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Design of an oral nanotherapeutic galantamine-loaded nanoemulsion composite system for the treatment of Alzheimer’s disease

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SIPHESIHLE NONTUTHUKO SITHOLE

DESIGN OF AN ORAL NANOTHERAPEUTIC GALANTAMINE-LOADED NANOEMULSION COMPOSITE SYSTEM FOR THE TREATMENT OF ALZHEIMER'S DISEASE



WITS
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A Dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the partial requirements for the degree of Master of Science in Medicine with specialization in Pharmaceutical Affairs.

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Johannesburg, 2024

DECLARATION

I, Siphesihle Nontuthuko Sithole, 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.



……

Signature

On this …….2nd.....day of ……AUGUST…… 2024

DEDICATION

This dissertation is dedicated to my parents, Octavia and Sibusiso Sithole; my grandmothers who are resting in heaven, Victoria Zuma and Victoria Khumalo; and my wonderful siblings. It is through their prayers and sacrifices, that I am where I am today. For that, I am eternally grateful.

Ngibonga oSithole, oMondise, oJobe kaMatshane. BaThembu!

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Despite the many challenges I've faced throughout this worthwhile journey, it would be a grave dishonour to not thank God for guiding, protecting and blessing me. The number of times I've felt despondent and discouraged, do not compare to the number of times He showed me grace.

To my friends and extended family members, who continuously motivated and supported me – thank you.

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ABSTRACT

Alzheimer's disease (AD) is a progressive disorder that degenerates neural pathways in the brain. Alzheimer's Disease continues to gain interest on new therapeutics with numerous pharmaceutical agents formulated to target the Central Nervous System (CNS) – namely the four acetylcholinesterase inhibitors which include galantamine, rivastigmine, donepezil and memantine. Nanotechnology focuses on the design and synthesis of nanomaterials which are functionally organised with at least one dimension of no more than 100 nm. The use of nanoparticles in medicine can be attributed to the fact that the human body contains numerous biochemical and biological mechanisms that occur at the nanoscale. Nanoparticles provide substantial support for the delivery of various macromolecules. Nanotechnologies are classified into different forms, which include nanotubes, nanofibers, polymeric nanoparticles, nanoemulsions, nanomicelles, and nanoliposomes. Alternative nanocomposites such as nanoemulsions are documented as highly selective delivery methods of dyes, lipophilic dyes and drugs of low molecular weight. Additionally, therapeutic agent-loaded nanoemulsions have revealed enhanced uptake of loaded therapeutic agents in degenerated cells significant to AD. The galantamine-loaded nanoemulsion in this study displayed desirable properties such as nanoparticulate size of 10.54 nm, 11.01 nm, 133.2 nm and 305.1 nm. Additionally, the galantamine-loaded nanoemulsion's drug release profile, which included a burst release of 66.31% over 6 hours and total controlled release of 68.07% in 24 hours, was also observed in this study. These promising results, as well as those obtained from further evaluations such as thermal stability and chemical interactions during formulation development, support the design of galanatamine-loaded nanoemulsion composite systems that can facilitate the treatment of AD.

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LIST OF ABBREVIATIONS

1. Alzheimer's Disease (AD)
2. Central Nervous System (CNS)
3. Blood Brain Barrier (BBB)
4. Fourier-Transform Infrared Spectroscopy (FTIR)
5. Differential Scanning Calorimetry (DSC)
6. High-Performance Liquid Chromatography (HPLC)
7. Poly Lactic-Co-Glycolic Acid (PLGA)
8. Polyethylene Glycol (PEG)
9. Mesenchymal Stem Cells (MSCs)
10. Neural Stem Cells (NSCs)
11. Polyamidoamine (PAMAM)
12. Polyvinyl Alcohol (PVA)

1 CHAPTER 1: BACKGROUND AND INTRODUCTION

1.1 Introduction

Alzheimer's Disease is a progressive disorder that degenerates neural pathways in the brain (Xie et al., 2022). Alzheimer's Disease is characterised by a progressive deterioration of cognitive function, memory loss (especially dementia), deterioration of visual ability and rational capacity (Haider et al., 2018, Salatin et al., 2016 and Shah et al., 2014). Globally, approximately 40 million people suffer from this chronic neurodegenerative disorder characterised by the progressive growth and aggregation of amyloid-beta and neurofibrillary tangles in the brain (Haider et al.,2018). Alzheimer's Disease continues to heighten the interest in new therapeutics with numerous pharmaceutical agents formulated to target the CNS, which is a complex microenvironment composed of the brain, spinal cord and complex neural networks (Harilal et al., 2019 and Wong et al., 2019).

For optimal neural functioning, the CNS is protected by the BBB (blood-brain barrier) which is an active interface that controls the movement of small active molecules and macromolecules from the bloodstream to the brain whilst selectively permitting non-toxic lipid-soluble molecules and oxygen to pass through (Chatterjee et al., 2019). Pharmaceutical agents are adversely challenged by the BBB from reaching the localised site of the neurodegenerative disease (brain). Effectively treating neurodegenerative diseases is a challenge due to efflux membrane transporters such as P-glycoproteins. These proteins are primarily distributed in the endothelial cells that line the BBB; and they prevent the accumulation of therapeutic agents and toxic compounds in the brain by actively pumping out these therapeutic drugs and toxins back into the bloodstream (Ding et al., 2021). However, other studies propose that the galantamine prodrug, Gal-2, inhibits the P-glycoprotein-mediated efflux from cultured cells by competing for the substrate binding sites – a finding that may potentially overcome challenges posed by these efflux membrane transporters on therapeutic agents (Zagorska et al., 2020).

The FDA-approved pharmacotherapeutic agents for the treatment of AD are the four acetylcholinesterase inhibitors including galantamine, rivastigmine, donepezil and memantine (N-methyl-D-aspartate receptor agonist) (Hara et al., 2019 and Xu et al., 2019). Memantine is mainly administered concurrently with donepezil (Gauthier et al., 2013). However, these therapeutic agents are principally administered as oral and transdermal formulations which pose a challenge of delivering adequate amounts of the therapeutic drug to the CNS due to their low bioavailability, first-pass effect in the liver, poor solubility and acidic pH in the stomach (Mendes et al., 2019, Malaiya et al., 2018, and Shadab et al., 2018).

Scientists have gained interest in oral nanoemulsions due to their non-invasive drug administration and shielding from enzymatic degradation (Singh et al., 2017 and Nasr, 2016).

Furthermore, oral nanotherapeutic delivery systems, specifically mucoadhesive systems, have proven to be advantageous because of properties such as effective adhesion to the intestinal mucosa and controlled release of the AD therapeutic into the bloodstream (Chauhan et al., 2019, Agrawal et al., 2018 and Haider et al., 2018,).

Galantamine has been observed to readily absorb into the bloodstream with an oral bioavailability of between 80 and 100 percent. Additionally, galantamine doses between 1.5 and 5 mg/kg have been hypothesised to appropriately reach brain levels and potentiate an optimal dose-response within documented allosteric ligands. Patient tolerability of the model drug increases following a controlled and sustained-release treatment regime. In addition to exhibiting a half-life of 7 hours, galantamine achieves a peak effect of inhibiting acetylcholinesterase activity approximately one hour after a single oral dose of 8mg in healthy individuals (Baakman et al., 2021).

Carbopol was identified as the mucoadhesive polymer of interest due to it's ability to facilitate the effective delivery of galantamine. Carbopol is a positively charged polymer that is used in drug-loaded nanoemulsions in order to enable their transportation to the localised site of the disease (Hamdi et al., 2023). Due to its numerous advantages such as biodegradability and biocompatibility, carbopol also improves the drug entrapment efficacy of nanoemulsions. Furthermore, the use of carbopol for brain targeting is appealing due to its ability to recognize and adhere to neural tissues effectively. Paracellular permeability of therapeutic agents is enhanced by mucoadhesive polymers due to their ability to potentiate the redistribution of major component proteins in the tight junctions of the BBB (Sarvaiya et al., 2015).

Despite the approval of galantamine for AD treatment along the mild to moderate spectrum, to the best of our knowledge, a therapeutic with nanoparticles of an advanced nature, carrying this nanodrug to improve its pharmacotherapeutic activity, has not yet been reported. This research work aims to employ a nano-emulsification strategy to formulate nanotherapeutic delivery systems, in the form of galantamine-loaded polymeric nanoemulsions, for the treatment AD.

1.2 Study Rationale and Motivation

Polymeric nanoemulsions are a favourable alternative in the formulation of advanced drug delivery systems for AD. This favourability can be attributed to (1) their systemic route of administration and nanometric size, which increases their capability of reaching any human organ; (2) their use in protecting drugs from degradation due to enzymes; (3) their ability to solubilise lipophilic drugs of high quantities; (4) their ability to produce a controlled, sustained drug release profile, leading to extended duration of action; (5) how they target BBB-specific

activities, thus improving the therapeutic's pharmacological activity at the CNS while reducing its side effect profile, thus improving patient's quality of life; and (6) how their preparation can be achieved through employing easily scalable and cost-effective methodologies (Mushtaq et al., 2023 and Fornaguera et al., 2015).

Whilst several oral pharmacotherapies for AD have been validated to improve patients' quality of life, only few AD patients respond to these therapies. Furthermore, existing therapies display severe side effect profiles, which include functional impairment and medical comorbidity (e.g. adverse effects of the gastrointestinal tract, headache and bitter tastes) (Fornaguera et al., 2015, Fazil et al., 2012 and Sahni et al., 2011). Hence the interest in developing oral drug delivery platforms to improve localised therapeutic effects of these drugs while decreasing their adverse effects.

Clinical trials comparing the long-term effects of galantamine vs. donepezil show that galantamine displays significant advantages when administered at daily doses of 24mg/day, with patient's reporting reductions in overall burden compared to donepezil. Furthermore, results show that patients receiving donepezil present with cognition scores that deteriorate significantly from the baseline in comparison to those of galantamine. Depending on the severity of the patient's AD, studies postulate that optimal doses for mild and moderate AD are 16 mg/day and 24mg/day, respectively. Despite the increase in side effects that patient's potentially experience with higher administrated treatment doses, the possible positive effect that galantamine may have on cognition and functioning in daily life should not be disregarded (Zhang et al., 2020).

Among various methodologies to prepare NPs (nanoparticles), in this study nano-emulsion templating from O/W nano-emulsions was employed and is an advantageous, versatile tool to prepare polymeric nanoparticles for diverse biomedical uses (Calderó et al., 2011). To the best of our knowledge, this is the first study that accounts for the encapsulation of GAL in polymeric nanoemulsions, specifically in carbopol nanoparticles. In *in vivo* therapies, small nanometric sizes could be advantageous in that they are not easily detected and eliminated from blood circulation by the immune system. Moreover, these particles easily cross the blood brain barrier (BBB) - a property that is required for optimal drug delivery and enhanced 4 therapeutic effects in treating AD (Jusril et al., 2022). Herein, the study aims to employ the nano-emulsification strategy to prepare galantamine-loaded polymeric nanoemulsions as alternative advanced drug delivery platforms to treat AD. Polymeric nanoemulsions have been formulated with biocompatible and biodegradable polymers using carbopol as the polymer and Tween80 as the surfactant. Carbopol is a biocompatible and biodegradable cationic polymer that will assist in the adhesion of the galantamine-loaded nanoemulsion to the intestinal

mucosa. This formulation of galantamine-loaded mucoadhesive nanoemulsion will aim to increase the bioavailability of the therapeutic drug on a neural level.

The perspective for this delivery system, is for the galantamine-loaded polymeric nanoemulsion of the nanoparticles to be orally administered, absorbed through the intestinal mucosa, distributed in the bloodstream and para/intracellularly pass through the epithelial cells of the BBB to the brain. Once successfully synthesised, the nanoparticles will be analysed for their physicochemical properties using Fourier Transformation Infrared (FTIR) Spectroscopy, to evaluate various chemical bond shifts, responsible for particle solubility and drug entrapment efficiency. Particle distribution and Zetasizer size of the polymeric nanoparticles will be determined. The designed polymeric nanoparticles will thus possess the potential of delivering galantamine with a lower drug-loaded dose, releasing the drug in a controlled manner, with higher affinity drug uptake at the BBB site.

1.3 Aim and objectives

This study aims to design and synthesise a biodegradable, oral nanoemulsion system for the controlled release of galantamine to the CNS. To achieve this aim, the following objectives will be conducted:

Objective 1: To design and synthesize a biodegradable galantamine-loaded nanoemulsion delivery system, of particles in the nanoparticulate range, using Tween 80, PEG 4000, polyvinyl alcohol (PVA) and carbopol.

Objective 2: To analyse the nanoparticles for their physicochemical properties and evaluate various chemical bond shifts using (FTIR) Spectroscopy and Differential Scanning Calorimetry (DSC).

Objective 3: To determine the optimal dimension of the nanoemulsion by evaluating the Zetasizer size and particle distribution of the nanoemulsion.

Objective 4: To determine the release profile of galantamine using High-Performance Liquid Chromatography (HPLC).

1.4 Overview of this research report

Chapter 1 is the introductory component of the dissertation; and defines AD, it's pathophysiology and debilitating effects on individuals suffering from the disease. Various methodologies and administration routes for nanoemulsions exist, but studies that account for the encapsulation of galantamine in polymeric nanomemulsions still need further exploration. Therefore, a nano-emulsification strategy to formulate galantamine-loaded, polymeric

nanoemulsion is employed on this study. Lastly, this chapter outlines the aims and objectives of the study.

Chapter 2 provides a critical evaluation of therapeutic nanocomposite technologies for the treatment of neurodegenerative disease – expanding on their advantages and disadvantages in treating AD. This chapter also highlights the toxicological considerations that need to be assessed when comparing and selecting nanotechnologies. Furthermore, research prospects in the nanotherapeutic field are covered in this chapter – highlighting the significant progress expected in the development of multifunctional nanocarriers.

Chapter 3 describes the materials and methodologies; results and discussion for the synthesized nanoemulsion as well as the characterization and analysis of the nanoemulsion using Zetasizer, FTIR and DSC. The drug release profile employing the HPLC method was also discussed in this chapter.

Chapter 4 provides a conclusion and future recommendations.

2 CHAPTER 2: CRITICAL EVALUATION OF THERAPEUTIC NANOCOMPOSITE TECHNOLOGIES FOR THE TREATMENT OF NEURODEGENERATIVE DISEASES

2.1 Introduction

Nanotechnology focuses on the design and synthesis of nanomaterials which are functionally organised with at least one dimension of no more than 100 nm for use in medicine or industry (Huang et al., 2019 and Poovaiah et al., 2018). Due to the nanoscale of the engineered materials, a significant interaction with other biological structures is observed, thus enhancing the potential effectiveness of neurological disease treatment. Various nanomaterials are formulated in a manner that allows specific interaction with cellular subsets or molecules. In addition to their specificity, nanomaterials are multi-faceted; and with the incorporation of various features into their structure, simultaneous targeting, bioactivity, and imaging capabilities are observed (Patra et al., 2018, Hajipour et al., 2017 and Srikanth et al., 2012).

The use of nanoparticles in medicine can be attributed to the fact that the human body contains numerous biochemical and biological mechanisms that occur at the nanoscale. Nanoparticles provide substantial support for the delivery of various macromolecules such as nucleotides and proteins, and molecules for therapy or diagnosis (Martin-Rapun et al., 2014). The use of nanoparticles as carriers of therapeutic agents is a highly-researched and promising approach because of their ability to protect and stabilise the entrapped therapeutic drug. Additionally, improved drug efficacy, which can be observed through enhanced delivery and controlled release, is a defining characteristic of these nanoparticles. Associating the drug with a nanocarrier potentially reduces the often-debilitating side-effects elicited by increased levels of a drug in tissues other than the target site (Dai et al., 2018, Azzawi et al., 2016 and Martin-Rapun et al., 2014).

Nanocarriers can be identified by various shapes which include tubes, rods, and globular particles. This versatility in shape provides a multifunctional platform for nanoscale entities. Nanocarriers have been investigated for the advancement of targeted nanotherapeutics (Dai et al. 2018 and Chen et al., 2018), in addition to diagnostic devices applied in neurodegenerative and autoimmune diseases as well as in cancer. Nanocarriers are classified into many categories such as nanoparticles, nanoemulsions, dendrimer nanoparticles, nanomicelles, carbon nanotubes and nanogels (Wong et al., 2015). Distinct chemical and physical aspects uniquely differentiate nanocarriers, namely size, shape, functionality and modification of the surface and architectural characteristics of the core-shell structure. An understanding of the interactions that nanomaterials have with biological systems, as well as their physicochemical properties, is crucial in the development of nanotherapeutic applications. Intrinsic toxicity and immunogenicity are the two most undesirable effects which

can be attributed to the suboptimal modification of the surface of nanomaterials (Wong et al., 2015).

Lipid systems, liposomes and micelles to be precise, were documented as the first generation of therapy that was based on the incorporation and implementation of nanoparticles. Furthermore, inorganic particles like magnetic and gold nanoparticles can be incorporated into the development of these liposomes and micelles (Figure 1). It then follows that these nanosized inorganic particles created interest in treatment therapies due to their propensity to potentially protect drugs from gastrointestinal and enzymatic degradation while still maintaining a comparatively higher oral bioavailability. The ability of nanomaterials to stay in the blood circulatory system for an extended period to ensure the controlled release of drugs is an additional desirable characteristic of nanocarriers (Harilal et al., 2019).

Despite the significant role that nanocarriers play in drug delivery across the blood-brain barrier, the available research still highlights the need for optimised safety, specificity and efficiency. This review aims to critically evaluate and highlight the various nanocomposite technologies that can be employed in treating neurodegenerative diseases such as AD. Moreover, it will elucidate how nanotechnologies such as nanoemulsions, may offer solutions that yield results favourable to drug delivery to the CNS.

2.2 Classification of Nanotechnologies

Nanotechnologies are classified into different forms (Figure 2) which include nanotubes, nanofibers, polymeric nanoparticles, nanoemulsions, nanomicelles, and nanoliposomes (Singh et al., 2019, Fazil et al., 2011, Poovaiah et al., 2018, Siddiqi et al., 2018 and Zhao et al., 2018). Considering the uniqueness of neurodegenerative diseases, these sub-classifications of nanocomposite technologies each provide unique properties that individually potentially enhance the therapeutic effect of loaded therapeutic agents (Srikanth et al., 2012 and Khan et al., 2022). An important component of nanoparticles that should not be overlooked is stability. Nanoparticles are 3D polymeric encapsulation systems that are capable of being loaded with therapeutic agents thus making them a highly stable delivery systems (Huang et al., 2019 and Srikanth et al., 2012).

Nanoparticle functionality may be achieved further, by including targeting ligands or antibodies. In addition to nanoparticles, nanomicelles and nanoliposomes are explored as alternative CNS-targeted drug delivery systems. Structurally, the latter two nanomaterials are dissimilar— nanomicelles contain a phospholipid monolayer with a hydrophobic core, whereas nanoliposomes comprise of a lipid bilayer structure that is similar to that of a vesicle (Huang et al., 2019 and Martin-Rapun et al., 2014).

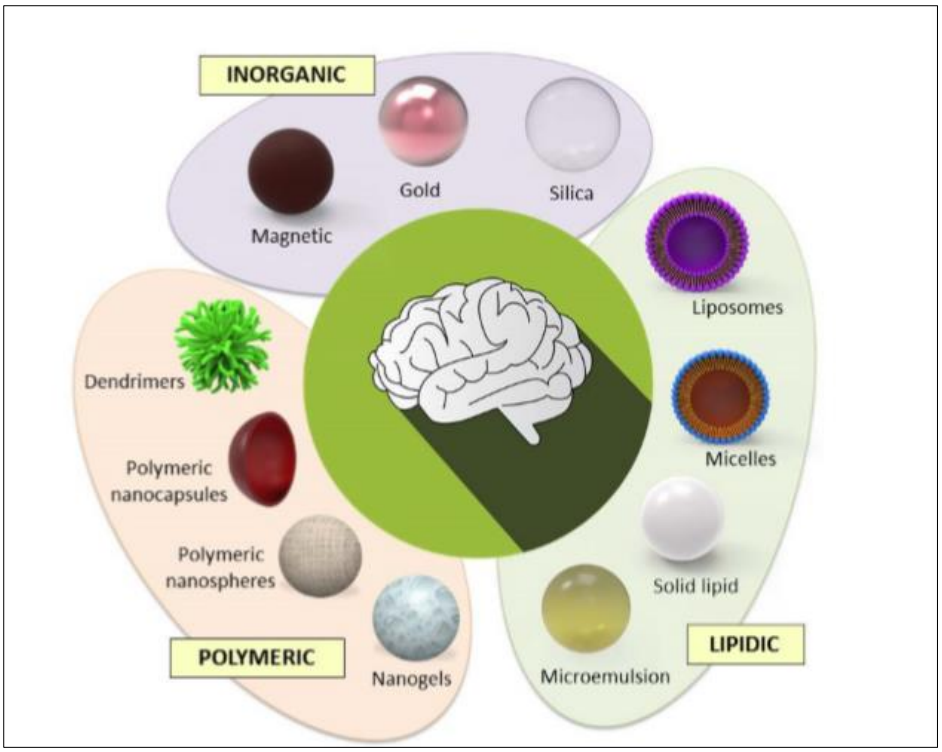


Figure 1. Image showing the sub-classification of lipidic, polymeric and inorganic nanomaterials commonly used in nanotherapy. Image acquired from Harital et al., 2019.

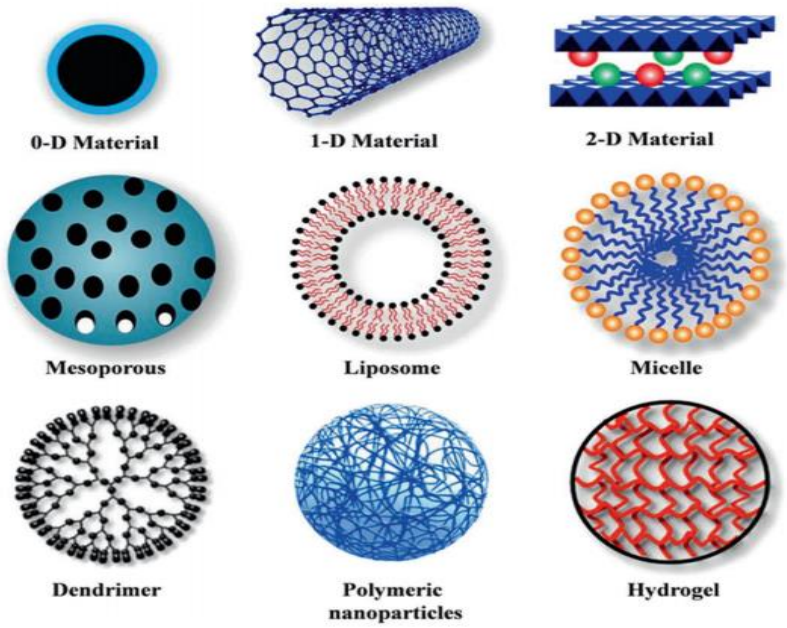


Figure 2. Nanomaterials encompass various classifications, with polymeric nanoparticles and nanoemulsions being the most commonly researched for neurodegenerative disease treatment. Image acquired from Siddiqi et al., 2018 and Ross et al., 2018.

Nanomaterials that are structured as long, linear arrangements are classified as nanofibers. These nanofibers can self-assemble and provide necessary structural guidance and support to neighbouring cells. Nanogels, particularly, are composed of nanofibers that can be organized into 3D scaffolds which assist in organizing transplanted cells as well as promoting cell adhesion and proliferation. Alternative nanocomposites such as nanoemulsions are documented as highly selective delivery methods of dyes, lipophilic dyes and drugs of low molecular weight (Wahba et al., 2016). Additionally, therapeutic agent-loaded nanoemulsions have revealed enhanced uptake of loaded therapeutic agents in degenerated cells significant to AD (D'Arrigo, 2017).

The maximum concentration of a therapeutic agent in the brain is achieved as a result of nanoparticles exhibiting enhanced fluidization of the blood-brain barrier, consequently increasing the degree of permeability which subsequently allows entry of drug molecules through the tight junctions of the endothelial cells. Moreover, the p-glycoprotein efflux system is inhibited; and the nanoparticles undergo selective endocytosis— resulting in the eventual release of the loaded drug (Fazil et al., 2011). The numerous variations in available nanomaterials allow for the effective selection of a nanoscale material with characteristics that are best suited to the challenges posed by an individual CNS disorder. An extensively investigated nanomaterial in medicine is polymeric nanoparticles, due to their low levels of toxicity, substantially lower biodegradability and higher biocompatibility. However, engineering these polymeric nanoparticles into target-specific and stable systems has proven to be an obstacle (Carradori et al., 2017). As a result, only a small number of cases have clinically succeeded in using polymeric nanoparticles for the treatment of neurodegenerative disorders (Poovaiah et al., 2018).

FDA-approved polymeric nanoparticles such as poly lactic-co-glycolic acid (PLGA) are one of the most commonly used in nanomedicine. PLGA is well documented for being highly biodegradable – a component that is imperative in effective drug delivery systems. In a study conducted by Rai and Yadav, 2019, blood clearance and biodistribution of etoposide-loaded PLGA nanoparticles in the range of 100 nm – 160 nm was evaluated. For long-term circulation, the 100 nm nanoparticles were more effective in bypassing the liver metabolism. This could also be aligned to the lack of surface modification of these 100 nm nanoparticles. However, despite these favourable results, PLGA molecules degrade by bulk erosion. The negative implications that this has on the drug-loaded nanoparticles include premature exposure of the therapeutic agent to harsh biological environments. Furthermore, water can penetrate these PLGA nanoparticles thus affecting the release profile of the drug. These obstacles may be overcome by incorporating surface-eroding nanoparticles such as polyanhydride. Overall, the

use of these polymeric nanoparticles is preferred because of easy fabrication, stability, safety and exhibition of controlled drug-release (Poovaiah et al., 2018).

2.3 Nanocarriers and Alzheimer's Disease

Table 1. Properties of nanocarriers used for treatment of AD.

Nanocarrier	Properties i.e size shape, charge etc.	Advantage in treating AD	Disadvantage in treating AD	References
Dendrimers	A core that projects symmetrical arms that diverge radially	Low viscosity, increase in the therapeutic activity of biologically active compounds attached to dendrimers	High non-specific cytotoxicity	Aliev et al., 2019
Nanoemulsions	Nanocolloidal system composed of emulsified oil and amphiphilic water, nanomaterials range in size from 20 – 500 nm	Enhanced absorption of the drug, thermodynamically stable	Creaming, lower stability	Kale et al., 2017
Nanomicelles	Formed when 50 – 100 amphiphilic nanomolecules, dispersed in liquid phase, aggregate	Ability to use their core to solubilise drugs, can increase drug permeability through blood-brain-barrier	Poor physical stability *in vivo*	Singh et al., 2019 and Hettiarachchi et al., 2019
Nanotubes	Cylindrical in shape and have a diameter of 1 – 100 nm	Can influence neuronal growth, is electroconductive and flexible	Potential toxicity	Xiang et al., 2020
Nanogels	Crosslinked networks that combine ionic and non-ionic chains	Uptake of nanogels is higher in the brain than in the liver and spleen	If even small amounts of surfactant remain in the nanogel formulation, they can have toxic effects in the body	Poovaiah et al., 2018 and Ahmad et al., 2017
Liposomes	Spherical and consist of steroids and phospholipids in the nanometric scale of 50 – 450 nm	Stabilise drugs, enhance biodistribution and can be used with both hydrophilic and hydrophobic therapeutic agents	Low solubility, short half-life,leakage and fusion of enveloped drug	Hernandez et al., 2022
Nanosuspensions	Loaded with crystalline drug particles which are stabilised by surfactants that are non-ionic or with lipid mixtures	High loading capacity and simplicity, increased efficacy, sustained drug release	High energy techniques,	Poovaiah et al., 2018 and Ahmad et al., 2017

Magnetic nanoparticles have been used for the diagnosis and treatment of neurodegenerative diseases. Magnetic delivery of therapeutic agents to the target site can be achieved, ideally, by the magnetic field directing the magnetic nanoparticles directly to the affected tissues (Zhang et al., 2018). Predominantly used in hyperthermia-based treatments and biomedical applications, magnetic nanoparticles such as iron oxide have been well documented. Iron oxide is biodegradable, biocompatible and superparamagnetic. Literature has reported the use of these iron oxide nanoparticles as carriers of drugs for the treatment of AD; and the results obtained were promising (Zhang et al., 2018, Siddiqi et al., 2018 and Nazem et al., 2011).

Some cases have reported the difficulty of magnetic nanoparticles effectively crossing the BBB to reach the target site in the brain. However, this can be bypassed by appropriately modifying the surface of these nanoparticles with compatible molecules. Gold nanoparticles are an alternative approach that has reached a broader application due to easier reproducibility and synthesis which can be attributed to their shape and size. Light sources such as near infrared light penetrate biological tissues without damaging the target and surrounding tissues with ionization (Siddiqi et al., 2018 and Zhang et al., 2018). This light source is commonly used in photothermal ablation where gold nanoparticles penetrate the target area and the near infrared laser is applied on the nanoparticles, thus destroying the cells in the target area through thermalisation. Nazem et al., 2011 highlight this light-induced therapy as a promising strategy for the destruction of Aβ fibrils in AD.

A nanomaterial that has been found to successfully target the brain is the micelle. Micelles consist of a spherical lipid monolayer ranging between 10 – 100 nm in size. A limitation that is common with micelles is that of requiring stabilisation sterically. However, to combat this, micelles are merged with polymers such as polyethylene glycol (PEG) to obtain a rigid outer shell. On the contrary, solid lipid nanoparticles are made of lipid crystals (Wong et al., 2015). Limitations of these nanomaterials are low drug loading capacity and difficult drug release once the lipids have crystallised. Nanosized lipid carriers are comprised of liquid and solid lipids whose crystal structure allows for more of the therapeutic drug to be encapsulated. In turn, this offers favourable delivery of the drug to target areas in neurodegenerative diseases. A more common and widely researched nanoparticulate system in nanomedicine is micro- and nanoemulsions. Due to favourable characteristics, namely colloidal thermodynamic stability, nanoemulsions can be spontaneously formulated through the process of mixing water, lipids, cosurfactants and surfactants in a suitable proportion. Substantial amounts of therapeutic agent can be encapsulated in these nanoemulsions (Siddiqi et al., 2018, Nazem et al., 2011 and Zhang et al., 2018).

2.4 Targeting strategies to improve delivery of nanocarriers

The main obstacle that nanocarriers need to overcome in neurodegenerative treatment, is traversing the blood-brain barrier and delivering the therapeutic agent. Mullis et al., 2018 explain the positive implications of employing targeted drug delivery methods, and discuss ligands that have been used in recent studies to enhance nanocarrier-mediated brain delivery. Such ligands can improve transcytosis of the nanocarrier through the blood-brain barrier using either cell-mediated, receptor and adsorptive transcytosis (Poovaiah et al., 2018 and Fonseca-Santos et al., 2015).

Nanoparticles exhibit a number of unique characteristics that have distinguished them as promising carriers for drug delivery (Hersh et al., 2016). Additional characteristics such as controlled drug release profile, multi-functionality and site-specific targeting add to the uniqueness of these nanoparticles. Possessing a readily modified surface, nanoparticles can thus effectively traverse the BBB to deliver drugs to target areas (Hersh et al., 2016). The mechanisms by which the abovementioned nanoparticles cross the BBB are not completely understood; however, numerous classes of nanoparticles such as polymeric, lipid and metallic nanoparticles have been depicted to traverse the BBB and enter the brain through various endocytic mechanisms (Hersh et al., 2016).

Cell-based drug delivery involving the implementation of immune cells and stem cells as therapeutic carriers is an alternative, exciting option for enabling therapeutic delivery across the BBB. The cell types that have been evaluated as therapeutic carriers are mesenchymal stem cells (MSCs)and neural stem cells (NSCs) (Hersh et al., 2016). These two cell types have demonstrated the ability to cross the BBB and migrate into the CNS via the inflammation-mediated pathway. The MSCs and NSCs present with advantages that make them an attractive option for drug delivery to the CNS (Hersh et al., 2016). This is due to their effective delivery of an assortment of therapeutics such as cytokines, enzymes, genes and nanoparticles. Furthermore, both immune cells and stems cells are naturally recruited to sites of tissue damage and inflammation – a common feature of diseases of the CNS (Hersh et al., 2016).

Despite the immune cells and stem cell's ability to cross the BBB, the evident limitations and challenges of cell-mediated delivery cannot be overlooked. The principal problem presented by the cargo on cell carriers is potentially toxic effects and as a result, the carrier needs to be safeguarded from its carrier or the therapeutic agent must be non-toxic to the carrier (Hersh et al., 2016).

2.5 Polymeric Nanoparticles

Nanoparticles have been employed for their potential in delivering drugs to the brain (Ahmad et al., 2017 and Chowdury et al., 2017). They are particles made of polymers, of natural or artificial composition, ranging in size between 10 – 100 nm. When in contact with fluids of a biological nature, polymeric nanoparticles exhibit higher stability than their colloidal carrier counterparts. Moreover, the nature of these nanoparticles allows the attainment of properties such as controlled and sustained drug release (Azzawi et al., 2016, Bamrungsap et al., 2012 and Gu et al., 2015 and). Preformed polymers or monomers are used to synthesize nanoparticles during its polymerization. Depending on the nanotechnology employed to manufacture, nanospheres or nanocapsules may be synthesized. Nanospheres are matrices that are dense and in which drugs are dispersed. Conversely, nanocapsules contain a liquid core that is encapsulated by a polymeric shell (Rai et al., 2019, Karthivashan et al, 2018 and Ramalhao et al., 2019).

2.6 Dendrimers

Dendrimers are monodispersed structures that are disc-shaped (Nazem et al., 2011 and Siddiqi et al., 2018). They consist of a core that projects symmetrical arms that diverge radially (Siddiqi et al., 2018 and Chowdury et al., 2017). These projections are referred to as dendrons (Aliev et al., 2019). Between these dendrons, cavities exist, which store a wide variety of molecules of hydrophilic, hydrophobic and amphiphilic nature (Aliev et al., 2019 and Nazem et al., 2011). Compared to their linear counterparts, dendrimers have low viscosity and the hydrophilic functional groups presented on their surfaces result in increased solubility in water, which is a fundamental aspect in ensuring high reactivity during surface modification (Aliev et al., 2019 and Saeedi et al., 2018). Commonly used dendrimers include polyamidoamine (PMAM) and polypropyleneimine (Aliev et al., 2019 and Ahmad et al., 2017). Literature has shown an increase in the therapeutic activity of biologically active compounds attached to dendrimers when compared with the equivalent free drug. This is known as the “dendritic effect” (Aliev et al., 2019).

2.6.1 Polyamidomamine Dendrimers

Research has shown that PAMAM dendrimers prevent amyloid deposits from forming They also present with hydrolytic properties similar to those of existing aggregated proteins. In a study where cells were incubated with these PMAM dendrimers, Aβ resistance to hydrolysis was lessened. This indicates that the dendrimers can both inhibit amyloid aggregation as well as remove present, toxic aggregated proteins. Furthermore, the projection of the dendrons is

an essential feature of the dendrimers as it is an essential tool in effectively combating the Aβ aggregates (Aliev et al., 2019, Harilal et al., 2019 and Kannan et al., 2014).

2.7 Liposomes

Discovered in 1960 by Alec Bangham, liposomes are biodegradable, biocompatible nanomaterials that have been used in cosmetic and pharmaceutical industries to transport an array of molecules; and they are a widely studied system for drug delivery (Wong et al., 2015 and Fazil et al., 2011). They are spherical and consist of steroids and phospholipids in the nanometric scale of 50 – 450 nm. Their membrane structure is similar to that of cell membranes and they facilitate desirable levels of drug incorporation. Researchers have proved that liposomes stabilise drugs, enhance biodistribution and can be used with both hydrophilic and hydrophobic therapeutic agents (Niu et al., 2018).

2.7.1 Classifications of Liposomes

Liposomes are classified into four categories. Conventional liposomes are composed of a lipid bilayer that can create neutral, cationic or anionic cholesterol and phospholipids. Hydrophilic and hydrophobic materials can fill the lipid bilayer and aqueous space respectively. “Classical” and “stealth” liposomes are nanometric vesicles that contain an aqueous core enclosed in a lipid bilayer (Ross et al., 2018 and Panahi et al, 2017). These liposomes are similar to the composition of a cellular bilayer and they could contain hydrophobic drugs within the lipid membranes. Several limitations are presented by liposomes as they are a target of the mononuclear phagocyte system, which subsequently clears these nanosized particles from the blood circulatory system.

In order to achieve steric equilibrium, the surface of liposomes may be incorporated with PEG thus creating PEGylated liposomes (Aalinkeel et al., 2017). A third type of liposome exists and is classified as the ligand-targeted type. Here, ligands such as carbohydrates, peptides and antibodies are attached to the liposomal surface. Lastly, theranostic liposomes are an amalgamation of the three liposome types described above and they usually consist of nanoparticles that exhibit imaging, therapeutic and targeting components (Patra et al., 2018, Niu et al., 2018, Poovaiah et al., 2018, and Panahi et al., 2017).

2.7.2 Synthesis of Liposomes

Typically, the synthesis of liposomes involves: a thin layer of hydration, mechanically agitating the particles, evaporation of the solvent, injection of the solvent and lastly, solubilisation of the surfactant (Singh et al., 2019). An important aspect to highlight is that the entrapped therapeutic agents will only become bioavailable once released. Therefore, the accumulation

of liposomes at target sites is important so that bioavailability of the drug within the required therapeutic window is achieved. Macrophages, astrocytes and microglia in the CNS readily take up liposomes. Liposomes containing PEG tend to accumulate faster in the brain when the blood-brain barrier is in a compromised state (as observed in diseases such as autoimmune encephalomyelitis) (Singh et al., 2019). Interestingly, PEGylated liposomes combined with monoclonal antibodies to glial fibrillary acidic proteins expressed altered brain penetrance.

2.7.3 Liposome vesicle optimization

The blood-brain barrier will only allow particles of a certain size to traverse through its endothelial cell wall. Therefore, liposomes need to be nanosized (100 nm) or microsized for the desired neurotherapeutic effects to be achieved (Ross et al., 2018). Studies have shown the advantages of liposomes ranging from 100 – 140 nm in size. These include longer half-life in the circulatory system while avoiding plasma proteins (Ross et al., 2018). Conversely, liposomes of a larger scale, 250 nm, are cleared twice as fast as the smaller sized nanoliposomes. Despite the lower propensity for the 100 nm liposomes to be cleared from the blood circulatory system, they do; however, have a storage capacity that is limited, and this leads to inefficient encapsulation of the drug (Ramalhao et al., 2019).

2.7.4 Drawbacks associated with liposomes

The drawbacks of using liposomes as drug delivery systems remain eminent. One of these drawbacks is increased cost when these liposomes are used (Ross et al., 2018). However, because these nanomaterials assist the drug to reach its target site while maintaining a relatively effective dose, concerns regarding cost can be reserved. As with most orally administered drugs, liposomes can undergo degradation in the acidic and hypotonic gastric environment (Ross et al., 2018). In turn, suitability for oral delivery is compromised. To avoid the mononuclear phagocyte system, researchers suggest administering larger doses rather than smaller doses at regular frequencies (Ross et al., 2018).

2.8 Nanotubes

Nanotubes are described as structures that are cylindrical in shape and have a diameter of 1 – 100 nm (Ross et al., 2018 and Wang et al., 2018). They are new generation nanomaterials that aim to treat neurodegenerative disease such as AD (Siddiqi et al., 2018 and Zare et al., 2021). However, the biological safety of these nanomaterials remains a concern as previous literatures continue to question its negative effects such as toxicity in organelles and cellular structures as well as its lack of biodegradability (Fazil et al., 2011). Despite this concern,

researchers have documented the successful use of acetylcholine, with precisely controlled doses, to effectively treat AD. Once the nanotubes have entered the brain, they enter the neuronal lysosomes where they release the loaded acetylcholine. Release of acetylcholine is achieved as a result of the markedly higher polarity and hydrophilicity in the pH of the lysosomes. An interesting component of nanotubes that Fazil et al. (2011) have highlighted, is its ability to target two organelles of different pharmacological activity. Lysosomes are the pharmacological target organelles, whereas mitochondria are the cytotoxic target organelles. At low dosing regimens, nanotubes preferentially enter lysosomes; however, in large doses, mitochondria absorb these nanotubes; thus, enhancing mitochondrial toxicity and increasing the overall toxicity of using the nanotube approach for the treatment of neurodegenerative diseases (Fazil et al., 2011).

2.9 Nanoemulsions

Nanoemulsions are a nanocolloidal system composed of emulsified oil and amphiphilic water, with the incorporation of a stabilizing agent to increase overall stability. The surfactant acts as the stabilising agent and this renders the system metastable and isotropic (Sharda et al., 2023). Thermodynamic stability of nanoemulsions can be attributed to the conglomeration of surfactants, oil, cosurfactants and water (Bonferoni et al., 2019, Harila et al., 2019 and Kale et al., 2017). These nanomaterials range in size from 20 – 500 nm and produce a translucent or transparent dispersion as depicted in Figure 3. Nanoemulsions are suitable for drugs that are lipophilic and require transportation to the brain. Therefore, the selection of the oil component of the nanoemulsion is important. Preparation can either be through high or low energy methods (Figure 4).

2.10 Advantages and disadvantages of nanoemulsions

Advantages of nanoemulsions include: 1) solubilisation of hydrophobic drugs as well as those that are soluble in oil; 2) enhanced absorption of the drug; 3) as well as the ability to mask bitter tastes and odours of therapeutic agents. Moreover, scaling up of the nanoemulsion is fairly easy because of the ability to formulate them spontaneously. Nanoemulsions are also thermodynamically stable. On the contrary, disadvantages of nanoemulsions include lower stability compared with other dosage forms; shorter shelf-life; creaming and phase inversion (Kale et al., 2017).

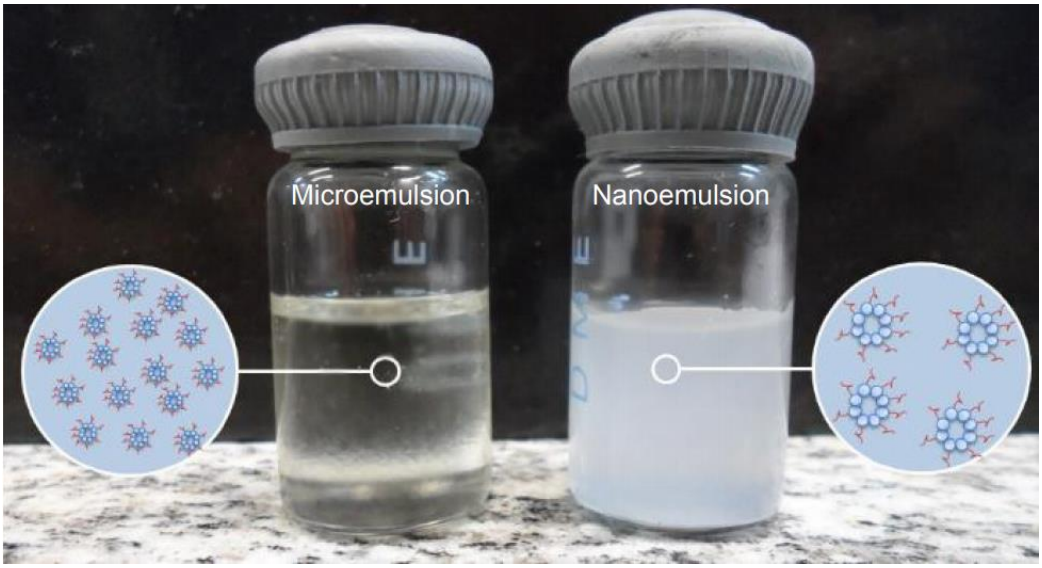


Figure 3. Nanoemulsions, due to their nanometric size, appear as translucent preparations, when compared to microemulsions. Image acquired from Fonseca-Santos et al., 2015.

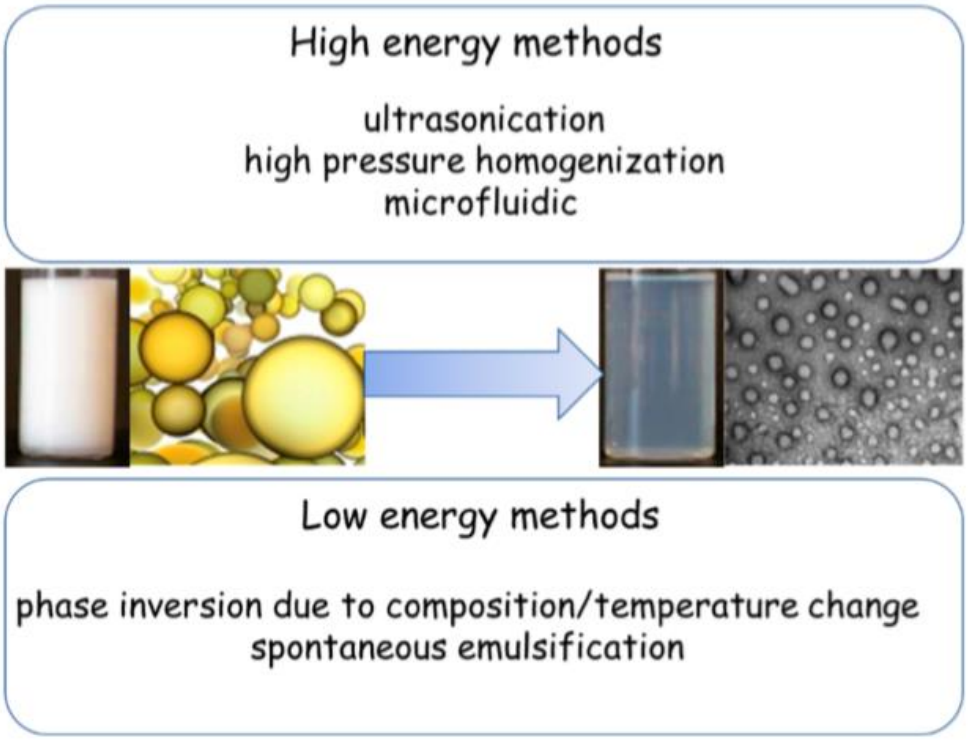


Figure 4. Image depicting the various high and low energy methods typically employed in the preparation of nanoemulsions. Image acquired from Bonferoni et al., 2019.

2.11 Nanogels and nanosuspensions

Nanogels are formed when polymers form crosslinked networks that combine ionic and non-ionic chains such as polyethyleneimine, PEG or polyacrylic acid. Additionally, polymeric

materials have been used in the engineering of tissues and the delivery of drugs to their target sites. Nanogels carry low molecular weight drugs across the blood-brain barrier (Poovaiah et al., 2018). Although nanogels are still in the early stages of development, the results of a study conducted by Vinogradove et al. revealed that the uptake of the nanogel was higher in the brain than in the liver and spleen. Nanosuspensions are nanomaterials that are also used for the delivery of therapeutic agents through the blood-brain barrier. They are loaded with crystalline drug particles which are stabilised by surfactants that are non-ionic or with lipid mixtures. Additionally, their loading capacity and simplicity are impressive (Poovaiah et al., 2018 and Ahmad et al., 2017).

2.12 Nanomiceles

Micelles are formed when 50 – 100 amphiphilic nanomolecules, dispersed in liquid phase, aggregate. They consist of a hydrophilic head and a hydrophobic tail. This formation of nanomicelles enables drugs that are hydrophobic to be entrapped in the micellar core (Patra et al., 2018 and Poovaiah et al., 2018). Micelles are typically in the smaller range of nanomaterials, with a diameter of 5 – 20 nm. Their ability to use their core to solubilise drugs makes them good carriers for the delivery of a drug to the brain. In a study conducted on endothelial cells of the bovine brain, poloxamers such as Pluronic p85 were shown to increase the permeability of micelles through the brain-blood barrier. The results can potentially be extrapolated to show that permeability of the drug through the human brain is significantly enhanced, as the results in the study revealed a 19-fold increase in permeability when Pluronic micelles were used (Hettiarachchi et al., 2019 and Singh et al., 2019).

2.13 Toxicological consideration of nanotechnologies

Despite the significant role that nanoparticles play in effectively delivering therapeutic agents to target areas, the potential toxicological effects that they may impose should not be overlooked (Zhao et al., 2018 and Fazil et al., 2011). To ensure that nanomedicine remains a reliable and sustainable treatment modality, one must fully understand the physicochemical, molecular and physiological processes of nanoparticles (Carro et al., 2019). As discussed, the eminent toxicity of the accumulated drug in the blood circulatory system remains an important topic to consider when selecting nanomaterials. Nanoparticles interact with tissues, cells and fluids starting from the entry route through many possible pathways before reaching the intended target area. Transdermal application, oral and intravenous administration are three of the most widely used drug delivery routes. To enter cells, the nanoparticles can undergo endocytosis (Table 1), patocytosis, potocytosis and pinocytosis (Gu et al., 2015 and Fazil et al., 2011).

Table 2. Table comparing the transport mechanism of nanoparticles when entering cells

Nanoparticle transport mechanism	Description	Reference
Carrier-mediated transcytosis	Numerous vital materials that are required in the brain, are allocated specific transporters. These transporters are involved in the active uptake these vital materials across the BBB. Therapeutic agents can thus utilise the aforementioned transporters for delivery to the brain.	Ross et al., 2018 and Wong et al., 2015
Receptor-mediated transcytosis	A concept that employs BBB endothelium receptors with corresponding ligands to trigger internalization. Enhanced penetration of the brain can be achieved through the incorporation of these ligands with therapeutic agents	Ross et al., 2018 and Wong et al., 2015
Cell-mediated transcytosis	Through endocytosis, drugs can be delivered across the BBB into endothelial cells.	Ross et al., 2018 and Wong et al., 2015
Adsorptive transcytosis	A mechanism that utilises modified, cationic biological macromolecule for interaction with the anionic BBB. This process is based on the electrostatic attraction.	Ross et al., 2018 and Wong et al., 2015

After endocytosis, the nanomaterial that has been engulfed is transported to endosomes where it then undergoes hydrolytic enzymatic degradation in the lysosome. Once the nanoparticles have been administered, they have the potential to permeate capillaries in the body and distribute to tissues (Gu et al., 2015). Nanoparticles that pass through biological membranes and epithelia may negatively affect any cell. However, they are anticipated to filter through the liver and kidney in order to be excreted. Gold and copper nanoparticles, compared to larger materials, have exhibited the potential to travel through organ systems and cross the blood-brain barrier. Despite this, gold and copper nanoparticles can induce oxidative stress and produce free radicals that can disturb the cell membrane of endothelial cells (Gu et al., 2015 and Fazil et al., 2011).

2.14 Conclusion

Despite research demonstrating the use of nanoparticles to effectively transport drugs across the blood-brain barrier for AD treatment, the need to optimise safety, efficiency and specificity still exists. Although the nanoparticulate system may successfully pass through the blood-brain barrier, the prediction that it will fully elicit a therapeutic effect should not be definitively concluded without considering that the biological activity of this system may or may not be retained (Ovais et al., 2018, Fazil et al., 2011 and Krol et al., 2013). Once an increased knowledge of nanoparticles and their distribution in the human body is obtained, the application of this nanotechnology will further expand in the future. Researchers expect that once imaging modalities that provide an in-depth understanding of the exact targets and fates of these molecules are available, the application of such nanotechnologies will rapidly increase.

Studies of nanomaterials and their toxicology in humans, particularly in neurodegenerative disease such as AD, have been limited to small scale and short-term exposures, thus better evaluations and studies considering larger cohort populations still need to be conducted. The focus should be on the long-term effects of nanomaterials in humans and the environment. Treatment strategies currently used do not fully address AD because of the nature and physiological factors of this neurodegenerative disease. Nanotechnology may offer innovative solutions to this, with nanoemulsions and dendrimers being the two drug delivery systems that may yield results favourable to drug transport to the CNS.

Overall, nanotechnology has garnered interest in the delivery of neurotherapeutic agents. Amongst the nanocarriers described, nanoparticles play a substantial role in delivering drugs to the brain because of their pharmacokinetic attributes. Such nanoparticles may be altered to the required shape, size and surface modification; and if designed appropriately, efficient drug localisation coupled with sustained release can be achieved. However, the nanotechnologies discussed in this review all contribute to the general advancement of therapeutic drug delivery systems. With favourable research results, significant progress is expected in the development of multifunctional nanocarriers that will target more than one aspect of brain pathology.

3 CHAPTER 3: DEVELOPMENT OF A GALANTAMINE LOADED-NANOEMULSION COMPOSITE SYSTEM

3.1 Introduction

Galantamine is a neuroactive drug, approved for the treatment of AD (Barnabas et al., 2020). Due to its acetylcholinesterase inhibitor properties, galantamine has the ability to modulate receptors of a nicotinic nature. Carbopol is a polymer with impressive mucoadhesive properties and is derived from acrylic acid (Rani et al., 2022 and Popova et al., 2021). Research highlights that nanoparticles integrated in emulsions of a surfactant-polymer composition, display impressive stability compared to emulsions typically stabilized by polymers and/or surfactants.

Furthermore, galantamine displays substantial advantages when it is administered at postulated optimum daily doses of between 16 mg/day and 24 mg/day. Additionally, controlled drug release encourages a treatment regimen that improves patient compliance and a more tolerable side effect profile (Kalola et al., 2023). The drug release study employed in this research work, also highlighted the satisfactory total drug release profile of just over 68% - congruent with observations reported in other studies (Khatavkar et al., 2012). The strategic employ of Tween 80, PVA and PEG 4000, was substantiated by the available literature that similarly elucidated the impressive water absorption, oxygen resistance and biodegradability of PVA. It was thus envisaged that the formulation of a galantamine-loaded nanoemulsion with mucoadhesive properties, would facilitate improved pharmacotherapeutic activities such as controlled and sustained drug release.

3.2. Materials and Methodologies

3.2.1 Materials

Galantamine, Soybean, Tween 80, PEG 4000, PVA and Carbopol (low molecular weight) were all purchased from Sigma–Aldrich (St. Louis, MO, USA).

3.2.2 Galantamine loaded nanoemulsion synthesis

Using a magnetic stirrer, 50 mg/ml of PEG 4000 was dissolved in acetone. This solution was then stirred on low for 20 minutes until the PEG 4000 was dissolved. In a separate beaker, distilled water was added to 30 mg/ml of PVA and magnetically stirred on low for 8 minutes, at a temperature of 37°C. Subsequently, the PVA-distilled water solution was then added to the PEG 4000-acetone solution, and magnetically stirred for an additional 5 minutes. Once a clear solution was obtained, galantamine was then dissolved in 4% w/v of acetone. The solution was allowed to magnetically stir for an additional 7 minutes at a temperature of 37°C. Thereafter, the solution was homogenized at 10 000 rpm for 2 minutes. Finally, the

galantamine-loaded nanoemulsion was achieved by adding 25% w/v water, soybean oil and Tween 80 to the homogenized solution.

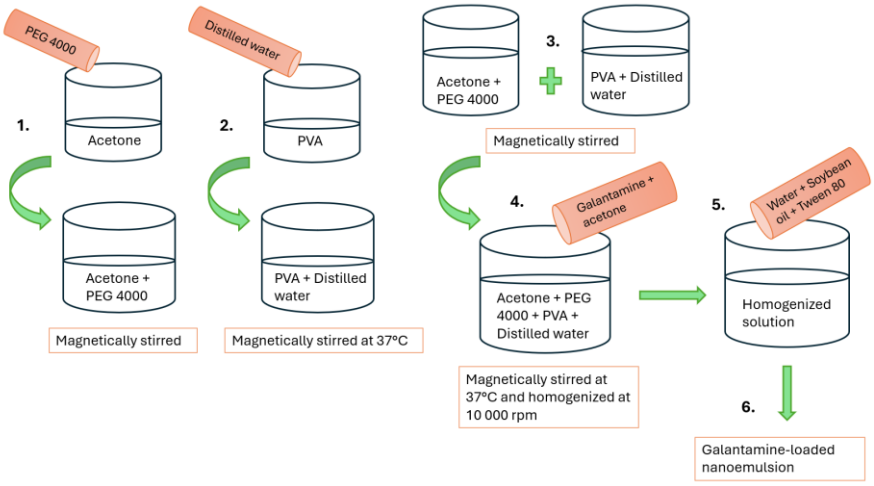


Figure 5. Image depicting the galantamine loaded nanoemulsion formulation process.

3.2.3 Chemical analysis of starting materials and nanoemulsion using FTIR

The Perkin Elmer Spectrum 2000 FTIR Spectrometer was employed, applying a single-reflection diamond MIRTGS detector (Perkin Elmer Spectrum 100, Llantrisant, Wales, UK). Samples of galantamine, plain nanoemulsion, galantamine loaded nanoemulsion, carbopol, PVA, PEG 4000, Tween 80 and soybean oil were analysed at a wavelength range of 4000 – 650 cm^{-1}. Spectra of all the samples were collected for assessment of specific functional groups and transitions due to chemical interactions during formulation development.

3.2.4 Thermal analysis using Differential Scanning Calorimetry (DSC)

The thermal stability of the nanoemulsion was evaluated through the utilization of DSC. Data of the following samples was obtained using the Mettler Toledo, DSC, STARe System, (Swchwerzenback, ZH, Switzerland system): Galantamine, Carbopol, PEG 4000, PVA and plain nanoemulsion. The aforementioned samples were heated from 0-400 °C, at 20°C/minute intervals, thus producing thermographs of thermal flow versus temperature.

3.2.5 *In-vitro* Galantamine release from the nanoemulsion

Nanoemulsion samples, which were refrigerated overnight, were sufficiently magnetically stirred. A predetermined amount of the release medium/phosphate buffered saline solution (PBS) (50 ml) was added to 3 separate beakers. Three snake skin membranes were prepared and 2 ml of the galantamine loaded nanoemulsion was drawn and inserted into each of the 3 snake skin membranes. Additionally, 1 ml of the PBS was added to each of the aforementioned membranes. Aliquots of 3 ml were withdrawn from the release medium at 0, 1, 2, 3, 4, 6 and 24 hour time periods. In order to maintain the release medium volume, 3 ml of fresh PBS was added to the three beakers after each extraction.

3.3 Results and Discussion

3.3.1 Fourier Transformation Infrared Spectroscopy of the galantamine-loaded nanoparticles

Samples were analysed using the Fourier Transformation Infrared Spectroscopy (FTIR) to confirm the presence of the functional groups in plain nanoemulsion, galantamine loaded nanoemulsion, pure galantamine, Carbopol, PVA, PEG 4000, Tween 80 and soybean oil. Depicted in Figure 5 are the FTIR spectra of the aforementioned nanoemulsion components. The pure galantamine and carbopol FTIR spectra, displayed peaks at 3560.15 and 3132 cm^{-1} due to the stretching vibration of the N-H and O-H bonds, respectively (Farissi et al., 2017). Furthermore, other studies showed that absorption bands detected at these wavelengths are congruent with single bonding (Nandiyanto et al., 2023). Absorption peaks at around 1688 – 1750 cm^{-1} could be attributed to C=O bond stretch, evident in the FTIR spectra for PVA, PEG 4000 and Tween 80. The PEG 4000 FTIR spectrum exhibited peaks at wavelengths 1449.19 cm^{-1} and 2926.31 cm^{-1} that could be attributed to the stretching vibration of C=C and C-H bonds, respectively. These results were similar to evidence provided in previously conducted research (Franca et al., 2022). Conversely, soybean oil displayed absorption peaks at 2853.9 and 2923.33 cm^{-1}, which is congruent with C-H stretching vibrations. No significant changes were observed between 2500-1800 cm^{-1}, where C-H and C=O functional groups are typically present.

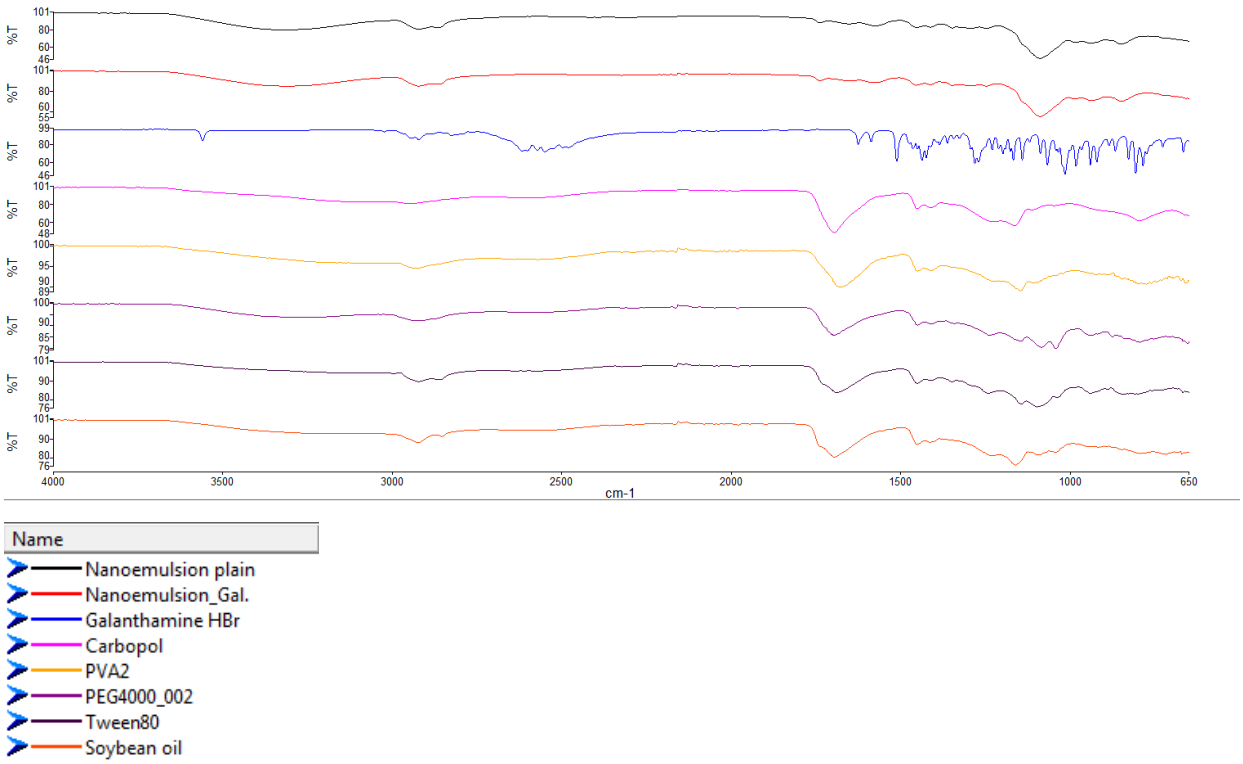


Figure 6. FTIR spectra of starting materials and the galantamine-loaded nanoemulsion.

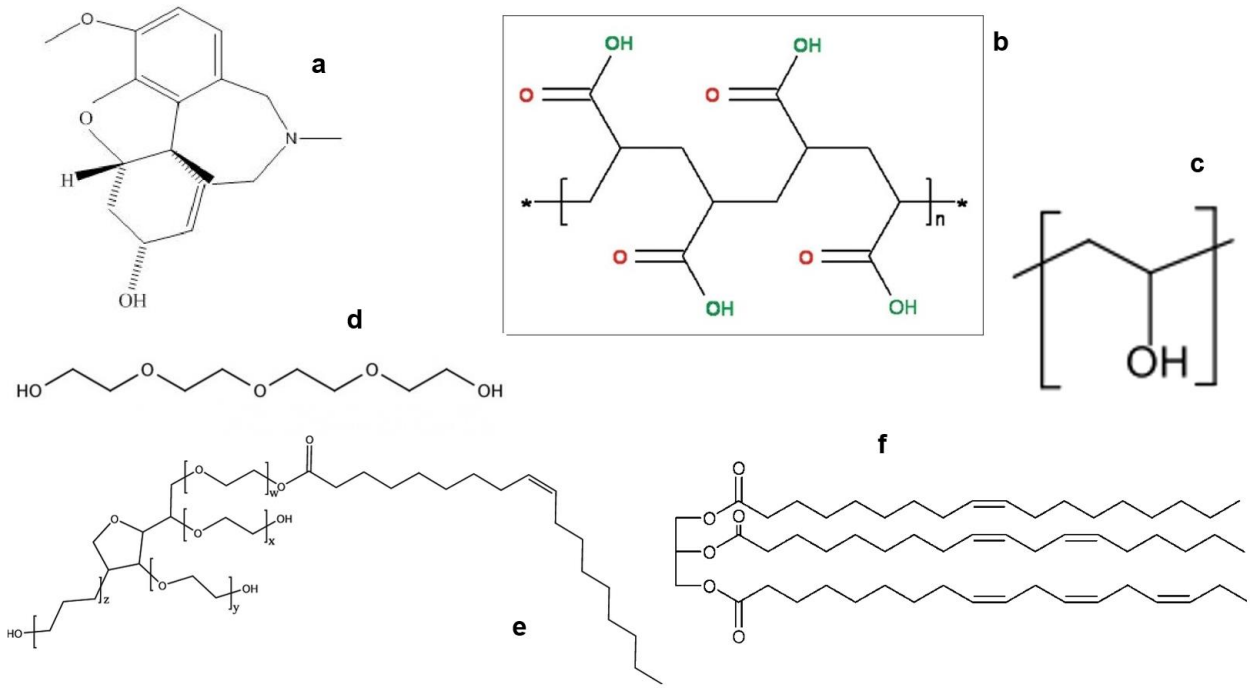


Figure 7. Chemical structures of (a) galantamine, (b) carbapol, (c) PVA, (d) PEG 4000, (e) Tween 8 and (f) soybean oil.

3.3.2 Thermal analysis of the biomaterials using Differential Scanning Calorimetry (DSC)

The DSC thermogram of galantamine displayed a characteristic endothermic peak at 280°C, which could be attributed to it reaching its melting point. The sharp exothermic peak observed at 285°C evidently disappeared in the galantamine-loaded nanoemulsion, thus verifying the encapsulation of galantamine into the carbopol nanoparticles (Hanafy et al., 2015). Thereafter, decomposition could be observed as documented in other studies (Nanaki et al., 2020).

The DSC thermograms for PEG 4000, carbopol and PVA all displayed endothermic peaks at 60°C, 230°C and 228°C, respectively. These DSC thermograms results could be accredited to possible loss of water that was bound and absorbed (Hanafy et al., 2015). Conversely, carbopol showed a minor exothermic peak at 120°C which may be due to its melting point. The galantamine nanoemulsion DSC thermogram displayed several minor exothermic peaks, visible at 210°C and again at 390°C, possibly due to the breakdown of ionic interactions between the cationic carbopol and anionic nanoemulsion agents.

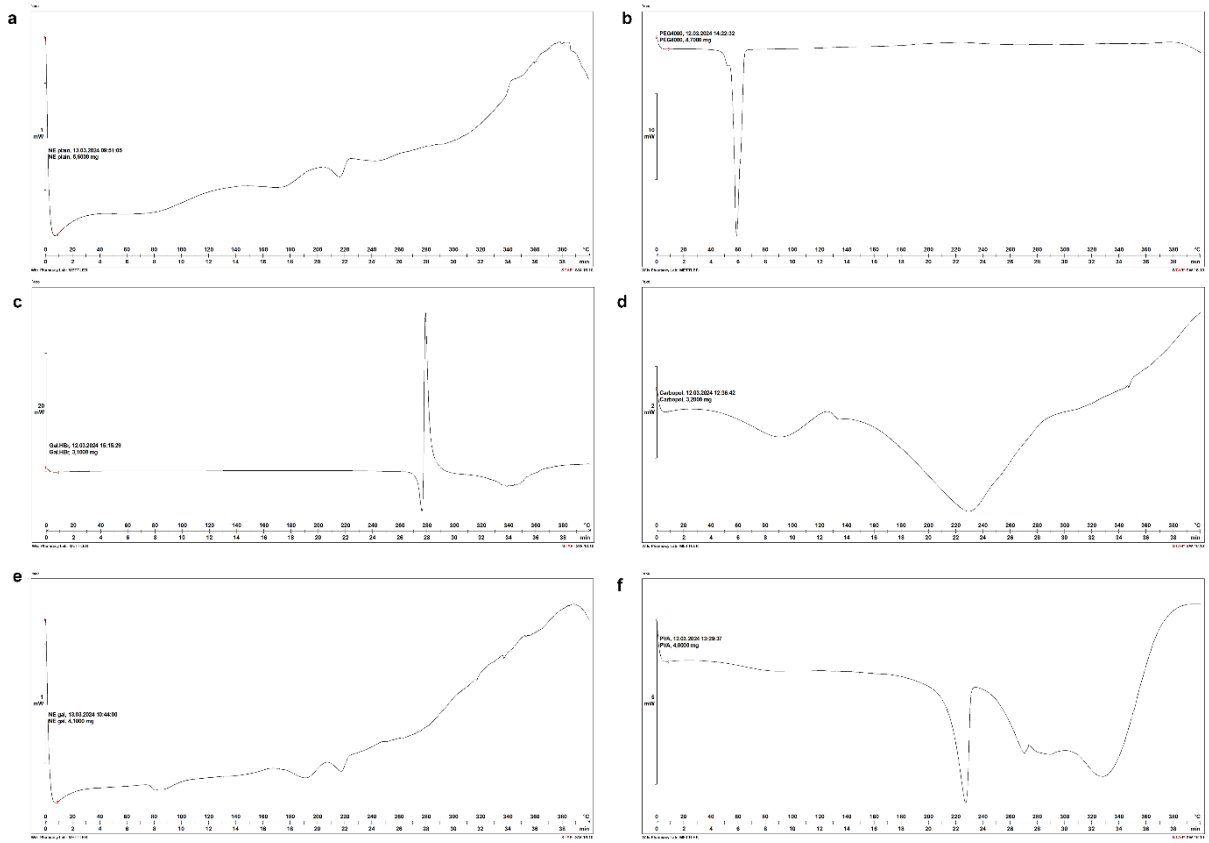
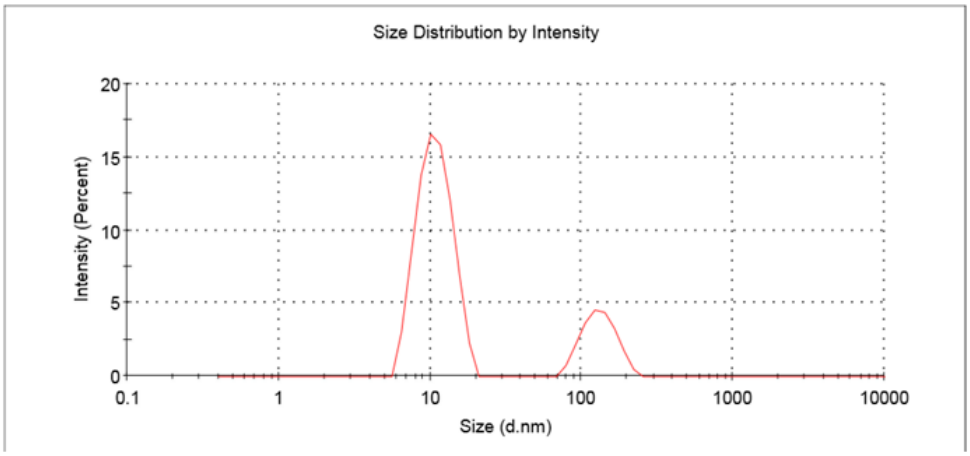


Figure 8. DSC thermograms of (a) plain nanoemulsion, (b) PEG 4000, (c) galantamine, (d) carbopol, (e) galantamine nanoemulsion and (f) PVA.

3.3.3 Analysis of the nanoemulsion stability employing Zetasizer

Nanoemulsion samples were collected and developed to assess the Zetasizer size, particle size distribution and properties. The nanoemulsion samples (0.2 ml) were dissolved in 2 ml of distilled water, at room temperature conditions of 25°C. Each sample was filtered using a 0.2 µm filter and transferred into a cuvette. Typically, nanoemulsion droplet sizes range between 10-100 nm (Algahtani et al., 2022) and the particle size distributions obtained in the investigations herein, average that of 10.54 nm, 11.01 nm, 133.2 nm and 305.1 nm. The larger particle size distributions could be attributed to a portion of the nanoparticles agglomerating due to the attractive forces that exist between them (Ashraf et al., 2018).

a



b

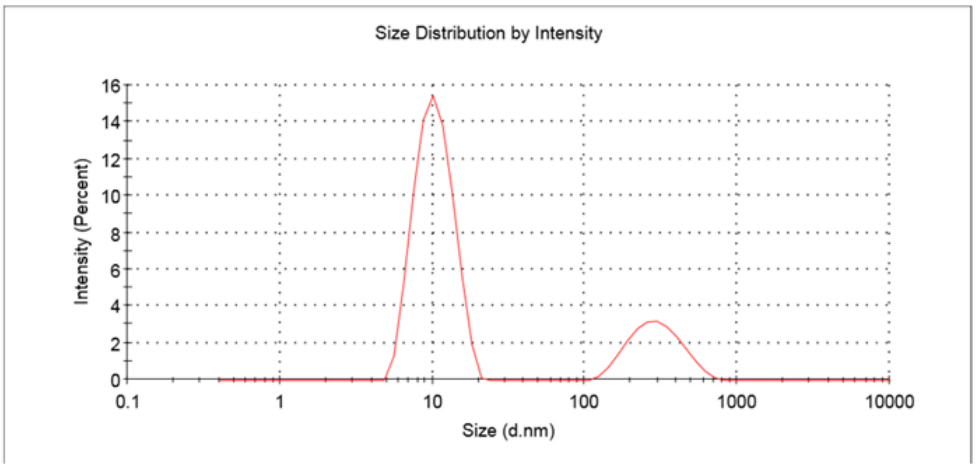


Figure 9. Zetasizer results of the galantamine nanoemulsion, depicting particle size distribution results and the observed variability in the larger particle size distributions.

3.3.4 Nanoemulsion Drug Release HPLC Study

Galantamine cumulative release from the nanoemulsion is demonstrated in Figure 9. At the 1-, 2-, 4- and 6-hour intervals, 26,94; 42,82; 58,19 and 66,31% of the galantamine was released. An evident burst release was observed between the 1–6-hour time intervals. However, the galantamine release profile then followed the desired controlled release between the 6–24-hour time intervals. Studies suggest that the initial burst release may be attributed to some of the galantamine molecules being presented on the nanoparticulate surfaces (Hanafy et al., 2015 and Janjic et al., 2017). Conversely, the controlled release could be attributed to the interaction between galantamine and carbopol. A sustained drug release profile of 68.07% was achieved.

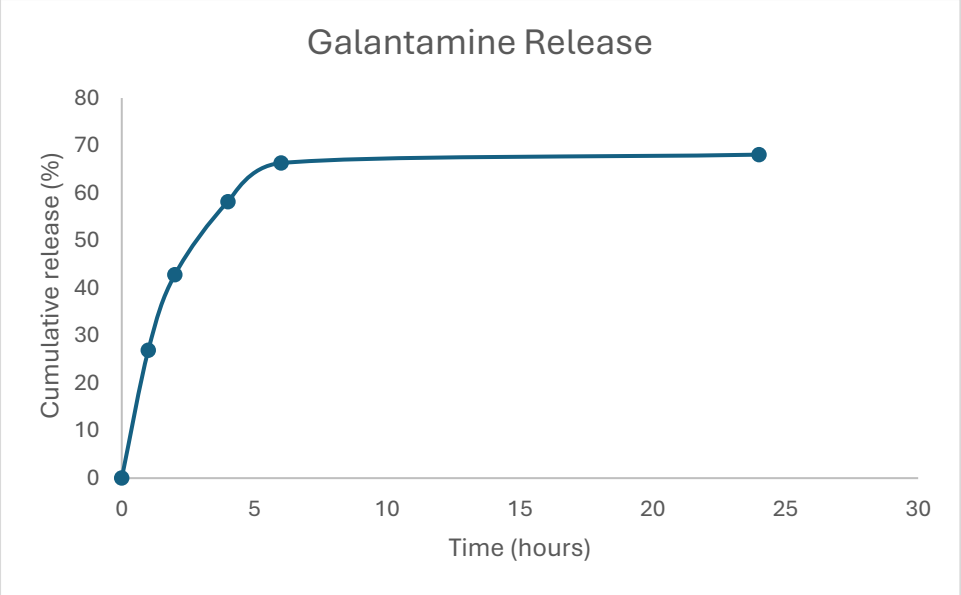


Figure 10. Graph showing cumulative galantamine release.

4 CHAPTER 4: CONCLUSIONS AND RECOMMENDATIONS

This study established the design of a galantamine-loaded nanoemulsion composite system for the treatment of AD, by strategically selecting surfactant and polymeric agents that have been researched and shown to elicit properties of a desirable nature. Despite the numerous neurotherapeutic nanotechnologies that exist, nanoemulsions have proven to be of increasing interest due to their desirable properties (Kale et al., 2017 and Hersh et al., 2016). The concept of incorporating the mucoadhesive polymer, carbopol, allowed for the nanoemulsion to present with mucoadhesive properties as well as consistency within the nanoemulsion (Hamdi et al., 2023 and Ullah et al., 2023), which essentially facilitated the effective delivery of galantamine and enable its transportation to the localised site of the disease.

An integral characteristic of nanoemulsions is their nanoparticulate size, which was evident in the results obtained during the Zetasizer analysis. Despite the forgiving nanomaterial particle size range of 20-500nm reported in other studies (Altammar, 2023 and Fonseca-Santos et al., 2015), the desired nanoparticle size is ideally that <200nm. Upon visual observations of the formulated nanoemulsions, stable nanoemulsion characterizations could be observed such as lack of creaming and cracking (Jaiswal et al., 2014).

Despite exploring the design of a galantamine-loaded nanoemulsion composite system and establishing its various physicochemical properties, it would be beneficial to include additional studies such as transmission electron microscopy, rheological properties, mucoadhesive strength analysis and cytotoxicity evaluation. The mucoadhesive strength analysis study

would essentially assess the mucoadhesive and properties of the cationic polymer, in this scenario, carbopol.

The results obtained from this study would elucidate whether the galantamine-loaded carbopol nanoemulsion adheres to the intestinal mucosa. If so, the anticipated result would thus be the increased bioavailability of galantamine on a neural level. Additionally, cytotoxicity studies would aid in observing cell reproduction and growth, including any morphological effects that the investigational nanotherapeutic has on the tissue cells *in vitro*. Moreover, the observations made in this study highlighted proof of concept that a galantamine-loaded, nanoemulsion mucoadhesive composite system could be formulated and further developed and improved, to employ the desired pharmacotherapeutic properties in neurotherapy.

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