Development of a Nanogel Carrier System for Oral Antidepressant Therapeutics

By

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A Dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the requirements for the degree of Master of Pharmacy

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Johannesburg, 2022

DECLARATION

I Fadzai Patricia Mutingwende declare that this is my own work. It has been submitted for the degree of Master of Pharmacy in the Faculty of Health Sciences in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any University.

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This……13th……day of ……June……..….2022

RESEARCH OUTPUTS

PUBLICATIONS

1. Fadzai P. Mutingwende, Pierre P. D. Kondiah, Philemon Ubanako, Thashree Marimuthu and Yahya E. Choonara. Review of Advances in Nano-Enabled Platforms for the Treatment of Depression. Polymers 2021, 13, 1431.
2. Fadzai P. Mutingwende, Pierre P. D. Kondiah, and Yahya E. Choonara. Design, Synthesis and optimization of a Paroxetine-Loaded mPEG-Chitosan Nanogel for enhanced Oral Delivery. To be submitted for publication to Nanomaterials, 2022 (I.F: 5,07)

DEDICATION

This work is dedicated to my mommy, my aunt, and my brother.

All that I am today, I owe you.

ACKNOWLEDGMENTS

My study journey has been very long and when I look back, I cannot avoid but see God's hand in it all. I am most grateful to the Almighty God who guarded me and put the right people in my path throughout my studies. These people not only supported me financially, but they have helped me build my self-confidence too. These people have become part of me, and I am most grateful.

Special thanks to Mrs Ann and Mr Shaun Baron for giving me a gift no one will ever take away from me. I cannot thank you enough. My mother Rumbidzai Mutingwende, my aunt Anesu Mupfigo and my brother Komborero Mutingwende, thank you for being my biggest cheerleaders and my pillar of strength.

Thank you; to my Supervisors, Professor Yahya Choonara and Professor Pierre Kondiah, for your guidance and encouragement during my study, to Professor Pradeep Kumar, Dr. Thashree Marimuthu and Professor Lisa du Toit, for assisting throughout the academic path and to the technical staff; Mr. Sello Ramarumo, Mr. Kleinbooi Mohlabi, Mr. Bafana Temba and Ms. Tshidi Mosete for always assisting with the procurement of chemicals and technical support. A special thanks to Prof Choonara and Prof Kondiah's NRF research grants, for the running expenses of this project.

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ABSTRACT

Depression is mental disorder that is life threatening as well as a leading cause of disability globally and contributes greatly to the global burden of diseases. It is characterized by suicidal thoughts, persistent sadness, poor concentration, and a lack of interest in previously rewarding or enjoyable activities. According to World Health Organization (W.H.O) (study undertaken in 2021), more than 280 million people suffer from depression. The selective serotonin reuptake inhibitors (SSRI) remain the first line therapy. They come with drawbacks such as delayed therapeutic onset due to extensive first pass metabolism and other side effects such as weight gain. This results in reduced patients' compliance, and they cannot be used under emergency conditions as it takes two or more weeks for therapeutic onset to be achieved. In the present research, an oral nanogel drug delivery platform was synthesised and loaded with paroxetine hydrochloride hemihydrate. Chitosan (CS) and methoxy polyethylene glycol (mPEG) were employed as the biopolymers and were ionically crosslinked with TPP to attain mPEG-CS nanogel carrier system. Paroxetine loaded mPEG-CS nanogel carrier system was synthesised to minimize the drawbacks, as the nano system exhibits characteristics that minimize the extensive first pass metabolism and to improve the solubility of paroxetine owing it the hydrophilicity properties of mPEG. The freeze-drying method was used to formulate the paroxetine loaded mPEG-CS nanogel carrier system bio-platform. A Box-Behnken experimental design was employed for optimizing the particle size and drug encapsulation efficiency (DEE). The chemical integrity and the thermal properties of the nanogel were determined using FTIR, TGA ^{1}H NMR, XRD and DSC. The morphology and size of the nanoparticles were confirmed using the SEM and zeta sizer, respectively. The optimized paroxetine loaded mPEG-CS nanogel carrier system exhibited a release of 99 % over 24 hours and entrapment efficiency of 77.90 %. *In vitro* analysis and Franz diffusion studies were done using the UV vis and the *in vitro* cytotoxicity analysis was carried out using a PC12 cell line (neuronal cells) and Caco 2 cells (intestinal cells). The *in vitro* cytotoxicity studies proved that the nanoformulation showed acceptable cytotoxicity on PC12 (murine brain-derived) cells. This study proved that paroxetine-loaded mPEG-CS nanogel carrier system improves the solubility of the drug and decreased the cytotoxic effect when compared to pure paroxetine. Hence, mPEG-CS nanogel carrier system may be considered for the delivery of paroxetine to treat depression.

LIST OF EQUATIONS

Calibration equation:	$y = 0.0102x + 0.0001$; R^2=0.9996	(Equation 3.1)
Encapsulation Efficiency %	$= \frac{weight\ of\ drug\ in\ the\ nanogel}{weight\ of\ drug\ loaded} \times 100$	(Equation 3.2)
Loading Efficiency %	$= \frac{weight\ of\ drug\ loaded}{weight\ of\ drug+copolymer} \times 100$	(Equation 3.3)
Enthalpy of change	$= \frac{Total\ area\ under\ the\ curve}{Sample\ weight}$	(Equation 3.4)
Crystallinity index	$= \frac{Sum\ of\ areas\ under\ crystalline\ peaks}{Total\ area\ under\ crystalline\ and\ amorphous\ peaks}$	(Equation 3.5)
WU (%)	$= \left(\frac{Wh-Wd}{Wd}\right) \times 100$	(Equation 3.6)
Cell viability (%)	$= \frac{Absorbance\ read\ in\ treated\ cells}{Absorbance\ in\ control\ (untreated\ )cells} \times 100$	(Equation 3.7)

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Figure 3.14: Cell viability evaluation of Caco-2 cell line treated with mPEG-CS nanogel carrier system, paroxetine, paroxetine loaded mPEG-CS nanogel carrier system, 0.1% ethanol and 5-Fluorouracil (10 µg/ml) after 72 hrs. The error bars lie within the graph ranges, therefore are not visible.

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LIST OF ABBREVIATONS

ATR-FTIR – Attenuated Total Reflectance-Fourier Transform Infrared

BBB – Blood Brain Barrier

Caco-2 -Colon Carcinoma

CNS – central nervous system

CS – Chitosan

CYP2D6 - Cytochrome P450 2D6

DSC- Differential Scanning Calorimetry

GIT - Gastrointestinal tract

HPA - Hypothalamic-pituitary-adrenal

mPEG – methoxy polyoxyethylene glycol

MTT- Methyl Thiazoly Tetrazolium

Mw- Molecular Weight

NMR- Nuclear Magnetic Resonance

PBS- Phosphate-Buffered Saline

PDI- Polydispersity Index

RES - reticuloendothelial system

SEM- Scanning Electron Microscopy

TGA- Thermogravimetric Analysis

TPP- tripolyphosphate

XRD- X-ray Diffraction

CHAPTER 1

INTRODUCTION

1.1 Background into Nanotechnology System for Depression

Depression is mental disorder that is life threatening, and a leading cause of global debilitating conditions. According to WHO, more than 280 million people suffer from depression [1]. Depression leads to suicidal thoughts, with approximately 800 000 people committing suicide on a yearly basis [2]. Depression is caused by several factors including social, psychological and biological conditions, in some cases a result of pathological illness [2] [3]. The signs and symptoms of depression include low self-esteem, loss of appetite, low energy, self-harm and lack of motivation. According to research that was conducted by Verduijn et al., 2015, depression is induced by the reduction in production of monoamines such as serotonin, and a rapid increase in production of tryptophan catabolites that are toxic to the brain [3]. These brain transformational events result in peripheral inflammation, and it has been discovered that inflammatory mediators alter monoamine neurotransmission and glucocorticoid receptor resistance [4] . Depression is also caused by hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis and low levels of Vitamin D [3].

Antidepressants such as paroxetine and sertraline are currently used as first line therapy for depression. However, earlier studies have indicated that about 30 percent of the population are being undertreated and a delayed onset of several weeks for a therapeutic effect, may contribute to reduction in patient compliance [5]. The delayed therapeutic effect is due to the fact that the antidepressant goes through extensive hepatic first pass metabolism. Other drawbacks of antidepressants include various side effects, such as erectile dysfunction, dry mouth, sleeping disorders, nausea and weight gain. The severity of side effects may also be due to the frequency of high doses [6]. Therefore, there is need to develop better drug delivery system such as nanotechnology, with fewer side effect profiles, and improved efficacy of antidepressant therapy.

Nanotechnology drug delivery system has the potential of improving the drug bioavailability, absorption and reducing side effects. In addition, nano-medicine have gained momentum for oral drug delivery, due to reduction of particle size thus increasing surface area, drug stability in the gastrointestinal tract (GIT), increased oral bioavailability and cellular uptake [7]. According to literature nanoparticles have the potential of reducing the first pass metabolism of the encapsulated drug due to their unique uptake mechanism hence improving the drug efficacy [8] [9] [10]. The nanotechnology system uses biopolymers that are biocompatible with the biological system such as methoxy polyoxyethylene glycol **(**mPEG), chitosan (CS) and

alginate. The use of nanoformulation has proven to be effective for the treatment of depression owing it to their size that permits them to pass through the BBB.

Chitosan, has gained attention in the pharmaceutical field due to its desirable biological properties that include, low toxicity, biodegradability and biocompatibility. Chitosan nanoparticles are promising carriers for the delivery of hydrophobic drugs [11]. Furthermore, chitosan have been reported to form gel-like micro particles and nanoparticles when cross linked with TPP (tripolyphosphate) [12]. PEGylation of bio platforms enhance the solubility of the drug and prolong blood circulation time, reduces first pass metabolism, escaping the phagocytosis of macrophages and avoid reticuloendothelial system (RES) clearance [11] [13]. In addition it has been reported that nanoparticles coated with PEG have increased drug folds in the brain due to the unique properties of PEG [14] .

Previous studies that were conducted using nanogel technology for intranasal administration, demonstrated limited side effects, and improved bio-efficacy with none or limited first pass metabolism. However, the materials used when preparing the nanogel for the nasal route of administration came with drawbacks which included damage of the ciliary layer, shrinkage of the mucosal layer and damage of epithelial cell. Due to this drawback the nasal route of administration cannot be used though it has the potential to improve the bio-efficacy of antidepressant [15].

The aim of this study was to improve the therapeutic effectiveness of the anti-depressant drug, paroxetine using a nanogel system. Nanogels have demonstrated the potential in increasing bio-efficacy of drugs in low dose regimens, due to their ability to provide sustained drug release kinetics and reduce the intensity of hepatic first-pass metabolism. Furthermore, these nanoparticles can target the brain and circumvent the BBB owing it to their size which is less than 200 nm [15] [16]. Nanogels are hydrogel materials, with a three-dimensional network in the nanoscale size. Nanogels can be crosslinked with polymer materials, possessing networks of high drug loading capacity, allowing them to swell and release drug accordingly. Nanogels can either be made from synthetic polymers or biopolymers or a combination of both [15]. The physicochemical features of nanogels can be altered by amending their chemical properties which include porosity, degradability, particle charge, particle size and amphiphilic nature. Nanogels differ in shapes and are composed of a core-shell-corona or core-shell structure, with crosslinked networks suitable for structural integrity. Nanogels are hydrophilic, high drug loading capacity and they are biocompatible, display stimuli-responsive behaviour, which shields the loading bioactive substance from degrading. They are also useful for targeted

delivery. These properties allow them to be suitable carriers for biomedical applications, such as drug delivery platforms [15].

The BBB interferes with the effective delivery of therapeutic agents to the central nervous system (CNS), hence hindering transportation of drugs through the endothelial capillaries to the brain [15]. It has been reported that, nanogel carrier systems, have significant potential as a drug delivery platform, for the management of brain conditions, including migraine, depression, Alzheimer's disease and schizophrenia [15].

In this research, paroxetine was employed as a model antidepressant drug, for the treatment of depression. Paroxetine exhibits drawbacks such as delayed therapeutic outcome due to extensive hepatic first pass metabolism, weight gain and erectile dysfunction. The first pass metabolism reduces the bioavailability of paroxetine by 30-60 % [17]. This drug was encapsulated and delivered in functionalized nanogel system, to specifically target the BBB. The designed system has the potential of overcoming various sides effects associated with high drug loaded doses, and decreasing the dosing frequency, by shielding against the hepatic first pass metabolism. Hydrophilic mPEG biopolymer and CS crosslinked, were employed as biomaterials, for the synthesis of paroxetine-loaded nanogel. The biopolymers were ionically crosslinked with TPP.

1.2. Rationale and Motivation of the Study

Current antidepressant formulations on the market have been reported to have many side effects including nausea, anxiety, weight gain (if taken for more than 6 months), suicidal thoughts (when escalated), low oral bioavailability, as well as a delayed onset of therapeutic effectiveness due to extensive hepatic first pass metabolism. Due to their delayed therapeutic onset, low oral bioavailability and limited efficacy, they cannot be used in suicidal patients and in cases of emergency, which require a rapid onset of action. Considering that antidepressants are prescribed on trial and error basis (due to major or minor depression), rapid onset to increase the effectiveness of the drug and improve patient compliance, is a key factor of concern [5]. Oral route of administration is most convenient for patients; however, it comes with the drawbacks mentioned above, hence the need to come with a strategy that will minimize the drawbacks such as employing nanomedicine. Nanotechnological systems, employing nanogels, have great potential of improving therapeutic efficacy, and significantly lowering side effect profiles [5]. Paroxetine formulation that is currently on the market is highly potent, however it is highly CYP2D6 metabolised. The first pass metabolism decreases the bioavailability to a therapeutic dose of approximately 30-60 %. Thus, less drug will be delivered to the CNS and approximately 64 % of the dose will be excreted in the urine. Furthermore, it

is administered in high doses (20-60 mg) hence the risk of side effects will be increased, and the formulation has delayed therapeutic effect. In this study, nanogel technology was employed to improve the therapeutic effect of paroxetine. Nanogels have potential to protect loaded drugs in the formulation, from passing through first pass metabolism and they are specific to the targeted organ owing to the biopolymers that were used during the synthesis and their size [18] [19] .

Nanogels have high drug loading capacity and swelling features, which enable them to absorb large quantities of fluid without structural variation. These amphiphilic polymers will influence drug dissolution, adhere to mucosal tissue membranes, and transiently increase drug absorption [20]. Furthermore, nanogels do not pass-through renal clearance/ elimination easily, due to their size, which enables them in having a longer half-life circulation. Nanogels have the capacity of carrying molecules with different charges, owing to their crosslinked electrolyte and amphiphilic properties [21]. The nanogel system will bypass the first pass metabolism of paroxetine, shielding the drug molecule from biodegradation, as seen in Figure 1.1 [20] hence resulting in improved drug efficacy and therapeutic onset.

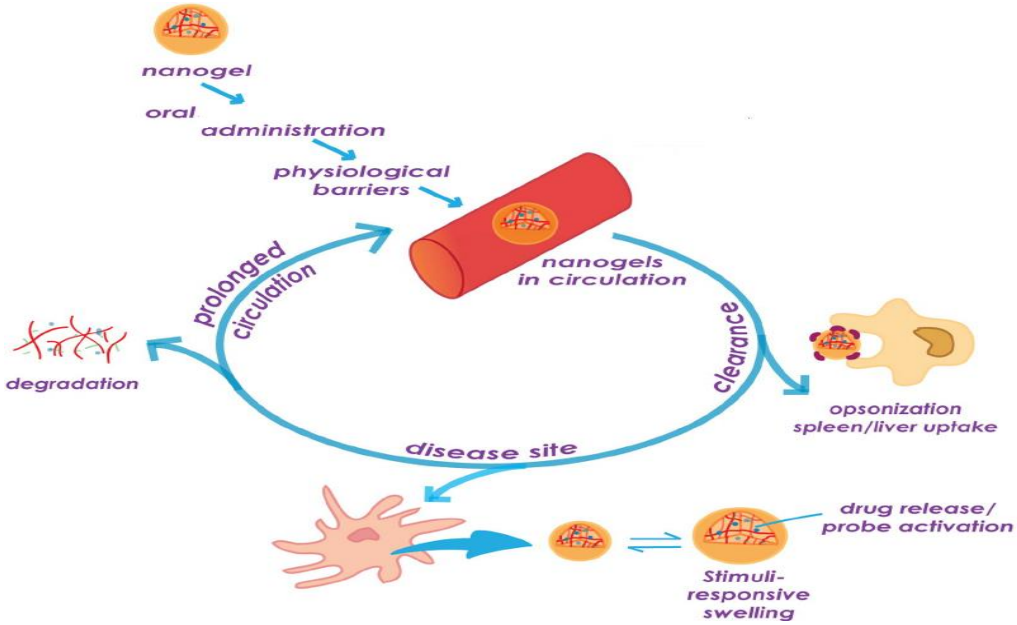

Figure 1.1: Diagram representing the increased half-life of the drug, due to the nanogel synthesised formulation [20] .

In the current study, paroxetine was loaded into the nanogel using the swelling method. mPEG biopolymer was incorporated on the hydrophilic surface, with hydrophobic crosslinked chitosan, as the inner hydrophobic core. mPEG possesses modified swelling properties, which improves the solubility, permeability and increases the half-life of the drug. It also reduces the

metabolic first pass metabolism [19] [22] . The copolymeric mPEG-CS nanogel carrier system were prepared by ionic gelation technology and they are self-assembled nanogel owing it to the unique properties of chitosan when exposed to aqueous or polar media as shown in Figure 1.2 [22].In addition, it is reported that mPEG-CS tend to aggregate spontaneously in aqueous conditions due to their unique properties [23]. The gelation properties are due to the electrostatic interaction between the copolymers and the crosslinker. In the GIT the nanogel tend to swell and degrade facilitating its absorption. The degradation in the small intestines is due to the present of enzymes such as lysosomes [24] [25]. Owing it to the mucoadhesive properties of CS and the hydrophilic properties of mPEG the drug absorption and permeation respectively will be enhanced in the small intestines. CS has the ability to directly attach to the GIT surface hence increasing the cellular uptake. Moreover, owing it to the positively charged moieties of CS, it has the ability to interact with the tight junction of the small intestines epithelial cell and thus modulate permeation and absorption of the nanoparticles through the interstitial space between the epithelial cells [26].

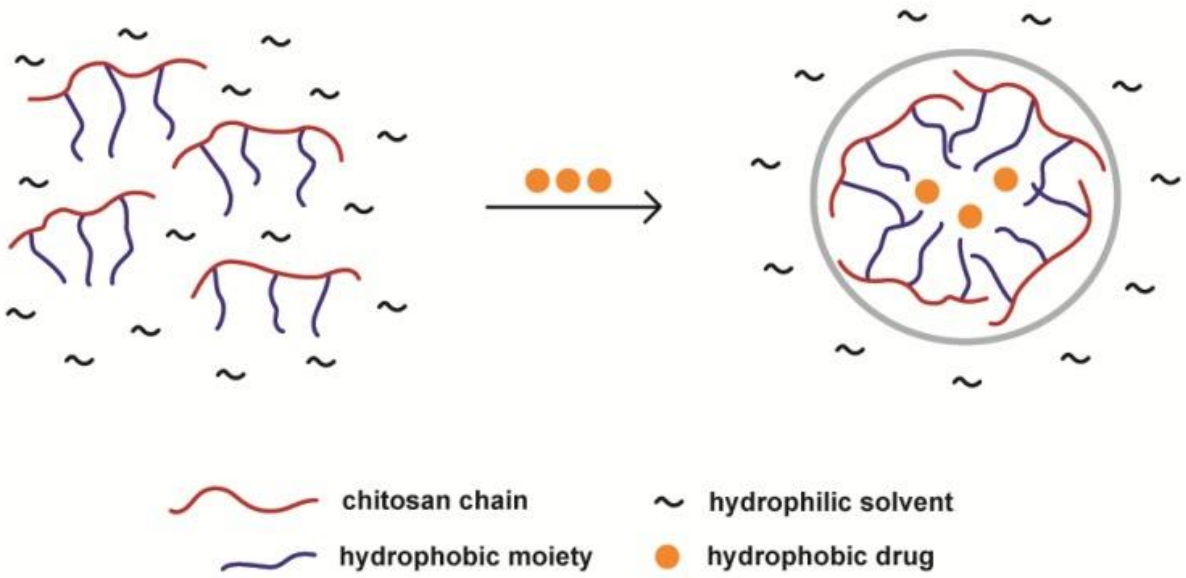

Figure 1.2: Shows the self-assembly properties of the CS biopolymer and its ability to encapsulate the drug.

1.3. Possible Therapeutic Applications of the Delivery System

Synthesising nanoformulation for oral drug delivery of antidepressants is currently an area of concern, as the nanotechnology system has gained momentum due to its unique properties. The oral nanogel carrier system was analysed, loading paroxetine which works by blocking the serotonin reuptake transporter hence increasing the concentration of synaptic serotonin levels. The mPEG-CS nanogel carrier system displayed better dissolution profile for both *in*

vitro and *ex vivo* studies compared to chitosan nanoparticles. The permeation studies of paroxetine loaded mPEG-CS nanogel carrier system were done using the intestinal tissue, using the Franz diffusion studies and it demonstrated a significant permeation efficient. In addition, the cytotoxicity studies of the bioplatform and the paroxetine loaded mPEG-CS nanogel carrier system were undertaken using PC12 cell line and Caco-2 cell line, on both cell lines the paroxetine proved to be toxic, and the mPEG-CS nanogel carrier system exhibited high cell viability. Physiochemical studies were undertaken on the synthesised mPEG-CS nanogel carrier system and on pure biopolymers to confirm their properties and to confirm if the mPEG-CS nanogel carrier system was successfully crosslinked and loading of the model drug.

1.4. Aim and Objectives of the Study

The aim of the study was to develop an oral mPEG-CS nanogel carrier system to improve therapeutic efficacy of the model antidepressant paroxetine.

The following objectives were defined for the study:

1. To develop a nanogel carrier system using crosslinked methoxy polyethylene glycol (mPEG) and CS, incorporated with drug-loaded paroxetine.
2. To evaluate the pharmaceutical stability (physicochemical) of the nanogel carrier system, using Scanning Electron Microscopy (SEM), Attenuated Total Reflectance- 9 Fourier Transform Infrared (ATR-FTIR), Nuclear Magnetic Resonance (NMR), Differential Scanning Calorimetry (DSC) Thermogravimetric Analysis (TGA) and X-Ray Diffraction (XRD).
3. To determine particle size and zeta potential of the mPEG-CS nanogel carrier system for to enhance oral- and neurobioavailability across the BBB.
4. To determine drug release profile of the paroxetine loaded mPEG-CS nanogel carrier system *in vitro* and the evaluation of the permeation studies.
5. To analyse the cytotoxicity and cell viability of the nanogel formulation, on a PC 12 cell line and Caco-2 cell line.

1.5. Novelty of the Delivery System

The purpose of the research was to synthesise a self-assembled nanogel carrier system with minimized side effects such as hepatic first pass metabolism, and to synthesise a bio platform that can cross the BBB. mPEG and CS were crosslinked with TPP, loaded with paroxetine. The crosslinker (TPP) gives the nanogel properties when crosslinked with CS due the inter- and intra-crosslinking between the positively charged amino groups of CS and negatively charged phosphate groups of TPP. The paroxetine was encapsulated in the core of mPEG-

CS nanogel carrier that will be taken orally. The nanotechnology technique will come with the following benefits:

- The size of nanogel drug delivery system allows it to penetrate through the BBB.
- CS improves the mucoadhesive properties of the copolymer
- Nanotechnology system owing it to mPEG shields the hepatic first-pass effect hence improving the therapeutic efficacy and fastening the onset of action
- mPEG improves the solubility of CS and paroxetine

The mPEG-CS nanogel carrier system was evaluated for physiochemical properties. The cytotoxicity studies were done using the PC 12 cell line and the caco-2 cell line.

1.6. Overview of the Dissertation

This thesis covers all the research from synthesis of the formulation to *in vitro*, *ex vitro* and *ex vivo* critical evaluation of the drug delivery system.

Chapter 1 gives a comprehensive overview of the rational and motivation for undertaking this research, thereby describing the mechanism of action of the delivery system, as well as research previously undertaken in the field. The aims and objectives of the study are clearly explained, ensuring clarity and analytical sequence throughout the study.

Chapter 2 a critical review of different biopolymers and nanocarriers that can be employed for drug delivery of antidepressants. This chapter covers different route of administration that can be used for antidepressants drug delivery and research that were previously undertaken using the nanotechnology system, stating their outcomes.

Chapter 3 focuses on the synthesis and evaluation of the self-assembled mPEG-CS nanogel carrier system formulation and the paroxetine loaded mPEG-CS nanogel carrier system bioplatform for their properties that includes physicochemical behaviours. *In vitro* drug release studies as well as *ex vivo* studies employing intestinal tissue from a rat were also conducted. The cytotoxicity of the formulation was also investigated using PC 12 cell line and Caco-2 cell line.

Chapter 4 concludes this thesis, summarizing the outcomes of the research and conferring the limitations and recommendations.

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CHAPTER 2

Advances in Nano-Enabled Platforms for the Treatment of Depression

2.1. Introduction

Depression is a common mental disorder that is characterized by a persistent feeling of sadness, low self-esteem, disturbed appetite, suicidal thoughts, insomnia and loss of interest [1]. Depression is caused by several aspects which include pathological effects, social activities such as drug and alcohol abuse and biological factors [2]. According to research done by the World Health Organization (W.H.O) in 2017, more than 300 million people (approximately 4.4 % of the world's population) suffer from depression; [1] making it one of the top two causes of disability-adjusted life years currently [2]. Pathological causes of depression include a chemical imbalance in the brain, energy metabolic decline and alteration in body hormones [3]. According to the serotonin hypothesis, depression is a result of dysfunctional serotonergic activities [4] which results in reduced serotonin levels in the brain. Several classes of antidepressant therapy that are currently on the market include selective serotonin reuptake inhibitors (SSRI), tricyclic antidepressants, serotonin-norepinephrine reuptake inhibitors and monoamine oxidase inhibitors. SSRI such as paroxetine and fluvoxamine are first-line treatment options in adults with depression, albeit with several contraindications [5] .The side effects of current medication include delayed therapeutic onset, low bioavailability, erectile dysfunction, weight gain, dry mouth, nervousness, and insomnia. Some currently approved antidepressant drugs pass through extensive first-pass metabolism which results in reduced oral bioavailability [5]. The time taken by the drug to reach the saturation point is usually prolonged, resulting in delayed therapeutic onset and reduced therapeutic efficacy. Furthermore, as the bioavailability is low, higher doses are required, leading to an increased prevalence of side effects. The therapeutic effect is also limited because of the presence of the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB). Traditional medicines have a limited capacity of crossing the BBB and BCSFB [6]. According to current research, nanotechnology-based delivery platforms can be employed to ameliorate the above-mentioned limitations [7,8]. The uses of nanomedicine, biopolymers and nanocarriers have gained significant attention on overcoming these gaps [7]. Nano-based drug delivery strategies offer various advantages in the treatment of chronic diseases by site-specific and targeted delivery, thereby improving the efficacy of approved formulations [7]. Additionally, nanoparticles can improve plasma bioavailability profiles, further enhancing a sustained delivery of antidepressants, resulting in reduced side effects on account of lowered dosing frequencies. Nanomedicine has been used to overcome the limitations of the BBB, as they penetrate through it due to their small size ≤ 100 nm [9].

Furthermore, nanoparticles can target specific receptors enabled by complexation to ligands such as transferrin and glutathione for improving therapeutic efficacy [9, 10,11]. In this review, we discuss different drug carriers, ligands and biopolymers that can improve the bioavailability and therapeutic efficacy of antidepressants by reducing undesirable side effects and dosing frequencies, to achieve safe, desired clinical outcomes.

2.2. Designing Sustained-Release Formulations for the Delivery of Antidepressant Drugs

Biopolymer-based nano-drug delivery systems have been shown to lower antidepressant drug toxicity and dosing frequency, as they can be designed to exhibit controlled drug release [12]. According to literature, drug solubility, specificity, improved bioavailability, bio-distribution and controlled release of antidepressant drugs have been achieved through the use of biopolymer-based drug delivery [12]. Nanocarriers influence the bioavailability and therapeutic efficacy of antidepressants depending on the route of administration used. They can enhance the drug stability, encapsulating rate, drug solubility and circulation time of antidepressants in the body [13].

2.2.1. The Oral Route of Antidepressant Drug Delivery

The oral route of administration is the route of choice for the delivery of chronic therapy due to good patient compliance, low cost and ease of administration. However, enzymatic barriers in the gastrointestinal tract harm the effectiveness of oral drug delivery. The oral route of delivery limits drug half-life due to the hepatic first-pass effect and other factors, resulting in reduced drug efficacy [14, 15]. The BBB is a complex physiological barrier, which hinders the penetration of substances into the brain. To improve neuro-availability, biopolymers can be employed to improve drug permeability through the BBB. Biopolymers such as polysaccharides present attractive strategies for the oral delivery of antidepressants due to their desired characteristics which include; sustained drug release profiles, small size, stability, biodegradability, biocompatibility and limited toxicity [16] .

A study was conducted in which venlafaxine was delivered in a copolymeric platform composed of sodium alginate and hydroxypropyl methylcellulose. The main focus of the study was to improve the oral bioavailability of venlafaxine as it is freely soluble in water, undergoes first-pass metabolism, has a narrow absorption window and exhibits a short half-life [14]. The results showed a sustained release profile, improved oral bioavailability and mucoadhesive properties of the antidepressant; resulting in improved drug efficacy [14]. The drug diffusion release in the above-mentioned study was dependent on the swelling behavior of the biopolymer and the concentration of sodium alginate employed. A high concentration of sodium alginate resulted in controlled release hence improved half-life [14]. Another study

showed that sodium alginate delays gastric emptying, hence increased drug circulation time when imipramine was encapsulated into sodium alginate-based nanoparticles for oral delivery [17]. Moreover, using chitosan to improve the oral bioavailability of escitalopram demonstrated reduced first-pass metabolism, as illustrated in Figure 2.1 [18]. The results showed reduced hepatic first-pass metabolism, with shielding from enzymatic degradation. The *in vitro* study which was done using a dialysis membrane exhibited improved drug circulating time in the body, hence sustained release over 24 hours. The oral bioavailability of the antidepressant was improved due to the mucoadhesive properties of the biopolymer. The drug release was 98.4 ± 1.07%, the particle size ranged from 60 -115 nm and the neuro- availability of the antidepressant was improved. The size and biodegradability properties of chitosan affect its bioactivity on the BBB; hence drug-loaded chitosan nanoparticles can penetrate through the BBB for drug delivery against neurological disorders.

Chitosan nanoparticles are also biocompatible, biodegradable, mucoadhesive and nontoxic, which makes them suitable for designing drug delivery vehicles for the treatment of neurological conditions [18, 19]. Erum et al. (2016) formulated and evaluated fluoxetine hydrochloride (HCL) microspheres using chitosan and arabinoxylan as the biopolymers. These biopolymers are known to be non-toxic, biocompatible, biodegradable, and have improved entrapment efficiency. The cationic property of chitosan allows it to react with polyanions giving rise to polyelectrolyte complexes with arabinoxylan biopolymers. The percentage yield was improved to 93% and the drug entrapment rate was improved to about 77%. *In vitro* studies proved that chitosan improved the drug release of the antidepressant due to swelling properties of the biopolymers. Hence, a copolymer of chitosan and arabinoxylan can be employed during the formulation of fluoxetine HCL oral microspheres in a 2:1 ratio, respectively, to obtain a sustained release profile. Moreover, chitosan has a high drug entrapping efficiency and the percentage yield is directly proportional to the concentration of the biopolymers [20]. This might result in improved neuro-bioavailability of the antidepressants.

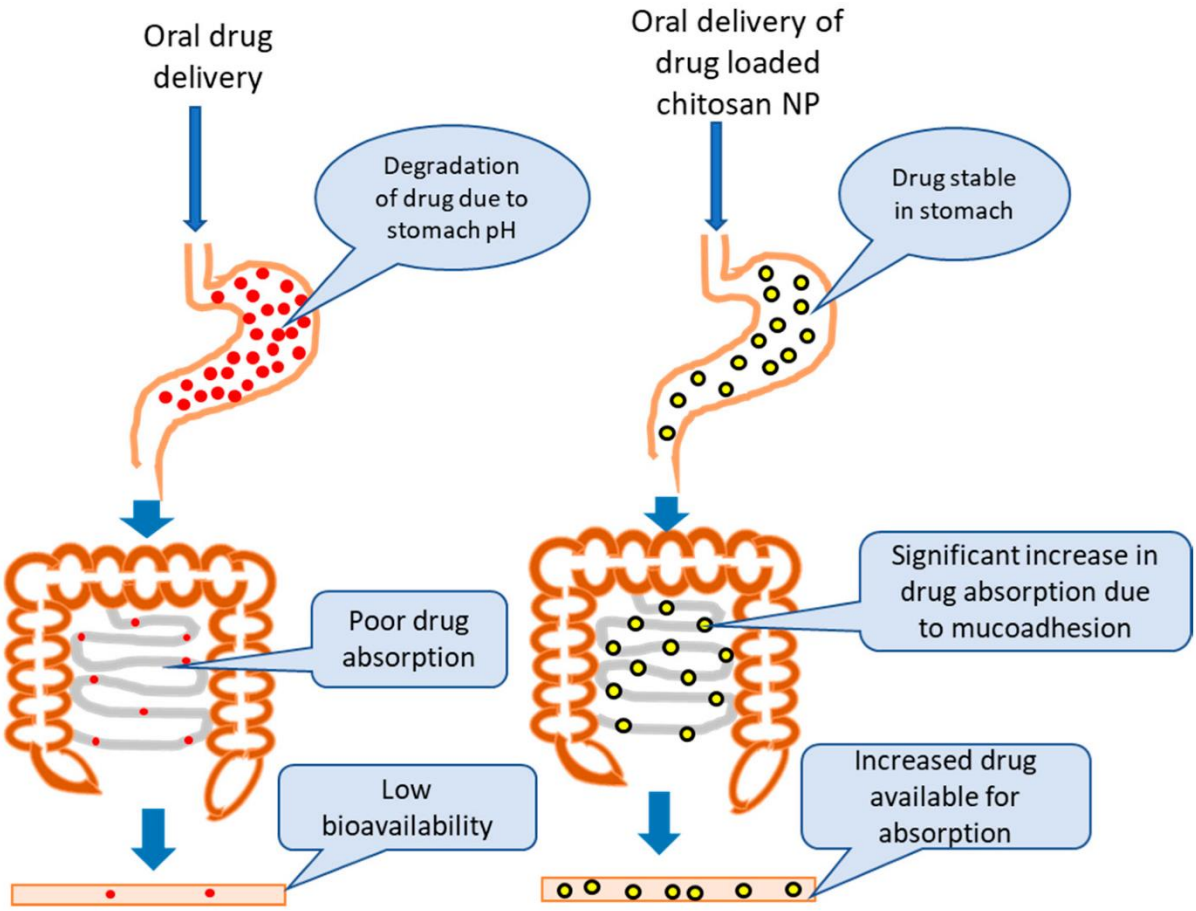

Figure 2.1: Chitosan improves drug oral bioavailability**.** *In vivo* comparison of oral bioavailability of a chitosan-based nanoparticle and pure drug to investigate the effect of chitosan-based nanoparticles on improving the drug availability for absorption via the intestinal epithelium. Adapted and modified with permission from [21]. Copyright (2019) MDPI.

Moreover, PEGylated oral nanoparticles exhibit enhanced half-life, oral bioavailability, and water solubility of bioactive molecules, reduced hepatic first-pass metabolism and premature leaking of the bioactive agent. PEGylation also increases the molecular weight of the drug, hence slowing down its clearance rate in the kidney. Figure 2.2 summaries the properties of PEGylated nanoparticles [22, 23]. PEGylated polymeric nanoparticles penetrate the brain better, as compared to nanoparticles coated with polysorbate 80 [24]. This is because PEG is covalently attached to the polymer, preventing the pre-leakage of the bioactive agent from the PEGylated polymeric nanoparticles whilst polysorbate 80 is prone to adsorption [24].

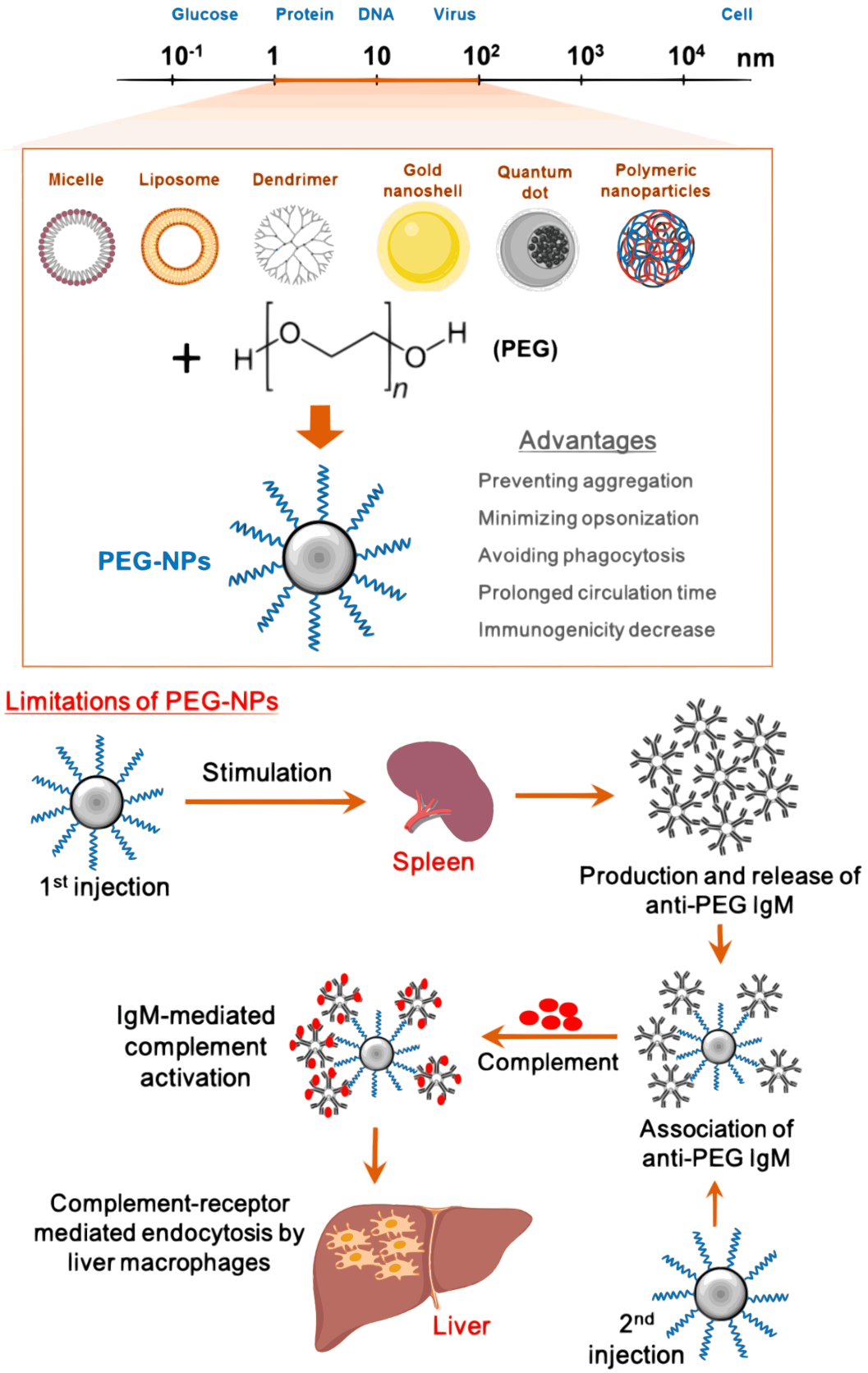

Figure 2.2: Effect of PEGylation. Polyethylene glycol (PEG) is conjugated to nanoparticles (NPs) surface to form PEG-NPs, providing several advantageous properties within a drug

delivery system. The diagram also indicates the limitations associated with PEGylating of nanoparticles. Adapted and modified with permission from [22]. Copyright (2020) MDPI.

Abourehab et al. (2018) conducted a study where they synthesized self-assembled biodegradable polymeric micelles to improve dapoxetine-loaded PEG-PLGA delivery of the antidepressant nanomicelles across the BBB. The study showed that PEGylation might protect the drug-loaded nanoparticle from uptake by the reticulo-endothelial system, hence improving the oral bioavailability and the circulation half-life of the drug. The extended circulation half-life allows more absorption of loaded PEG-PLGA via the bloodstream. The study further demonstrated that PEGylation of the nanoparticles improves the rate of accumulation of the antidepressant in the brain and its absorption rate on account of reduced first-pass metabolism. Furthermore, the improved half-life ameliorates the accumulation of the antidepressant, hence increasing its cerebral concentration [25].

2.2.2. Intranasal Route of Administration of Antidepressants

The intranasal route of administration has gained momentum in enhancing the efficacy of antidepressants due to diminished first-pass metabolism, circumvention of the BBB and non-invasiveness [26, 27]. The intranasal route transports drugs to the CNS through the olfactory and trigeminal nerve pathways [28]. Poly(lactic-co-glycolic acid-Chitosan (PLGA-CS) nanoparticles possess biocompatible, mucoadhesive, bio-adhesive and biodegradable properties which support the prolonged circulation of the loaded nanoparticles in the nasal cavity and reduced nasal mucociliary clearance. To investigate the mucoadhesive properties of desvenlafaxine (DVF) loaded PLGA-CS nanoparticles on the brain, Tong et al. (2017) conducted a study in which they incorporated DVF into a CS and PLGA copolymeric platform. The *in vitro* studies displayed a sustained release profile for over 24 hours. When PLGA-CS nanoparticle loaded with DVF were intranasally administered in *in vivo* depression rat animal models, the bioavailability was improved (56.35 %) when compared with the bioavailability of the free drug (23.70 %). Chitosan possesses the ability to enhance paracellular transport through epithelial tight junctions due to its interaction with the protein kinase C pathway in mucosal epithelial cells. The findings demonstrated that the entrapping efficacy, neuro-bioavailability, and uptake of the antidepressant into the brain, and efficacy were improved. Moreover, DVF/PLGA-CS nanoparticles displayed improved brain targeting efficacy and uptake of DVF by the brain [27]. Chitosan biopolymer lowers the rate of mucociliary clearance and opens the tight junctions between cells rapidly, which facilitates drug transport across the nasal membrane to the brain by the paracellular route. The small size of chitosan nanoparticles improves their transport across the nasal mucosa. In a previous study, venlafaxine-loaded chitosan nanoparticles were formulated to investigate the ability of

venlafaxine-loaded chitosan nanoparticles to enhance drug delivery to the brain via intranasal administration, thereby improving the treatment of depression. The *ex vivo* studies that were carried out using a Franz diffusion cell on the porcine nasal mucosa, resulted in enhanced uptake of venlafaxine-loaded chitosan nanoparticles by three-fold, relative to the free venlafaxine solution.

The *in vivo* studies that were conducted in rat animal models resulted in increased bioavailability. The data showed that the intranasal route of administration can improve the uptake of venlafaxine-loaded chitosan nanoparticles by the brain [29]. Venlafaxine-loaded alginate nanoparticles have also been synthesized and characterized for the nasal route of administration [30]. When the particles were analyzed *in vitro*, they displayed a sustained release profile over a period of 24 hours. The *ex vivo* studies that were done using porcine nasal mucosa showed that venlafaxine-loaded alginate nanoparticles significantly increased the permeation of venlafaxine as well as the mucosal absorption rate. The *in vivo* studies demonstrated that the mucoadhesive properties of venlafaxine/alginate nanoparticles also improved the concentration of the venlafaxine in the brain. The researchers concluded that alginate-loaded nanoparticles can improve the therapeutic effect of antidepressants [30]. Furthermore, venlafaxine-loaded alginate nanogels have been formulated and characterized for the treatment of depression. The formulated venlafaxine-loaded nanogel was stable at pH 5.4 ± 0.3 and also in the nasal cavity. The efficacy and safety of the venlafaxine-loaded nanogel were investigated using sheep nasal mucosa membrane. The drug accumulation release was found to be 96.96 ± 0.13 %, and there was no evidence of tissue damage. The improved permeation of the antidepressant was attributable to the interaction of negatively charged sialic acid residues on the cell membrane and tight junctions of the mucosal epithelial cells and positively charged amino group on the alginate biopolymer resulting in the opening of tight junctions. The *in vivo* studies conducted with rats indicated an improved concentration of the drug in the brain, hence improved efficacy of the antidepressant. This study showed that venlafaxine-loaded nanogels can improve drug delivery of antidepressants and are safe for intranasal administration [31]. However, clinical studies need to be done to prove the safety and efficacy of the delivery system.

2.2.3. Parenteral route for the delivery of antidepressants

The parenteral route of drug delivery possesses attractive attributes such as the avoidance of hepatic first-pass metabolism, improved bioavailability and reliable doses; thereby enhancing the efficacy of antidepressants [32]. For example, sertraline-loaded chitosan nanoparticles have been formulated and characterized for the treatment of depression [33, 21]. A single dose of sertraline nanoparticles was intravenously administered into a rabbit via the marginal ear vein. The half-life and the entrapment rate of the sertraline nanoparticles were improved. The

plasma bioavailability of the loaded nanoparticles quadrupled when compared with the pure drug on account of the mucoadhesive properties of the chitosan. The data showed that chitosan-loaded nanoparticles prolonged the circulation period of sertraline and enhanced its plasma bioavailability [33]. L–tyrosine-loaded nanoparticles have also been synthesized, characterized and administered to rat models for the treatment of depression. The size of the loaded nanoparticles was found to be ±141.80 nm and the entrapping rate was 87.45 %. The *in vitro* studies of L-tyrosine-loaded nanoparticles also showed a sustained release profile of ± 86.65 % over 48 hours. The study proved the safety of the nanoparticles an improved drug efficacy of ± 86.65 %. The data indicated that the parenteral administration of L-tyrosine-loaded nanoparticles improves its efficacy [34]. The study did not report any toxicity caused by the nanoparticles, prompting the need for further clinical and *in vivo* studies to be conducted. Summary of biopolymers discussed is presented in Table 2.1.

Table 2.1: Summary of the application of biopolymers using different routes of administration

Biopolymers	**Benefits**	**Disadvantages**	**Drug**	**References**
Oral				
sodium alginate HMC	Sustained release from microcapsules due to swelling properties of sodium alginate/hydroxypropyl methylcellulose copolymers at pH 7.4 and improved bioavailability due to mucoadhesive properties of sodium alginate when they bind to the epithelial mucous membrane lining.	Drug release is dependent on the concentration of the biopolymers	Venlafaxine	[14]
Sodium alginate	Increased circulation period enabled due to the mucoadhesive properties of sodium alginate which allows a delay in gastric emptying.	Solubility of alginate is dependent on pH of the solvent	Imipramine	[17]
Chitosan	Improved sustained release and circulation. Enhanced permeation due to mucoadhesive properties of chitosan which allows the nanoparticles to bind with the mucosa via the ionic interaction	Solubility of chitosan is affected by pH.	Escitalopram	[18]
Chitosan-Arabinoxylan	Improved entrapping rate due to the swelling properties of the biopolymers. Sustained release from the microspheres due to swelling properties of chitosan/ arabinoxylan copolymer under acidic conditions of pH 1.2, due to	Encapsulation rate is directly proportional to the concentration of chitosan	Fluoxetine HCL	[20]

	protonation of the free amine groups on the copolymers			
PEG-PLGA	Improved circulation, half-life and bioavailability due to amphiphilic copolymers of PEG-PLGA.	PLGA is not stable on its own	Dapoxetine	[25]
Intranasal				
Chitosan-PLGA	Sustained release profile due to hydration and swelling properties of CS/PLGA copolymers. Enhanced drug uptake rate and bioavailability as a result mucoadhesive and cationic properties of chitosan which increases the retention time of the nanoparticles in the nasal passage	PLGA cannot be stabilized by chitosan on its own	Desvenlafaxine	[27]
Chitosan sodium TPP	Amplified drug intranasal uptake and bioavailability as a result of mucoadhesive properties of chitosan and the interaction of cationic charges on the chitosan and anionic charges on the tight junction of the mucosal epithelium cells	Solubility of chitosan is affected by pH	Venlafaxine	[29]
Alginate	Higher mucoadhesive properties and permeation and sustained release. Enhanced therapeutic efficacy.	Covalent cross linking can result in cell toxicity	Venlafaxine	[30]
Nanostructured lipids	Increased drug release and drug efficacy due to improved residential time of the nanoparticles in the nasal cavity due to HPMC biopolymer.	Requires a stabilizer	Venlafaxine	[31]
Parenteral				
Chitosan	Improved half-life, entrapping rate and bioavailability owing it to mucoadhesive, encapsulation efficacy and delayed clearance properties of chitosan	Solubility of chitosan is affected by pH.	Sertraline	[21], [33]
Polycaprolactone	Enhanced entrapping efficiency and sustained release.	More efficient with hydrophobic drugs Requires a stabilizer	L –tyrosine	[34]

2.3. Nanocarriers Employed as Therapeutic Delivery Platforms of Antidepressants

Nanocarriers possess attractive properties which include a high surface-area-to-volume ratio, controlled drug release, targeted delivery, multi-functionality, and a great potential for surface modification [35 ,36]. Moreover, their nano size has conferred on them the ability to penetrate the BBB and target the brain—rendering them desirable for neurotherapy and diagnosis. Nanocarriers can be employed to enhance drug solubility, circulating time, stability, and the biocompatibility of antidepressants targeted to the brain [35, 37]. Moreover, nanocarriers minimize hepatic first-pass metabolism and protect bioactive agents from hydrolytic and enzymatic degradation [38]. They show great potential in improving antidepressant drug delivery due to their characteristics [35]. The use of nanocarriers to improve the efficacy of delivery systems of antidepressants has gained increased attention among researchers [35].

2.3.1. Dendrimers

Dendrimers are nano-sized artificial macromolecules with monodispersed structures and hyperbranched synthetic polymer systems [39]. Dendrimers have garnered significant interest from researchers as drug carriers for several neurological disorders due to their attractive properties which include increased half-life, rapid cellular entry, high drug loading capacity, improved delivery efficiency, biocompatibility, targeting ability, stability and reduced side effects [12, 40]. Furthermore, they can be used to deliver both hydrophobic and hydrophilic drug molecules and can maintain drug levels in a therapeutically desired range [12]. Dendrimers can be modified with linkages and conjugated with specific ligands to improve biocompatibility and enhance targeted delivery to the CNS. In a previous study, poly(amidoamine) dendrimers crosslinked with PEG hydrogel was used as a nanocarrier for the antidepressant, venlafaxine [41]. The *in vitro* results displayed sustained drug release of the antidepressant due to the swelling properties of the nanocarrier, and reduced drug toxicity due to a decreased dosing frequency. The data indicated that the incorporation of PEG hydrogel improved the sustained release profile of the drug and stability of the nanoparticles [41].

2.3.2. Nanogels

Nanogels are three-dimensional nanoscale hydrogel materials that are formed by chemically or physically crosslinking, hydrophilic or amphiphilic polymer networks. Nanogels have a high capacity of retaining water without being dissolved or denatured, thereby maintaining an intact structure [36, 42]. They have a large surface area, protect bioactive agents from premature leakage, and can be employed to deliver bioactive agents which includes antidepressants in a controlled release manner when stimulated (Figure 2.3) [43, 44]. Nanogels possess desirable properties for the delivery of antidepressants. They are biodegradable, non-

immunogenic, have a high entrapping rate, drug loading capacity, permeability and are highly biocompatible due to their hydrophilic features [42]. The size of nanogels allows them to penetrate the smallest capillary vessels, hence improving their circulation in the blood and thereby enhancing the bioavailability of the contained drug [42]. The drug release mechanism from nanogels involves degradation of the nanogel structure, simple diffusion, pH- or temperature-induced changes. Nanogels can improve the delivery of antidepressants because their properties can be altered to deliver drugs at targeted sites; leading to diminished side effects and enhanced therapeutic outcome [45, 42].

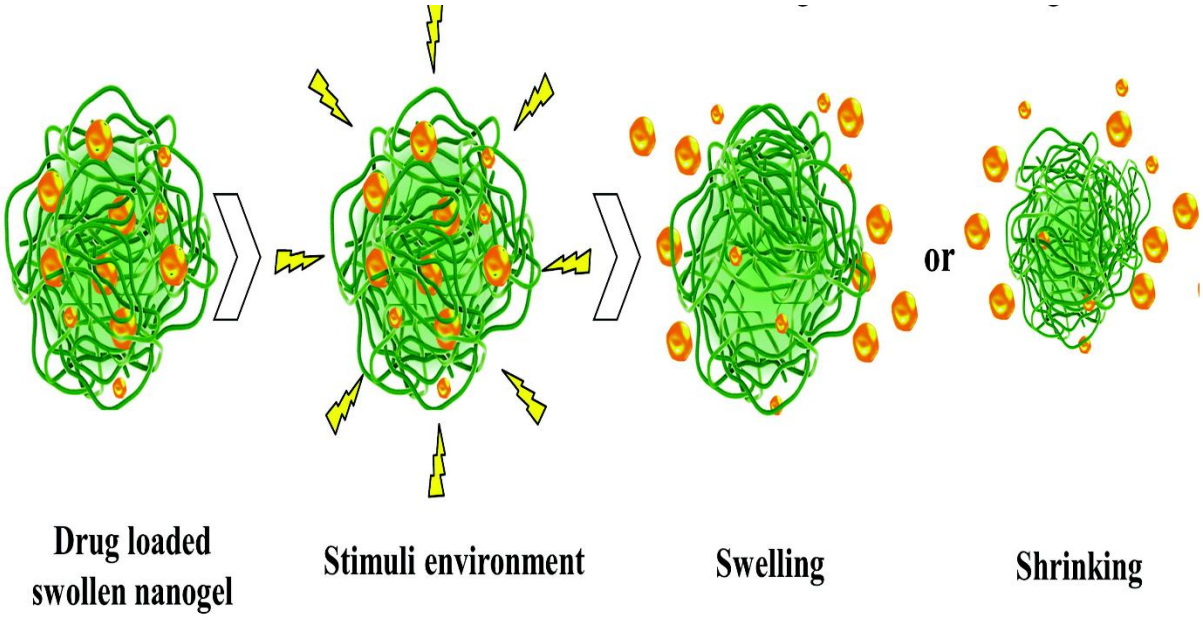

Figure 2.3: Drug release from a nanogel network under stimuli environments. The swelling or shrinking process of nanogel under stimuli environment to attain the controlled release. Adapted from [44] Copyright (2017) Taylor and Francis.

A few studies have evaluated the effectiveness of nanogels as delivery systems for antidepressant drugs. In one study, formulated venlafaxine-loaded nanogels showed an improved drug encapsulation of 88 ± 4.163 %. The *in vitro* analysis used to investigate the drug release displayed a sustained release profile [31]. Moreover, the nanogel displayed a rapid onset with a long duration of action compared to the pure drug solution. The formulation showed good stability with particle size and zeta potential of 150 nm and -8.08 mV, respectively [31]. The *ex vivo* studies indicated that the permeation rate of the venlafaxine-loaded nanogel had improved [31]. In another study, paroxetine- and duloxetine-loaded nanogels were formulated to enhance their drug release profiles. The *in vitro* studies of the

loaded nanogels displayed a sustained release profile with duloxetine's release profile higher by 10 %. The study proved that biocompatible nanogels can be used to design formulations for the sustained release of antidepressants and have the potential of maintaining long-term antidepressant activity [46].

2.3.3. Polymeric Micelles

Polymeric micelles are nanocarriers that are formed by self-association of amphiphilic block copolymers in aqueous solutions [47]. They can be used to deliver oral antidepressants which are poorly water-soluble. Polymeric micelles possess important properties that can improve the aqueous solubility, stability, bioavailability and half-life of the oral antidepressants [48]. Furthermore, micelles have other properties which include controlled delivery of hydrophobic drugs, target specificity, low toxicity, biodegradability, biocompatibility and their nano size [49, 50]. They also display a slow rate of dissociation which increases the retention time of the loaded drug. The hydrophilic shell stabilizes and supports the hydrophobic core in the aqueous medium hence improving the solubility of the biopolymer in the medium, while the hydrophobic core protects the drug [49]. Moreover, micelles can protect the drug from interfering with serum proteins, non-targeted cells, harsh conditions of the gastrointestinal tract (GIT) and facilitate safe transportation through the GIT. Nanomicelles also improve drug absorption through the GIT mucosa giving credit to their enhanced permeability. Due to these properties, micelles can be used to deliver drugs to the brain using non-toxic polymers. According to another study, polymeric micelles present an attractive potential for enhancing the sustained release of antidepressants.

Polymeric micelles can also enhance the permeability of the BBB through copolymer interaction with cell membranes that improve membrane fluidity, inhibit P-glycoprotein and multidrug efflux transporters. In the above study, *ex vivo* studies were carried out on bovine intestines, while rat animal models were used for *in vivo* studies to investigate the delivery of dapoxetine in a polymeric nano-micelle across the BBB. The *ex vivo* studies displayed that the permeation rate was found to be 91.27 % ± 7.64 %. Brain cells from three rats used for the investigation showed that polymeric micelles loaded with the antidepressant displayed high kinetic stability, improved drug solubility and oral bioavailability of the encapsulated dapoxetine by 2.7 folds [25]. The results indicated that polymeric micelles enhanced the distribution of dapoxetine into the brain matrix and reduced its elimination rate due to a delay in residency time. The data suggested that dapoxetine-loaded polymeric micelle formulations improved both delivery across the BBB and oral bioavailability of the drug [25].

However, polymeric micelles possess drawbacks which include, low drug loading capacity and poor drug release from the nanomicelles if the drug particles are too large [9]. They are also prone to premature drug leaking due to low drug incorporation stability, which might decrease

drug bioavailability. Furthermore, their ability to show controlled release requires certain proprieties such as low chain mobility core and high thermodynamic and high kinetic stability in a viscous medium [51]. Drugs with a high diffusion coefficient are unsuitable for incorporation into nanomicelles as they tend to display an immediate release and not the desired sustained release profiles [51].

2.3.4. Nanoliposomes

Nanoliposomes can be defined as nanoscale bilayer lipid vesicles. They can improve drug permeability through the BBB hence a high concentration of antidepressants can be delivered considering that the BBB is highly selective [52]. Nanoliposomes are made up of phospholipids with an aqueous reservoir which gives them the ability to have a high encapsulation rate [53]. The nanoparticle lipid bilayer is compatible with the lipid layer of the BBB because both layers are similar physiological membranes. The similarity in both membranes confers a positive impact on the BBB permeability of the drug. Nanoliposomes are compatible with both hydrophobic and hydrophilic drug molecules. Besides, they show several characteristics which make them good drug carrier systems for CNS conditions which include biodegradability, biocompatibility, improved intracellular uptake and solubility of the bioactive agents, and reduced toxicity [54, 55]. Nanoliposomes can impart controlled drug release resulting in improved therapeutic efficacy and reduced side effects. Moreover, they reduce the rate of first-pass effect in the liver [53]. Nanoliposomes can also be complexed with ligands to improve the specificity; thereby resulting in enhanced bioavailability and reduced undesirable side effects. They have been shown to protect bioactive agents from degradation, hence increasing oral bioavailability. According to literature, nanoliposomes have successfully improved the oral bioavailability of various compounds such as lipophilic and hydrophobic bioactive agents [56]. Notwithstanding, nanoliposome carrier systems display several limitations such as poor stability in aqueous environments due to their mechanical structure, high cell-penetrating ability, and increased chances of serum protein binding. Considering that the nanoliposomes have poor stability under physiological conditions, oral drug delivery would also be complicated [53].

To our best knowledge, no research has been published on the use of nanoliposomes as nanocarriers for the delivery of antidepressant therapy. However, nanoliposomes have been used for the delivery of drugs in other neurological conditions such as Alzheimer's disease. Alzheimer's disease is a neurodegenerative disease characterized by the accumulation of toxic proteins in the brain [57]. According to a study that was done, nanoliposomes can increase the penetration of rivastigmine through the BBB. The *in vivo* studies proved that the nanoliposomes have the potential of protecting the drug from the enzymatic and pH degradation, hence increasing the therapeutic efficacy. The *ex vivo* studies that were done

using the Madin-Darby Canine Kidney (MDCK) cell line showed improved permeation of the drug [58]. Rotman et al. (2015), synthesized glutathione PEGylated liposome for the delivery of anti-amyloid antibodies against Alzheimer's disease. The bioavailability of the antibody and target specificity was improved because of size and surface modification of the nanoliposomes. The *in vivo* studies that were conducted using mouse animal models proved that nanoliposomes can cross the BBB and they can be retained for a longer period, enhancing the neurological bioavailability [59].

2.3.5. Carbon Nanotubes (CNT)

Carbon nanotubes are molecules that comprise a single sheet of carbon atoms rolled up into a cylindrical shape. CNTs possess chemical and structural properties that render them good drug carrier systems for drug delivery to the CNS [60],[61]. CNTs show high biocompatibility and solubility which are determined by certain parameters that include size, physical properties and morphology of the modified molecules. These parameters determine the therapeutic outcome as they affect the biocompatibility of the molecule with the body [6]. CNTs can entrap high drug volumes owing it to their spherical shape and high surface area to volume ratio [62]. They also shield the drug from degradation during transportation and release it either through a chemically- or electrically controlled release. CNTs have low solubility several in solvents compatible with the biological milieu and it is hard to maintain high quality with negligible impurities [63]. CNTs permeability into the brain cells is dependent on temperature; with higher temperatures leading to decreased permeability [9]. To our best knowledge, CNTs have not yet been investigated as nanocarriers for antidepressants delivery. Notwithstanding, they have been researched for other neurological conditions such as Alzheimer's and Parkinson's diseases [64]. In one study, single-walled nanotubes were synthesized for the targeted delivery of dopamine into the brain of parkinsonian mice [64].

Parkinson's disease is a neurodegenerative disorder in which there will be low levels of dopamine in the brain. The study aimed to improve the permeation of dopamine, target delivery and to improve neurological bioavailability. PEGylation of carbon nanotubes improved feasibility and therapeutic efficacy of dopamine. PC 12 cell line was used for *ex vivo* analysis. PC 12 cell line was used due to its properties which include the ability to take up and release dopamine. The *ex vivo* proved that the carbon nanotubes have a potential of enhancing the permeation of dopamine due to their size of less 200 nm and surface modification. The pre-clinical study showed that small doses of carbon nanotubes (25 mg/kg) are safe for delivery in parkinsonian mice when using the parental route of administration [64] .

2.3.6. Solid-Lipid Nanoparticles (SLN)

SLNs are lipid-based and can overcome the limitations exhibited by the other colloidal carriers, due to good physical stability and excellent drug release profiles [65]. Also, SLNs are biodegradable, easy to synthesize, non-toxic and display controlled release properties. Due to their attractive characteristics, SLNs possess the potential to improve the efficacy of antidepressant drug delivery. SLNs display enhanced stability, improved bioavailability, improved epithelial permeability, prolonged half-life, enhanced permeability through the BBB and reduced toxicity [15],[66],[67]. Furthermore, SLNs can be used to deliver both hydrophilic and lipophilic drugs, making them versatile drug delivery vehicles. They also have a large surface area due to their nano-sized feature, resulting in an improved absorption rate. The physicochemical properties of SLNs such as surface charge, size, lipophilicity and surface property can be modified to enhance the penetration of SLNs across the gastrointestinal membrane (see Figure 2.4). They also improve the oral bioavailability of drug molecules due to decrease in hepatic first-pass effect through the use of emulsifiers [15]. Several studies have shown that SLNs could increase the oral bioavailability and therapeutic efficacy of antidepressants [15]. Venlafaxine is a substrate of P-glycoprotein with lowered permeability through gastrointestinal and BBB. In one study, venlafaxine-loaded SLNs administered to mice via the oral route demonstrated a 1.5 fold higher concentration of the drug from SLNs in the brain and plasma when compared with venlafaxine alone. This data proved that SLNs can enhance the oral bioavailability of venlafaxine and its accumulation in the brain [15]. The SLNs also showed reduced P-glycoprotein-mediated efflux of venlafaxine, hence improving the penetration of the venlafaxine-loaded SLNs through the BBB [15]. Moreover, an *in vivo* study using mice indicated that SLN nanocarriers enhance the oral uptake of antidepressants by accessing the lymphatic system, hence improving oral bioavailability [68]. Overall, the data showed that SLNs can be used to improve the efficacy of antidepressants [68]. In another research study where the antidepressant, duloxetine was encapsulated in SLNs, the oral bioavailability of the drug was improved owing to reduced first-pass metabolism of the duloxetine-SLN system when compared with duloxetine only [69] . The drug-nanoparticle formulation was stable under acidic media and it displayed improved pharmacological properties *in vivo*. The *in vivo* studies that were done using mice proved that the SLN enhanced the release profile and neuro-bioavailability of the antidepressant. The nanoparticles also displayed a sustained release profile in *in vitro* [69].

Currently, no SLNs have been clinically approved as drug carriers for CNS conditions. Although many *in vitro* and preclinical studies have been carried out on SLN-mediated drug delivery, clinical trials are still limited [15]. The paucity of clinical trials on SLNs might be due to insufficient *in vitro* and preclinical data to prove their efficacy and biocompatibility. On the

negative side, SLNs show lipid particle growth, are prone to gelation and have a poor incorporation rate which can be affected by the molecular weight of the types of compounds involved [70]. The loading capacity of SLNs can either be improved or decreased by the length of the hydrocarbon chain, depending on the physico-chemical properties of the drug. This might result in low oral bioavailability if the entrapment efficiency is low. The stability and specificity can also be affected by lipids, surfactants and co-surfactant used [71]. In other cases, the diseases or condition might become under-treated because the drug molecule is released very slowly. Sometimes, the drug molecule delays accumulating in the targeted organ due to prolonged drug circulation in the body [15].

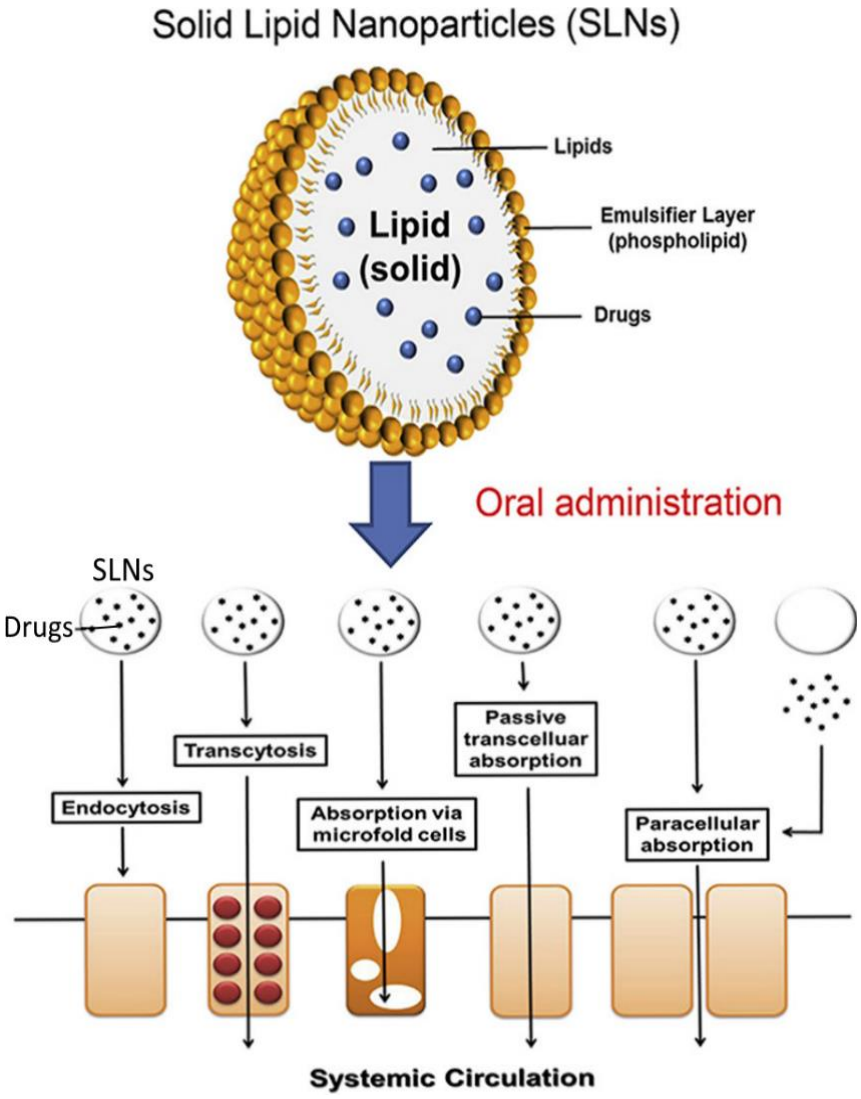

Figure 2.4: Mechanism of oral solid lipid nanoparticles (SLN) using different routes of delivery. Encapsulation of the lipophilic moiety of phospholipids in the lipid matrix and the absorption of drugs across the gastrointestinal tract. Adapted and modified with permission from [15] Copyright (2017) Science direct.

2.3.7. Polymeric Nanoparticles

Polymeric nanoparticles are sub-micron particles composed of active pharmaceutical substances encapsulated within or adsorbed onto polymers [72]. Due to their nano size, they have a high potential of being taken up by cells and they can penetrate blood capillaries. This leads to improved bioavailability as a result of an increased rate of drug accumulation at the target organs. The specificity of antidepressants can be amended by conjugating a ligand covalently to the polymeric nanoparticles [23]. As a drug carrier, it displays sustained drug release, biodegradability, prolonged duration, ability to deliver peptides, proteins and genes through the oral route of administration and high stability during storage [23, 73]. Moreover, polymeric nanoparticles can cross the BBB via receptor-mediated endocytosis [74]. In one study where the antidepressant effect of L-tyrosine-loaded polymeric nanoparticles was investigated, enhanced therapeutic efficacy and drug safety were observed [34]. Another study demonstrated that when desvenlafaxine was encapsulated in PLGA-CS-loaded polymeric nanoparticles the mucoadhesive properties and the retention time of the antidepressant in the nasal cavity were increased; thereby improving the circulation time of the drug [27]. Moreover, encapsulated escitalopram in polymeric nanoparticles composed of chitosan and tripolyphosphate biopolymers have been used to enhance the drug release profile. The *in vitro* study conducted using a dialysis membrane displayed a sustained release profile of up to 98.4% drug release from the loaded polymeric nanoparticles and about 78.6% for the pure drug over a period of 24 hours. The encapsulation rate of the antidepressant was improved to about 79%. The researchers concluded that polymeric nanoparticles can be used for sustained drug release of antidepressants [18].

2.3.8. Magnetic Nanoparticles

Magnetic nanoparticles (MNPs) are generally spherical and crystalline nanoparticles that are composed of elements with unpaired electrons such as iron (Fe), nickel (Ni) and chromium (Cr) which confer magnetic properties on them. Their magnetic properties are harnessed for drug delivery through the application of an external magnetic field. Iron oxide is the most employed core because it exhibits high physiological stability and is easily removed through the endogenous iron metabolic pathway [75]. On account of their small size, MNPs can easily penetrate the brain matrix by temporarily creating pores in the BBB endothelium. The size and magnetic properties of synthesized MNPs are dependent on the physiological characteristics of the targeted organ [76]. Including their magnetic properties, the attractive characteristics of MNPs which include biocompatibility, low toxicity, easily modifiable surfaces have sprouted interest in drug delivery research [77]. Furthermore, since they can bind to several compounds such as drugs, antibodies and proteins, they can be directed to different receptors using an external magnetic field [77]. Despite mounting *in vitro* and *in vivo* data that indicate the

potential applications of MNPs and other nano formulations, only a very few clinical trials have assessed their efficacy and safety on CNS conditions such as depression [77]. A study that was done using iron oxide nanoparticles proved that they are biocompatible and highly biodegradable under *in vivo* conditions. Interestingly, after metabolism, the iron can easily be incorporated into erythrocytes to form a part of hemoglobin, making it an added advantage [75]. *In vivo* studies that were done using rats to investigate the effects of iron oxide nanoparticles on depression treatment indicated that iron oxide nanoparticles are beneficial in reducing the symptoms of depression [78].

In another study, paroxetine and duloxetine-loaded nanogels were formulated to investigate the effect of MNPs on the efficacy of the antidepressants. The study showed that MNPs enhanced the release of the antidepressant. Magnetic fields induced stress on the nanoparticles, and this resulted in improved swelling properties of the nanogel. The group concluded that the use of magnetic nanoparticles could enhance the drug loading capacity and the sustained release profile of the formulation [46]. A previous study displayed that MNPs might be cleared by macrophages before reaching the targeted receptor or organ and the nanoparticles tend to aggregate due to strong magnetic interactions [77] which might result in increased toxicity and tissue damage. Moreover, in the absence of surface coating, the MNPs are prone to oxidation which may lead to the loss of magnetic field properties. However, aggregation can be prevented by coating the MNP with biopolymers such as PEG and chitosan, which stabilize the nanoparticles. This might result in a reduction of antiparticle surface interaction [79]. Summary of nanocarriers discussed is presented in Table 2.2.

Table 2.2. Summary of nanocarriers

Type of nanocarrier	Drug delivery Characteristics	Structure	Drawbacks	References
Dendrimers	Rapid cellular entry, high drug loading capacity, improved half-life, biocompatibility	Highly branched, Monodisperse structure,	Non-degradable in physiological environment, Large particle size	[12, 40]
Nanogels	Large surface area, high entrapping rate, biocompatible, high loading capacity,	Hydrogels, cross-linked hydrophilic polymer networks,	Physically cross-linked nanogels are less stable	[42, 43]
Polymeric micelles	Increased half-life, solubility and stability, biodegradable, biocompatible	Amphiphilic Block copolymers,	Low drug loading capacity, Premature leaking,	[48, 49 , 50, 72]

Nanoliposomes	Enhanced encapsulating rate, biocompatible, biodegradable, improved intracellular uptake	Lipid vesicles, amphiphilic phospholipids	poor stability in aqueous	[53, 55]
Carbon nanotubes	Improved cell-penetrating ability, biocompatibility, high drug entrapping rate,	Tubular morphology, two or more layers, allotropes of carbon	Mechanism is not known, too small, low solubility, permeability can be affected with temperature	[6, 62]
Solid Lipid Nanoparticles	Excellent drug release profile, stable, biodegradable, large surface area	Spherical structure,	Poor incorporation rate, prone to gelation, loading capacity depends on length of the hydrocarbon chain,	[15, 66]
Polymeric nanoparticles	High cell-penetrating rate, prolong duration, biodegradable, enhanced stability,	Spherical shape,	Easily eliminated in the bloodstream	[23, 73]
Magnetic nanoparticles	High stability, biocompatible, improve drug targeting	Spherical structure, crystals.	Easily eliminated from the body, prone to aggregation	[75, 77]

2.4. Surface Modification of Nanoparticles for targeted delivery of antidepressants

Nanotechnology systems have gained considerable attention over the past years due to their attractive properties which include a high surface-area-to-volume ratio [80];[81]. However, nanoparticles possess limitations such as instability when exposed to biological fluids and lack of specificity [80]. There is therefore a pressing need to modify the nanoparticle-based drug delivery systems to improve drug efficacy. Surface modification of nanoparticles has gained vast attention due to the important properties conferred on the particles which include improved drug specificity, nanoparticle circulation time, safety, biocompatibility and solubility of hydrophobic drugs [82, 83, 84]. Most nanoparticles that are prepared from hydrophobic polymers are hydrophobic by default [85]. Hence, it is important for them to be modified accordingly and targeted to the required sites. This can be done by coating the nanoparticles with biopolymers such as PEG and complexing of them with ligands such as transferrin [86]. Coating of nanoparticles stabilizes them with no significant change on particle size). In addition, coating of nanoparticles with hydrophilic polymers improves the circulation time, while the conjugation of nanoparticles with a ligand also improves the specificity of antidepressants (Figure 2.5) [85],[87] thereby improving the bioavailability and efficacy of the antidepressants.

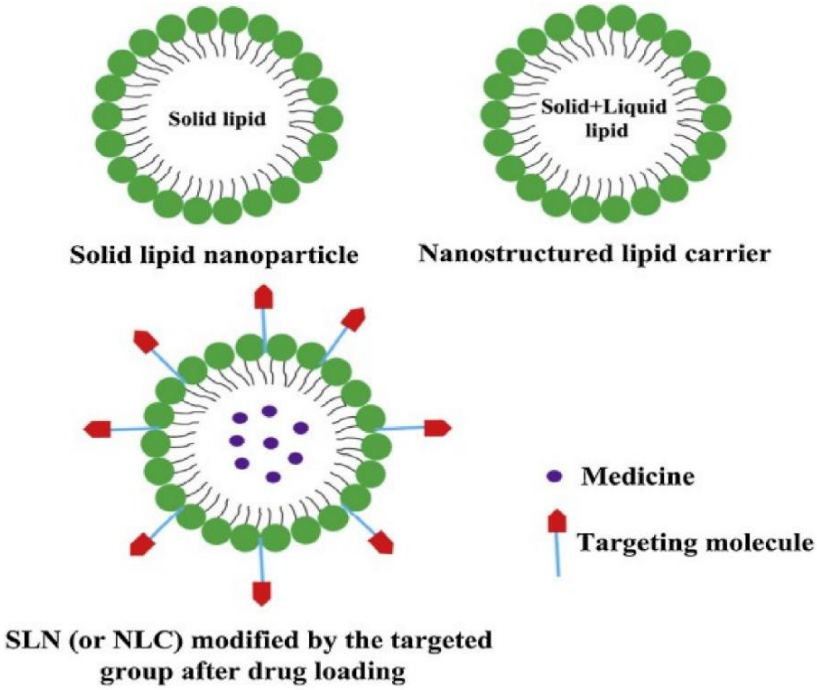

Figure 2.5: Surface modification of solid lipid nanoparticle**.** Modified SLN after conjugation of nanoparticles with ligands, to improve drug specificity and stability. The oral and neurobioavailability are improved and the nanoparticles exhibit a sustained release profile. Adapted from [87] Copyright (2019) Science Direct

2.4.1. Use of Ligands to Improve the Specificity of antidepressants and to enhance Neuro Bioavailability

To achieve an optimal therapeutic outcome from an antidepressant drug delivery system, several factors should be considered. These include the solubility of the drug in physiological fluids, target specificity, the molecular weight of the drug and its particle size [88]. A limited concentration of antidepressants bind to targeted receptors; hence, employing targeting ligands such as transferrin, Apolipoprotein-E (Apo-E) and angiopep-2 can improve the potency and therapeutic outcome, by targeting the desired receptors and reducing the dose [89]. Administering a lower dosage might reduce the side effects while receptor-targeted delivery could enhance bioavailability in the targeted organs. Ligand-complexed drugs enable the recognition and targeting of specific receptors of the BBB. Consequently, this results in optimal drug delivery and prevents the drug from harming healthy tissues or inadvertently targeting other receptors. Moreover, due to the large surface area to volume ratio of nanoparticles, multiple ligands can be complexed in a process known as multivalent functionalization, thereby

improving the binding affinity [89]. Moreover, efficient ligands for targeted drug delivery should have high binding infinity for the targeted receptors and the ability to penetrate to the targeted site [78]. For example, angiopep-2, can easily penetrate the BBB and has a high affinity for brain cells. A study by Masserini (2013), showed that complexing Angiopep-2 to nanoparticles coated with PEG caused a high accumulation of the drug delivered into the brain matrix [89, 90].

Also, for effective antidepressant drug delivery and desired therapeutic outcome, the intrinsic drug needs to exceed the minimum threshold. Hence, an adequate number of ligands need to be complexed to the drug so that they can bind to sufficient receptors to enable receptor-mediated drug delivery to exceed the minimum threshold within a short period. This improves the therapeutic onset of the antidepressant and reduces its toxicity [7].

2.5. Toxicity of Nano-Based Antidepressants

As discussed above, nanotechnology offers a remarkable potential to improve the therapeutic effect of antidepressants. Nevertheless, nano-drug delivery systems have been reported in several studies to cause neurotoxicity [73]. For example, *in vitro* studies and clinical trials, indicated that nanoparticles exhibit minor or major toxicity effects depending on the degree of toxicity of the biopolymers [73]. In an independent study, the degradation of other biopolymers such as PLGA could also induce cell damage due to the generation of an acidic medium which was dependent on the amount of polymer administered [35, 91]. Another study showed that drug burst release of some oral bioactive agents from SLN nanocarriers might result in toxicity [15]. To assess the impact of nanoparticles on healthy brain cells, Teleanu et al. (2018) showed that nanoparticles tend to accumulate in specific brain regions accessible to neural cells such as neurons and microglia, leading to neurotoxicity and cell damage. Furthermore, iron oxide and gold nanoparticles have been reported as potential neurotoxic materials. For example, daily exposure to iron oxide nanoparticles can impair nerve conduction and synaptic transmission, resulting in inflammation and neural apoptosis [92]. Neural inflammation may breakdown the BBB which may result in cerebral edema and nerve cell dysfunction [93]. Hence, it stands to reason that more studies need to be done to improve the safety of nanomedicines.

2.6. Conclusion and Future Perspectives

The use of nano-based drug delivery platforms has garnered considerable interest in the treatment of depression [94],[80]. This review has summarized different nanocarriers and the route of administration that can be used to improve the efficacy and safety of the delivery of antidepressants. Currently prescribed antidepressants exhibit adverse effects such as delayed therapeutic onset, low bioavailability and undesirable side effects. Nano-based drug

delivery strategies possess attractive attributes to overcome the afore-mentioned limitations. These include sustained drug release, high drug specificity, increased bioavailability of the drug, improved drug absorption rate due to mucoadhesive properties of the biopolymers, enhanced drug delivery, systematic enhancement and low drug cytotoxicity [69]. Biopolymers protect drugs from undesirable phenomena which includes first-pass effect and harsh stomach conditions and can allow the permeation of the drug through the BBB due to their sizes and other properties. A critical appraisal of scientific literature has shown that the use of ligands enhances drug delivery by improving the drug concentration at the targeted receptors or organs. By 2016, 51 nanomedicines for various conditions were FDA approved and 77 were under clinical trials for other disease conditions [95]. *In vitro* and *in vivo* studies that were done using nanomedicines demonstrated positive outcomes in the treatment of depression, though more *in vivo* studies still need to be done to ensure the safety of the biopolymers and the nanocarriers [69]. Nanocarriers have the potential of increasing the therapeutic index hence enhancing drug efficacy. To our best knowledge, no nanomedicines have been approved for the treatment of depression to date. Nevertheless, several studies have shown that nanomedicines may improve the therapeutic efficacy of antidepressants. These observations warrant further research and clinical translation of nanomedicines against depression. Moreover, the safety of nanomedicines needs to be considered especially when they are prescribed for chronic diseases. This would ensure the complete degradation and nontoxicity of the biopolymers.

2.7. References

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CHAPTER 3

Design and Synthesis of a Paroxetine-Loaded mPEG-Chitosan Nanogel for Oral Delivery

3.1. Introduction

Depression is among the most prevalent mental illnesses, estimated to affect 280 million people worldwide [1]. Previously reported, World Health Organisation (W.H.O) had forecasted that depression will be the second highest disease to threaten human health and increase the economic burden by 2020 [2]. It is associated with low self-esteem, suicidal thoughts, hopelessness, changes in eating patterns, and insomnia [3] . Depression is caused by several factors such as biological factors, pathological effects and social activities which includes alcohol and drug abuse. According to monoamine- deficiency theory, depression is caused by a reduction in levels of neurotransmitters such as dopamine, serotonin and norepinephrine in the central nervous system (CNS) [4]. Currently, there are different classes of antidepressants on the market; selective serotonin reuptake inhibitors (SSRI) being the first-line therapy. Paroxetine (SSRI) acts by inhibiting the serotonin reuptake transporter (SERT) hence restoring the balance of serotonin in the synaptic cleft. Furthermore, it can inhibit the reuptake of norepinephrine. Paroxetine hydrochloride hemihydrate (hereafter referred to as paroxetine) can be used to treat other conditions such as generalized anxiety, mood disorders and post-traumatic stress disorder (PTSD) [5].

In addition, paroxetine can be administered orally, and this helps with ease of administration, improved patient compliance, and reduced cost of therapy. However, the oral route of administration is limited by a delayed therapeutic outcome, insomnia, nervousness, weight gain and if used for more than 6 months due to alterations in 5HT2C receptor activity, and increment in appetite and carbohydrate cravings as serotonin regulate their intake and is the most often manipulated neurotransmitter in depression treatment [6] [7] [8] [9]. Furthermore, paroxetine has poor solubility in water, and it goes through an extensive first-pass effect when administered orally with inactive metabolites. According to the literature, approximately 64 % of paroxetine is excreted in the urine [10]. The drug also has a delayed therapeutic onset due to the impact of extensive first-pass metabolism. Since the therapeutic effect is only obtained after approximately 2 weeks of continuous administration, this reduces patient compliance [11].

In addition, the blood-brain barrier (BBB) presents an obstacle and hinders the neuroavailability of the drug within the brain [12]. As a result, the time taken to reach the saturation point will be prolonged with a delay in therapeutic onset and a reduction in

therapeutic efficacy. Hence higher doses of the drug, which are known to cause undesirable side effects are required to obtain an effective therapeutic outcome [13].

The use of nano-based therapeutics can improve the oral- and neuro-bioavailability of antidepressants and minimize undesirable side effects. Biopolymers such as chitosan (CS), methoxypoly(ethylene glycol) (mPEG) and alginate (ALG) have been used to synthesize nanosystems to overcome the limitations of poor drug bio- and neuro-availability. CS has excellent properties such as mucoadhesiveness, biocompatibility, bioactivity, biodegradability, and self-assembly in aqueous solution. The mucoadhesion has the added advantage of improving the effectiveness of delivering drug to the brain by prolonging tissue contact time. However, CS is limited by poor solubility and mechanical properties, hence its use in biomedical applications are relatively limited. To overcome this limitation, mPEG can be employed to improve the solubility of CS and for paroxetine loading due to its hydrophilic flexibility, low immunogenicity, biodegradability, biocompatibility, and low toxicity. PEGylation of CS by blocking the cations on the surface of CS has the potential of minimizing phagocytosis by RES cells; resulting in a prolonged half-life of the nanosystem in the systemic circulation [14] [15]. In addition, PEGylating has proven to reduce the hepatic first-pass metabolism, as a result, the accumulation rate of antidepressant drugs in the brain can be improved. Thereby, the saturation point will be obtained faster with an improved therapeutic onset [16] [17] [18] [19].

Therefore, in this study, a self-assembly nanogel carrier system (nanoparticles) was designed and synthesized using a mPEG crosslinked onto CS (mPEG-CS) as a biopolymer platform to improve the oral- and neuro-bioavailability, mucoadhesivity, GIT stability and reduce the hepatic first-pass effect and side effects profile of the model antidepressant drug, paroxetine. In addition, CS nanoparticles were synthesised to assess the influence of mPEG on improving drug solubility thus encapsulation efficiency and dissolution. The use of mPEG has been shown to improve drug stability in the systemic circulation, reduce renal clearance, reduce hepatic first-pass metabolism, and enhance the pharmacokinetic behaviour of nano-enabled drug delivery systems [20][21]. Nanogels are three-dimensional nanoscale hydrogel materials that are formed by either crosslinking, amphiphilic or hydrophilic polymer networks. Nanogels have important advantages for the delivery of antidepressant drugs such as paroxetine such as a high drug loading capacity. Owing to their small size, drug-loaded and functionalized nanoparticles embedded within nanogels can have prolonged circulation times and cross the BBB for superior drug bioavailability [22] [23].

3.2. Materials and Methods

3.2.1. Materials

Chitosan (CS) low molecular weight (CAS 9012764), methoxypoly(ethylene glycol) (mPEG, MW=2kDa) (CAS 9004744), succinic anhydride (CAS 108305), sodium tripolyphosphate (TPP) (CAS 7758294), dialysis membrane (MWCO =3.5kDa) (68035), PBS tablets (pH 7.4), mannitol, tween 80 (CAS 9005656), acetic acid (CAS 64197)and Ham's Nutrient Mixture F12 (N6658) were purchased from Sigma-Aldrich (St Louis, MO, USA). Paroxetine hydrochloride hemihydrate (ID 110429351) was purchased from Leap-Chem. Donor Equine Serum was purchased from Hyclone (Utah, USA). Fetal Bovine Serum (FBS) was purchased from Capricon Scientific (South America, USA). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay kit was obtained from Roche Diagnostics GmbH (Mannheim, Germany). PC 12 cells and Caco-2 cells were purchased from Cellonex, Separations (Randburg, South Africa). All solvents and reagents were used without further purification.

3.2.2. Synthesis of the of Self-assembly mPEG-CS Nanogel Carrier System

3.2.2.1. Synthesis of the of Crosslinked Self-assembly mPEG-CS Nanogel Carrier System

The self-assembly mPEG-CS nanogel carrier system was firstly synthesized using the freeze-drying method. Briefly, CS and mPEG were ionically crosslinked using sodium tripolyphosphate (TPP).

Initially mPEG was activated to mPEG-COOH. mPEG (10 g) was weighed, and it was melted at 60 ˚C for 15 minutes. Thereafter 0.50 g of succinic anhydride was added into the mPEG solution. The mixture was mechanically stirred overnight at 60 ˚C. The reaction was then precipitated with diethyl ether and filtered to remove any excess solvent. The solid was dried at room temperature in a fume hood to obtain mPEG-COOH. CS (1 g) was then dissolved in 100 mL of 0.50 % $^{v}/_{v}$ glacial acetic acid and left to stir overnight at room temperature. The previously synthesized mPEG-COOH was then added to the CS solution and the mixture was mechanically stirred overnight. The crosslinking of mPEG and CS was obtained by adding 0.5 % $^{w}/_{v}$ TPP dropwise to the solution (while stirring vigorously) in the ratio 1:5 TPP:mPEG-CS nanogel carrier system solution, respectively. After 2 hours mannitol was added and the mixture was then dialyzed against deionised water for 48 hours at room temperature. The resultant solution was lyophilized (Freezone 12 lyophilizer, Labcono, Kansas City, MO, USA) after frozen at –80 ˚C to obtain solid mPEG-CS nanogel carrier system. The mPEG-CS nanogel carrier system biopolymers were prepared in the ratio 1:1.

CS nanoparticles were synthesised as a stand-alone system inorder to understand how mPEG affects the drug release profile and encapsulation efficient of mPEG-CS nanogel carrier system thus improving solubility. CS nanoparticles were synthesised using the ionic gelation technique employing TPP as the crosslinker. CS (200 mg) was dissolved in 20 mL of 0.50 % ($^{v}/_{v}$) glacial acetic acid and left stirring overnight at room temperature. The crosslinking of CS was obtained by adding 0.50 % of TTP dropwise under vigorous stirring, in the ratio of 1 TPP : 5 biopolymer. The mixture was dialysed against deionised water for 48 hours at room temperature to remove unreacted substances and glacial acetic acid (organic solvent). The resultant solution was frozen at –80 ˚C and lyophilized to obtain the CS nanoparticles.

3.2.3. Determination of Chemical Structure Integrity of the Self-Assembled mPEG-CS Nanogel Carrier System

FTIR analysis was conducted on drug-free and drug-loaded mPEG–CS nanogel carrier system samples to assess the chemical integrity, structure and stability of the synthesized compounds and the nanogel. Infrared spectra were acquired using a FTIR spectrometer (PerkinElmer Inc., Waltham, MA, USA) with samples processed by a universal attenuated total reflectance (ATR) polarization accessory, at a resolution of 4 cm^{-1}, with in a spectrum range of 4000 to 650 cm^{-1} in which 20 scans were done per sample. The study was done to confirm the crosslinking and to ascertain the structural changes [24].

^{1}H NMR studies were further undertaken on the lyophilised mPEG-CS nanogel carrier system employing a Bruker AVANCE II 500 MHz (Bruker Avance Biospin, Germany) to determine the crosslinking and the chemical shifts. The mPEG-CS nanogel carrier system was dissolved in heavy water (D_2O) and it was evaluated at 25 ˚C.

3.2.4. Optmisation of the Particle Size, Zeta Potential and Encapsulation Eficiency of the CS Nanoparticles and the Self-Assembled mPEG-CS Nanogel Carrier System

Nine formulations were produced in accordance with a Box-Behnken experimental design approach to optimize the drug encapsulation efficiency (DEE), particle size (PS) and zeta potential (ZP) by varying the concentration of TPP and CS. The concentration of paroxetine and mPEG were kept constant. For measuring the particle size and zeta potential, initially lyophilized paroxetine-loaded mPEG-CS nanogel carrier system samples were accurately weighed and placed in polytops. For all 9 formulations, the lyophilized nanogel was produced by dissolving in deionized water (1 mg in 5 mL deionized water) and sonicated. The sonicated samples were filtered before suspending for analysis. The sample particle size and zeta potential were measured employing a Zetasizer NanoZs instrument (Malvern Instruments

(Pty) Ltd., Worcestershire, UK) and the temperature was maintained at 25 °C for the duration of the analysis.

For DEE, the *in vitro* release of paroxetine was assessed on 9 formulations with a 20 mg paroxetine load. A sample of each formulation was immersed in 100 mL buffer (PBS and tween 80; 2% $^{v}/_{v}$ pH 6.80) independently. The DEE was measured by UV-vis spectroscopy (PerkinElmer Spectrum 100, Llantrisant, Wales, UK) at wavelength 294.90 nm [25]. The calibration equation was employed to determine the concentration and mass of the encapsulated drug. The formulations were measured in triplicate.

3.2.5. Preparation of Self-Assembled Paroxetine loaded mPEG-CS Nanogel Carrier System and Paroxetine loaded CS Nanorpaticles

The equilibrium swelling method was used for paroxetine loading into the mPEG-CS nanogel carrier system [26]. Briefly, 80 mg of paroxetine and 160 mg of mPEG-CS nanogel carrier system (ratio 1:2 respectively) were dissolved separately. Paroxetine was dissolved in ethanol and water was added to the mPEG-CS nanogel carrier system. They were then mixed and sonicated for 1 hour; the mixture was left stirring overnight. The sample was later dialyzed against deionised water to remove any free paroxetine and excess organic solvent. The paroxetine-loaded mPEG-CS nanogel carrier system was obtained under solid phase by lyophilization.

Paroxetine was loaded into the CS nanoparticles by dissolving 40 mg paroxetine in ethanol and deionised water was added to 80 mg of CS nanoparticles. Paroxetine solution was added slowly to CS nanoparticles solution and sonicated for an hour [27]. The mixture was left to stir overnight. The sample was dialyzed by dialysis membrane (MWCO: 3.5 kDa) against deionised water to get rid of the unloaded paroxetine and the organic solvents. The paroxetine-loaded CS nanogel was obtained under solid phase by lyophilization.

3.2.6. Determination of Paroxetine Encapsulation Efficiency within the Self-Assembled mPEG-CS Nanogels Carrier System and CS Nanoparticles

A sample of paroxetine-loaded mPEG-CS nanogel carrier system and paroxetine-loaded CS nanoparticles were immersed in 100 mL buffer (PBS and tween 80; 2 % $^{v}/_{v}$ pH 6.8) independently. The DEE was evaluated by measuring the UV absorbance (PerkinElmer Spectrum 100, Llantrisant, Wales, UK) at wavelength 294.90 nm [25]. Equation 3.1 was used to determine the concentration and mass of entrapped paroxetine. The samples were measured in triplicate and the DEE was determined using Equations 3.2 and 3.3 [24]:

Calibration equation: $y = 0.0102x + 0.0001$; R^2=0.9996 (Equation 3.1)

Encapsulation Efficiency % = $\frac{weight\ of\ drug\ in\ the\ nanogel}{weight\ of\ drug\ loaded} x100$ (Equation 3.2)

Loading Efficiency %= $\frac{weight\ of\ drug\ loaded}{weight\ of\ drug+copolymer}\ x\ 100$ (Equation 3.3)

3.2.7. Determination of Particle Size and Zeta Potential of the Self-Assembled mPEG-CS Nanogel Carrier System

The lyophilized nanogel was dissolved in deionised water (1 mg in 5 mL of deionised water) and sonicated. The sonicated samples were filtered before suspending them for analysis. The sample particle size and zeta potential were determined by dynamic light scattering and photon correlation spectroscopy at a fixed angle of 90 ˚C on a Zetasizer NanoZs instrument (Malvern Instruments (Pty) Ltd., Worcestershire, UK), particle size analyser and the temperature was maintained consistence at 25 ˚C for the duration of the analysis. The nanoparticle size and zeta potential of a drug-free and paroxetine-loaded nanogel carrier system were measured.

3.2.8. Surface Morphological Analysis of the Self-Assembled mPEG-CS Nanogel Carrier System

The surface morphology of the lyophilized mPEG–CS nanogel was analysed using SEM (Phenom™ Scanning Electron Microscope, FEI Company, OR, USA). The lyophilized nanogel was sonicated after being redispersed in deionised water. A drop of a sonicated mPEG-CS nanogel carrier system was suspended on an aluminium stub and left to dry in a fume hood overnight. The sample was coated with carbon and Au/Pd. The sample was then analysed at a voltage of 2 kV. The study was undertaken to confirm the surface morphology and size of the mPEG-CS nanogel carrier system.

3.2.9. Determination of Thermal Stability of the Self-Assembled mPEG-CS Nanogel Carrier system

TGA (TGA 4000, PerkinElmer, Llantrisant, Wales, UK) was employed to analyse the thermal properties of the drug-free and drug-loaded-mPEG-CS nanogels carrier system well as the pristine CS and mPEG as well as the pure drug. This was undertaken to determine the thermal stability and weight loss of mPEG-CS nanogel carrier system. The technique was used to evaluate the degradation nature of the biopolymers and the mPEG-CS nanogel carrier system [24]. The analysis was carried out at a heating rate of 10 ˚C per minute, at a temperature range of 30 - 900 ˚C under constant nitrogen gas flow. The graphs were plotted as % weight against temperature.

3.2.10. Determination of the Thermal Stability of the Self-Assembled mPEG-CS Nanogel Carrier System

Differential scanning calorimetry (DSC) (Mettler Toledo, DSC1, STAR System, Switzerland) was used to evaluate the thermal degradation and enthalpy changes of mPEG-CS nanogel carrier system and their crystalline behaviour. The crystallinity nature of the nanogel carrier system plays an important role in dissolution [28]. The drug-free and drug-loaded nanogel, pristine polymers and native drug ranging from 5-10 mg were analysed using sealed aluminium crucibles heated over a temperature range of 0–200 ˚C at a heating rate of 10˚C per minute. The inert nitrogen gas atmospheric conditions were maintained, with a flow rate of 20 mL per minute. The curves were plotted as heat flow vs temperature. Equation 3.4 was used to calculate the enthalpy changes [29].

Enthalpy of change = $\frac{Total\ area\ under\ the\ curve}{Sample\ weight}$ (Equation 3.4)

3.2.11. Determination of the Crystallinity of the Self-Assembled mPEG-CS Nanogel Carrier System

The degree of crystallinity of pristine mPEG, CS and the mPEG-CS crosslinked nanogel carrier system were analysed using X-ray diffraction. XRD spectra were recorded on a benchtop MiniFlex 600 (Rigaku, Japan) diffractometer using *CuK*α radiation. CS, mPEG and mPEG-CS nanogel carrier system was analysed at a voltage of 20 kV and a current of 20 mA, 2 theta scan range of 0 – 60 ˚C at a scan rate of 15 ˚C per minute. Equation 3.5 was used to calculate the crystallinity index of the biopolymers and the crosslinked mPEG-CS nanogel carrier system [30].

Crystallinity index = $\frac{Sum\ of\ areas\ under\ crystalline\ peaks}{Total\ area\ under\ crystalline\ and\ amorphous\ peaks}$ (Equation 3.5)

3.2.12. Swelling Properties of the Self-Assembled mPEG-CS Nanogel Carrier System

The swelling properties of the lyophilized mPEG-CS nanogel carrier system was analysed using PBS at pH 6.80. The lyophilized mPEG-CS nanogel carrier system was prepared by weighing 7mg into a polytop and added 0.70 mL of PBS. The hydrated samples were left for 24 hours in an orbital shaking incubator, maintained at 37 °C, and set at a speed of 50 rpm. The excess water was removed by drying the nanogel with paper towel and weighed. The study was done to prove that a nanogel carrier system was formulated as nanogels swell in aqueous conditions. The swelling properties were determined using Equation 3.6 [31].

$WU\ (\%) = \left(\frac{Wh-Wd}{Wd}\right) x\ 100$ (Equation 3.6)

Where, WU%, Wh and Wd represent the percentage fluid uptake, the weight of the mPEG-CS nanogel carrier system in hydrated(swollen) and dried state, respectively.

3.2.13. *In vitro* Analysis of Paroxetine Release from the Self-Assembled mPEG-CS Nanogel Carrier System and CS Nanoparticles

The *in vitro* release of paroxetine from the mPEG-CS nanogel carrier system and CS-nanoparticles were conducted using the dialysis method [32]. The release analysis of paroxetine was carried out in two different buffers as simulated gastric fluid (pH 1.20) and simulated intestinal fluid (pH 6.80) at 37 ˚C using a dialysis membrane (MWCO=3.5 kDa). Equivalent amounts of paroxetine-loaded mPEG-CS nanogel carrier system and CS nanoparticles were transferred to dialysis tubing and immersed in 100 mL PBS at the different pH values (1.20 and 6.80). The samples were incubated in an orbital shaker (LM-530-2. MRC Laboratory Instruments Ltd., Hahistadrut, Holon Israel) at 37 ± 0.5 ˚C, with 50 mpm. The release medium (5 mL) was withdrawn for analysis and replaced with the same quantity of fresh buffer at scheduled time intervals (0.5, 1, 2, 4, 6, 8, 12, 16, 24 hrs). Sink conditions were maintained throughout the analysis. UV-vis spectroscopy was employed to evaluate the absorbance of the samples at a wavelength of 294.90 nm. All studies were conducted in triplicate.

3.2.14. *Ex Vivo* Permeation Studies through rat Intestinal tissue

The *ex vivo* study was undertaken using rat intestinal tissue with ethical waiver approved for the study. Rats were sacrificed and intestinal segments were collected promptly after the animal was sacrificed, rinsed twice with normal saline and kept in 10% formalin. The prepped small intestine was setup in a vertical jacketed Franz type diffusion cells (5 mL acceptor volume) with 5 mm diameter orifice (0.20 cm, 2 area) for the permeation study using a Franz Diffusion Cell apparatus. The donor and acceptor chamber filled with PBS (pH 6.80) were separated by the intestinal tissue and was maintained at 37 ± 0.1 °C for 30 minutes, to equilibrate the tissue specimen prior to running the experiment. Thereafter, DPBS in the donor compartment was carefully removed and replaced with samples of the paroxetine-loaded mPEG-CS nanogel carrier system and CS nanoparticle dispersion at pH 6.80. At predetermined intervals, aliquots (5 mL) were withdrawn and instantly replaced with fresh PBS (5 mL) to maintain sink conditions. A small magnetic stir bar set at 700 rpm ensured homogeneity throughout the investigation. Followed by analysis using the UV vis spec at 294.90 nm and paroxetine quantified using a calibration curve. All the studies were conducted in triplicate.

3.2.15. Cytotoxicity Analyses of the Paroxetine-loaded mPEG-CS Nanogel Carrier System on a PC 12 Cell Line

The drug-free and paroxetine-loaded mPEG-CS nanogel carrier system were investigated for their effects on the viability of PC12 cells, a cell line of rat pheochromocytoma origin. The PC12 cell line displays neurological characteristics [33]. The cells were cultured in Ham's F12 media containing Donor Equine Serum (DES) (15 % $^{v}/_{v}$), FBS (2.50 % $^{v}/_{v}$) and Penicillin-streptomycin (1% $^{v}/_{v}$). The cells were seeded (80 000 cells/mL) into 96 plates (90 µl per well), then incubated in a humidified incubator overnight under 5% carbon dioxide atmosphere at 37 °C. Thereafter, cells were treated with 10 µl of mPEG-CS nanogel carrier system paroxetine, paroxetine-loaded mPEG-CS and ethanol at different concentrations (39, 78, 156 µg/mL). The mPEG-CS nanogel carrier system and the paroxetine-loaded mPEG-CS nanogel carrier system were dissolved in PBS and the native drug was dissolved in ethanol. The positive control wells were treated with 10 µg/mL of 5-Fluorouracil, while the negative controls were treated with 10 uL of 0.1 % ethanol. The plates were then incubated for 48 and 72 hrs. After 48 hrs and 72 hrs respectively, 10 µl of the MTT reagent (Roche Diagnostics GmbH, Mannheim, Germany) was added to each respective well and the plates were incubated for 4 hrs. After 4 hrs, 100 µl of the MTT solubilizing agent was added into each well and incubated overnight. The absorbance of the plates was measured to determine the percentage of cell viability at a wavelength of 570 nm using Multilabel plate Reader (VICTOR X3, PerkinElmer, MA, USA). The results are presented as % cell viability (% CV) and Equation 3.7 was used to calculate the %.

$$\text{Cell viability (\%)} = \frac{Absorbance\ read\ in\ treated\ cells}{Absorbance\ in\ control\ (untreated\ )cells} \times 100 \qquad \text{(Equation 3.7)}$$

3.2.16. Cytotoxicity analyses of the Paroxetine-loaded mPEG-CS Nanogel Carrier System on a Caco-2 Cell Line

A human colon carcinoma cell line, cancer coli-2 (Caco-2) was purchased from Cellonex, Separations (Randburg, South Africa). The mPEG-CS nanogel carrier system and the paroxetine-loaded mPEG-CS nanogel carrier system nanogel were prepared to investigate their effects on the viability of Caco-2 cell line. The cells were cultured in Dulbecco's Modified Eagle's media (DMEM) containing 10 % ($^{v}/_{v}$) fetal bovine serum (FBS) and 1% ($^{v}/_{v}$) Penicillin-streptomycin. For this experiment, cells were seeded (80 000 cells/mL) into 96 plates (90 µl per well), then incubated in a humidified incubator overnight under 5% carbon dioxide atmosphere at 37 °C. Thereafter, cells were treated with 10 µl of mPEG-CS nanogel carrier system, paroxetine, paroxetine loaded mPEG-CS and ethanol at different concentrations (39, 78, 156 µg/mL). The mPEG-CS nanogel carrier system and the paroxetine

loaded mPEG-CS nanogel carrier system were dissolved in PBS and paroxetine was dissolved in ethanol. The positive control wells were treated with 10 µg/mL of 5-Fluorouracil, while the negative controls were treated with 10 uL of 0.10 % ethanol. The plates were then incubated for 48 and 72 hrs. After 48 hrs and 72 hrs respectively 10 µl of the MTT reagent (Roche Diagnostics GmbH, Mannheim, Germany) was added to each respective well and the plates were incubated for 4 hrs. After 4 hrs 100 µl of the MTT solubilizing agent was added into each well and incubated overnight. The absorbance of the plates was measured to determine the percentage of cell viability at 570 nm using Multilabel plate Reader (VICTOR X3, PerkinElmer, MA, USA). The cytotoxic studies were carried out on two different cell lines to prove that the formulated nanogel carrier system is nontoxic to the intestinal cell line and the brain cells.

3.3. Results and Discussion

3.3.1. Assessment of the Chemical Integrity of the Self-Assembled mPEG-CS Nanogel to Confirm the Crosslinking

CS was crosslinked to mPEG through the formation of an amide bond using TPP as a coupling agent. The FTIR spectra of CS, mPEG COOH and mPEG-CS nanogel carrier system are presented in Figure 3.1. The spectrum of CS reveals distinctive absorption bands at 1648 cm^{-1} (amide 1) and 1578 cm^{-1} (-NH_2 bending). The 1732 band shown on mPEG COOH indicate that mPEG was activated successfully. Regarding Figure 3.1, the CS amide peaks slightly shifted to 1633 cm^{-1} and 1547 cm^{-1} respectively on the mPEG-CS spectrum. This shift was due to the amide bond formed between the amide and NH_2 group on CS and the carboxylic group on the mPEG-COOH. The 1547 cm^{-1} (amide 2) peak further confirms the amide bond formation between carboxyl end of mPEG-COOH and amine groups of CS. The observed increment in absorbance intensity of the 1578 cm^{-1} peak of mPEG-CS in comparison to the pure CS peak, might be due to attractive intermolecular interaction between CS and mPEG. In addition, the increment in absorbance intensity of peaks at ≈ 1146 cm^{-1} and 2932 cm^{-2} indicate C-O-C stretch of mPEG the CH_2 groups [23] [21]. The obtained data also displayed that the amino groups of CS were substituted by mPEG groups. In addition, the results proved that mPEG was crosslinked on CS successfully as shown in Figure 3.1.

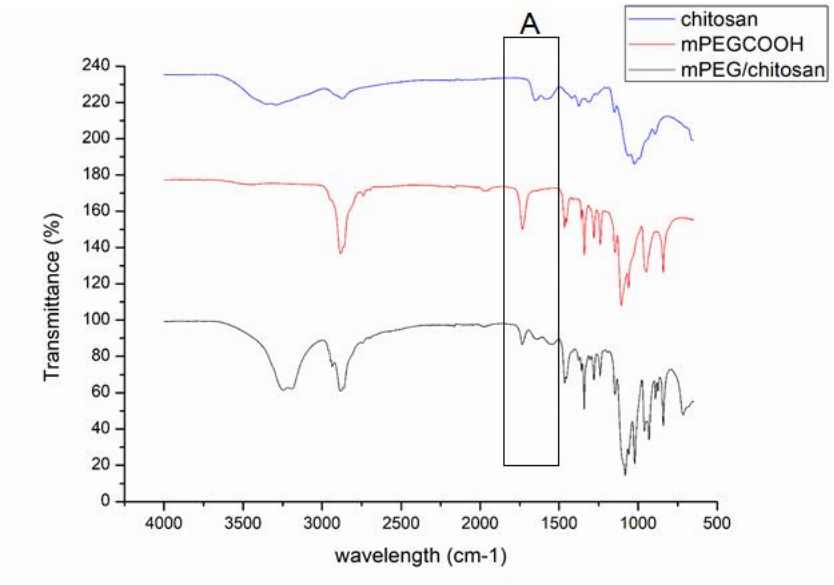


Figure 3.1: FTIR spectra of CS, mPEG COOH and mPEG-CS; “A” highlights the positions affected by crosslinking

The ^{1}H NMR of lyophilized mPEG-CS nanogel carrier system was done to further confirm the chemical structure and the cross-linkage of the mPEG-CS nanogel carrier system. As shown in Figure 3.2, a peak at around 1.87 ppm represents $–CH_3$, acetyl group of CS [23]. The peaks around 3.45 to 3.90 ppm displays the methane ring and methylene protons of CS saccharide units, and methoxy and $C(O)OCH_2$ functional groups of MPEG [23] [34] . The $–CHNH_2$ and $–COCH_3$ functional groups of CS are identified in the region of 2.05 ppm and 3.10 ppm. The peaks at 2.00 ppm and 3.45 ppm are attributed to methylene protons and $–OCH_3$ groups of mPEG. The peaks at 3.32 ppm, 3.60 ppm and 4.30 ppm exhibit the presence of oxyethylene, methoxy and $C(O)OCH_2$ functional groups of mPEG. Peak 4.62 ppm is attributed to hydrogen at the amine group (NH-CH2-O) confirming that CS and mPEG were successfully crosslinked [23] [34] [35] .

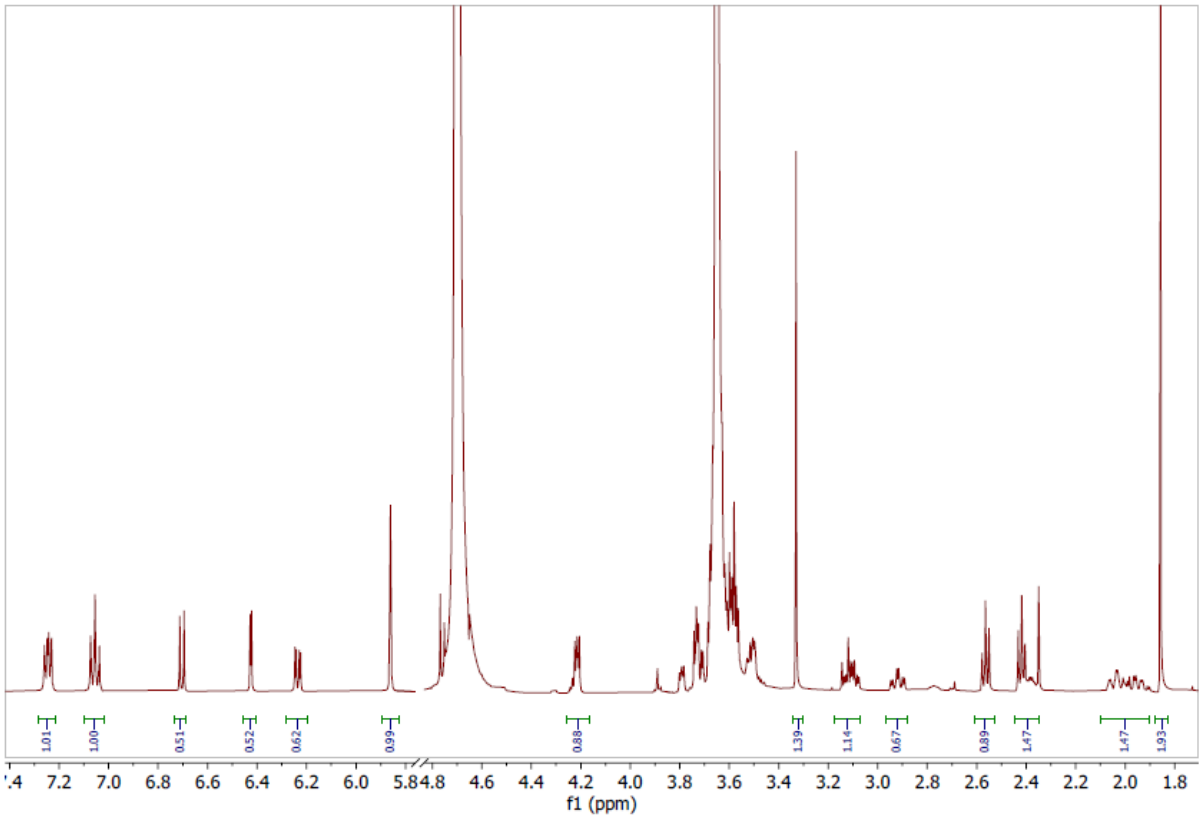


Figure 3.2: The ^{1}H NMR spectra of mPEG-CS nanogel carrier system in D_2O

3.3.2. Morphology Evaluation of Self-Assembled mPEG-CS Nanogel Carrier System

In this study, SEM confirms the spherical morphology of the mPEG-CS nanogel carrier system (Figure 3.3). The mPEG-CS nanogel carrier system exhibited a smooth surface giving credit to the crystalline nature of CS [23]. The SEM further proved the nanosize of mPEG-CS nanogel carrier system and the moderate polydispersed state of the nanogel particles. Hence nanoparticles were successfully formed. The size of the nanoparticles was less than 200 nm which is favourable for the BBB membrane.

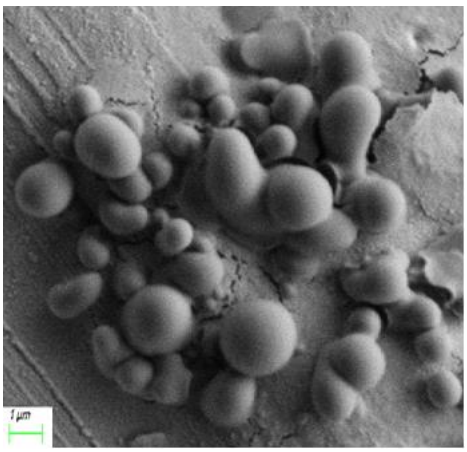


Figure 3.3: Morphology of paroxetine-loaded mPEG-CS nanogel carrier system as observed using Scanning Electron Microscopy at a magnification of 19.89 KX and EHT of 2.00 kV

3.3.3. Assesment of the Thermal properties of Self-Assembled mPEG-CS Nanogel Carrier System

TGA was used to determine the thermal decomposition of mPEG-CS nanogel carrier system and it also provides information about the decomposition pattern of the biopolymers and drug owing to the change in temperature [21]. Furthermore, TGA determines the changes in weight loss of mPEG-CS is shown in Figure 3.4. The initial weight loss around 100˚C was due to water loss that was loosely bound to the mPEG-CS nanogel carrier system. In addition, regardless of both biopolymers being hydrophilic, they are less hygroscopic, hence low water adsorption of mPEG-CS nanogel carrier system biopolymers may be due to its highly organised arrangement in the solid state, which reduces the adsorption of water [36]. The second weight loss at around 360 ˚C was a result of dehydration of sugar unit, bond cleavage of the dominant chain and decomposition of deacetylated and acetyl units of CS. As shown in Figure 3.4, mPEG-COOH displays low weight loss in the 2nd phase, indicating that it is thermally stable at this temperature range [36], [37]. The weight loss of mPEG-CS nanogel carrier system has 3 distinct phases as shown in Figure 3.4. The 3rd weight loss at around 410 ˚C was a result of the degradation of mPEG. From the results, paroxetine affects the physical and thermal properties of mPEG-CS nanogel carrier systems.

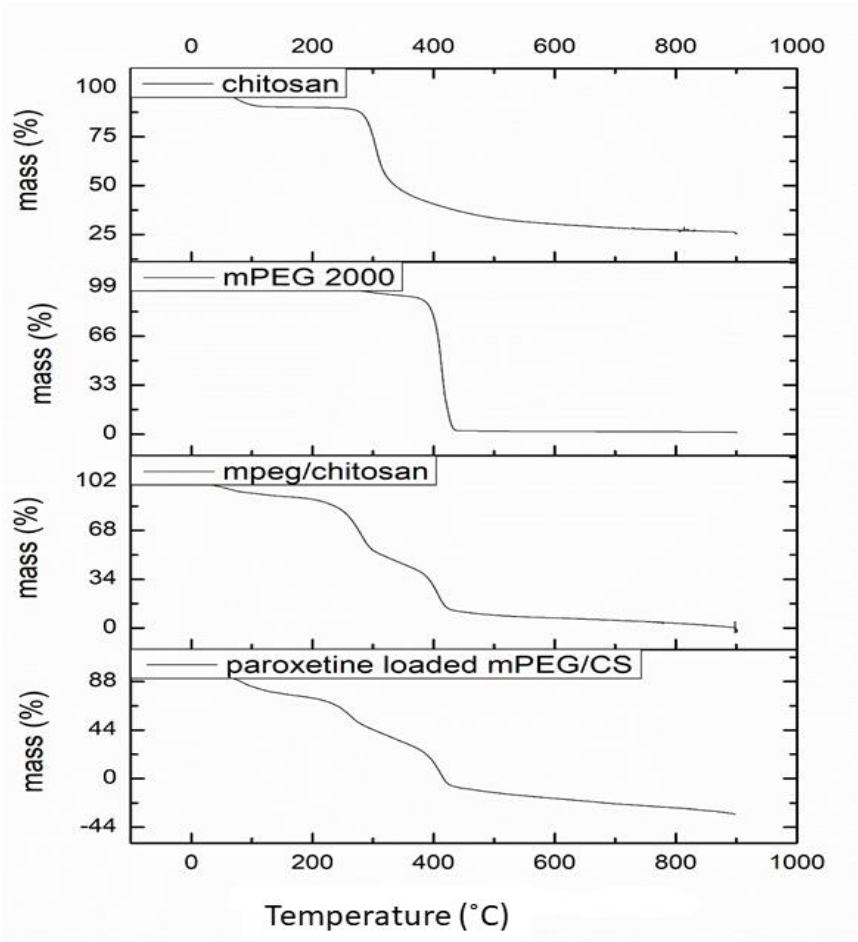


Figure 3.4: TGA graphs of CS, mPEG 2000, mPEG-CS nanogel carrier system and paroxetine loaded mPEG-CS nanogel carrier system

DSC was used to analyse the thermal properties of CS, mPEG, mPEG-CS nanogel carrier system, paroxetine, and paroxetine loaded mPEG-CS nanogel carrier system as shown in Figure 3.5. As evident below, all peaks obtained were endothermic. CS showed an endothermic peak at around 100˚C, this is due to the elimination of bound water. The peak on mPEG-CS nanogel carrier system graph at around 160˚C is due to liquid-liquid transition where mPEG side chains are acting as internal plasticizers [21]. For mPEG-CS nanogel carrier system the endothermic peak of mPEG slightly shifted to the left. The mPEG peak on paroxetine loaded mPEG-CS nanogel carrier system further shifted to the left. As shown in Figure 3.5, mPEG-CS nanogel carrier system has an endothermic peak at around 50˚C, which also confirms that mPEG-CS nanogel carrier system were successfully crosslinked [37]. In addition, the second peak at around 100 ˚C of CS became stronger, hence shifted to a higher temperature with DSC of mPEG-CS increasing, this proved that the crosslinking was rigid and the mPEG-CS nanogel carrier system is more stable than the individual biopolymers (Figure 3.5). The results proved that the introduction of mPEG on CS sidechains improved thermal decomposition. That means the mPEG segments disrupt part of the hydrogen bonds linking

CS chains and part of the CS domains. The CS domains are made up of strong interior intra-molecular hydrogen bond aggregation linking glucosamine units of CS, that can be disrupted by the positively charged action of $–NH_2$ groups in acidic aqueous environments [38] . The results also confirm that paroxetine has an impact on the physical properties and thermal properties of the biopolymers. The second peak of paroxetine loaded mPEG-CS nanogel carrier system slightly shifted to the left compared to mPEG-CS nanogel carrier system and the intensity of the peak also decreased.

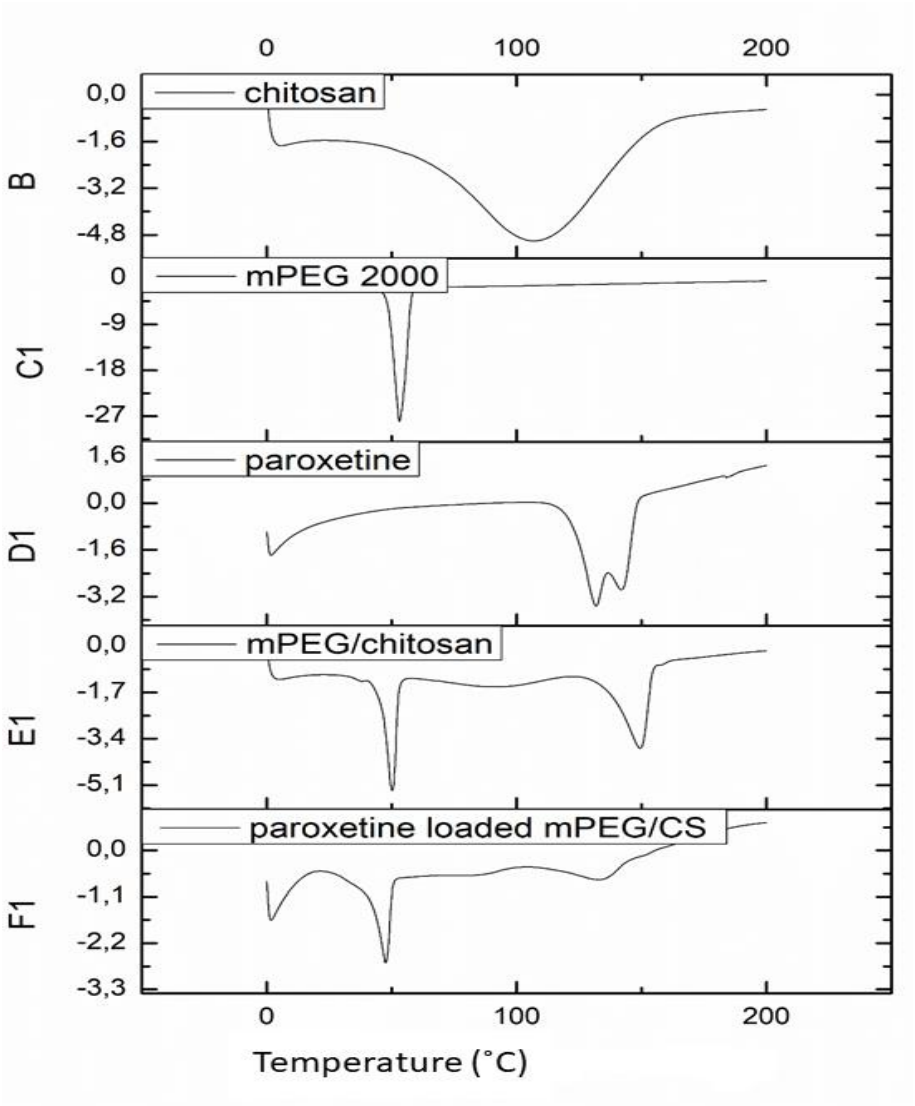


Figure 3.5: DSC graphs of CS, mPEG, mPEG-CS nanogel carrier system, paroxetine, and paroxetine loaded mPEG-CS nanogel carrier system

3.3.4. Evaluating the Crystallinity of the Biopolymers and Self-Assembled mPEG-CS Nanogel Carrier System

The crystallinity of the biopolymers was determined by the XRD. As shown in Figure 3.6, the mPEG-CS nanogel carrier system bio-platform graph exhibited a more intense semi-

crystalline peak as compared to pure CS. This further confirms the DSC and TGA results that the mPEG-CS nanogel carrier system bio-platform have improved thermal stability compared to pure CS. According to the results (Figure 3.6), pure CS exhibited low intensity peaks compared to the mPEG-CS nanogel carrier system bio-platform, which is taken as evidence of low crystallinity. mPEG-CS nanogel carrier system bio-platform displayed intense peak, at around 2θ ≈ 18 - 24˚, which are also detected in mPEG-COOH XRD patterns. The results also confirms that the biopolymers were successfully crosslinked and mPEG affects the crystallinity of CS [36].

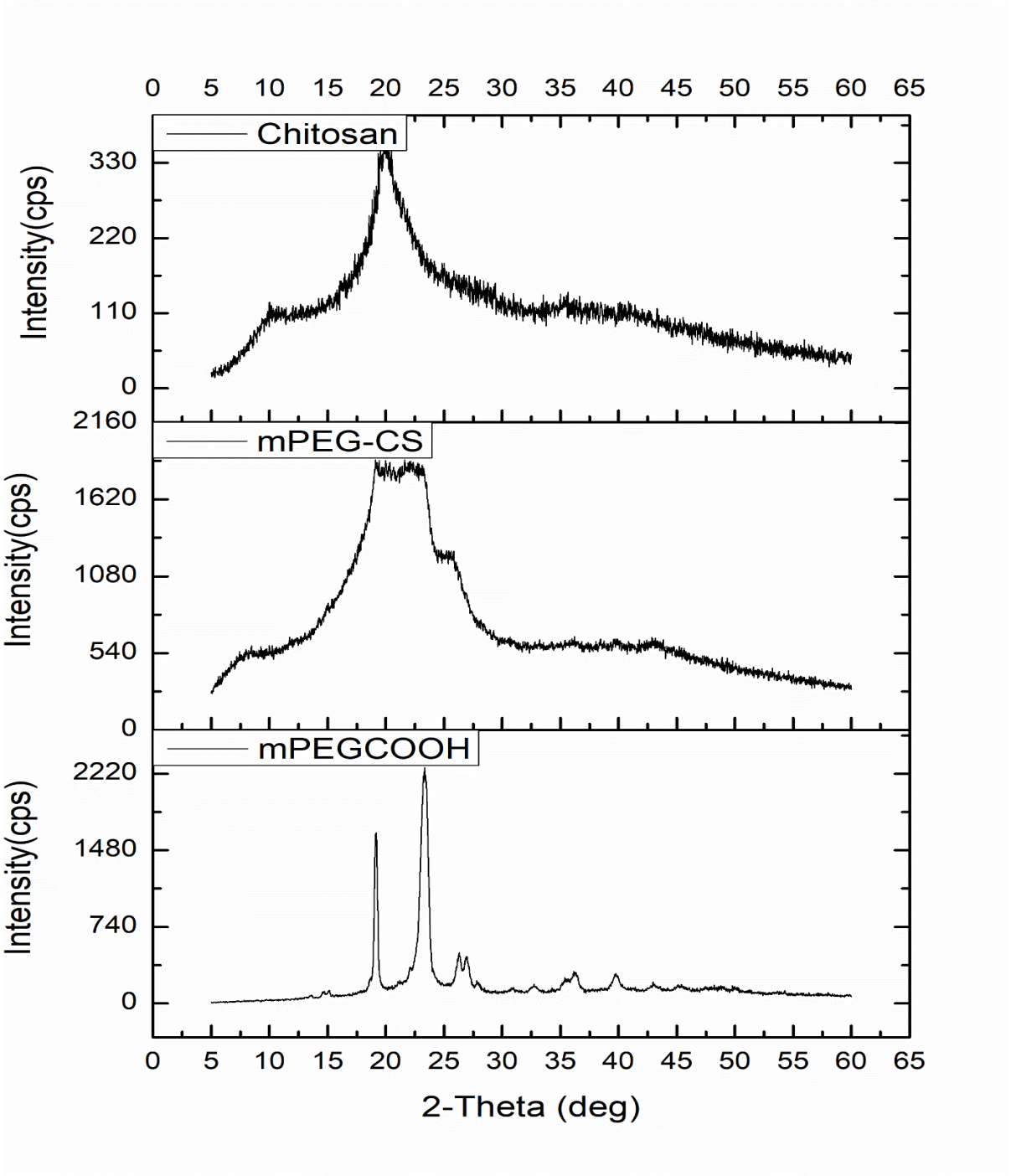


Figure 3.6: XRD analysis undertaken on CS, mPEG-COOH and mPEG-CS nanogel carrier system

3.3.4. Swelling Properties of the Self-Assembled mPEG-CS Nanogel Carrier System

Nanogels have the ability to swell in aqueous media, without dissolving in the aqueous environment due to their nature. They are formed by crosslinking biopolymers with swelling properties and high-water holding capacity [39]. In the current study, when PBS (pH 6.80) was added to the lyophilized sample, it swelled (Figure 3.7) and the swelling % of the mPEG-CS nanogel carrier system was 1299 %. The swelling properties of the mPEG-CS nanogel carrier system bioplatform was governed by both mPEG side chains and the gelling properties of CS when crosslinked with TPP. The mPEG-CS nanogel carrier system bioplatform have the ability to hold the water due to the present of the intramolecular hydrogen bonds [40]. The swelling properties also proves that a nanogel formulation was synthesised successfully.

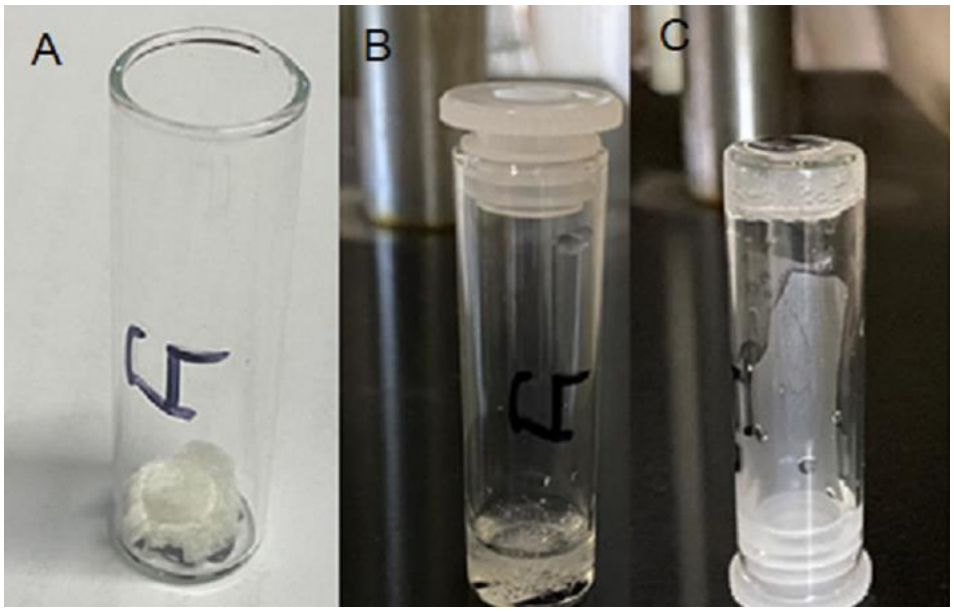


Figure 3.7: Shows the lyophilised and hydrated samples of the mPEG-CS nanogel carrier system respectively. A-Lyophilized sample, B-hydrated sample, and C-hydrated sample

3.3.5. Experimental Design and Optimization using Box-Behnken Design

Initially 9 formulations were optimised to get the desired particle size and to analyse the impact of varying biopolymer concentration and the crosslinker % on particle size, zeta potential and encapsulation efficiency %. Box-Behnken experimental design was generated using Minitab® V15 statistical software (Minitab® Inc., PA, USA), for designing an optimum formulation. mPEG (200 mg) and paroxetine (10 %) were constant in all the 9 formulations, whereas CS (100 – 300 mg) and TPP 0.5 % (20 – 100 % ratio of TPP solution to mPEG-CS solution) were

used as the variables. The formulation variables were evaluated for their effect on average particle size, zeta potential and PDI as shown in Table 3.1. Particle size is an important aspect when targeting the blood brain barrier. According to the results obtained, it was established that crosslinker % and varying the biopolymer concentration have an impact on the nanoparticle size and zeta potential. As the % of the cross-linker increased, the particle size also increased. According to the literature, many studies have been done to evaluate the impact of crosslinking CS nanoparticles with different concentration of TPP, on the nanogel particle size. The studies also confirmed that an increment in percentage of TPP have an influence on the particle size. This is due to the particle precipitation and aggregation caused by the TPP ions [41]. The precipitation might be due to free TPP ions which are available to bridge existing micro- and nanogels which will result in aggregation of the mPEG-CS nanogel carrier system [41] [42]. Furthermore, it was also concluded the particle size was directly proportional to concentration of CS, as the concentration of CS was increasing the particle size was also increasing. This might be attributed to the hydrophobic properties of CS. According to the results, F6 had the most desired particle size, which was less than 200 nm, hence have potential of crossing the BBB.

It was also concluded that as the % of the cross-linker (TPP) increases, the zeta potential decreases hence it becomes less positive or negative. The decrease in zeta potential might be attributed to dissociation or ionization of surface groups such as amino, carboxyl and the phosphate groups [43]. Therefore, from the obtained data, it can be concluded that formulation F6 is highly stable because they have less ionic charges. The previous researchers reported that nanoparticles with a zeta potential of ± 30 mV are less prone to aggregation, as they will be more stable, hence formulation F6 will be less prone to aggregation [43]. The polydispersity index of F6 was 0.26, which indicated monodisperse stable system [44].

Table 2.1**:** Summary of the average particle size, zeta potential and PDI of paroxetine loaded mPEG-CS nanogel **carrier system**

Formulation	**Chitosan**	**Cross-linker %**	**Particle size (d.nm)**	**Zeta potential**	**PDI**
1	200	100	238.80	-16.60	0.38
2	300	60	260.00	4.32	0.25
3	100	20	233.80	23.00	0.45
4	200	60	151.50	20.20	0.48
5	300	100	313.60	-4.04	0.06
6	200	20	144.50	32.60	0.26

7	100	100	257.60	4.37	0.32
8	300	20	235.90	12.80	0.29
9	100	60	250.80	16.20	0.36

The encapsulation efficiency % was evaluated on all Box-Behnken design formulations (9 formulations) to determine the effect of varying the variables concentration on the drug encapsulation efficiency. According to the optimisation data in Table 3.2, as the concentration of TPP and CS increases the encapsulation % of the drug decreases, presumably as a result of inhabitation of the stability of ionic bond due to increase in polymer concertation [43]. In addition; the decrease in encapsulation efficiency % might be attributed to the contending interaction between TPP and paroxetine for CS binding [45]. F3 further confirms the effect of mPEG on improving the solubility of the nanoparticles due to its hydrophilic nature.

Table 3.2: Summary of encapsulation efficiency % and drug loading efficiency of the loaded nanogel

Formulation	**Chitosan**	**Cross linker %**	**Encapsulation efficiency %**	**Drug loading efficiency %**
1	200	100	71.80 ± 0.24	6.52
2	300	60	76.37 ± 0.74	6.95
3	100	20	94.51 ± 0.15	8.60
4	200	60	75.82 ± 0.16	6.90
5	300	100	65.20 ± 0.14	5.92
6	200	20	79.67 ± 0.43	7.25
7	100	100	90.30 ± 0.59	8.23
8	300	20	83.51 ± 0.22	7.60
9	100	60	91.21 ± 0.50	8.30

It can be concluded that the optimum ratio of CS and mPEG (1:1), and 0.50 % TPP (20 %) led to the formation of desired size and zeta potential of the loaded nanoparticles. However, the encapsulation efficiency % was low for F6 compared to the other formulations. For the working formulation F6 parameters were used due to its desirable particle size as we are targeting the BBB membrane and the desirable particle size need to be less than 200 nm.

3.3.6. Particle Size Analysis of Unloaded and Paroxetine Loaded mPEG-CS Nanogel Carrier System

Nanogel nanocarriers exhibit enhanced permeability of the medicine across the BBB, owing it to their size which is less than 200 nm [46]. In the current study, the average particle size of the loaded and unloaded mPEG-CS was 145.40 nm and 167.80 nm respectively and the PDI were 0.356 and 0.360 respectively as shown in Table 3.3. The particle size of the loaded nanogel carrier system was slightly higher than the unloaded due to the drug that was entrapped into the matrix of the mPEG-CS nanogel carrier system. The PDI indicates the good homogeneity and dispersity of the nanoparticles [47]. The zeta potential of the unloaded drug was +36 mV as shown in Table 3.3. The particle size of the loaded mPEG-CS nanogel carrier system was less than 200 nm, hence the nanogel have the potential of penetrating through the brain through the BBB membrane, as it is within the range acceptable range of less than 200 nm [48]. The achieved size of the nanoparticles also indicates that the cellular uptake will be effective, and they can overcome biological barriers [23]. The physiochemical characteristics of nanomedicine are vital in determining the stability and physiological function of the drug-loaded nanoparticles. The particle size plays a vital role in mucosal and cellular uptake. Furthermore, the zeta potential has an impact on the ability of nanoparticles to escape from endolysosomes, permeation and mucoadheviness of the nanoparticles. The zeta potential +34.7 mV have the potential of preventing aggregation hence improving the stability of the paroxetine loaded nanoparticles [49] [43]. In addition, mPEG crosslinked to CS leads to more sterically stable paroxetine loaded nanogel and avoid protein binding, hence improving the therapeutic effect by improving the circulation period [23]. The zeta potential has an impact on the stability and crossing of the nanoparticles through the BBB passage. The positively charged nanoparticles will be attracted to the negatively charged cell on the BBB membrane, which is advantageous. In this research, the zeta potential was +34.70 mV, hence the paroxetine loaded mPEG-CS nanogel carrier system have the potential of crossing BBB passage and the nanoparticles are less prone to aggregation. As a result, the stability of paroxetine loaded mPEG-CS nanogel carrier system will be improved [50].

Table 3.3: PDI, size and zeta potential of mPEG-CS nanogel carrier system formulation

Formulation	Polydispersity Index (PDI)	Particle size (d.nm)	Zeta potential (mV)
Unloaded mPEG-CS	0.36	145.40	36.00

Loaded mPEG-CS	0.36	167.80	34.70

3.3.7. Drug Loading and Release Behaviour of Paroxetine Loaded mPEG-CS Nanogel Carrier System and Paroxetine loaded CS Nanoparticles

Incorporation of drug molecules into nanogels is a vital aspect, as it influences the nanocarrier that will be administered for medication. In the current study, the drug release studies were undertaken in pristine paroxetine, paroxetine loaded CS nanoparticles and paroxetine loaded mPEG-CS nanogel carrier system to prove the effect of mPEG in improving the solubility of molecules.

In the present work, the entrapment of paroxetine loaded mPEG-CS nanogel carrier system and paroxetine loaded CS nanoparticles were 77.90 ± 0.1 % and 20 ± 0.1% respectively. The crosslinking of the mPEG-CS nanogel carrier system with TPP have an impact on the drug entrapment efficiency. The crosslinking between TPP and the amine groups of mPEG-CS nanogel carrier system and the ionic interactions of paroxetine with mPEG-CS nanogel carrier system gives room for the biopolymers to entrap the paroxetine within the matrix core. However, the hydrophilic surface of the mPEG-CS nanogel carrier system hinders the significant impact of drug entrapment efficiency as it adsorb the drug into the surface of the mPEG-CS nanogel carrier system [23]. On the other hand, the entrapment of paroxetine loaded CS nanoparticles was low compared to paroxetine loaded mPEG-CS nanogel carrier system. This is due to reduced entanglement of CS efficiency with paroxetine, as a result of interaction between the positively charged amines groups on the CS chain and the TPP anions [51]. *In vitro* release studies were carried out on paroxetine loaded CS nanoparticles and paroxetine loaded mPEG-CS nanogel carrier system to evaluate the effect of pegylation.

The *in vitro* behaviour of loaded mPEG-CS nanogel carrier system and paroxetine loaded CS nanoparticles were carried out in PBS (pH 6.80) and of paroxetine loaded mPEG-CS nanogel carrier system was also carried out in PBS (pH 1.20) at 37 ˚C to mimic the pH of small intestines and the stomach, respectively. At pH 6.80 the loaded nanoparticles showed a sustained release owing it to the swelling properties of the nanogel. In addition, the sustained release maybe a result of the nature of the nanogel, thus the degradation mechanism and permeation of mPEG-CS nanogel carrier system biopolymers and CS nanoparticles. The drug release of paroxetine loaded mPEG-CS nanogel carrier system, paroxetine loaded CS nanoparticles and pristine paroxetine after 24 hrs were 99 %, 76 % and 69 % respectively. The results further confirms that pegylation enhances drug release owing to increased hydrophilicity of nanoparticles by mPEG. The release of drug from the nanogel after 2 hrs at

pH 1.20 and 6.80 were 16 % and 21 % respectively as shown in Figure 3.8, confirming that there is minimum release in the stomach hence maximum release will be in the small intestines. This indicates that more drug release will happen in the small intestines hence drug absorption will be improved, and the drug release is pH dependent. The drug release results showed that there was a burst release of drug from both pH 1.20 and pH 6.80. The bursts release might be due to paroxetine that was loosely bound on the surface of the nanogel and the hydrophilic effect of mPEG [52]. The constant release displays that paroxetine was successfully incorporated in the matrix of the biopolymer after crosslinking it with TPP [23] . The biopolymer crystallinity also plays a role in drug release. In the previous studies, they reported that the biopolymers that are highly crystalline are more prone to immediate release owing it to microchannel structure that could be formed. This result in large surface area of the copolymeric matrix hence the drug will be easily released [28]. However, in the current study, it was proved that the mPEG-CS nanogel carrier system was semi-crystalline hence the drug release was sustained. In addition, the sustained release is attributed to swelling of the mPEG-CS nanogel carrier system and the rate at which the copolymeric matrix was degrading, hence releasing the drug [28]. The results also confirm that mPEG improved the hydrophilicity of paroxetine loaded mPEG-CS nanogel carrier system (Figure 3.8). In addition, the hydrophilic mPEG have the potential of forming a hydrated outer shell which hinders the nanogel from being rapidly uptaken by RES [34]. The solubility of paroxetine improved by 30 % over 24 hr period as shown in Figure 3.8.

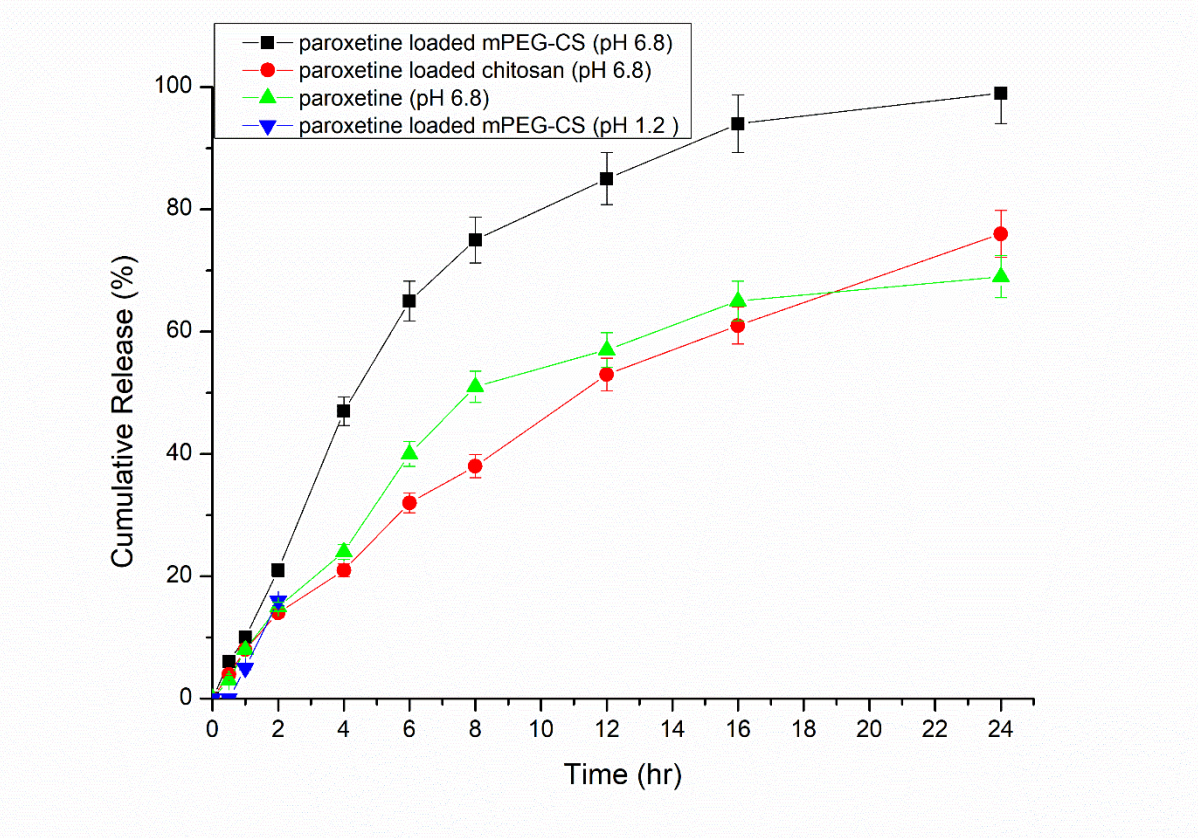


Figure 3.8: Drug release profile of paroxetine mPEG-CS nanogel carrier system at pH 1.20 and 6.80 and of pure paroxetine and paroxetine loaded CS nanoparticles at pH 6.80 (n=3)

3.3.8. *Ex-vivo* Release of Paroxetine from Self-Assembled Paroxetine loaded mPEG-CS Nanogel Carrier System and Paroxetine loaded CS Nanoparticles

In the study undertaken, the rat intestine was used for determining the permeation of paroxetine loaded mPEG-CS nanogel carrier system and paroxetine loaded CS nanoparticles across the intestinal barrier. As evident in Figure 3.9 the permeation of paroxetine loaded mPEG-CS nanogel carrier system (97 %) was significantly higher than paroxetine loaded CS (71 %) nanoparticles (p-value < 0.0001, α=0.05), as also proven by the dissolution results. Owing it to mPEG, water can easily penetrate into the paroxetine loaded mPEG-CS nanogel carrier system due to the present of mPEG brushes that are more hydrophilic as a result, the nanogel are more flexible and they can penetrate through the intestinal barrier more easily compared to paroxetine loaded CS [53]. Furthermore, the interaction that happens between CS biopolymer and the phosphate anions of crosslinker TPP weakens the surface charge of CS , resulting in reduction of CS's aqueous solubility [54] .

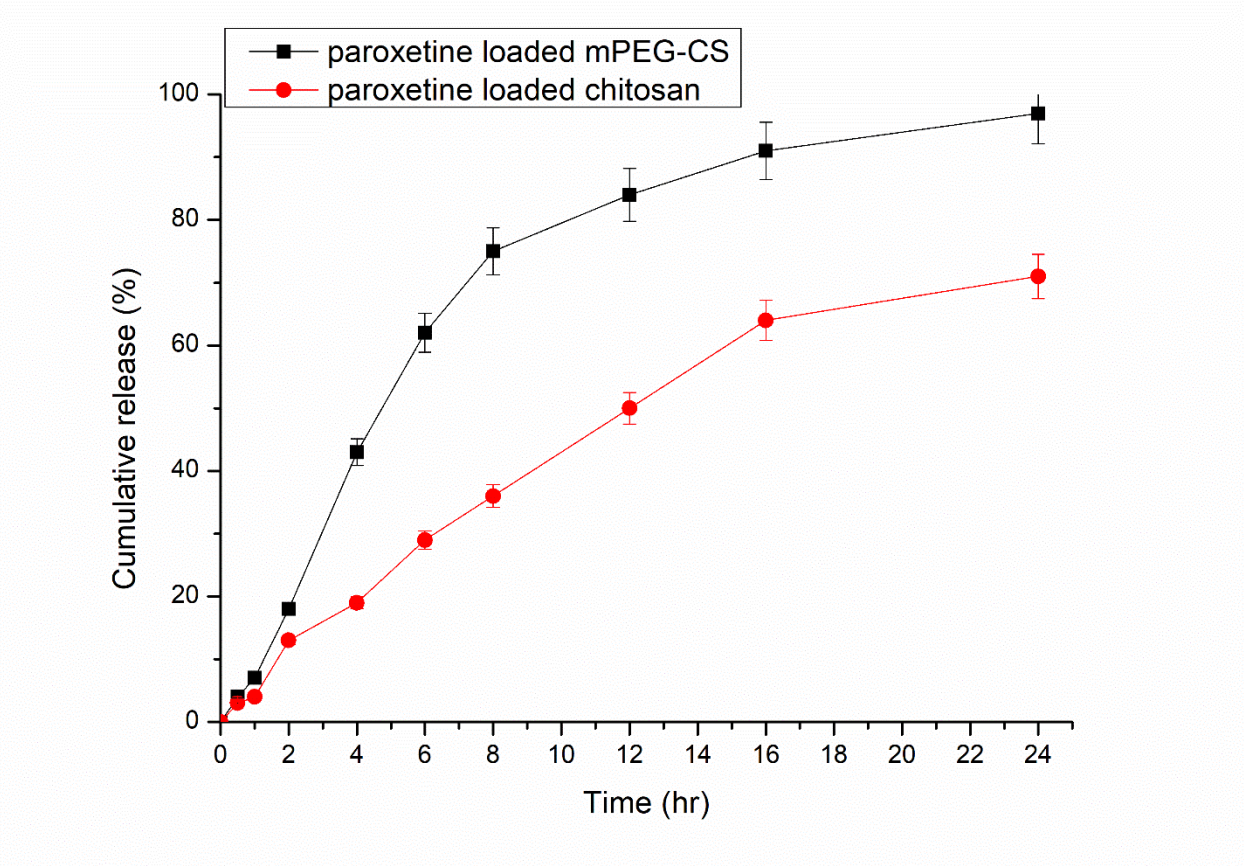


Figure 3.9: Franz diffusion drug release profile of mPEG-CS nanogel carrier system and CS nanoparticles (n=3).

3.3.9. *In-vitro* Cytotoxic Assessment of mPEG-CS Nanogel Carrier System and Paroxetine loaded mPEG-CS Nanogel Carrier System on PC 12 cell line

Nanoparticles formulated for CNS need to be safe and non-toxic to the brain cells. The *in-vitro* cell studies were done to investigate the biocompatibility of paroxetine-loaded mPEG nanogel. The percentage cell viability was calculated for 48 hrs and 72 hrs respectively and summarised in graph form in Figure 3.10 and Figure 3.11. The mPEG-CS nanogel carrier system showed cell viability of above 80% for all the concentration and both time frames. This low toxicity of the mPEG-CS nanogel carrier system demonstrates that the unloaded mPEG-CS nanogel carrier system may be biocompatible with brain tissue, hence proving the neuro-compatibility of mPEG-CS nanogel carrier system. To support this observation, low molecular weight CS is known to be non-toxic to the brain cells owing to its properties [55]. In addition, according to the research that was done using mPEG biopolymer for delivery of α-asarone to the brain, the *in vivo* studies showed no toxicity [56]. Paroxetine displayed significant decrease in live PC 12 cell density at high concentration, thus paroxetine toxicity is dependent to the concentration as shown in Figure 3.10 and 3.11. In addition, it is also evident that the cell viability was

reduced from 100 % (control) to 17% and 2% at 156 µg/mL 48 hrs and 72 hrs respectively, confirming neurotoxicity. The cytotoxicity might be due to paroxetine anticancer activities that have been reported in previous studies; and PC12 cells can be classified as neuronal cancer cell line [57]. Nevertheless, further research needs to be done to unravel the mechanisms involved in paroxetine-mediated cytotoxicity [58]. From 78 µg/mL the toxicity of the drug was reduced; hence paroxetine is safe to use at low doses for both time frames. The paroxetine loaded mPEG-CS naogel carrier system for both 72 hrs and 48 hrs showed dose-dependent toxicity at 156 µg/mL. The toxicity might be due to the anticancer activities of the paroxetine drug [59]. The loaded nanomedicine proved to have improved safety and efficacy at 156 µg/mL as shown in Figure 3.10 and 3.11. As it was the drug released per mL over 24 hrs and it is within the range of the actual dose recommended for patient administration [60]. In addition, the percentage cell viability at 156 µg/mL was high as shown in Figure 3.10, 3.11 and 3.12. Ethanol (0.10 %) was used as a negative control and displayed the expected low levels of cytotoxicity (Figure 3.10 and 3.11). The positive control (5-Fluorouracil (10 µg/mL) induced a high level of cytotoxicity; proving the validity of the MTT assay used. Figure 3.12 also indicates the morphology of the treated and untreated PC12 cell line at different periods. Many dead/dying cells can be observed at high concentrations of the drug (Panel B of Figure 3.12), due to the drug possessing inherent anticancer activity. The mPEG-CS nanogel carrier system and paroxetine loaded mPEG-CS nanogel carrier system showed low cytotoxicity on PC12 cells (Figure 3.10, 3.11 and 3.12), indicating biocompatibility. Evaluation of cytotoxicity using the MTT assay and corresponding light microscopy images showed that paroxetine-loaded mPEG-CS nanogel carrier system showed acceptable cytotoxicity on PC12 cells and indicate that the formulation is biocompatible. Both microscopy images and the MTT assay proved to have high % cell viability for the paroxetine loaded mPEG-CS nanogel carrier system at 156 µg/mL as compared to the positive control (5-FU).

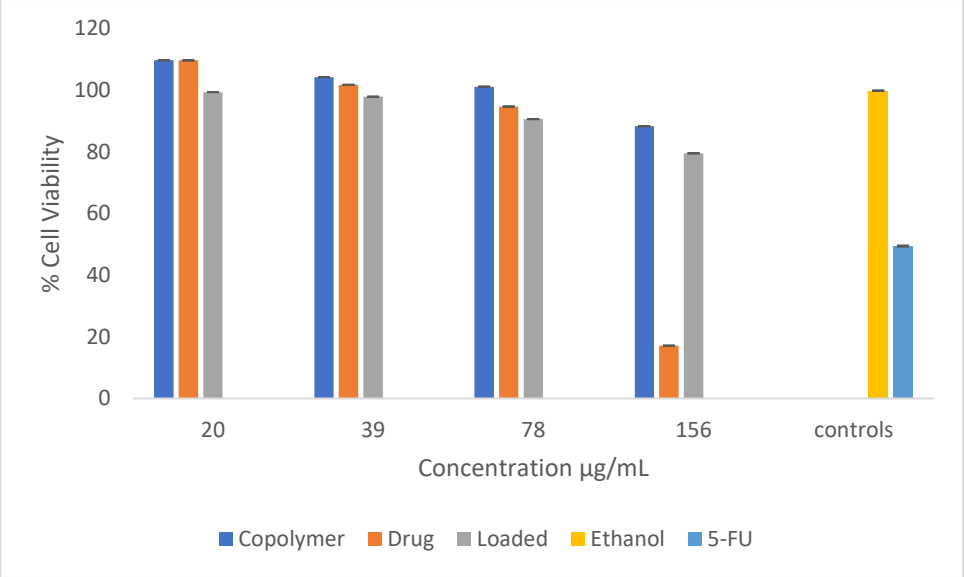


Figure 3.10: Cell viability evaluation of PC 12 cell line treated with mPEG-CS nanogel carrier system, paroxetine, paroxetine loaded mPEG-CS nanogel carrier system, ethanol (0.10 %) and 5-Fluorouracil (10 µg/mL) after 48 hrs (n=3). The error bars lie within the graph ranges, therefore are not visible.

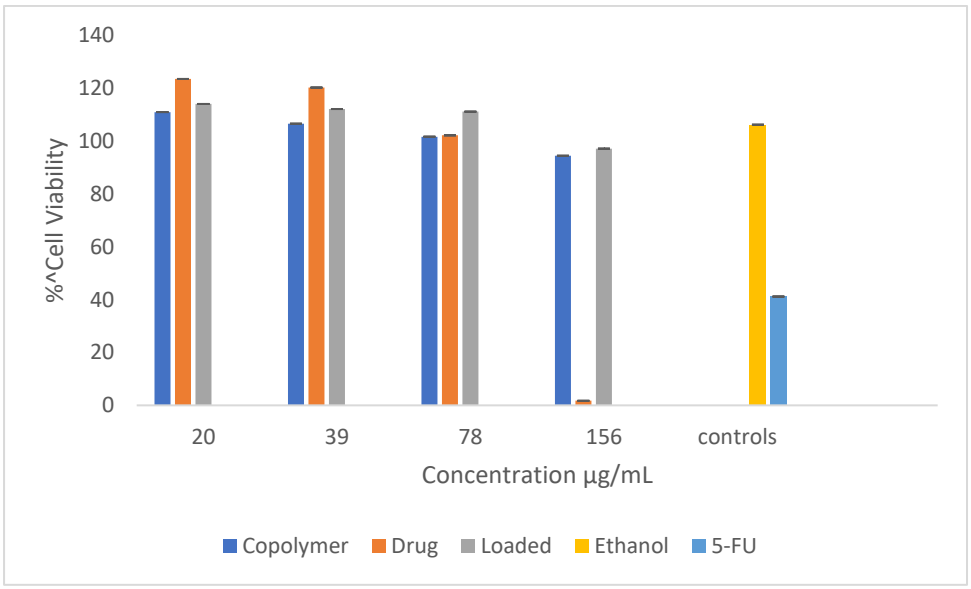
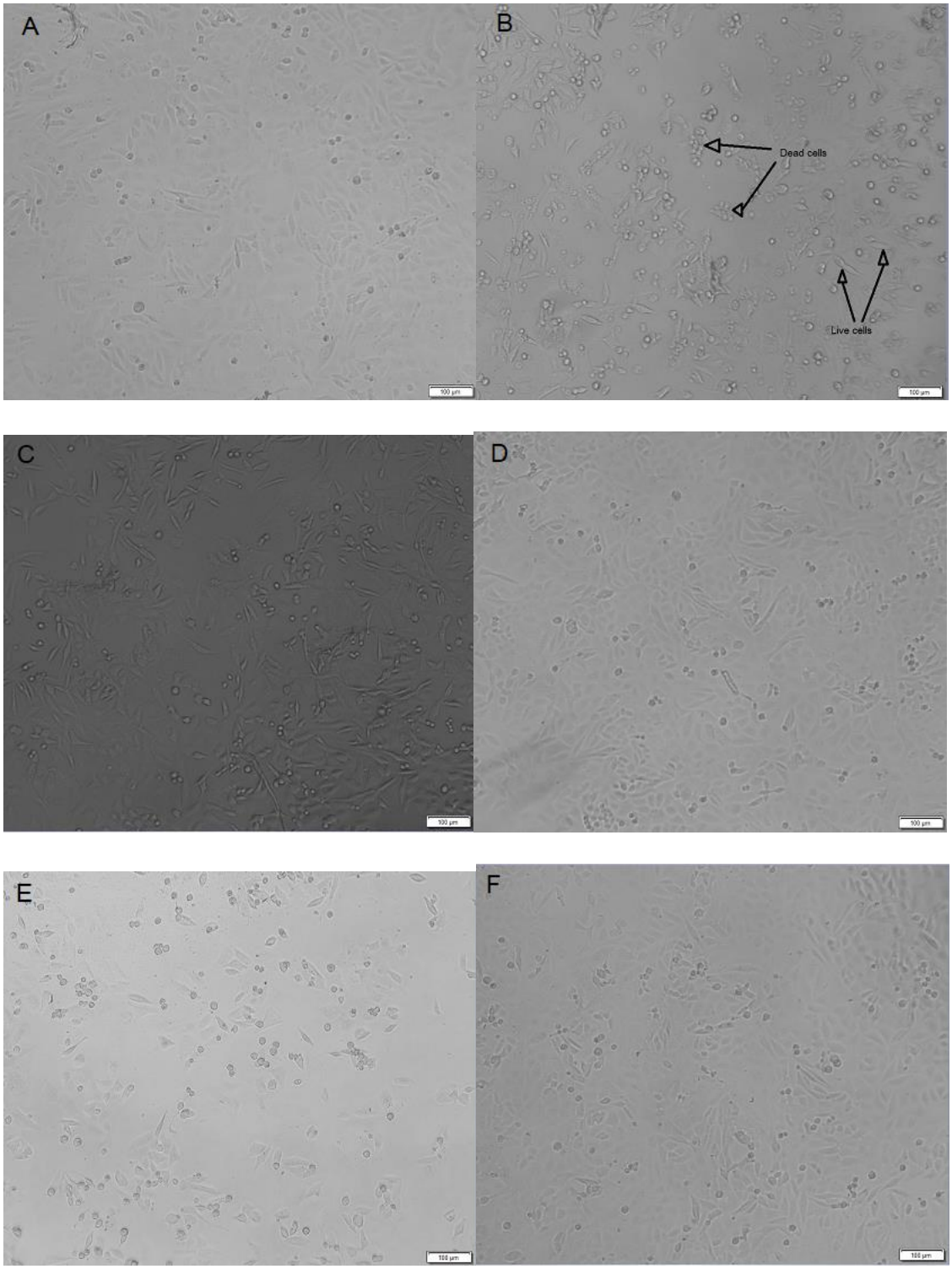


Figure 3.11: Cell viability evaluation of PC 12 cell line treated with mPEG-CS nanogel carrier system, paroxetine, paroxetine loaded mPEG-CS nanogel carrier system, 0.1% ethanol and 5-Fluorouracil (10 µg/mL) after 72 hrs (n=3). The error bars lie within the graph ranges, therefore are not visible.


A
B
Dead cells
Live cells
C
D
E
F

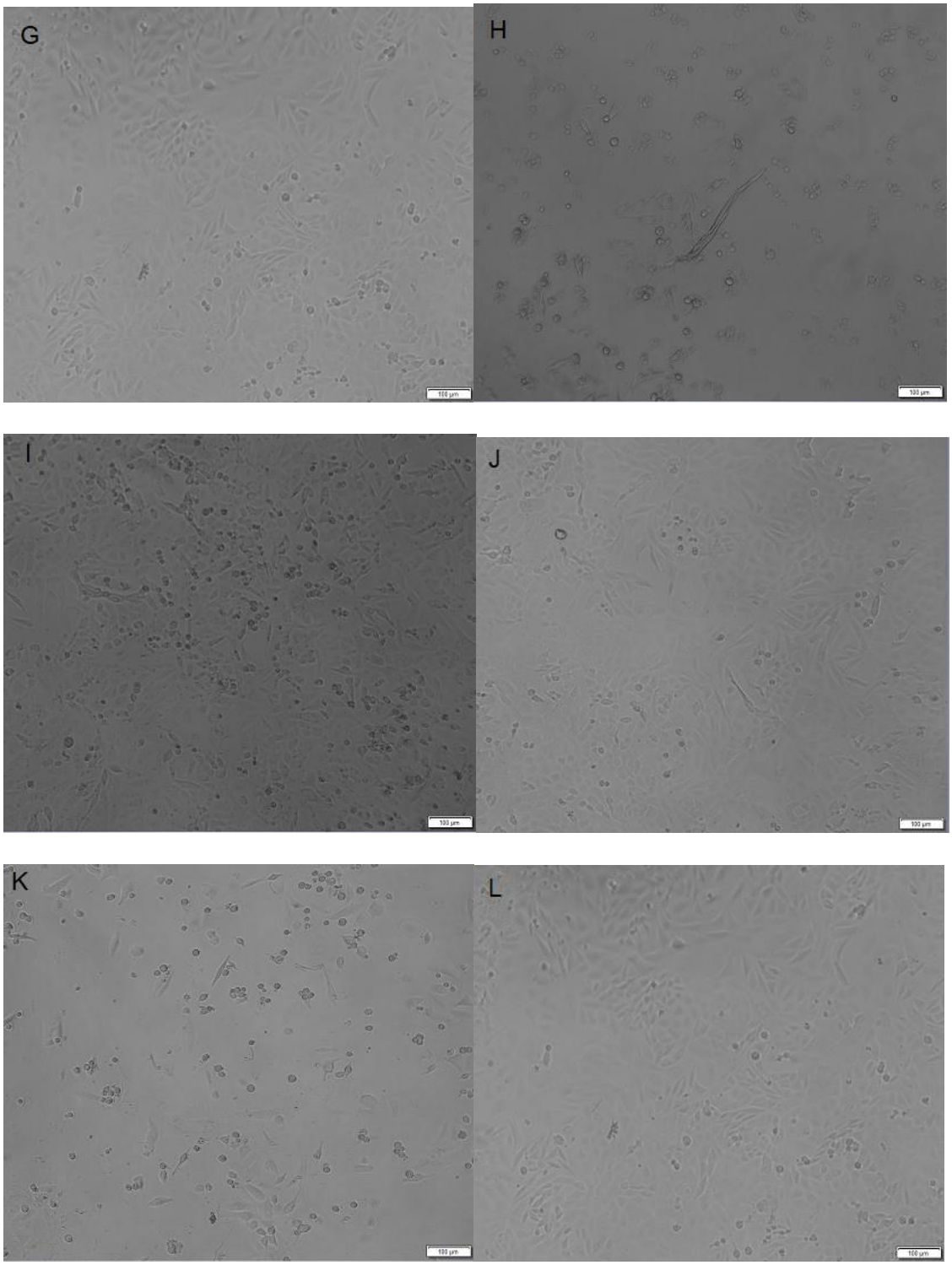


Figure 3.12: Light Microscope images (X10, Olympus CKX53, Tokyo, Japan) of PC 12 cell line at 156 µ/mL at different time frames A-F (48 hrs), G-L (72hrs). A-Copolymer-mPEG-CS nanogel carrier system, B-Paroxetine (156 µg/mL), C-Paroxetine-loaded mPEG-CS nanogel carrier system, D-0.10 % ethanol, E-5-FU (10 µg/mL), F-untreated cells, G-copolymer-mPEG-CS nanogel carrier system, H-Drug, I-Loaded, J-Ethanol, K-5-FU, L-untreated

3.3.10. *In-vitro* Cytotoxic Assessment of mPEG-CS Nanogel Carrier System and Paroxetine loaded mPEG-CS Nanogel Carrier System on Caco-2 cell line

The cytotoxicity of paroxetine, mPEG-CS nanogel carrier system and paroxetine loaded mPEG-CS nanogel carrier system was in investigated on Caco-2 cells. The Caco-2 cells were treated with the nanoformulation and drug (96 to 156 µg/mL) containing medium for 48 hours and 72 hours respectively, and MTT assay was used to investigate cell viability. As shown in Figure 3.13 and Figure 3.14, the drug and the paroxetine loaded mPEG-CS nanogel carrier system were toxic to the cell line for both time frames, different concentrations. Nonetheless, the blank mPEG-CS nanogel carrier system showed cell viability of more than 80 % on all the concentrations and both time frames, confirming that it is nontoxic. In addition, one of the vital properties of biodegradable nanoparticles is that they should not affect the cell viability [53]. This proved the biocompatibility of blank mPEG-CS nanogel carrier system and demonstrated that the blank formulation did not contribute towards the reduction in cell viability of Caco-2 cell line. The toxicity of the drug on Caco-2 cell line was significant.

The current study proved that the blank nanogel formulation is not significantly toxic to the Caco-2 cell line whereas the drug proved to be toxic to the cell line for both time frames (p-value < 0.0001, $\alpha=0.05$). This might be due to the anticancer activity of paroxetine as previously reported though the mechanism is still under research to date, globally [61]. According to the research that was conducted on human colorectal cancer cells (HCT116 and HT-29), paroxetine exhibited cytotoxicity behaviour on both cell lines and the *in vivo* studies that were conducted on thymic nude mice bearing HT-29 cells resulted in suppressed tumour growth [61]. The researchers concluded that the mode of action of paroxetine might be through the compromisation of AKT, ERK and p38 activation and induction of JNK and caspase-3 pathways as a result of inhibition of two major receptors, tyrosine kinases - MET and ERBB3 [61].

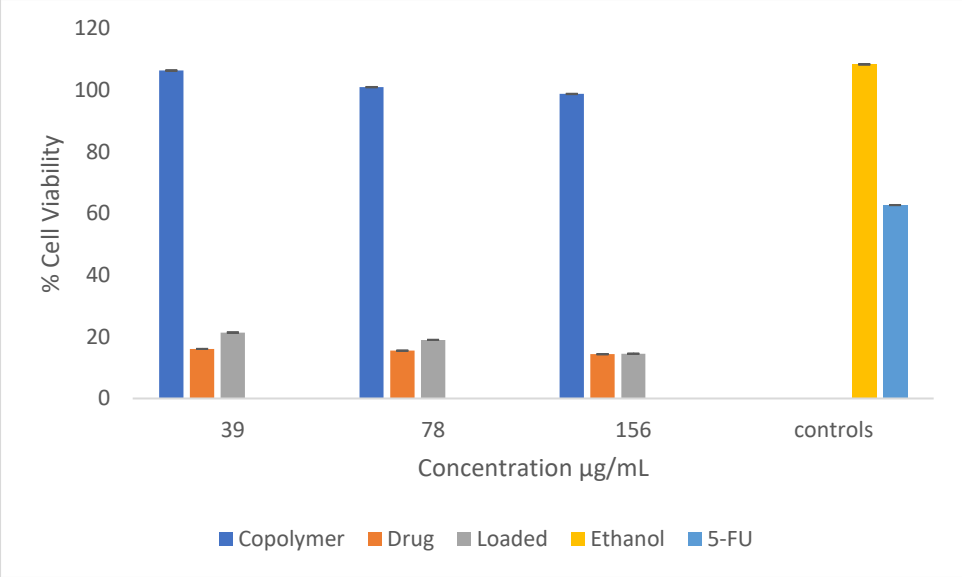


Figure 3.13: Cell viability evaluation of Caco-2 cell line treated with mPEG-CS nanogel carrier system, paroxetine, paroxetine loaded mPEG-CS nanogel carrier system , ethanol (0.10 %) and 5-Fluorouracil (10 µg/mL) after 48 hrs (n=3). The error bars lie within the graph ranges, therefore are not visible.

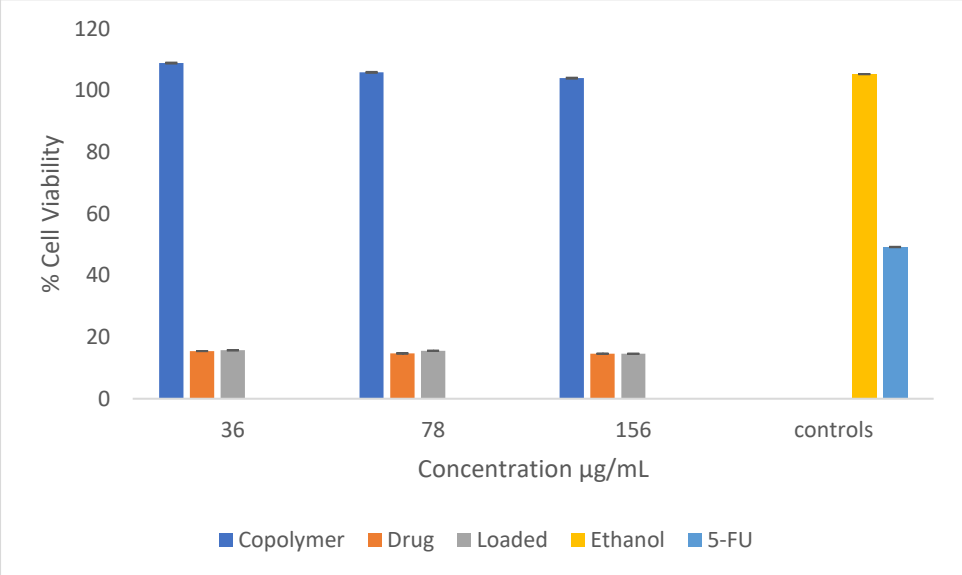


Figure 3.14: Cell viability evaluation of Caco-2 cell line treated with mPEG-CS nanogel carrier system, paroxetine, paroxetine loaded mPEG-CS nanogel carrier system, 0.10 % ethanol and 5-Fluorouracil (10 µg/mL) after 72 hrs. The error bars lie within the graph ranges, therefore are not visible.

3.4. Conclusion Remarks

In the present study, CS was successfully crosslinked to mPEG and the paroxetine-loaded mPEG-CS nanogel carrier system exhibited low, acceptable toxicity levels, thereby suggesting

good biocompatibility. The self-assembled mPEG-CS nanogel carrier system were successfully formulated using the freeze-drying method. The mPEG-CS nanogel carrier system was synthesised using the ionic gelation method. The CS was used to stabilize the nanogel, owing to its mucoadhesive properties. The obtained mPEG-CS nanogel carrier system was analysed using FTIR, TGA, DSC and SEM to determine the chemical structure and interactions. The drug release and the permeation studies of paroxetine loaded mPEG-CS nanogel carrier system were analysed using UV-vis, and it showed a release of 99 % and 97 % respectively over 24 hrs and an encapsulation efficiency of 77 %. The particle size of the nanogel also confirmed the nanosize of the nanoparticles, which have the potential of enhancing oral- and neurobioavailability across the BBB. The formulated blank and paroxetine loaded mPEG-CS nanogel carrier system showed acceptable levels of toxicity (above 80 %); over a period of 48 and 72 hrs, hence indicating biocompatibility on PC 12 cell line. In addition, on Caco-2 cell line; the blank mPEG-CS nanogel carrier system proved to be biocompatible with the cell line. However, the pristine drug and the paroxetine loaded mPEG-CS nanogel carrier system proved to be cytotoxic over a period of 48 and 72 hrs. The paroxetine loaded mPEG-CS nanogel carrier system (on PC 12 cell line) showed toxicity only at high concentrations, inherently due to the anticancer activities of the paroxetine drug. The formulation improved the efficacy of the loaded nanogel and the solubility of the drug. With regards to the findings of the research, it can be concluded that paroxetine-loaded nanogel nanoparticles have the potential of improving the drug delivery system for the treatment of depression.

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CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

4.1. Conclusion

The dynamic and rapidly advancing field of nanotechnology system has led to numerous innovations, such as nanogel drug delivery system. The study of nanogel carrier system for oral delivery system encompasses a great deal of innovative research, striving for novel site-specific drug release properties, displaying significant potential for a new era of oral dosage forms in oral antidepressant therapeutics. The self-assembled paroxetine loaded mPEG-CS nanogel carrier system was developed to shield the hepatic first pass metabolism, to improve the solubility of the drug and to minimize the side effects associated with the drug. In addition, to minimize the time taken to reach the therapeutic onset due to reduction of hepatic first pass metabolism. This might also lead to low dosages as the hepatic first pass metabolism will be minimized, hence the undesirable side effects will be minimized. Chitosan and methoxypoly(ethylene glycol) mPEG biopolymers were utilized for developing a nanogel carrier system. The biopolymers were successfully crosslinked with TPP using an ionic gelation method. The nanogel was a self-assembled nanogel owing it to the properties of the biopolymers employed. Paroxetine was employed as the antidepressant drug; hence it was loaded into the mPEG-CS nanogel carrier system.

Extensive characterisation of the self-assembled mPEG-CS nanogel carrier system was also undertaken to determine the thermal stability and the physicochemical properties of the synthesised mPEG-CS nanogel carrier system.

The comparative studies that were done using paroxetine loaded mPEG-CS nanogel carrier system and paroxetine loaded chitosan nanoparticles, displayed that paroxetine loaded mPEG-CS nanogel carrier system have better encapsulation efficiency and dissolution profile compared to paroxetine loaded chitosan nanoparticles. Furthermore, optimization studies that were done using Box-Behnken proved to have the desirable particle size and zeta potential at 1:1 ratio of mPEG and chitosan, and 20 % of 0.5% TPP. Moreover, for the *ex vivo* permeation studies (Franz diffusion studies); paroxetine loaded mPEG-CS nanogel carrier system exhibited a favourable dissolution profile. Due to the mucoadhesive properties of chitosan and mPEG that further enhance mucus-penetrating properties of chitosan-based nanomaterial.

Cell viability studies were conducted using the PC 12 cell line and the Caco-2 cell line. The *in vitro* cytotoxic studies established that the mPEG-CS nanogel carrier system and paroxetine loaded mPEG-CS nanogel carrier system are biocompatible with the PC 12 cell line. At high concentration the drug proved to be toxic to the cell line. On Caco-2 cell line the pristine drug

and paroxetine loaded mPEG-CS nanogel carrier system formulations proved to be toxic to the cell line whereas the unloaded mPEG-CS nanogel carrier system was biocompatible with the cell line. We concluded that the unloaded mPEG-CS nanogel carrier system was nontoxic. The toxicity behaviour of the drug was linked to the cancer activities of the drug though the mechanism is not yet known. It can be concluded that the mPEG-CS nanogel carrier system carrier system demonstrated greater evidence of improving the solubility of the drug, efficacy, and the desired size for crossing the BBB. Furthermore, due to size and properties of the nanogel nanoparticles, the first pass metabolism have the potential of being minimized resulting in improved therapeutic onset and reduced side effects due to reduction in the dosage strength compared to the current dosage form which takes about 4 to 6 weeks for the therapeutic onset to be achieved due to extensive hepatic first pass metabolism. In conclusion, the treatment of neurological conditions such as depression comes with drawbacks which can be overcome with nanotechnology system

4.2. Future Outlook and Recommendation

The science of nanotechnology system is currently among the most fascinating areas of research for the treatment of neurological conditions. The current study focused on formulating a nanogel carrier system to improve the therapeutic efficacy of the antidepressants, hence improving the patients' compliance. The future research needs to establish ways to minimize undesirable side effects like weight gain, none other than reducing the dosage strength.

In addition, future research needs to focus on other approaches such as surface modification thus attaching a ligand, because the approach has potential of improving the targeting and the ability of the loaded nanogel to penetrate multiple biological barriers, such as the blood brain barrier. This will also result in improved therapeutic efficacy. Furthermore, they can also focus on finding an alternative of modifying the particle size of mPEG-CS (ratio 2: 1 respectively) considering that the formulation had a high encapsulation rate.

The i*n vitro* cytotoxicity studies undertaken in the current study, displayed toxicity for the pristine paroxetine and the paroxetine loaded mPEG-CS nanogel carrier system on the Caco-2 cell line. Hence it is vital for future research to focus on improving the biocompatibility of the loaded nanogel and to understand the mechanism underpinning the anticancer activities of paroxetine. In addition, it is also recommended that other studies such as the animal studies must be done to further confirm the neuro-biocompatibility of the paroxetine loaded mPEG-CS nanogel carrier system bio-platform and to ascertain therapeutic efficacy of the paroxetine loaded nanogel bio-platform in shielding the hepatic first pass metabolism. *In vivo* studies are recommended for the synthesised nanogel system to analyse the degradation products in depth of the formulation in an animal model, as this would be required to be evaluated in a

preclinical model. There is still potential for the nanotechnology system to be exploited to its fullest extent for the potential advanced therapeutic approaches.

As positive remarks of studies undertaken, we concluded that nanogel carrier system can be designed to increase the therapeutic effect of the antidepressants, however, since this novelty has been developed with extreme specifications of optimal drug loading and release kinetics, significant effort would be required to eliminate the limitations which are minimally displayed in this research.

5. APPENDICES

5.1. Animal Ethics Clearance Waiver

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

ANIMAL RESEARCH ETHICS COMMITTEE
Registration number: AREC-101210-002

Date: 25/10/2021

Certificate reference: Waiver 25-10-2021-O
Category: O
Applicant: Fadzai Mutingwende (1113854)
Department: Department of Pharmacy and Pharmacology (WITS)
Tel: 011 717 2552; **Email**: 1113854@students.wits.ac.za

Re: Waiver from the Animal Ethics Research Committee of the University of the Witwatersrand

This letter is to confirm that Fadzai Mutingwende, MSc student under the supervision of Prof. Yahya E Choonara (Department of Pharmacy and Pharmacology), does not require full Animal Ethics Research Committee clearance to undertake the work titled **'Development of a nanogel carrier system for oral antidepressant therapeutics"**.

Reason for waiver
The project is using intestinal tissue collected from one adult male Sprague–Dawley rats euthanized by the WRAF for *ex vivo* permeation of rat's intestinal tissue to drug-loaded nanoparticles.

Details of the study
After animal euthanasia the intestine segments will be used for permeation experiments. Appropriate sections of intestine will be mounted in vertical jacketed Franz type diffusion cells (5 mL acceptor volume) with 5 mm diameter orifice (0.20 cm2 area). To equilibrate the tissue will initially be placed at 37 ± 0.1 °C between donor and acceptor chambers both filled with DPBS solution at pH 6.80. Afterward, DPBS solution will be carefully removed from the donor compartment and replaced with drug-loaded nanoparticles dispersion in DPBS pH 6.80 (1 ml; drugs concentration 0.20 mg/ml). At scheduled time intervals, aliquots (200 µl) will be withdrawn from the acceptor chamber followed by analysis of the filtrate spectrophotometrically at 229 nm and Paroxetine quantified using a calibration curve. Experiment will be carried out in triplicate at 37 ± 0.1 °C for 8 h under continuous agitation in an orbital shaker. At the end of each *ex vivo* permeation experiment, the amount of drug entrapped into intestinal tissue will be quantified by organic solvent extraction. Intestinal sections will be removed from Franz cells and washed with DPBS at pH 6.80 in order to remove the eventual drug remained on its surface. Subsequently, colon specimens will be stirred in 2 mL of organic solvent DMSO overnight at 37 °C. Extracted liquor will be transferred into a flask and analysed spectrophotometrically at 229 nm.

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De Mohac, L.M., Caruana, R., Pavia, F.C., Cavallaro, G., Giammona, G. and Licciardi, M., 2019. Multicomponent solid dispersion as a formulation strategy to improve drug permeation: A case study on the anti-colorectal cancer irinotecan. Journal of Drug Delivery Science and Technology, 52, pp.346-354.

The individual covered by the waiver is Fadzai Mutingwende (Wits).

Please contact me should you require further information.

Yours sincerely

Prof Frederic Michel
Chair: Animal Research Ethics Committee
University of the Witwatersrand

5.2. Review Paper 1

polymers

MDPI

Review

Advances in Nano-Enabled Platforms for the Treatment of Depression

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Abstract: Nanotechnology has aided in the advancement of drug delivery for the treatment of several neurological disorders including depression. Depression is a relatively common mental disorder which is characterized by a severe imbalance of neurotransmitters. Several current therapeutic regimens against depression display drawbacks which include low bioavailability, delayed therapeutic outcome, undesirable side effects and drug toxicity due to high doses. The blood–brain barrier limits the entry of the drugs into the brain matrix, resulting in low bioavailability and tissue damage due to drug accumulation. Due to their size and physico-chemical properties, nanotechnological drug delivery systems present a promising strategy to enhance the delivery of nanomedicines into the brain matrix, thereby improving bioavailability and limiting toxicity. Furthermore, ligand-complexed nanocarriers can improve drug specificity and antidepressant efficacy and reduce drug toxicity. Biopolymers and nanocarriers can also be employed to enhance controlled drug release and reduce the hepatic first-pass effect, hence reducing the dosing frequency. This manuscript reviews recent advances in different biopolymers, such as polysaccharides and other nanocarriers, for targeted antidepressant drug delivery to the brain. It probes nano-based strategies that can be employed to enhance the therapeutic efficacy of antidepressants through the oral, intranasal, and parenteral routes of administration.

Keywords: drug delivery; antidepressants; biopolymers; nanocarriers; nanomedicines; biomedical nanotechnology

Citation: Mutingwende, F.P.; Kondiah, P.P.D.; Ubanako, P.; Marimuthu, T.; Choonara, Y.E. Advances in Nano-Enabled Platforms for the Treatment of Depression. *Polymers* **2021**, *13*, 1431. https://doi.org/10.3390/polym13091431

Academic Editors: Alessandro Pistone and Daniela Predoi

Received: 16 March 2021
Accepted: 19 April 2021
Published: 29 April 2021

1. Introduction

Depression is a common mental disorder that is characterized by a persistent feeling of sadness, low self-esteem, disturbed appetite, suicidal thoughts, insomnia and loss of interest [1]. Depression is caused by several aspects which include pathological effects, social activities such as drug and alcohol abuse and biological factors [2]. According to research done by the World Health Organization (W.H.O) in 2017, more than 300 million people (approximately 4.4% of the world's population) suffer from depression [1] making it one of the top two causes of disability-adjusted life years currently [2]. Pathological causes of depression include a chemical imbalance in the brain, energy metabolic decline and alteration in body hormones [3]. According to the serotonin hypothesis, depression is a result of dysfunctional serotonergic activities [4] which results in reduced serotonin levels in the brain. Several classes of antidepressant therapy that are currently on the market include selective serotonin reuptake inhibitors (SSRI), tricyclic antidepressants, serotonin-norepinephrine reuptake inhibitors, and monoamine oxidase inhibitors. SSRI such as paroxetine, vilazodone, and fluvoxamine are first-line treatment options in adults with depression, albeit with several contraindications [5]. The side effects of current medication include delayed