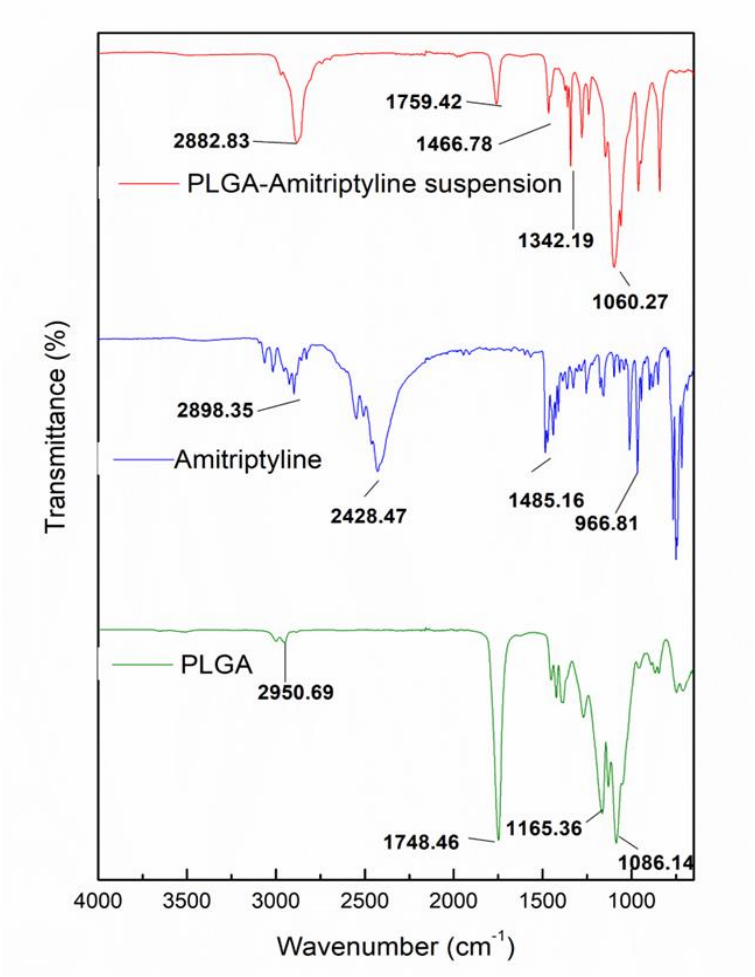


Figure 3.5 FTIR spectrum curves for AMT-PLGA nanosuspension, AMT and PLGA. The nanosuspension was analysed in a liquid form.

3.3.4 Thermal analysis using DSC

To analyze the physiochemical interaction between the polymer and the drug encapsulated, differential scanning calorimetry is employed. The distinctive peaks of various compounds are visible in DSC. The melting point of native amitriptyline is represented by the prominent and sharp endothermic peak at 198°C in its thermogram. This is also confirmed by DCS thermogram reported by Gao et al, 2013. The nanoformulation of AMT-PLGA showed a distinctive endothermic peak between 60ºC and 75ºC (Jana *et al*., 2014). The peak observed for PLGA between 290ºC – 350 ºC, indicates degradation of the sample (Zhang *et al.,* 2016). Degradation peaks typically appear because the sample is releasing heat as the material undergoes chemical changes. A material is more thermally stable when there are no degradation peaks in the DSC thermograms. More stable materials are those that show little degradation over a broad range and retain their structural integrity. AMI-PLGA and AMT display sharp endothermic peak. This is an indication that there is a high concentration of the polymer, which suggests that the drug is entrapped in the nanoparticle.

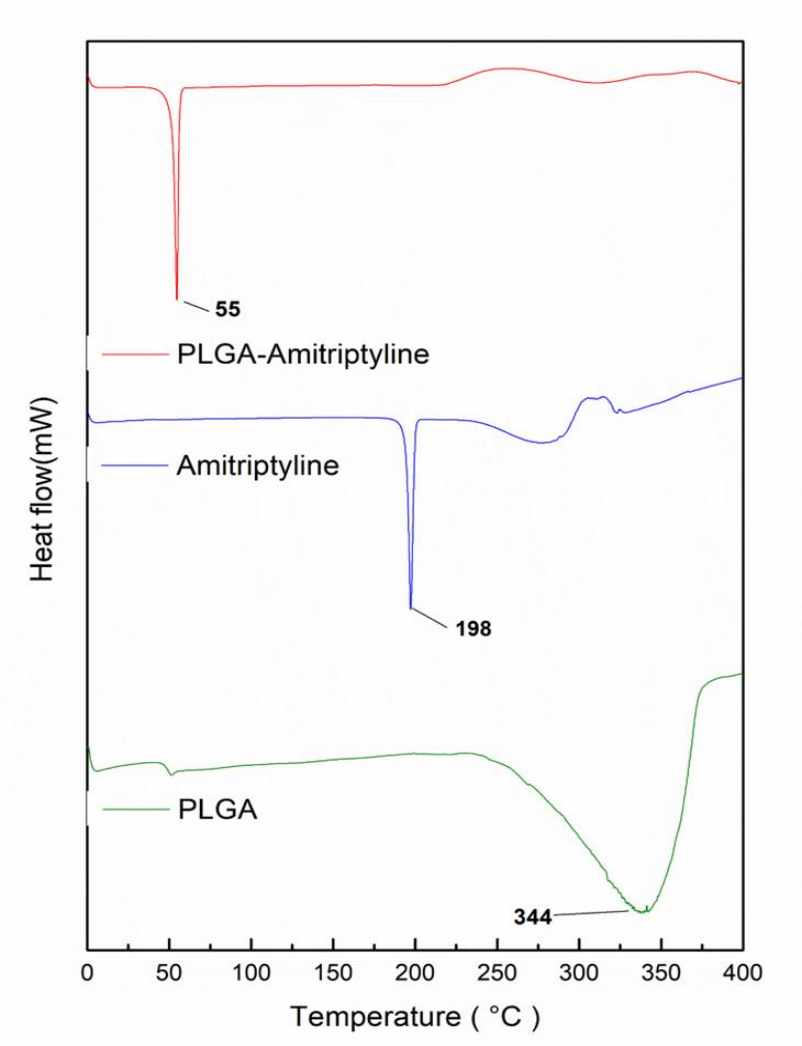


Figure 3.6 Differential Scanning Calorimetry thermograph for AMT-PLGA nanosuspension, AMT and PLGA

3.3.5 Calibration curve

The λmax of amitriptyline was found at 254nm. Based on the λmax, the calibration curve was obtained (Figure 3.7). This curve confirms that there is a linear relationship between concentration of the drug and the absorbance (R2 = 0,9999) i.e., an increase in the concentration of the drug results in an increase in the respective absorbance (Abbas et al., 2017).

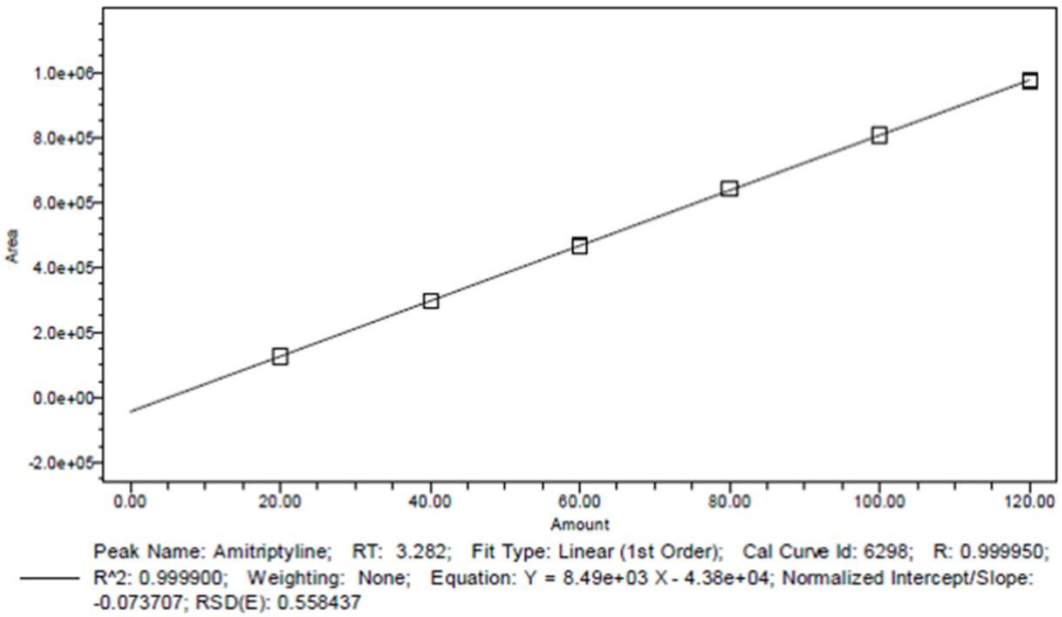


Figure 3.7 Calibration curve of AMT HCl using a wavelength of (λmax) 254nm

The drug release profile of amitriptyline exhibited a burst release of approximately 60% in the first hour, followed by a gradual release up to approximately 68% within 8 hours. This correlates with the formulations that have been reported (Mohebali *et al.,* 2021). The maintenance dose for amitriptyline ranges between 50-100mg daily. The drug release for AMT ranged between 60-68% which was 60-68mg within one to eight hours. This shows that the release is within the daily dose. This amitriptyline release releases the same amount as other delivery systems i.e. film-coated tablets. However, the current nanosuspension makes use of materials that will enable the drug to cross the BBB, thus increasing the bioavailability and reducing drug loss that could arise from first pass metabolism.

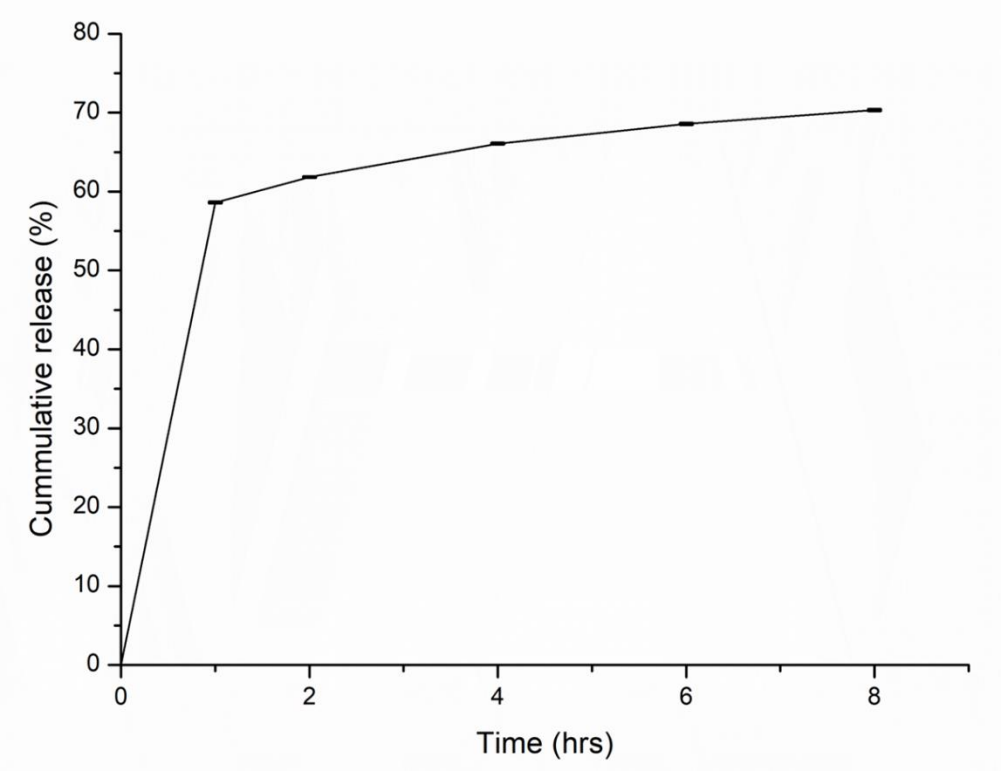


Figure 3.8 amitriptyline release from the nanosuspension

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CHAPTER 4

CONCLUSION AND RECOMMENDATION

4.1 Conclusion and recommendations

Depression affects people of all ages worldwide. This study highlighted the need for innovative drug delivery strategies for depression. Most antidepressants are administered orally. Following oral administration, a drug undergoes extensive hepatic metabolism which decreases the bioavailability of the drug in the systemic circulation and the drug's ability to reach the target site. Sublingual delivery allows the drug to directly enter the systemic system and bypass hepatic metabolism. One promising approach to enhancing treatment outcomes and patient quality of life is the creation of advanced drug delivery systems can deliver desirable dosage in the brain to treat depression. These innovative approaches have the potential to fill the unmet clinical gaps and enhance the treatment of depression and related mood disorders by improving medication efficacy, safety, and patient compliance. By increasing the drug's solubility, stability, and bioavailability, innovative drug delivery strategies may be able to increase treatment efficacy.

Long-term maintenance of therapeutic drug levels in the body is possible with controlled release formulations, which also lessen dosage frequency and systemic system fluctuations while enhancing therapeutic outcomes. Controlled release formulations can lessen systemic side effects and minimize off-target effects related to traditional antidepressant therapy. The incorporation of AMT to the delivery system can improve its effectiveness and promote targeted delivery to the brain. The BBB must be crossed by a nanoparticulate system smaller than 200 nm. In this study, the synthesized amitriptyline-loaded nanosuspension had a size of 137.7 nm, which suggests that they may penetrate the BBB (Tong *et al., 2017)*. Thermal studies of AMT-PLGA showed improved stability for AMT-PLGA compared to AMT alone. FTIR characterization confirmed amitriptyline's' successful incorporation in PLGA which can enhance the drugs' ability

to penetrate the BBB and improve its neuroavailability. During the drug release studies 60-68% of amitriptyline was released after 8 hours.

To this day, sublingual nanoparticulate drug delivery systems for depression remain a research and development area rather than a widely available treatment option. More research must be conducted to provide more direction for current investigations, even though this technology has the potential to increase drug efficacy and decrease side effects.

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