DESIGN OF AN ORAL IONIC NANOEMULSION FORMULATION FOR TARGETED TREATMENT OF MIGRAINES

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DECLARATION

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

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ABSTRACT

Migraine is the third most prevalent disorder and is regarded as the seventh-highest cause of disability worldwide. It is prevalent globally and significantly affects quality of life. Current strategies to treat migraine include the use of analgesics such as aspirin and non-steroidal anti-inflammatory Ibuprofen commonly formulated in conventional drug systems. The oral drug delivery system is the most popular and convenient route to administer various dosage forms for systemic therapy. However, several molecules have poor stability in gastrointestinal fluid and possess limited ability to cross the Blood Brain Barrier (BBB). To overcome the BBB in order to allow central nervous system delivery, several strategies have been explored. The nanotechnology based drug delivery system approach can results in improved bioavailability, increased drug solubility and improved permeability. A nanoemulsion is an advanced mode of drug delivery system that can be developed to overcome the major drawbacks associated with conventional drug delivery systems. They are designed to address some of the problems associated with conventional drug delivery systems such as low availability and noncompliance. Stability, solubility, absorption, bioavailability and site targeting are often challenges experienced by therapeutic agents orally administered. An oral drug formulation in a nanoemulsion system can improve the bioavailability, rapid absorption and effective relief compared to a conventional oral solid dosage and therefore there is an urgent need to design an ionic nanoemulsion for fast effective relief of migraine pain. Nanoemulsions can be characterised using various methods such as zeta potential, zeta size and particle size/size distribution by dynamic light scattering.

The purpose of this study was to synthesize and characterize Ibuprofen (IBU) loaded PLGAPVA nanoparticles in an oral ionic nanoemulsion formulation, and to evaluate their potential for transport of Ibuprofen to the CNS via oral delivery path for improved efficacy with reduced side effects. IBU-loaded PLGA-PVA nanoparticles was prepared by the solvent emulsion evaporation method and synthesis was confirmed by analyzing the physicochemical properties including Scanning Electron Microscopy (SEM), Fourier Transformation Infrared Spectroscopy (FTIR), zetasizer, Ultraviolet (UV) spectroscopy and Thermogravimetric Analysis (TGA). SEM of the nanoparticles explored the morphology in terms of shape and pore distribution of the nanoparticles. The size, charge and polydispersity index (PDI) of the synthesized nanoparticles were evaluated utilizing the zeta-sizer and the nanoparticles were found to be in the size range of 140 ± 23, 50 nm, with polydispersity index (PDI) of 0.190 and with a zeta potential of -53.01 (±10) mV. A drug loading efficacy of 78% was attained by the nanoparticulate formulation. IBU release studies showed a constant release over a 24-hour period. The synthesized IBU-PLGA-PVA nanoparticles were dissolved in two pH environments simulating the stomach pH 1, 2 and intestinal pH 6, 8. At pH of 6.8 26% of IBU was released from the copolymeric nanoparticles, whereas only 25% was released at pH 1.2. This illustrates the shielding effects of the PLGA-PVA nanoparticulate formulation on the IBU in an acidic environment of the stomach. FTIR results exhibited the formation of nanoparticulate structure with comparable peaks between the polymers, IBU and the PLGAPVA nanoparticles. IBU compared to PLGA-PVA nanoparticles showed peaks at 3320 attributed to -C-O vibration, at 3328 attributed to -N-H stretching and -CH_2 bending at 2950. TGA thermal studies indicated that the PLGA-PVA nanoparticulate structure increased the stability of IBU. In addition, HEK 293 neural cells were treated with IBU loaded PLGA-PVA

copolymeric nanoparticles and evaluated for cytotoxicity utilising a 3-(4, 5-dimethylthiazole-2yl)-2, 5-diphenyltetrazolium bromide dye (MTT) assay and absorbance measured at 570 nm employing a multimode microplate reader. From the MTT assay analysis conducted, the results indicated that the IBU-PLGA-PVA- nanoparticles were less toxic to the HEK-293 cells compared to free Ibuprofen. The combined trials and results from the synthesis of IBU-PLGAPVA nanoparticles, showed evidence that these nanoparticles can be utilized as potential invaluable formulation for oral drug delivery of Ibuprofen with improved bioavailability and rapid relief of migraine at a low dose for a longer period of time.

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LIST OF ABBREVIATIONS

BBB	Blood Brain Barrier
CSF	Cerebrospinal Fluid
CNS	Central Nervous System
DMSO	Dimethyl Sulfoxide
FDA	Food and Drug Administration
FTIR	Fourier Transformation Infrared Spectroscopy
HCL	Hydrochloric Acid
HEK	Human Embryonic Kidney
MTT	3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyltetrazolium bromide dye
NP	Nanoparticle
PBS	Phosphate Buffered Saline
PDI	Poly dispersed index
PLGA	Poly (lactic-co-glycolic acid)
PVA	Polyvinyl Alcohol
SEM	Scanning Electron Microscopy
SLN	Solid Lipid Nanoparticles
T	Transmission
TGA	Thermogravimetric Analysis
USP	U.S Pharmacopeia
UV	Ultraviolet
WHO	World Health Organization

OVERVIEW

Chapter 1: Introduces the rationale of the study profiling migraine. According to the Global Burden of Diseases Survey, migraine is the third most prevalent disorder and seventh in terms of disability worldwide. Therapeutic agents targeting a site for clinical efficacy experiences challenges from low solubility; minimum absorption; low bioavailability decreasing their potential of reaching the desired target site. In this chapter, challenges to cross the BBB experienced by therapeutic agents are also discussed, such as molecular size and composition, lipophilicity and ability to be taken up by transport systems. Polymer nanomedicine is briefly described and presented as one of the mechanisms that can be used to overcome the BBB. This chapter summarises the results from the analyses conducted for proof of concept. The aims and objectives with motivation of the study are also explained.

Chapter 2: A comprehensive literature review on nanotechnological strategies employed to enhance bioavailability of therapeutic agents targeted to the brain is presented in this chapter. The challenges of the gastro-intestinal system before absorption and the restrictions at the BBB site before internalization into the brain are further described. Unlike nutrients and oxygen, most molecules and therapeutic agents have limited access through the BBB. The focus is on design of a drug delivery system for treatment of migraine and innovative mechanisms that have been employed to ensure therapeutic efficacy are outlined. Several strategies and nanotechnological delivery vehicles such as polymeric micelles, nanosuspensions, dendrimers, and nanogels are discussed including several transport mechanisms through the BBB.

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

Chapter 4: Provides a conclusion and future recommendations in which the study can be enhanced. Efficacious PLGA nanoparticles loaded with Ibuprofen were formulated, with significantly positive results from the physicochemical characterization, dissolution analysis

and in vitro studies. Cellular viability studies showed low toxicity and potential biocompatibility to physiological tissue. Further studies should be conducted on the combination of these Polymers, their behaviour *in vivo* and stability over time

CHAPTER 1
INTRODUCTION

1.1 Introduction

The Global Burden of Disease range the migraine condition as the third most prevalent disease and it is also regarded as the seventh highest cause of disability in the world (1). Migraine headache is a debilitating neurologic disorder, symptoms such as intense headaches and gastrointestinal disturbances describe the disorder (2). This type of headache has a duration of pain that can last up to three days with symptoms such as one-sided deep pain in the head, photosensitivity and sound sensitivity as well as gastrointestinal effects such as gastroenteritis. The main cause of migraine is not known; some people have frequent migraine attacks due to hereditary factors (3). The immediate goal in management of migraine is to achieve rapid pain relief. Numerous treatment options of migraine have proven to be efficacious in trials with placebo (4).

Currently, analgesics, anti-inflammatories and ergotamines as well as opioids are used for treatment and management of migraine headaches. Analgesic drugs such as paracetamol and aspirin and anti-inflammatory drugs such as ibuprofen are widely used. Most of the drugs used for treatment of migraine are formulated for oral delivery. Treatment in form of an oral liquid dosage for migraines can provide fast effective relief since it has a rapid onset of action as compared to solid dosage forms. An oral administration is the most preferred and convenient method to administer drugs for systemic absorption compared to injectable. An oral drug formulation in a nanoemulsion system can improve the bioavailability, rapid absorption and effective relief compared to a conventional oral solid dosage (5). Therefore, there is urgent need to design an efficient delivery system, such as an ionic nano-emulsion for fast effective relief of migraine pain.

A Nano-emulsion is an advanced drug delivery platform that can be designed to bridge the challenges encountered by commonly used drug delivery systems (6). Nanoemulsion, nanosuspensions and other different techniques for enhancement of solubility have since become the most promising tools in drug design recently to promote higher bioavailability (7). There are some studies performed on nanoemulsion preparation and these studies oral nanoemulsion delivery of aspirin-loaded and several nanoemulsion formulations generated by prototype utilizing the cavitation using ultrasound (8). In a study by Shafiq et al, it was reported that ramiprilat absorption was 2.94 times greater than conventional capsules and 5.4 times higher than suspension-formulated drug from a ramipril-loaded nanoemulsion and as a results, the system is commonly used in children and elderly patients (9).

A nanoemulsion refers to a heat stable system with two clear immiscible liquids, which are oil and water dispersed, and a surfactant molecule for stabilization (10). A nanoemulsion system, which consists of oil in water, is described as having small droplets of oil dispersed in an aqueous medium and the composition of w/o nanoemulsion is small droplets of water spread in an oily medium (11). These emulsions are thermodynamically stable of isotropic dispersions. Some of the advantages of a nanoemulsion are increase of drug absorption, enhanced bioavailability and the ability to incorporate both lipophilic and hydrophilic drugs.

Nanoemulsions can be prepared by using two types of methods, which include high and low energy methods. In the high-energy method, large disruptive forces are in non-equilibrium systems, while the low energy method are non-destructive and cause no damage to encapsulated molecules (12). In the high-energy method, a nanoemulsion is prepared by using mechanical energy input utilizing a homogenizers with high pressure and a stirring it at a higher shear with generators with ultrasound (13). This method requires high energy supplied in a brief period. High_shear stirring use employs high_energy mixers and rotostator systems to prepare the nanoemulsion system. An emulsification process with ultrasound in acoustic field form interfacial waves, which mixed the two phases together, and the ultrasound produced an acoustic cavitation resulting in breakdown of microbubbles due to changes in fluctuation, because of sound waves. This study explores the synthesis and characterization of nanoemulsions as a promising platform for the rapid relief of migraine headaches, thus improving patients' quality of life. This study aims to design an oral ionic nanoemulsion formulation with a rapid absorption and high bioavailability of drug to overcome setbacks of conventional oral solid dosage formulations such as tablets and capsules. The ionic nanoemulsion formulation of ibuprofen will be used in this study for further analysis of pharmacokinetic properties

1.2 Rational and motivation of this study

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

Therefore, controlled release properties of an oral nano delivery system will be beneficial for fast and effective treatment of migraine headache. Drugs formulated in tablets, capsules and powders delivered via an oral system formed in a solid form are used in the treatment of migraine in adults. Although these dosage forms provide an effective relief, their effects are not as immediate compared to liquid dosages. Oral solid dosage forms require disintegration and dissolution before drug permeability can occur.

Nanoemulsions provide a unique system for oral systemic release of drugs in the body and can be used to formulate lipophilic drugs consisting of a surface area that is higher and an energy that is free for effective transport. Nanoemulsions do not display creaming which is inherent, sedimentation, coalescence and flocculation challenges. Some of the reasons of employing nanoemulsions as a delivery treatment mechanism are increase in effectiveness of the drug at the onset of action, enhancing bioavailability of the drug, cell permeability, tagging of cell and tissue line and lastly imaging (14). Therapeutic agents should not be toxic and irritant and as a result damage human and animal cells. (15). The aim of this study is to design an oral ionic nanoemulsion for targeted treatment of migraine headache for fast effective relief from pain and is not currently available on the market for adult use. The formulation and administration route of nanoemulsions will be the key parameters to ensure that the drug molecules are clinically effective and reaches the targeted area with minimum side effects. The formulation will be subjected to physicochemical characterization as well as pharmacokinetic evaluation.

Following successful synthesis of the copolymeric nanoparticles, they will be analysed for their physicochemical properties using (FTIR), to assess the degree of crosslinking, evaluating various chemical bond shifts, responsible for particle solubility and drug entrapment efficiency. The size particle and zeta potential of the polymeric nanoparticles will be determined for surface charge receptor interactions, thereby designing an ionic equilibrated system, for high affinity receptor binding. The designed polymeric nanoparticles will thus possess the potential of delivering Ibuprofen with a lower drug-loaded dose, releasing drug in a controlled manner, with higher affinity drug uptake at the BBB site. As size of the particles affect drug release properties, this nanoemulsion formulation aims to deliver a controlled therapeutic profile, due to the incorporation of polymers ‘’Polyvinyl alcohol (PVA) and (PLGA)’’. The biodegradable polymers display controlled pharmacokinetic properties. The nanoemulsion will also be tested for stability profiles, thus subjecting the formulation to increased humidity, temperature and atmospheric pressure variations.

The prospect for this delivery system is for the nanoemulsion to be orally administered, absorbed through the intestinal mucosa, distributed in the blood stream and para/intracellularly pass through the epithelial cells of barrier between the brain and the blood when travelling to the brain, as illustrated in Figure 2.

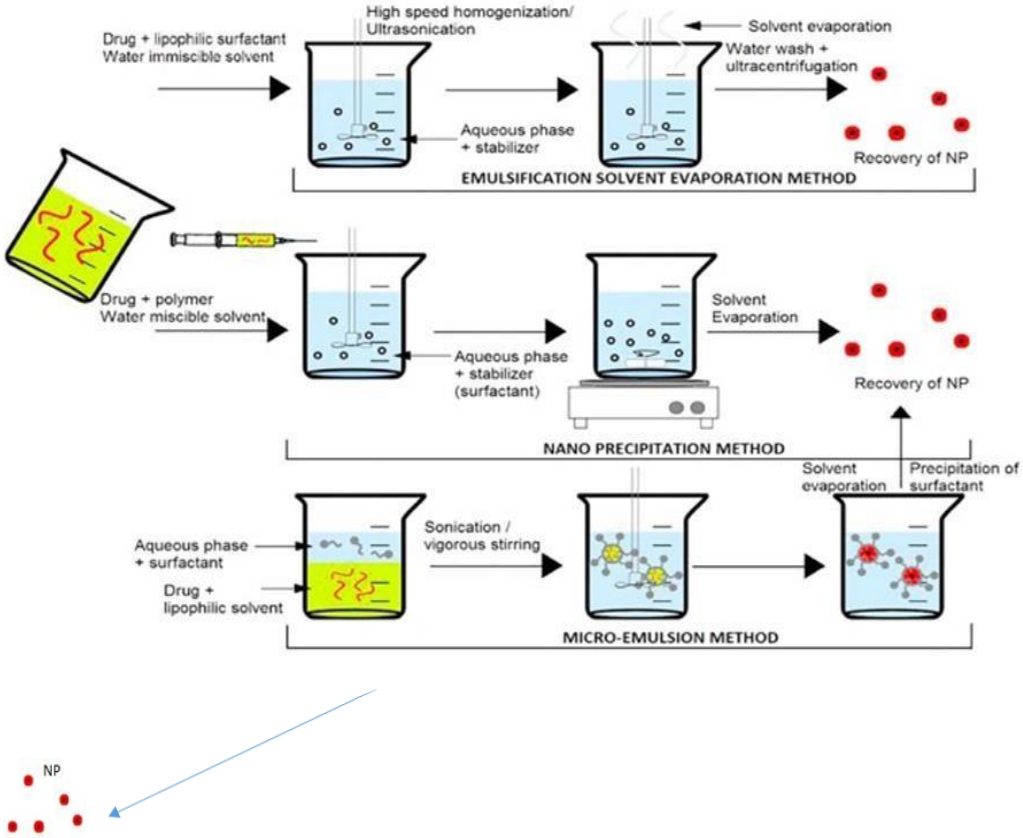


Figure 1: Schematic representation of the proposed process to prepare an oral ionic nanoemulsion platform for treatment of migraine

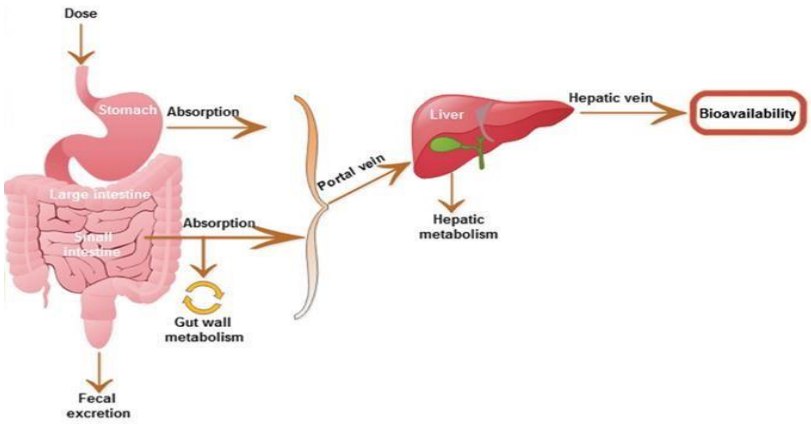


Figure 2: Schematic representation of pharmacokinetic process of the proposed drug

1.3 Aim and Objectives

1.3.1 Aim

End goal of the research is to design an oral ionic nanoemulsion drug delivery system, for controlled release of analgesic therapy employing ibuprofen, for the treatment of migraines

1.3.2 Objectives

1.3.2.1 To design a biodegradable Ibuprofen-loaded, nanoemulsion delivery system, using PVA and PLGA polymers, for particle size targeting range of 70- 90nm.

1.3.2.2 To evaluate the physicochemical properties of the nanoemulsion, employing Fourier Transformation Infrared (FTIR) spectroscopy, to characterize the composition structure at a molecular level and the extend of crosslinking of the polymer matrix.

1.3.2.3 To evaluate particle size and zeta charge analysis, to determine the uniformity and surface charge distribution of the nanoemulsion.

1.3.2.4 To determine drug entrapment efficiency and determine the drug release profile of Ibuprofen *in vitro*, employing a USP 2 apparatus.

1.3.2.5 To undertake thermal and morphological evaluation, evaluating the glass transition nature of the nanoparticulate delivery system and particle conformation properties respectively.

1.3.2.6 To evaluate the cytotoxicity of Ibuprofen-loaded nanoparticles on HEK-293 cells line by conducting an MTT assay.

The rationale of the study was introduced in this chapter profiling migraine. Migraine was defined and its therapeutic agent described concerning their clinical efficacies. Nanomedicine was introduced as one of the mechanisms that can be used to overcome challenges experienced by therapeutic agents used to treat migraine. Lastly, the aims and objectives with of the study were explained.

CHAPTER 2 LITERATURE REVIEW OF NANOTECHNOLOGICAL STRATEGIES AND BRAIN TARGETING

2.1. Background

Migraine is characterised by mild to severe episodes of headaches that are often throbbing and frequently unilateral. A migraine headache is best understood as a primary brain disorder that is characterized by an episode of attacks that can last up to 72 h'' (16). Neural events result in blood vessels dilation, which in turn cause throbbing and pain experienced during migraine attacks. Migraine attacks usually start from adolescence and is more common among women. The lifetime prevalence of migraines is at least 18% (17).

Migraine headaches often start with a slow, irritating pain at the back of the head and often worsen when one perform extreme activities such as running. Most often, duration of the migraine may last up to four hours to three days when is severe. A migraine headache may affect one side and later affect another side. One may experience migraine headache at least two to four days in a month and while some patients experience migraine for few days to a week. Migraine headaches are typically accompanied by dizziness, nausea, extreme sensitivity to lights, noises, and smells, lack of appetite and bowel dysfunction.

There are four phases of migraine, which differ with timeline and are associated with several symptoms and neurological disturbances observed during all phases of the disease. In chronological order, prodrome is the first stage, which can last few hours to days, experiencing nausea, depression, fatigue and irritability as most common symptoms followed by aura then the main headache and lastly the postdrome. In the aura phase visual disturbances, temporary loss of sight as well as numbness can be experienced, and this phase occurs for 5-10 minutes. The typical headache, which can last between 4-72 hours, is experienced with symptoms such as throbbing, nausea, vomiting, burning, insomnia, nasal congestion, and neck stiffness. Lastly, the postdrome phase lasts between a day to two days, and the inability to concentrate, fatigue, lack of comprehension are some of the symptoms experienced.

During the first phase of migraine in the early stages, fatigue, nausea and reduced vision may be experienced and this may be a sign of need to medicate immediately to avoid the migraine from exacerbating. Identifying the root cause as well as medicating in the early stages of migraine can assist in prevention of migraine in some people. Evaluation of timeline can assist in assessing the risk factors that can contribute to effects experienced following the postdrome phase and different migraine phases and symptoms.

The prevalence of migraine cannot be based on gender, culture or socioeconomic status specific. In the early 700 B.C migraine, episodes have been recorded (18). According to the World Health Organization, migraine headache is among the health related causes and is ranged the 19th cause. It is estimated that migraine affects an estimated 12% of the population and higher worldwide (18). More women than men experience migraine headaches and women carry a stronger genetic predisposition for the condition (19). In toddler boys around the age of 5 years and toddler girls aged between 12 -13 years there are cases of high migraine phases peaks. The aura phase is more prevalent and peaks in boy toddlers aged 10 -11 and teenager girls aged 14 -17 years (20). New cases were uncommon among males in their late 20s. Women in their ages between 40 and 50 years have more chance of experiencing migraine vertigo, suggested by a Hsu et al (21).

2.2 Pathophysiology of migraine: Mechanisms and Hypotheses

A migraine headache can greatly affect the quality of life. It is not the same as the usual headache, which most people experience. Pathophysiology of migraine is based on the anatomy and physiology of structures of the cranium that produces pain, which is integrated with knowledge of their central nervous system modulation. One of the most important aspects of the pathophysiology of migraine is the inherited nature of the disorder (9). Surrounding the large cerebral vessels, petal vessels, large venous sinuses and dura mater is the plexus of the large unmyelinated fibres that arise from the ophthalmic division of the trigeminal ganglion and the posterior fossa from the upper cervical dorsal roots (10). The key pathways for the pain are trigeminovascular input from the meningeal vessels, which passes through the trigeminal ganglion and synapses on second-order neurons in the trigeminocervical complex (11). These neurons, in turn, project through the quintothalamic tract, and after decussating in the brain stem, form synapses with neurons in the thalamus (12). There is a reflex connection between neurons in the pons in the superior salivatory nucleus, which results in a cranial parasympathetic outflow that is mediated through the pterygopalatine, otic, and carotid ganglia (13). This trigeminal–autonomic reflex is present in normal persons and is expressed most strongly in patients with trigeminal–autonomic cephalgias, such as cluster headache and paroxysmal hemicrania; it may be active in migraine (14).

Brain imaging studies suggest that important modulation of the trigeminovascular nociceptive input comes from the dorsal raphe nucleus, locus ceruleus, and nucleus raphe magnus (15).

Acute migraine attacks normally occur depending on the individual's inherent level of vulnerability (16). The higher the vulnerability the more attacks occur frequently. In the aura phase, there is focal neurological symptoms that can last up to an hour and symptoms such as visual, sensory or language disturbances may be experienced (17). After an hour of

experiencing the aura symptoms a typical migraine headache usually occurs with symptoms such as unilateral throbbing pain and nausea, vomiting and photophobia are usually associated with typical migraine headaches. If the migraine headache is not treated usually after 72 hours, the typical migraine headache can result in a resolution phase and this phase is characterised by deep sleep (18)

2.3 The Blood-Brain Barrier (BBB) pathophysiology and migraine

The blood-brain barrier (BBB) plays a very important role in maintaining the normal physiological function of Central Nervous System (20). With the rapid evolution of nanobiotechnology, nano-medicine has shown great potential in the therapeutics and diagnostics of neurological disorders, although the mechanism of many brain pathologies is still not fully exemplify, the leading reason is thought to be the BBB disruption (19) The BBB is formed by the endothelial cells lining the brain micro vessels under the inductive influence of neighbouring cell types within the neuromuscular unit including astrocytes and pericytes. The endothelium forms the major interface between the blood and the central nervous system and by a combination of low passive permeability and the presence of specific transport systems, enzymes and receptors regulate molecular and cellular traffic across the barrier layer (20). The BBB has a significant contribution to homeostasis and protection of the central nervous system but this barrier also limit therapeutic agents from crossing and this result in central nervous system treatment complications (21). There are several different methods and models to examine the BBB both in vivo and in vitro and these methods can provide important information about ways in which therapeutic agent works and these may lead to drug discovery, delivery and reduction of toxicity (22). There are several in vitro models now available to provide reliable predictions of penetration of drugs when testing central nervous system drug delivery (23). The use of nanocarriers has been seen to overcome the limitations of crossing the BBB.

The Central Nervous System (CNS) serves as central regulator of bodily functions including selective barrier in the brain, supply is through blood brain barrier. It plays a very important task in maintaining normal physiological function of CNS (22). If the speedily discovery and development of nanotechnology, there is a great growth in the discovery and development in nanomedicine and great chance has been discovered in the treatment and diagnostics of neurological disorders by employing nanomedicine. Although the mechanisms that underline several brain pathologies are not fully understood, disruptions of the BBB disruption have been thought to contribute to several neurological disorders (23).

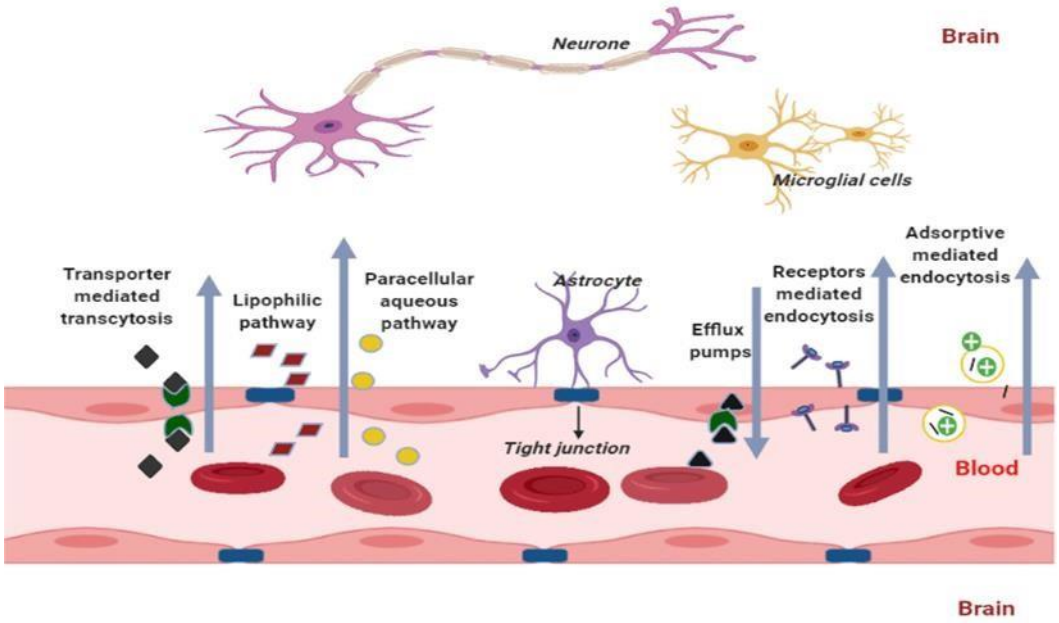


Figure 3: Transport pathways across blood brain barrier also illustrating BBB junctions and efflux pumps (23)

The cells called endothelia lines the small vessels of the brain, forms the blood-brain. ‘’The blood-brain barrier forms a very critical part in the protection including balance of the central nervous system. The BBB filter therapeutic agents and prevent some leading to complications during treatment (24). There are several different methods and models to examine the barrier though the *in vitro* system and *in vivo* system within the body and these methods can provide important information about ways in which therapeutic agents’ works and these may lead to drug discovery, delivery and reduction of toxicity (25).

There are many *in vitro* models now available to grant reliable predictions of penetration of drugs when testing central nervous system drug delivery (26). Nevertheless, there is a high accessibility to the brain with regards to nutrients and oxygen substances, the BBB prevents undesirable material to be transported through and it also with several ways, safeguard it from probable damaging substances such as bloodstream toxins. As a result, 98% of micro molecules and 100% of macro drug molecules greater than 500 Dalton (Da) are prevented from entering the brain allowing fewer drug molecules to penetrate through, based on their physico-chemical properties and molecular size. Lipinski’s rule described the characteristics required for molecules to gain excess through the BBB (27). This rule has proposed several predictive models for the qualitative assessment of drugs, which are not, absorbed easily (28). When there is more than 5 H-bonds donors, 10-H acceptors with a molecular weight of 500 da or more and the calculated Log P is not less than 5 then there is a greater chance of poor absorption. This rule is only for drugs that are non-substrates for active transporters and efflux proteins. The use of nanocarriers has been seen to overcome the limitation of therapeutics in crossing the blood-brain barriers.

Different models with physiological mechanisms can be utilized for delivery of the drug crossing the blood brain barrier changed in such way that kinetics and efficacy desired are achieved.

This study focuses on nanocarriers and the developed nanotechnologies for transporting pharmaceutical agents past the blood-brain barrier for improved therapeutic efficacy to target migraine in the brain area and assist in decreasing drug related side effects (Figure 2.1).

This objective of this literature review is to present recent advances in nanotechnological strategies as improved therapeutic intervention approaches for neurological diseases with particular focus on migraine. It also highlights major barriers to effective therapy. The reengineering of the polymeric nanomaterial as a drug nano-carrier system employed in crossing the BBB, drug therapeutic applications, and future prospects of the biomaterial are profiled.

2.3.1 Diseases of the Central Nervous System and treatment strategies employed

A complete range of common neurological disorders include meningitis, Parkinson disease, Alzheimer's disease, aneurysm, brain tumour, concussion, dementia, epilepsy, headaches and migraines, multiple sclerosis, psychiatric conditions (severe depression, obsessive-compulsive disorder), seizures and stroke (29). The treatment of migraine includes the use of drugs, which are not specific in treatment of migraine only, such as (NSAIDs). Drugs can arrive at the target site after being administered by oral route thereby requiring disintegration, through injections (intramuscular or intravenous) which are most rapidly released forms for drug delivery, as well as through nasal sprays, and suppositories (28). Currently, approaches to deliver drugs to the brain include non-invasive delivery systems for drug delivery e.g., nano therapeutics, invasive methods such as direct injections, and temporary disruption of the BBB are employed. Although, remarkable progress has been made in developing system of delivering drug for treating disorder of the central nervous system such as migraine, depression disorder, brain tumours, tuberculosis meningitis and HIV-neurocognive dementia, there is still a growing need for more innovative, safe and effective strategies(29). Some neurological disorders with corresponding therapeutic delivery strategies to combat them are concisely articulated in this section and in Table 2.1., respectively.

Table 1: Therapeutic strategies for brain drug delivery

Strategies	Advantages	Limitations	Reference
Viral vectors	Efficiency of the gene transfection is high brain is direct.	Endangerment, Injection of in the	(30)
Nanoparticles	Transportation to the brain is actively targeted;	Crossing the BBB	(31)
Exosomes	Transportation with gene to the brain; potential capacity to cross the barrier	Donor cells of exosome.	(32)
Active transporters employed for delivery BBB	Possible capacity to cross the barrier by blood systemic system	Aimed for micro molecules	(33)
Permeability enhancer of brain	Temporary open the BBB	Incomparable match among the findings of rodents and humans	(34)
Transportation through the penetration barrier as per condition of the disease	Ability to cross the barrier between the blood and brain	Evolution changes in barrier and processes knowledge shortcomings.	(34)
Routes of administration changes	Passes the barrier through nasal administration	Satisfactory at doses which are lower	(35)
Brain imaging/diagnostics nanoparticles	Improve imaging; passes the barrier along the peak-permeable barrier between brain and blood.	Pass barrier; recognize process changes in the barrier between the blood and brain	(36)

The currently available pharmaceutical treatments for migraine have adverse effects in some patients resulting in negative effect on patient compliance with continued treatment and high probabilities of relapse (38). Most common adverse effects of the treatment of migraine are gastrointestinal including diarrhoea or constipation, nausea or vomiting, dyspepsia and bloating. Other side effects include dizziness and nervousness. Apart from adverse effects, migraine drugs such as Ibuprofen face several challenges to reach the brain site of action and several strategies have been investigated.

A newer technique called transcranial alternating current stimulation was investigated by Haller N (37). This technique involves non-invasive introduction of gamma (40 Hz) frequencies. Transcranial Magnetic Stimulation (TMS), a non-invasive neurostimulation strategy which electrical stimuli is introduced by an electromagnetic coil, placed on the scalp and operated in

such a way that it produces a magnetic field, which have an effect on the firing of neurons (38). However, the treatment requires continuous therapy up to 25 repetitive sessions, with remission being a probability (39). There are also few studies conducted on the long-term effects of treatment using Transcranial Magnetic Stimulation TMS, although it has less side effects and more tolerable than ECT (39). A clinical trials review performed by Loo C (40) investigating the efficacy of TMS for treatment of depression found that with the extension of the treatment course efficacy has shown to be greater. The studies reviewed were limited in that they had small sample groups and the studies lasted to a maximum time of 2 weeks. The TMS treatment showed great effect over placebo but no significant clinical effect (40). A more recent technique with more favourable side effects, easy to administer and more cost effective is called the ‘’Transcranial Direct Current Stimulation (tDCS) and differs to TMS in that it cannot directly activate the neurons but manipulates the membrane potential of the neurons’’ (39).

Chronic Deep Brain Stimulation (DBS) is another technique discovered in the recent years; the technique is however invasive although has proven favourable results. The treatment requires electrodes to be implanted in the brain and is a chronic application. The electrodes function to regulate abnormal impulses. Vagus Nerve Stimulation (VNS) is another invasive technique, in which electrodes are surgically implanted into the brain (39). The techniques mentioned above although showing potential for treatment still requires intensive studies on effectiveness of treatment especially long term, Invasive therapies pose a risk of exposure to toxic substances to the brain, safety profiles on long term exposure to magnetic and electric stimulation is not thoroughly understood, and greater population size would need to be studied to determine efficacy (39).

Tong et al (42) investigated the potential of PLGA-chitosan nanoparticles loaded with desvenlafaxine, which is used in the suppression of depressive symptoms, for improvement of pharmacokinetic, and pharmacodynamics profile. Nanoparticles have been used to provide maintenance of the concentration of drug in the treatment range, and increase their duration in the body, enhance stability, improve solubility and permeability of drugs. These nano formulations also have the capacity to transport other functional. The nanoformulations in Table 2.2 are applied in the delivery of diagnostic or remedial substances/molecules to the CNS. These nanoparticles are detailed in the nanotechnological strategies section 2.5.

Table 2: Neurotherapeutics and nano-technological strategies for brain delivery (41)

Drug	Nano-technological Action	Strategies	Reference
Doxorubicin	PEGylated nanoliposomes	Enhanced tumour inhibition by preventing in vivo Doxorubicin potential	(43)
Efavirenz	Lipid solid nanoparticles	Improved bioavailability and brain targeting human immunodeficiency virus	(42)
L-dopamine	Cyclodextrinnanosponges	Controlled drug delivery in Parkinson's diseases	(43)
Elvitegravir	poloxamer-PLGA nanoformulation	Delivery of elvitegravir across BBB with the aim of halt HIV neurocognitive disorder	(44)
Cornell dots	Silica nanoparticles with a fluorophore, PEG-coated	Malignant brain tumours imaging	(45)
Dexmethylphenidate HCL	Nanocrystals	To halt attention-deficit/hyperactivity disorder	(46)
L-dopa	Gold nanoparticles	Parkinson's disease	(47)
Pretomanid	oil-in-water nanoemulsion	Tuberculosis meningitis	(48)

2.3.2 Factors that affect pharmacotherapy aimed for treatment to the brain migraine site

Delivery of therapeutics into the targeted regions of the brain can result in complications. This may represents the most far reached issue in medical neurology treatment (49). The transportation of medicinal agents to the brain has been of concern with low bioavailability of drugs in the brain.

2.3.3 Gastric absorption of therapeutic agents and cell permeability

Polymeric systems can be employed to reduce and control degradation and instability to improve the uptake of water like and oil like drugs in the intestines. Ability to cross through the stomach and subsequently, following reaching the intestine, release the drugs. Some treatments are able to shorten transit time, resulting in incomplete uptake with a bioavailability

that is low. The mucoadhesive systems have potential benefits, such as increased local drug concentrations, which is favourable to absorption, improving the drug effectiveness by maintaining their plasma drug concentration, and in some cases specially restricting absorption to a particular site in the intestine (50).

Bioavailability is defined as a quantity of the drug substance that enters the bloodstream after absorption and hepatic metabolism (51). The gastric pH, which is 1.2, 5-7 in the small intestines and 6-7.5 in the colon affects drug stability. Moreover, the large surface area of the small intestines and its enzymes create another barrier of drug absorption. The bioavailability of drug substances depends on their physicochemical characteristics shown in Table 2.3. Low molecular weight hydrophobic drugs readily pass through the intestinal wall through transcellular pathways, whilst hydrophilic drugs require paracellular transport. For a drug to pass through the intestinal epithelium, aqueous solubility and cell permeability are essential.

2.4 The Blood Brain Barrier

The CNS is well protected from the entry of substances that may be harmful, however, this provides an ideal environment for neuro-disease to replicate independently whilst the therapeutics are circulating in plasma (52). CNS is protected by two biological barrier systems known as BBB and BCSFB. The surface area of the BBB is 5000 times greater compared to BCSFB and for therapeutics that are administered orally or intravenously the BBB is the most important route for absorption into the brain (54). Tightly sealed joints of capillary endothelial cells form the BBB. Endocytic vesicles and fenestrae that are typically numerous in other organs are particularly fewer in brain capillaries. The tight junctions in the BBB significantly reduce small molecules passage through passive diffusion into the CNS and less than 2% of therapeutic agents can penetrate the BBB.

The movement of bioactive substances through the BBB depend on size, configuration, surface charge, and chemical composition. Only molecules smaller than 4 nm can passively penetrate the epithelial cells of the BBB (5). The restrictive transport mechanisms that control the entry of essential substances would be required for molecules not meeting the passive diffusion characteristics (53). For an effective passive penetration of nonfunctionalized therapeutic drugs across membrane, Lipinski's rule of physicochemical properties must be applied, Figure 2.2. "The permeability of molecules through the BBB is highly impacted by their size and physicochemical properties, especially via passive diffusion. There are five key physiochemical parameters, which require enhancement to provide an increased permeability of the BBB as per a Lipinski's "rule of five", (27).

Table 3: Factors contributing to the permeability of therapeutics across the BBB and BCSFB

Therapeutic Agent	Molecular weight (Da)	Protein binding (%)	Lipid solubility	Log *P*	Efflux transporters	References
Doxorubicin	543.5	74-76%	low	-0.5	P-gp, MDR1, ABCB5	(37))
Efavirenz	315.7	> 99%	high	4.6	BCRP	(54)
Curcumin	368.4	-	high	3.29	MRP2, BCRP	(51)
Elvitegravir	447.9	97-98%	high	5.3	P-gp	(49)
Dexmethylphenidate HCl	233.3	12 -15%	moderate	2.25	-	(55)
Daunorubicin	527.5	50-60%	moderate	1.83	P-gp	(56)
α-mangostin (α-M)	410.5	-	high	7.7	ABCG2	(57)
Desvenlafaxine	263.4	30%	moderate	2.72	P-gp	(58))
Pretomanid	359.3	93%	moderate	2.75	-	(59)

There are various transport mediums across the BBB for the transportation of essential molecules, solutes, enzymes, proteins and nutrients for normal functioning of the brain (Figure. 2.2). Although small water-soluble molecules can move across tight junctions of the epithelial cells and hydrophobic molecules readily passing through the epithelial cells, many molecules such as glucose, proteins and amino acids cannot pass through the BBB and require transport mediums.

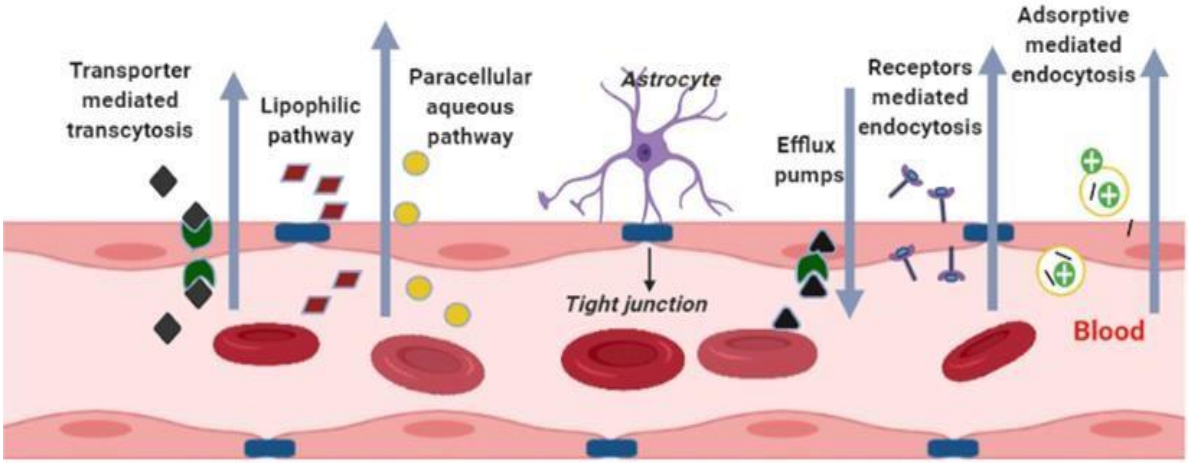


Figure 4: Carrier pathways across blood brain barrier also illustrating junctions and efflux pumps (60)

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

2.5 Limitations

Limitations of nanotherapeutics designed for oral CNS drug delivery include that when there is a change in size from micro particles to nanoparticles, these results in an increase in surface atoms numbers and decrease in size of the particle to a bigger extent. Interparticular friction and sticking are some of the shortcomings associated with larger surface area including small size particles.

The chemical reactivity of these particles result in augmentation formation as a result of an improved surface area, subsequently bring uncertainties with regards to the reactions of the particles at different conditions and its ability to pass through the cell membranes and invade the cells. The production of Reactive oxygen species (ROS) is brought up by an elevated level of chemical reactivity of nanoparticles, this may cause oxidative stress, inflammation, and damage to DNA, proteins and membranes, leading to toxicity.

2.5.1 Overcoming the Limitations

Nanostructures synthesized for brain delivery possess certain characteristics such as small size and high lipophilicity for permeability. These nanomolecules are limited by low solubility, rapid metabolism, and poor absorption. Traditionally, the main purpose of nano-strategies is to decrease normal tissue toxicity by improving drug specificity to target areas such as tumours (61). The effective non-invasive nano-strategies can enhance the permeability and retention

of therapeutics allowing passive accumulation in targeted areas such as tumour interstitium (61). However, suboptimal delivery is accomplished with most nanoformulations due to heterogeneities of vascular permeability, which limits nanoformulations penetration. Scientists have developed several strategies to overcome such hindrances, however, several of these strategies employed to deliver drugs to brain at the area required for action are classified under invasive and (non-invasive strategies. Some examples of the non-invasive strategies include aerosol sprays, metallic particles, liposomes, dendrimers and polymeric biodegradable particles (62).

2.5.2 Nanotechnological Strategies to combat the limitation of the brain delivery of therapeutics

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

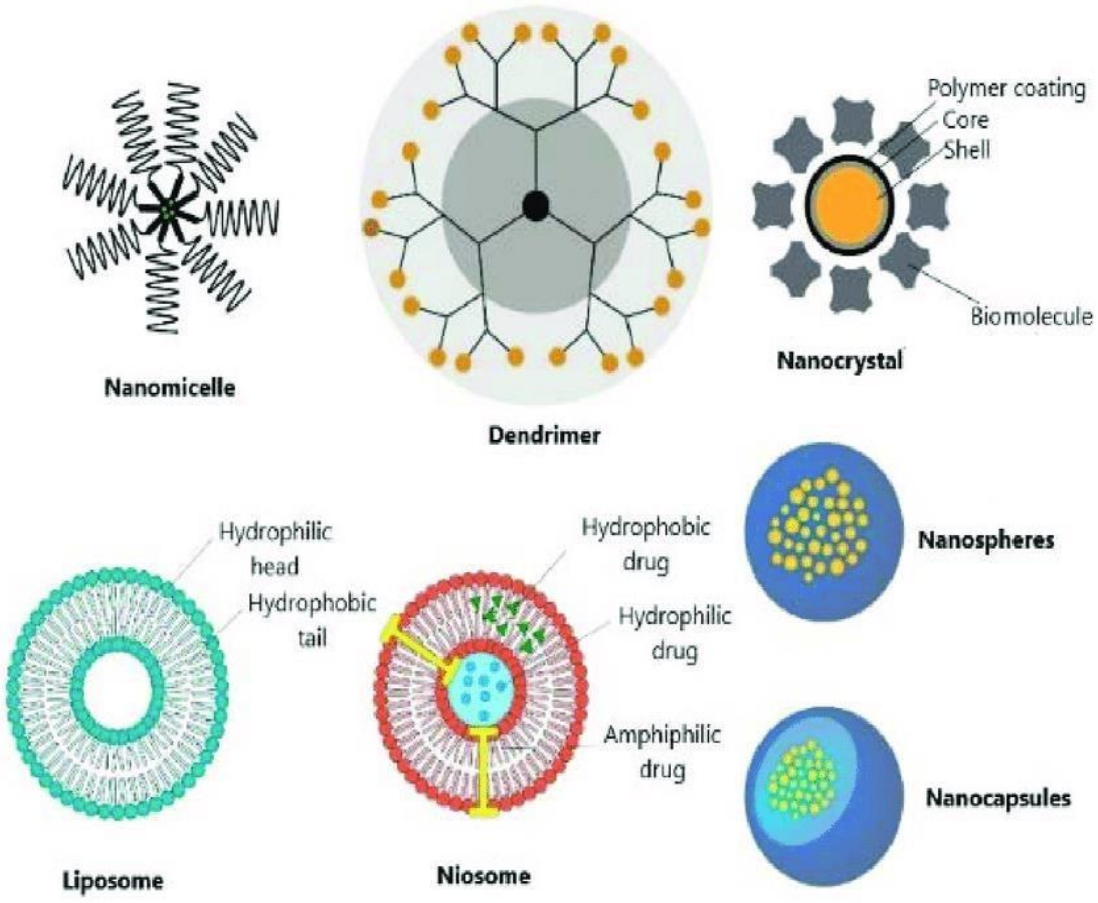


Figure 5: Nano-technological systems on basis of therapeutic delivery in brain targeting (63)

2.5.3 Self-assembled nano-constructs

The self-assembled nano-constructs consisting of amphiphilic block copolymers that are attracted by itself to build a core shell structure in the water solution. The water-dislike drugs can be added in the water dislike core part. Simultaneously hydrophilic shell makes the core stable by the whole system, which is soluble in water. The size of a self-assembled nanoconstruct is below 100 nm and usually have a lesser distribution to avoid rapid excretion through the renal, resulting on permeability of their build up in tumour tissues through the EPR effect. Additionally, non-specific interactions with biological components are restricted by their polymeric shell (64).

Drugs are loaded within self-assembled nano-constructs utilizing several methods such as direct dissolution process, solvent evaporation process, and the dialysis process. The copolymer and drugs combine in direct dissolution process in the water medium and resulting in micelles formation. An organic volatile solvent is utilized for dissolving the solvent in an evaporation process. Lastly, the co-polymer and drug in case of the dialysis process in the organic solvent are combined in the dialysis bag and then dialyzed with the formation of the micelle (65).

2.5.4 Dendrimers

Dendrimers are shaped in a globular shape and surface is functionalized in a well-maintained way, they are well bifurcated, with a single mix, well-pronounced and three-dimensional structures. Due to the globular shape, these structures are great potentials for delivery of drugs (66). They are able to carry both hydrophilic and hydrophobic drugs with loading capacity that is high due to their versatility (67).

Several therapeutic agents consist of numerous restrictions namely low water solubility and a limited blood circulation time, as a result it was found necessary to develop new drug delivery systems, which can deliver a drug efficiently to surmount BBB (68). These nanostructures are useful in prolonging the half-life of therapeutic agents, aid in solubility, permeability, stability of drugs and easily cross biological barriers. Dendronized polymers improve the loading of hydrophobic drugs.

There are two different ways the drug delivered by the dendrimers, firstly by the breakdown of the drug bonds called the covalent bonds with the dendrimer in the body depending of the enzymes availability and its suitability or favourable environment that could cleave the bonds and secondly by removal of the drug as a result to changes in the external environment like acidity or alkalinity, temperature (69).

The toxicity issues associated with this technological application in biological systems has remained a matter of concern until today. Dendrimers that are normally linear polymers such as polyethylene glycol (PEG), polyglutamic acid, polysaccharide, poly(allylamine hydrochloride) and N-(2-hydroxypropyl) methylacrylamide have been investigated as drug vehicles and accepted for clinical applications (70). Include examples of its use to treat migraine or, if not available, another neurological condition.

Polyamidoamine (PAMAM) dendrimers are one of the smallest and most precise nanomolecules available today, which have promising applications for the treatment of brain diseases.

2.5.5 Lipid nanoparticles

Lipid nanoparticles are an engrained formulation strategy to improve the drug delivery. They are vesicles of spherical form composed of phospholipids and steroids usually in the 50– 450 nm size range. These are considered as a better drug delivery vehicles since their membrane structure is different to the cell membranes and because they assist in incorporation of drugs in them (71). They have superior advantages over polymeric nanoparticulate system due to their lipid nature, permeability through the BBB more efficient than their polymeric counterpart has, but still require surface modification for enhanced permeation (72).

There are four sub-division of Lipid nanoparticles namely; conventional type lipid nanoparticles: these consists of a lipid bilayer that can make either anionic, cationic, or neutral cholesterol and phospholipids, which surrounds an aqueous core material (73). In this case, both the lipid bilayer and the aqueous space can be filled with hydrophobic or hydrophilic materials, respectively (74). PEGylated types: polyethylene glycol (PEG) is incorporated to the surface of lipid nanoparticles to achieve steric balance, ligand-targeted type: ligands like antibodies, carbohydrates and peptides, are linked to the surface of the lipid nanoparticles or to the end of previously attached PEG chains and theranostic liposome type: it is an amalgamation kind of the previous three types of liposomes and generally consists of a nanoparticle along with a targeting, imaging and a therapeutic elements (75).

Lipid nanoparticles have been studied to reduce side effects and improve the solubility of NSAIDs. For example, SLNs loaded with ibuprofen and a variety of matrix lipids, including stearic acid, tripalmitin, and trilaurin, have been demonstrated to increase the dissolution rate of ibuprofen. The SLNs containing trilaurin exhibited rapid ibuprofen dissolution within the first 30 min when tripalmitin was used. The dissolution rate increased, and all the ibuprofen could be released within 2 hour (76).

2.5.6 Nano crystalline particles

Nano crystalline particles are pure solid drug particles within 1000 nm range. There is no carrier attached to this particles and the surfactant or polymeric steric stabilizers are employed to stabilize this system. A nano crystalline particles suspension in a marginal liquid medium is normally improved by adding a surfactant agent. This surfactant agent is known as nanosuspension. The dispersing medium are mostly water or any aqueous or non-aqueous media including liquid polyethylene glycol and oils in a nanoemulsion system (77). Nano crystalline particles have certain characteristics that allows them to supersede certain difficulties like elevation of saturation solubility, elevation of dissolution velocity and improved glueyness to surface/cell membranes.

The process by which this small crystallines are made are separated into top-down and bottom-up approaches. The top-down approach includes, sono-crystallization, precipitation, high gravity controlled precipitation technology, multi-inlet vortex mixing techniques and limited impinging liquid jet precipitation technique (77). Nanocrystals in chitosan microparticles for pulmonary drug delivery of the hydrophobic drug. The nanoparticles were contrived for continuous release of the drug taking advantage of the swelling and muco-adhesive potential of the polymer. Ni et al found that inhalation efficacy might be conceded under the disease conditions, so more studies are needed to prove that this system has more potential (78).

2.5.7 Nanoemulsions

Nanoemulsions are thermodynamically stable surfactant molecules produced by simple emulsification method such as titration and by mixing oil, water, surfactant and co-surfactant. Curcumin is a drug used in the treatment of toxoplasmosis and can be prepared in a nanoemulsion to tackle the challenges of its poor water solubility and low bioavailability. Nanoemulsions is one of the promising drug delivery systems especially for those potent drugs whose clinical development failed due to their poor solubility. They provide controlled, sustained and targeted drug delivery. Nanoemulsions consist of oil in water emulsion with size of droplet ranging between 50 to 1000 nm (10, 42). The main purpose formulation of nanoemulsion is to deliver poorly soluble drugs to various sites at a rapid and effective rate.

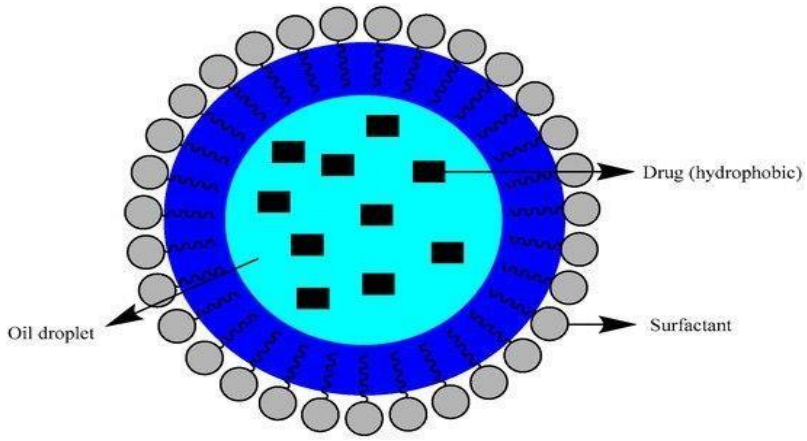


Figure 6: Nanoemulsion structure (7)

Formulation of nanoemulsion has many advantages such as an increase of drug absorption, enhanced bioavailability and the ability to incorporate both lipophilic and hydrophilic drugs and this makes them a future in improving the bioavailability od few drug (16). The constituents of a nanoemulsion are oil, aqueous phase and stabilizer.

The carrier of lipophilic active compounds, the lipid phase functions as the dispersed phase in the continuous phase (16). Three types of nanoemulsions are formulated depending on their components. First, the emulsion is comprised of oil dispersed in water (o/w), droplets of oil in a continuous water phase then secondly water dispersed in oil and this consist of droplets of water in a continuous phase of oil (w/o) and lastly a two-continuous phase which entails nanoemulsions consisting of microdomains of water and oil are interspersed inside the system. There are distinctive effects such as nano droplet size, great firmness with regard to stability, clear appearance and tunable rheology when it comes to nanoemulsions. The droplet diameter of the nanoparticles should be around 100 nm (14). The colour of the nanoemulsion is transparent. However, the milky white colour of nanoemulsion is because of controlling the droplet size. Nanoemulsions can be ruled comprising of a strong firmness with shelf life up for few years. Nanoemulsions are also comparatively less sensitive towards dilution and temperature. In nanoemulsion formulation, active drugs, additives and emulsifiers are included. There are different methods for preparation of nanoemulsion (13).

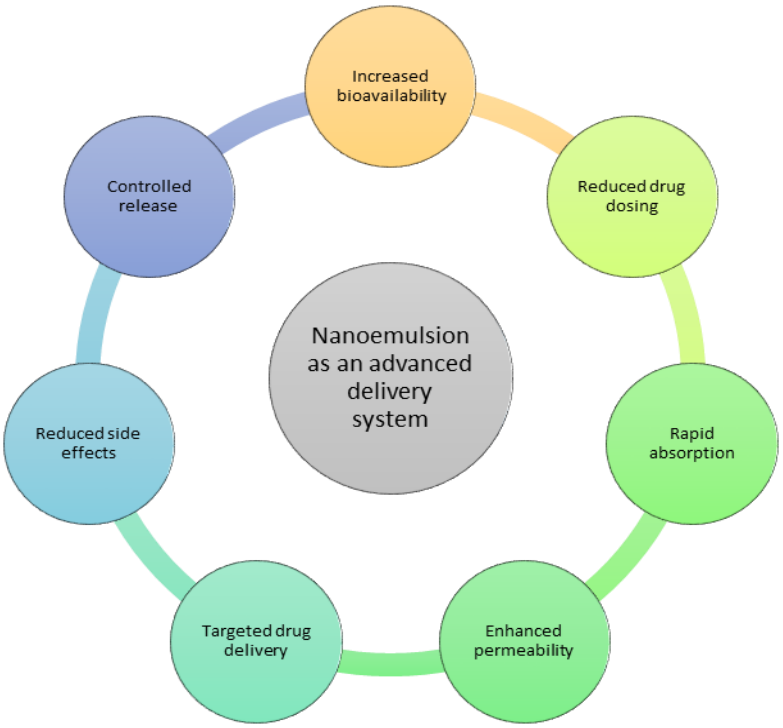


Figure 7: Features of nanoemulsion as an advanced delivery system

Nanoemulsions have small-sized droplets with greater surface area providing greater absorption. It can be formulated in variety of formulations such as creams, liquids, and sprays. It provides better uptake of oil-soluble supplements in cell culture technology. It helps to solubilize lipophilic drug and can be used in taste masking. Less amount of energy is required to produce nanoparticles formulated as a nanoemulsion. Nanoemulsion due to their high permeability level can be used in case of liposomes and vesicles substitute. It enhance drug bioavailability (5). It is does not irritant or cause toxicity in nature. Lastly, it is able to with stand physical instability when exposed to heat.

Several factors can be considered to successfully produce nanoemulsions with desired properties. These include ultralow interfacial tension, the concentration of surfactant and lastly, the surfactant must be flexible or fluid enough to promote the formation of nanoemulsion (79). Include examples of its use to treat migraine or, if not available, another neurological condition.

2.6 Transport Mechanisms for neurotherapeutics delivery into the brain

There are three different classes of endogenous transport systems, and these are namely, the Carrier Mediated Transport, Receptor Mediated Transport systems, and lastly Adsorptive Mediated Transport)

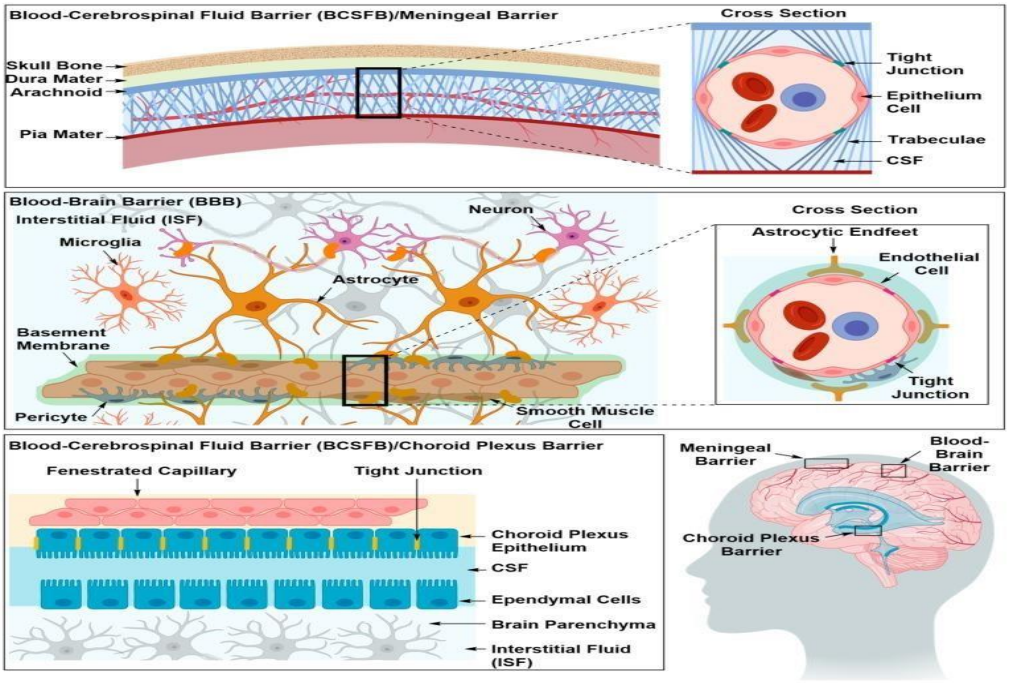


Figure 8: Possible drug pathways introduced into the nasal cavity and CNS, supplied with Olfactory and trigeminal nerve (26)

Barrier permeation allows drug transportation to the brain and is achieved via transporters in the barrier between the brain and blood and they are widely categorised into three types, including transporter-mediated delivery, adsorptive mediated transport, and receptor mediated transcytosis (Figure 2.6). The larger sum of nanosized formulations that can pass through the BBB utilize two main systems namely the RMT and AMT for delivery through the in the CNS consist of including a molecule which targets or bind to the blood molecules acknowledged by receptors presently at the brain cells called endothelium, mediating endocytosis or transcytosthe BBB (68). The archetypical approach used to enable the carriage of nanoparticles (Figure 2.6).

2.6.1 Carrier-mediated transport system

The body chemicals are included in carrier-mediated transport system and controls the two directional way of micro particle resources and vitamins among the blood and the brain (80).

2.6.2 Receptor-mediated transcytosis system

The receptor and TfR are included in the RMT systems as well as the carriers from the brain to the blood (80). The Receptor-mediated transcytosis system moderate the two-direction movement of sizeable-molecule peptides between the brain and the blood. The preferred size of the molecule drug and gene medicines are transported to the brain as they are allowed by the system.

2.6.3 The Adsorptive Mediated Transport (AMT)

The biological characteristics of the blood brain barrier consist of various transporters including glucose carriers, transferrin receptors, Nicotinic acetylcholine receptors, insulin receptors, lipoprotein receptors, amino acid carriers LAT1 by which several solutes including nutrients move across the BBB. Nanoparticles have ligands such as transferrin, nicotinic, integrin and use receptors for entry to the brain. AMT depends on ligand's static charge for interactivity with luminal exterior charges of the cells forming the barrier. Cells of the BBB contain negatively charged vesicles that attract cationic molecules.

2.7 Oral nanoemulsion for brain targeting specific to migraine

During literature review, it was noted that there are few to no studies conducted for oral nanoemulsion studies for brain targeting specific to migraine, this study will provide an importance advancement in the advanced oral treatment of migraine. In one study Samira Khani et al, developed a nanoemulsion drug delivery system to increase the oral bioavailability of mebudipine as a calcium channel blocker with very low bioavailability profile (81). The impact of nano-formulation on the pharmacokinetic parameters of mebudipine in rats was investigated. Nanoemulsion formulations containing ethyl oleate, Tween 80, Span 80, polyethylene glycol 400, ethanol and deionized water were prepared using probe sonicator. The optimum formulation was evaluated for physicochemical properties, such as particle size, morphology and stability. The particle size of optimum formulation was 22.8 ± 4.0 nm. Based on the results of this study, the relative bioavailability of mebudipine nanoemulsion was enhanced by about 2.6-, 2.0- and 1.9-fold, respectively, compared with suspension, ethyl oleate solution and micellar solution. In conclusion, nanoemulsion is an interesting option for the delivery of poorly water-soluble molecules, such as mebudipine. (81)

Studies on oral delivery of nanoemulsions employing the oral route are not common and there are has shown to avoid barrier and release the drugs into the CNS at a higher rate and extent (82). There is a better chance of reaching the cerebrospinal fluid (CSF) when nasal administration of drug is employed in administering into the nostrils and transported to the olfactory mucosa upon diffusion along the mucosa itself. There are three regions in the nasal cavity namely; the nasal vestibule, cavity proper or fossa, and lastly olfactory region'' (83) The intranasal route use has been utilized in previous studies and initiated to be a possible way of transferring therapeutic substances directly to brain targeted site of action by employing the trigeminal and olfactory nerve pathways across the nasal mucosa in order to bypass the BBB and its associated limitations. Intranasal administration gives advantage to drug delivery

because the drug is transported directly into the blood system and the brain resulting in the first-pass metabolism in the liver avoidance and bioavailability is significantly improved (76).

Administration of intranasal drugs is a conducive and suitable route for delivering drugs to the brain and CNS for the treatment of migraine headaches. Lipophilic compounds penetrate rapidly and effectively passing through the nasal membranes into the systemic circulation that shows the same pharmacokinetic behaviour to that of intravenous injection in terms of absorption with bioavailability of drugs up to a 100%. Because of this rapid action, lipophilic compounds hardly pass through the olfactory pathway in the nasal cavity.

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

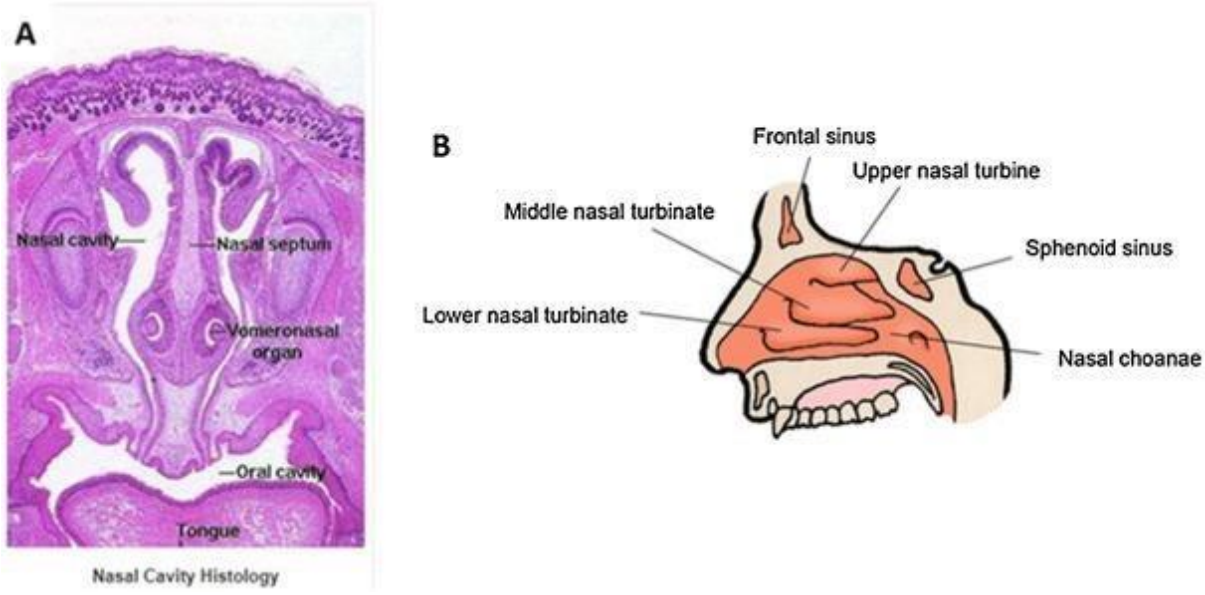


Figure 9: Anatomy of nasal cavity (84)

This present review discusses advancement of collection of prominent facts that embody the potential of nasal mucosa in targeted and site specific delivery of neurotherapeutic agents, mechanisms of drug absorption across nasal mucosa, metabolism of drugs in the nasal cavity and in brain tissue, various formulation strategies for enhanced brain targeting via nasal route, physicochemical factors affecting brain uptake of neurotherapeutics, various drug delivery systems explored to date for brain targeting through the nasal mucosa route and future opportunities. The list of positives and shortcomings of drug delivery via the nose to brain route are presented in Table 4.

Table 4: Advantages and limitations of intranasal drug administration to brain pathway (85)

Advantages	Disadvantages	Reference
Limited risks of infections	Damper for potent	(85)
Uncomplicated self-administration	Small volumes (25–200 µl in humans)	(86)
Relatively large absorption area (160 cm) in humans;	Operative mucociliary clearance	(85)
Sizeable olfactory epithelium area	Short retention time	(85)
Rapid absorption	Breaking down of the enzymatic by nasal cytochrome p450/peptidases/proteases.	(85)
Nasal submucosa profuse the vascular and lymphatic	Low permeability for hydrophilic drugs	(87)
There is no liver first-pass metabolism of the drugs	Absorption enhancers needed	(87)
Bypassing the blood brain barrier directly to deliver the drugs	Low nasal epithelial pH inter individual variability	(85)

2.8 Factors influencing nose to brain and its limitations

Nose to brain route usage has some restrictions, which should be accepted when designing novel therapeutics for administration via this route. Few restriction are noted on the quantity of the dose, a limit between 100 - 250 ml for liquids and powders 20 - 50 mg (in relation to the bulk density of the powder) resulting in this route as the only possible for potent drugs (88). Protection from degradation should be provided as well for drugs that are metabolized by nasal cavity enzymes and drug formulations must be non-irritant to the nasal cavity. Subsequently, delivery device is required to deliver drugs via the nose-to-brain route from a development point of view for nasal delivery.

Paths involving nerves joining the nasal passages to the brain and spinal cord are significant. Additionally, paths connecting the vasculature, CSF and lymphatic system are employed in carrying molecules from olfactory mucosa to the CNS. It is possible that a blend of these paths

is responsible; however, one of the paths may be the main contributor, which depends on the qualities of the neurotherapeutics, the attributes of preparations and the delivery tool utilized. Thus, the different modes of drug transport across the nasal olfactory epithelium include transcellular passive diffusion, paracellular passive diffusion, carrier-mediated transport, and transcytosis and efflux transport (89). The permeability at the site where the formulation is deposited and the area of the nasal cavity exposed affects nasal absorption of drugs. Absorption and permeability of drugs across nasal mucosa are influenced by various factors as illustrated in Figure. 2.7.

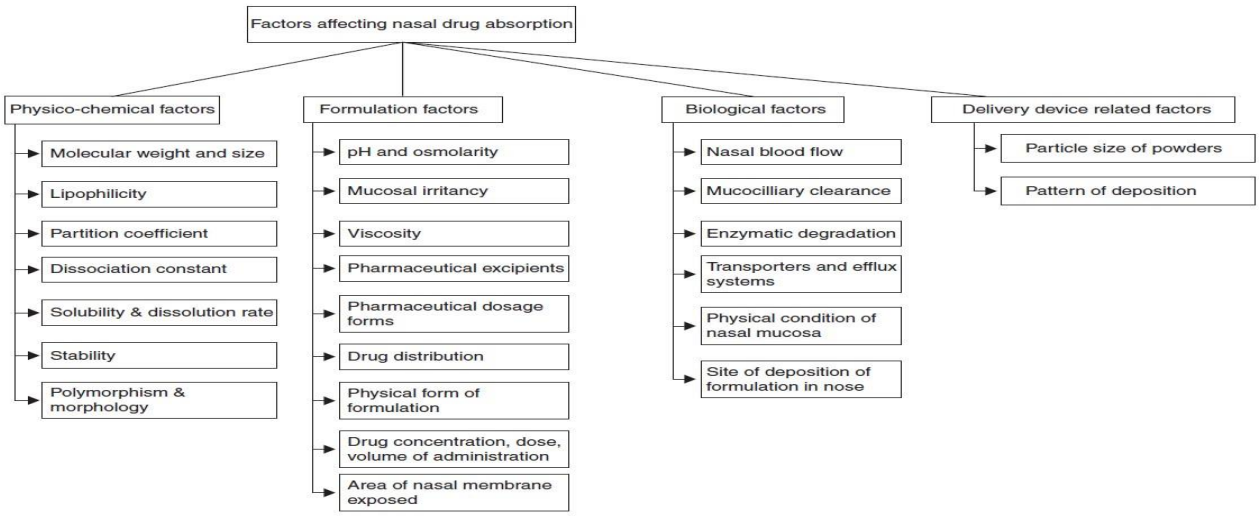


Figure 10: Factors that influence the absorption and permeability of drugs across nasal mucosa (86)

A variety of therapeutic agents loaded in various carrier systems are said to be utilizing the intranasal route of delivery for brain-targeted delivery. A brief summary of drug molecules, proteins, peptides, hormone and/or biological cells including stem cells and respective drug carrier systems delivered by direct nose to brain delivery route are illustrated in Table 2.5.

2.9 Drug delivery system patents employing the nasal route to the brain method

A high toxicity of the drug due to high concentration being present in the vicinity of the tumours due to delivery of the drugs via the nose. A study with a total participants (154), this involved 117 men and 81 women with primary glioblastoma multiforme, grade III astrocytoma (n = 26), and anaplastic oligodendroglioma (n = 5), the intranasal administration of perillyl alcohol, an antitumor agent, resulted in 19% survival in the cohort 4 years after dosing (77, 85) nose to brain. Figure 2.5 illustrates some nose to brain systems of drug delivery patents.

2.10 Shortcomings and adverse reactions

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

2.11 Other nanoparticles formulation for brain targeting specific to migraine

An innovative approach has been conceived by developing chitosan solid lipid nanoparticles (SLN) that contained sumatriptan succinate. This formulation is optimized in brain targeting to treat migraine. In the study, the optimization of the formulations was achieved by multi-level design factorial in order to obtain a minimize size particles with a high entrapment efficiency and drug concentrations. (3)

In the *in vivo* study, rats received a previously dispersed in deionized water, an oral formulation. The treatment with formulations showed a major availability of the formulation in the brain in comparison with controls. These results suggest that formulations orally administered consisting in hydrophilic drug Sumatriptan succinate, loaded in chitosan SLN, were able to cross the BBB, allowing the drug in exerting its pharmacological activity in the brain. Considering their data, nanoparticulate drug delivery systems might represent a future approach to cross the BBB and to improve brain targeting of medications in migraine therapeutics (90).

A nanoparticulate drug delivery system using poly (butyl cyanoacrylate) (PBCA) and bovine serum albumin linked with apolipoprotein Bovine serum albumin linked with apolipoprotein E3 (BSA-ApoE) was used in a study with the aim to evaluate the optimal therapeutic effect of the drug in migraine. Sumatriptan succinate was incorporated in the BSA-ApoE NPs and

compared with the same drug loaded polysorbate 80-coated optimized PBCA NPs to determine the brain uptake potential of these formulations (3).

New emerging classes of medications, including 5-HT receptor agonists (ditans), CGRP receptor antagonists (gepants) and receptor or ligand antagonists (monoclonal antibodies) are opening further options in therapeutics for acute and chronic migraine (3).

2.12 Concluding remarks for chapter two

The direction of Nanotechnology is headed towards advancement of delivery systems that provide safe and efficacious systems of delivery, that across BBB for the sufficient treatment of disorders of the CNS, in particular migraine. Bioavailability, minimization of adverse side effects and improved patient compliance are essentials components of drug delivery. Clinical migraine management relies on an officious drug delivery, which will deliver therapeutic agents to the localized site of action in the brain, at required concentrations and intervals with sustained drug release profile. Oral nanoparticulate system of delivery is particularly advantageous, as it provides non-invasive method of delivery, enhancing treatment of migraine disease with a fewer side effects.

The success of nano technological approaches has beneficial potential in effectively decreasing the percentage of active ingredient in a formulation and increasing drug circulatory retention time in the body. Receptor-mediated transcytosis aid in the transportation of nanostructures with specialized ligands such as transferrin, nicotine, and integrin discussed above, further enhance drug concentration in the CNS. Adsorptive Mediated transport that is dependent on surface charge is also responsible for successful availability of active drugs. Nose to brain delivery has been thoroughly investigated to offers an innovative and alternative potential for successful delivery of pharmaceutical agents, at lower doses, avoiding the hepatic system and providing instant action on targeted brain site. It would seem that oral nanotechnology for treatment of migraine incorporated in drug delivery is yet in its infancy, as there are not enough research and studies conducted previously for oral nanoparticles for treatment of migraine. Oral nanotechnological drug delivery system for treatment of migraine would present a worthwhile area of study, for safe, effective and efficient system that will revolutionize traditional medical therapy.

A comprehensive review on the literature of nanotechnological strategies, which are used to improve bioavailability of therapeutic agents targeted to the brain, was presented in this chapter. The chapter also highlighted challenges of the gastro-intestinal system before absorption and the restrictions at the blood brain barriers. Nanotechnological delivery vehicles such as polymeric micelles, nanosuspensions, dendrimers and nanogels were discussed including several transport mechanisms.

CHAPTER 3 DESIGN OF AN ORAL IONIC NANOEMULSION SYSTEM FOR TARGETED TREATMENT OF MIGRAINES

3.1 Introduction

Migraine is characterised by mild to severe episodes of headaches that are often throbbing and frequently unilateral. A migraine headache is best understood as a primary brain disorder that is characterized by an episode of attacks that can last up to 72 hours. Oral drug delivery systems are the most widely known and convenient route to administer various dosage forms for systemic therapy. However, some molecules display poor stability in gastrointestinal fluid and inability to cross the blood brain barrier. To overcome the barrier in order to allow central nervous system delivery, several strategies have been explored. Nanotechnology-based drug delivery systems demonstrate improved bioavailability, increased drug solubility and improved permeability.

However, because of the systemic use of Ibuprofen, side effects such as stomach ulcer, drowsiness and fatigue, dry mouth, altered sexual function are experienced. This often results in patient non-compliance, and possibility of relapse (91). Some of the most widely and frequently prescribed medications for migraine are naproxen, ibuprofen and diclofenac—the non–steroidal anti-inflammatory drugs (92). The main reasons for prescribing NSAIDs are to reduce pain, inflammation, osteoarthritis, rheumatoid arthritis, and musculoskeletal pain (93). Ulceration, bleeding in the stomach or intestine may result from long therapy of the oral ibuprofen. These adverse effects can occur at any time without warning symptoms especially for older patients. In In this study, Ibuprofen was used as the drug in the nanoemulsion formulation to reduce adverse side effects associated with the hepatic first-pass metabolism (94)

Ibuprofen (IBU), a NSAID, has a potent analgesic and antipyretic effects. Inflammation associated production of prostaglandins can be suppressed by IBU through the inhibition of cyclooxygenase-1 and -2 enzymes (95). Ibuprofen is absorbed rapidly and reaches peak plasma fast within 1 to 2 hours of ingestion and it is eliminated rapidly as it has a half-life of about 1.5 to 2 hours, which is prolonged in hepatic and renal diseases. In doses up to 1200 mg taken orally, IBU exhibits approximately linear kinetics. Biotransformation of IBU in the liver involves metabolism to well-characterized phenolic and carboxylic acid derivatives via cytochrome P450 (CYP) 2C8, CYP2C9, and possibly CYP2C19 activities, and to conjugates with glucuronic acid and taurine catalysed by uridine 5′-diphospho-

gluronyltransferases. Ibuprofen and its metabolites are eliminated through the kidneys, mainly as conjugates. Ibuprofen is also known to bind extensively to plasma proteins (95)

The rate of absorption in the stomach of Ibuprofen is accelerated by drug such as magnesium hydroxide, and this is due to changes in the gastrointestinal pH, without altering the extent of absorption or AUC (96).

The oral route of delivery is not always favourable, as some therapeutic agents encounter challenges of dissolution, permeability, and solubility in the gastro-intestinal tract (97). These factors hamper the bioavailability and efficacy of the therapeutic agent. The use of nanomedicine render an opportunity to achieve a higher efficiency in delivery of orally administered drugs (91).

Several nanotherapeutics delivery strategies for brain targeting have been developed including nanoemulsion, dendrimers, liposomes, polymeric micelles, nanogels, nanocapsules, vesicles, and carbon nanotubes. These nanoparticles have proven to be very successful in transporting drug molecules passing through the blood-brain barrier because of their small size less than 200 nm with improved solubility of drug, absorption and assist in decreasing drug related side effects. An immense potential to improve drug delivery can be achieved by employing these nanoparticle-based delivery strategies, as they can facilitate targeted drug delivery, controlled drug release, and improved stability.

This study set out to synthesize Ibuprofen loaded PLGA nanoparticles, formulated in a nanoemulsion and evaluate the potential for transport of Ibuprofen to the brain through the oral delivery route for improved efficacy with reduced side effects. PLGA, polymer fully known as Poly(L-lactideco-glycolide) (PLGA) (Fig. 2B) is approved for human use in the world's largest markets (98). It can be used employed as degradation protection for drugs in the stomach and can be incorporated with hydrophobic drugs (98).

Incorporation of Ibuprofen in the nanoparticulate formulation possesses an enormous potential for improving solubility and absorption of the Ibuprofen through the GIT wall. Ibuprofen-loaded PLGA nanoparticles formulation was synthesized by solvent emulsion evaporation method and synthesis was confirmed by physicochemical properties including (SEM), (FTIR), zetasizer, Ultraviolet, Thermogravimetric Analysis (TGA) for thermoanalysis. The synthesized IBU-PLGA nanoparticles were subjected into two pH environments simulating the stomach and small intestinal. Drug release was determined by dissolution and analysed by UV spectroscopy, and thermal analysis by Thermogravimetric analysis was conducted. In addition, HEK 293 neural then were treated with IBU-loaded PLGA nanoparticles finally

evaluated for cytotoxicity utilising a MTT assay reagent, assay and absorbance measured at a wavelength of 570 nm employing multimode microplate reader (Victor X3, Perkin Elmer, Massachusetts, USA) .

3.2 Materials and Methodologies

3.2.1 Materials

Ibuprofen, PLGA (low molecular weight), PVA (low molecular weight), chloroform, water (Milli Q), Ice and mannitol will be acquired from Sigma-Aldrich (St Louis, MO, USA). The other chemicals will be of analytical grade and utilized as received, these include Hydrochloric acid 1M and Phosphate buffer.

Magnetic stirrer, beakers, syringe (2 ml, 3 ml, 5 ml, 10 ml), needles 32G, Sonics (ultrasonic), centrifuge 5804, NE-300 just infusion syringe pump, freezer, settle plates, marker, Polytops and a pot. Immortalized human embryonic kidney cells (HEK 293) were purchased from Cellonex (Randburg, South Africa). 1XPhosphate-bufferred saline were all purchased from PAN Biotech (Bavaria, Germany). The MTT Cell Proliferation kit was procured from Sigma (MO, USA). Instruments that will be used include Ultrasonicator, pH meter, Zeta Potential Analyser, Ultraviolet–visible spectroscopy, FTIR spectrometer, Scanning Electron Microscopy and Thermogravimetric analyser and Microscopy for cell analysis are all purchased by University of Witwatersrand.

3.2.2 Synthesis of the Ibuprofen-PLGA nanoemulsion

The nanoparticles were synthesized by emulsification and solvent evaporation as described in Khani S et al (81). Ibuprofen was weighed, and 5 mg of the drug and 50 mg PLGA were dissolved in in 5 ml of chloroform to form an organic solution.

Aqueous solution of 10 ml of PVA 3% (w/w) (1.5 g in 50 ml) was prepared using a heated magnetic stirrer after which the PVA solution was brought to room temperature. The PLGA polymer solution was added dropwise using a syringe pump injected in a pure water-based solution of 3% of polymer, PVA 10 ml (0.167 as an organic solvent fraction), . The solution was emulsified for 1 minute at an amplitude of 75% (90 mm) in an ice bath utilizing Q sonica 500 Sonicator (Q Sonica, Newton). Ultrapure water (20 ml) was added to the mixture and an O/W emulsion was formed. The system was stirred with magnetic stirrer to evaporate the organic solvent at room temperature for 24 hours. The resulting mixture was centrifuged at 4750 rpm for 90 minutes. After centrifugation, the supernatant was separated. For removal of excess PVA, 10 ml ultrapure water was added and centrifuged at 4750 rpm for 30 minutes.

After centrifugation, the supernatant was separated and re-suspended by adding 20 ml of ultrapure water to the nanoparticles. Mannitol, at a concentration of 5%, was added as cryoprotectant and the formulation was stored in a freezer at -85 °C. Finally, the frozen nanoparticles were then lyophilized.

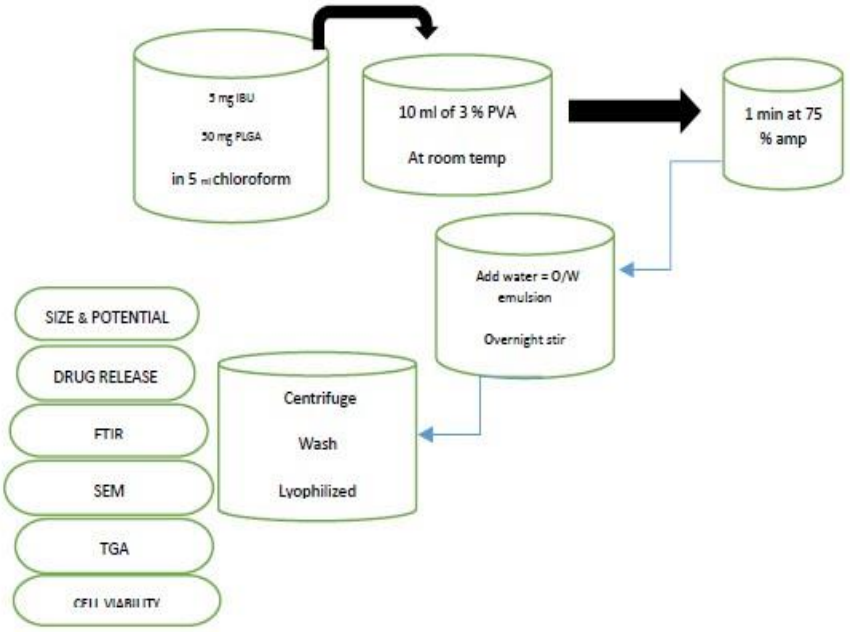


Figure 11: Schematic illustration of solvent emulsion evaporation synthesis and physico-chemical characterization of Ibuprofen-loaded PLGA-PVA nanoparticulate system.

3.2.3 Physiochemical analysis using Fourier Transformation Infrared (FTIR) Spectroscopy

The characteristics of the polymers and synthesized copolymer analysed using the (ATRFTIR) and the data were recorded. The characteristics of the polymers and synthesized copolymer, and possible chemical interactions between PLGA-PVA nanoparticles, loaded with ibuprofen were studied. Perkin Spectrum 2000 FTIR spectrometer was used studying characteristics of polymers and drug-loaded nanoparticles, it applied MIRTGS (Perkin Elmer).

Test samples were analysed at a resolution of 4 cm^{-1} using universal ATR polarization accessory. In a diamond crystal, samples were carefully placed and ran at 100 times to decrease the signal in the wavelength range of 4000–600 cm^{-1} at a constant pressure of 120 psi (99).

3.2.4 Establishment of the size of particle, Polydispersity Index and zeta potential

The characterization of the nanoparticles such as determination of size particle, zeta potential and size distribution, were performed by the use of the light scatter. Light scattering

angle of 90° and a temperature of 25 °C were utilized. Particle size was calculated as a z-average size ± SD, and polydispersity index was determined to analyse the width of the size distribution. In a beaker 100 µL of the formulation and 5ml of distilled water were mixed the solution was then filtered during 0.45 µm filter and analysed for particle size using the light scatter. Following that, the formulation was subsequently analysed for zeta potential.

3.2.5 Scanning Electron Microscopy (SEM) undertaken on the Ibuprofen-PLGA nanoparticles at various pH conditions

The prepared nanoemulsion was examined using the JEOL 840 SEM equipment (JEOL, Japan), under various magnifications at 20 keV. The formulation, in lyophilized form, was loaded on studs and sputter-coated with gold-palladium shadowing. Micrographs of the formulation were then critically evaluated; the surface morphology of the samples exposed to pH 1.2 and 6.8 phosphate buffer medium were determined, hence determining the pH sensitive nature and texture of the synthesized polymeric structures.

3.2.6 Thermal analysis undertaken on the nanoparticles employing Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) was performed on the Ibuprofen-loaded PLGA nanoparticles, PLGA nanoparticles and pure Ibuprofen, heated at 10 °C/min in an open aluminium pan, using nitrogen as a purge gas (flow rate of 25 mL/min). The percentage polymer mass loss over temperature heating conditions were evaluated

3.2.7 Determination of IBU loading efficiency of Ibuprofen inside the nanoparticle formulation

The concentration of Ibuprofen within PLGA nanoparticles was carried out using UV-VIS spectroscopy (100). Entrapment efficiency was calculated after determined the concentration. Samples were weighed, Ibuprofen-PLGA loaded (F1) and PLGA-only-loaded (F2). A mass of 10 mg from each formulation was weighed and mixed in phosphate buffer saline (10 mL) at pH of 6.8 and temperature of 37 ^{o}C. The suspension was then placed for 45 min at speed of 10 000 rpm under centrifuge. Using a filter with a 0.22 µm Millipore, the resultant solution was filtered through to remove the residue that may have not dissolved (58). The Calibration curve (Figure. 3.2.) was used to determine drug entrapment efficiency (DEE), at 265 nm utilizing the calculated linear Equations (1 & 2) where r^2 = 0.9981. The specific absorptivity can be used on the determination of drug concentration of released using the constructed standard calibration curve relating absorbance to concentration by Beer Lamberts Law of linearity (Equation 1).

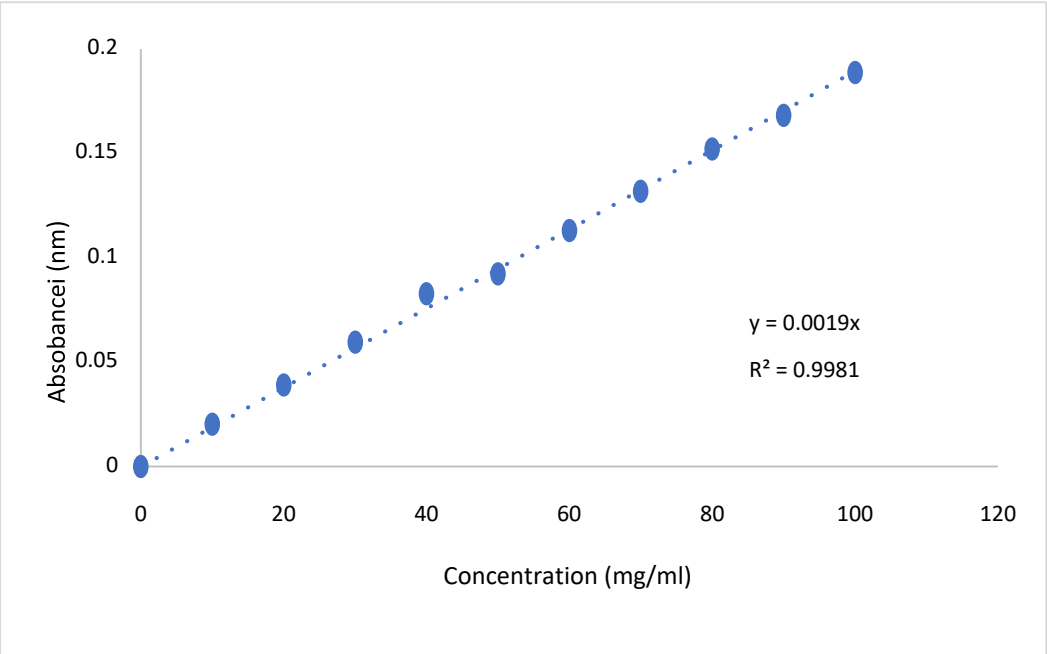


Figure 12: Calibration curve of Ibuprofen PBS pH 6.8 using Cecil 3021 scanning spectrophotometer at 265 nm (All instances; n= 3 and SD < 2.025)

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

Equation 1: Beer Lamberts Law of linearity

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

Equation 2: Ibuprofen Concentration

$$\text{Concentration of Ibuprofen} = y = mx + C \quad (2)$$

Equation 3: Drug Entrapment Efficiency

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

Equation 4: IBU loading Capacity

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

3.2.8 The analysis of nanoparticles *in ex-vivo* drug release

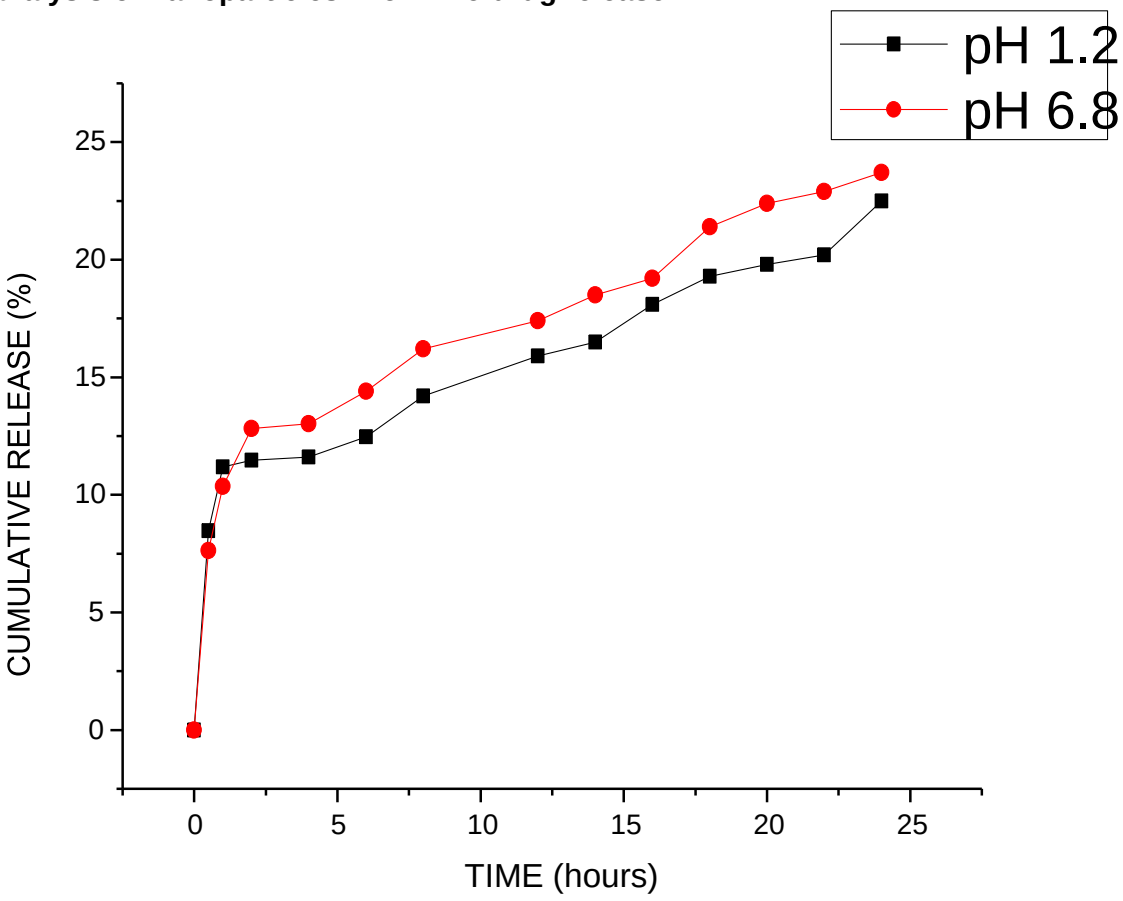


Figure 13: Ibuprofen release from Ibu-PLGA-PVA nanoparticles at simulated conditions of stomach pH and intestinal pH (1.2 & 6.8). Each individual point represent mean standard deviation (n=3)

Release studies of ibuprofen from PLGA nanoparticles in *ex-vivo* were determined by using a USP 2 apparatus operated at a paddle revolution of 50 rpm over a 24-h period. Ten milligrams of Ibuprofen-loaded PLGA nanoparticles was weighed, dissolved in 10 ml of 1X PBS and immersed in a dialysis membrane. The nanoparticles were evaluated in 0.1N HCL replicating the stomach gastric fluid that has a pH of 1.2, at a temperature of 37°C. A sample volume of 4 ml was withdrawn at different time points at half an hour initially the second hour and every two hours therefore and last after a day intervals at triplicate and 4 ml fresh HCL buffer was added to the remaining volume in the beaker. Thereafter, HCL was replaced with sodium phosphate at pH of 6.8 to replicating the small intestine conditions. A sample volume of 4 ml at triplicate was withdrawn at different intervals of half an hour initially the second hour and every two hours therefore and last after a day intervals and 4 ml fresh PBS buffer was added to the remaining volume in the beaker. An Ultraviolet spectrophotometer was employed to measure the

absorbance at wavelength between 265 nm, with standard curves obtained, reflecting results as a mean ± SD. HEK-293 cells are immortalized and are among the widely used cell lines in research (101). HEK-293 is a robust, fast-growing, and low-maintenance cell line with a variety of applications, including receptor signalling, cancer research, protein production, and CRISPR gene editing." Figure 3.2 with release curve in Figure 3.14 showing the concentration of Ibuprofen at different time intervals each point depicted as mean -+ SD (n=3).

3.2.9 Kinetic modelling of drug release of IBU-PLGA nanoparticles *in vitro*

Drug release kinetics were determined using Zero order release model, first order release kinetics, Fick's law of diffusion, Korsmeyer-Peppa's equation, and Hixson-Crowell equation, (Equations (5) - (9)). Statistics and Arithmetic were determined using Systat Sigma Plot software 12. The zero-order model (Equation (5) represents a stable release process.

Equation 5: The zero-order model represents a stable release process

$$Y = a_0 + \mathrm{K_0 t} \quad (5)$$

Equation 6: First-order release kinetics

$$Log\ Y = Log\ a_0 + \mathrm{K_1 t} \quad (6)$$

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

Equation 7: Fick's law of diffusion

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

Equation 8: Fraction of releasing the drug

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

Fraction of releasing the drug is represented by Mt/M and t for time, Mt represents quantity of the drug delivery at a time t, M∞ represent maximum quantity that can be released, t represents the period of release in hours, the kinetic constant is represented by k while the release component is represented by *n*.

Equation 9: Highest R2 value

$\sqrt[3]{Qa} - \sqrt[3]{Qa} = K_1 t$ (9)

The highest R^2 value determined from the kinetic model was chosen to represent the most appropriate model for authenticating the release of IBU from the nanoparticles.

3.2.10 *In vitro* cytotoxicity studies of drug-loaded (IBU-PLGA Nanoparticles)

3.2.10.1 Materials and reagents

Immortalized human embryonic kidney cells (HEK-293) were purchased from Cellonex (Randburg, South Africa). DMEM was purchased from PAN Biotech (Bavaria, Germany). The MTT Cell Proliferation kit was procured from Sigma (MO, USA) (101)

3.2.10.2 Cell line and culture procedure

The Ibuprofen (IBU), IBU-PLGA-loaded nanoparticle formulation and blank nanoparticles formulation, 5-Fluorouracil at 10 ug/ml as positive control and PBS as negative control compounds lastly the untreated cells were evaluated for cytotoxicity using the cell lines HEK293. HEK-293 was cultured in DMEM growth medium. The growth media was supplemented with 10% (v/v) Foetal Bovine Serum (FBS) and 1% (v/v) Penicillin-Streptomycin antibiotics. The cells were incubated at 37°C, and 5% CO_2 saturation under humid conditions until a confluence of 90% was reached, at which stage the cells were sub-cultured and allowed to reach the required confluence.

3.2.10.3 Subculture and seeding

Briefly, the spent growth medium in a T75 flask was aspirated and the cell monolayer was washed with 3 ml of 1X Phosphate Buffer Solution (pH=7.4). Next, 2 ml 1X trypsin-EDTA was added to cell monolayer and the flask was incubated at 37°C with a 5% CO_2 for 5 minutes in an incubator to allow for cell detachment. After detachment, the enzymatic process was stopped by adding 5 ml of fresh DMEM, pre-warmed at 37°C. The suspension produced of the cells was re-suspended, in a sterile 15 ml centrifuge tube the re-suspended cells were then transferred and lastly for 5 minutes centrifuged at 1500 rpm. Following the discarding the supernatant obtained from centrifugation, the pellet were then re-suspended in 1 ml of DMEM. Appropriate dilutions were made and noted for counting of cells using haemocytometer trypan blue exclusion method.

Equation 10: Calculation of percentage cell viability

$$\%\, Viable\ cells = \frac{Number\ of\ cells\ counted}{Number\ of\ Quandrants\ counted}\ x\ DF\ x\ 10^4\ cells/ml$$

4

HEK-293 cells were seeded in a 96-well plate at a density of 3.0 X10 cells per ml and a volume of 90 µl per well. Moreover, the 96-well plates were then incubated at 37ºC, 5% CO2 in a humidified atmosphere for 24 hours to ensure the adherence of the cells. Next, the cells were treated with 10 µl of Ibuprofen (IBU), IBU-PLGA-loaded nanoparticle formulation, blank nanoparticles and 5-FU as positive control making final concentrations of 100, 50, 25, 12.5, 6.25 and 3.125, 1.56 and 0.78 µg/ml. The cells were further incubated for 24 and 48 hours prior to microscopy and MTT cell viability assay.

3.2.10.3 Microscopy

Bright field images were captured in duplicates after 24 hours and 48 hours of treatment using the 20X objective of inverted light microscope (Olympus CKX53, Tokyo, Japan) and representative images were used.

3.2.10.4 Cell viability assay

The gauge of cell viability, proliferation and cytotoxicity can be assessed by conducting an MTT assay (50). After 48 hours of treatment, the MTT assay was performed. After 48 hours of treatment, 10 µl of the MTT solution prepared and added using a multichannel pipette. Following the addition of MTT, in an incubator for three hours the 96-well plates at 37ºC and 5% CO_2 under humid conditions were incubated. The formazan crystals that were formed as a result, these were then dissolved by adding 100 µl of the solubilizing agent and incubated overnight at 37ºC and 5% CO_2 for complete solubilisation. Triplicate wells containing only the growth medium and solubilisation reagent were used as the blank. The absorbance values were read at 570 nm with a reference wavelength of 620 nm using a multimode microplate reader. Mean values of the corrected absorbance measurements of each triplicate treatment were computed relative to the mean values of the control (set at 100% viability), treated with PBS only, and the data was plotted in a grouped bar chart.

Equation 11: Calculation of viable cell number.

$$\%\, Viable\ cells = \frac{Number\ of\ cells\ counted}{Number\ of\ Quandrants\ counted}\ x\ DF\ x\ 10^4\ cells/ml$$

4

3.2.11 Statistical Analysis

Origin software (version 8.5.0 SR1, Origin Lab Corporation, Northampton, MA, and USA) was used for processing and analyzing data for the preparation and characterization of nanoparticles and drug release studies. The mean standard error of three experiments was used to represent all results.

3.3 Results and Discussion

3.3.1 The synthesised nanoparticulate formulation utilizing FTIR spectroscopy characterization

The molecular composition and characteristics of the Ibuprofen-PLGA (IBU-PLGA) loaded nanoparticles were evaluated for their chemical characteristics. FTIR spectra of starting material PLGA, PVA and combinational blank PLGA-PVA nanoparticles are illustrated in Figure. 3.3. Comparable peak in PLGA and PLGA-PVA due to ester bonds is also observed at 2952. Figure. 3.4 illustrates the FTIR spectra of the blank nanoparticle, plain IBU and PLGAPVA nanoparticles. FTIR Spectral evaluation of Ibuprofen and Ibuprofen loaded nanoparticles shows comparable peaks the wavelength of 2952, 1702 first peak attributed C-O, while the second peak^{-1} is attributed to -CO stretch. Comparison of IBU-PLGA-PVA nanoparticles and PLGA (Figure. 3.3) depicts similarities in peaks at 2971 cm^{-1}. Comparing Ibuprofen FTIR spectra, depicted in Figure. 3.3 and the FTIR spectra for nanoparticles (Figure. 3.4.), they thus displayed distinct peaks noted at 3270 and 2632 cm^{-1}. The results of the FITR characterisation thus, indicate the synthesis of the complexed nanoformulation.

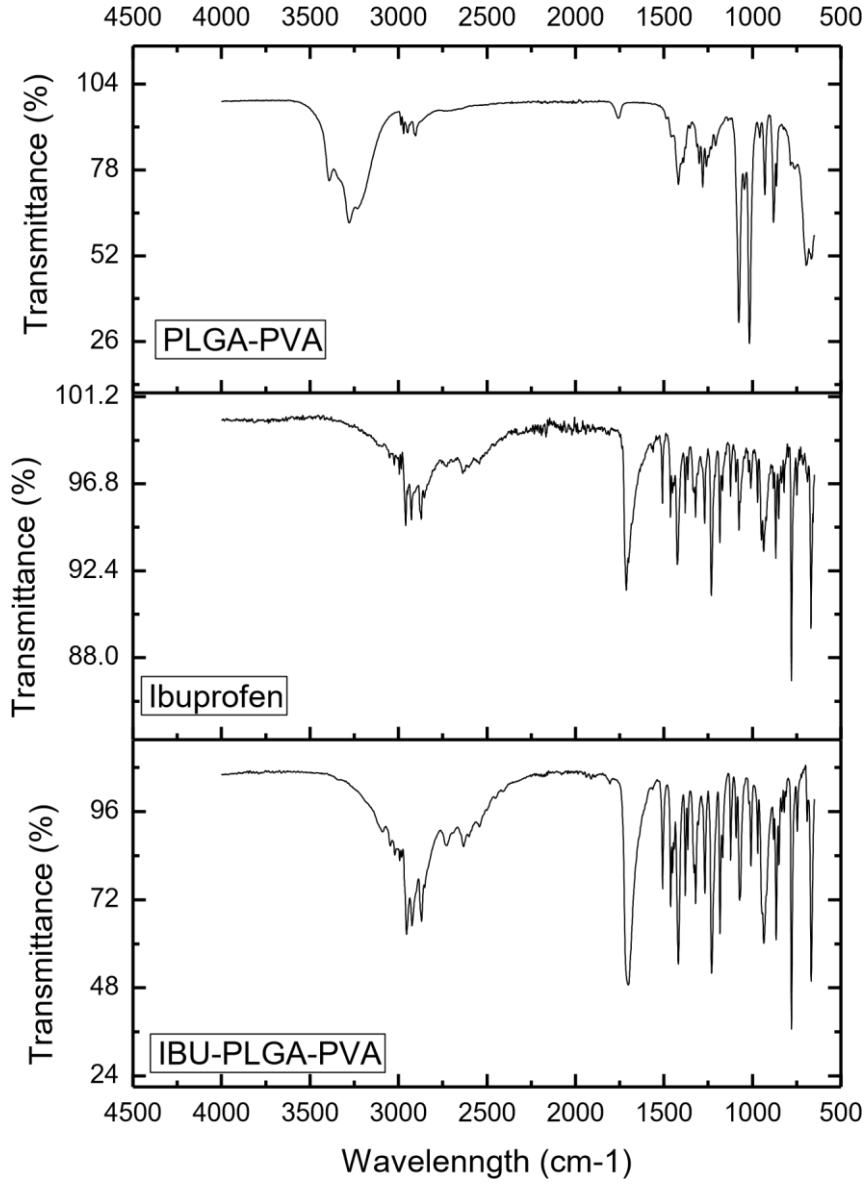


Figure 14: Fourier Transformation Infrared Spectra of Ibuprofen, PLGA-PVA nanoparticles and Ibu-PLGA-PVA nanoparticles

3.3.2 PLGA-PVA-IBU nanoparticles size, zeta potential and SEM morphological evaluation

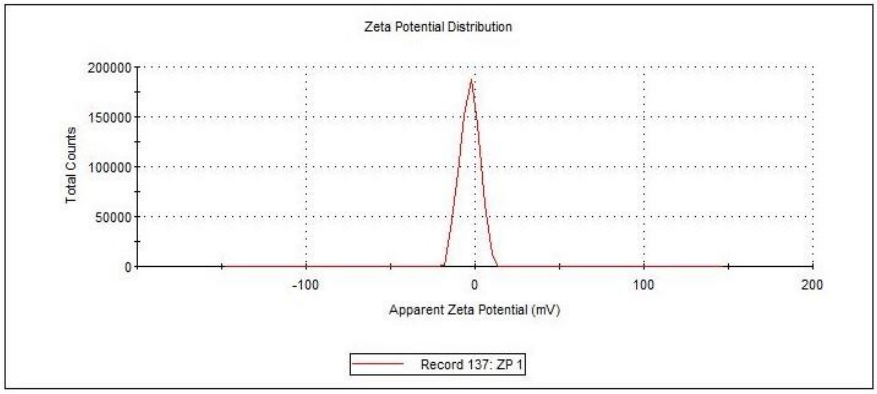
The PLGA-PVA (blank) nanoparticles and IBU-loaded nanoparticles were evaluated for size of nanoparticle and zeta potential employing the Zetasizer NanoZS analyser (Malvern Instruments Ltd. UK). The particle size and size distribution peaks are illustrated in Figure 5 with the size of the particle of 140 nm with polydispersity index (PDI) of 0.195. PDI less than 0.5 indicates good and narrow size distribution that is, perfectly uniform/homogenous sample dispersion and stability with respect to the particle size. The observed particle size for Ibuprofen-loaded nanoparticles in the first formulation, in Figure 6, is 128 nm with PDI of 0.190. The average particle size for drug-loaded nanoparticles was comparable to blank

nanoparticles, regardless of Ibuprofen-loading. Particle size of < 200nm is favourable for BBB crossing.

a

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -3.19	Peak 1:	-3.19	100.0	5.68
Zeta Deviation (mV): 5.68	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0155	Peak 3:	0.00	0.0	0.00

Result quality : Good



b

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -3.77	Peak 1:	-3.77	100.0	5.91
Zeta Deviation (mV): 5.91	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0210	Peak 3:	0.00	0.0	0.00

Result quality : Good

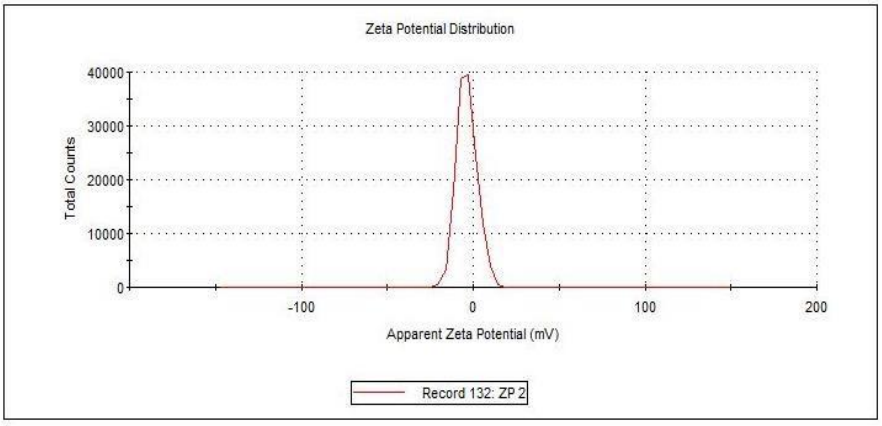
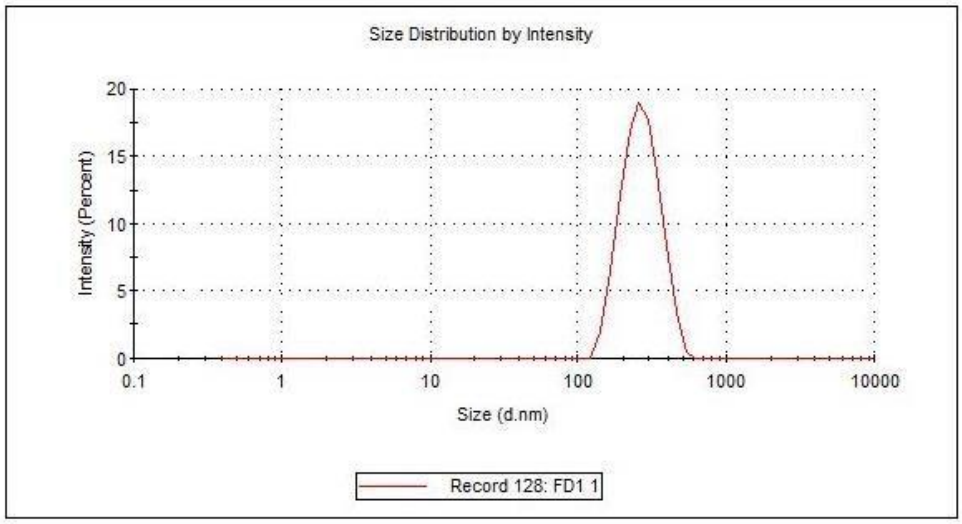


Figure 15: Zeta potential graph of (a) Blank nanoparticles and (b) Ibuprofen loaded nanoparticles

a

		Size (d.n...	% Intensity:	St Dev (d....
Z-Average (d.nm): 226.9	Peak 1:	272.5	100.0	77.42
PdI: 0.190	Peak 2:	0.000	0.0	0.000
Intercept: 0.861	Peak 3:	0.000	0.0	0.000

Result Good



b

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 277.0	Peak 1:	299.6	100.0	67.92
PdI: 0.048	Peak 2:	0.000	0.0	0.000
Intercept: 0.872	Peak 3:	0.000	0.0	0.000

Result quality : Good

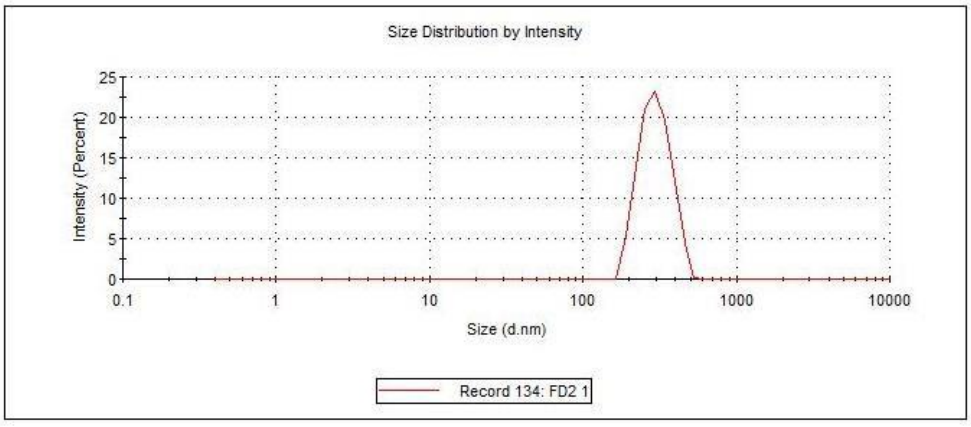


Figure 16: Particle size distribution graph of (a) Blank nanoparticles and (b) Ibuprofen-loaded nanoparticles.

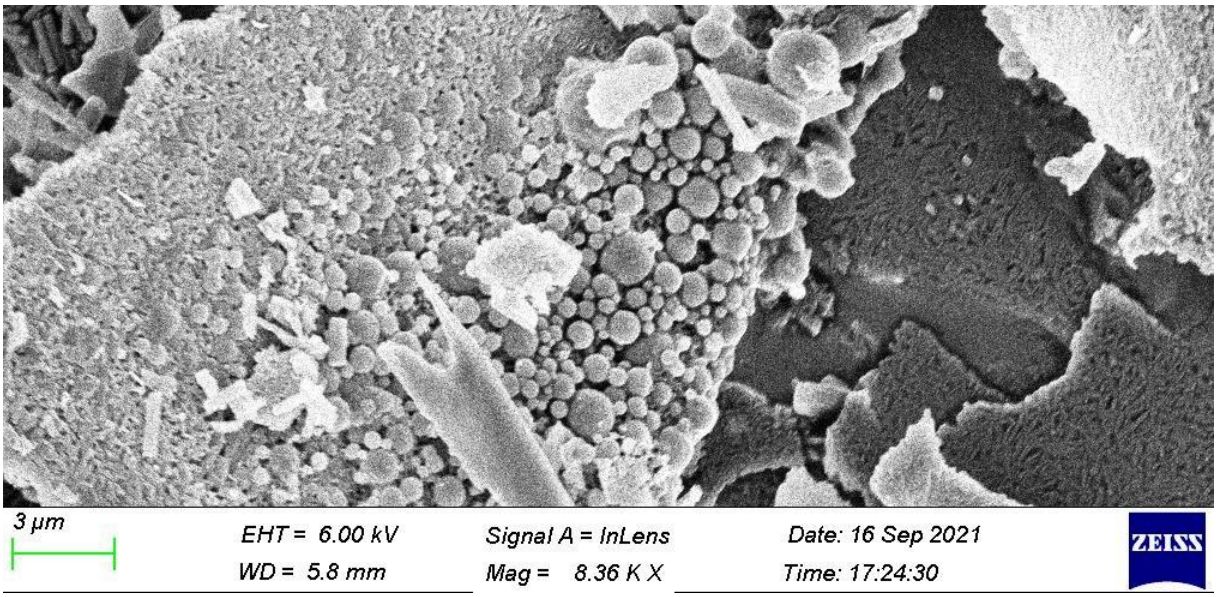


Figure 17: Scanning electron micrograph (SEM) of self-assembled Ibu-PLGA-PVA nanoparticulate formulation in dry state

Surface Morphology was investigated and characterized utilizing SEM. The size of the particle compared with Surface Emission Morphology images (Figure15); illustrate particles shaped like spherical with a small width in size distribution and particle size less than 100 nm. This observation shows a corresponding result compared to Zetasizer. The nanoparticles also show various agglomerated particles, this indicates good interaction between the nanoparticles.

The concentrations of the polymers used also affected the level of stability due to interference of cohesion (94) although aggregation of nanoparticles was also observed to be dependent on the concentration of nanoparticles used during characterization and application (102), at high concentration nanoparticle aggregated whilst small concentrations were evenly dispersed as shown with SEM image (Figure. 15). A positive charge provided by the PLGA improve the interaction with negative cell membranes.

3.3.3 Thermogravimetric analysis

Thermal stability studies were conducted as evaluation of integrity and changes in the mass of the polymeric nano-formulation during application of thermal stress. Thermal analysis is greatly utilized method for the study of stability of a pharmaceutical substance. Ibuprofen is known to be thermally unstable. Analysis was conducted to examine the magnitude of change in thermal stability properties of Ibuprofen incorporated in the nano-formulation. Utilizing the TGA, the thermal degradation of PLGA-PVA-loaded-IBU nanoparticle generated the thermogram showed in Figure 3.8 (red solid).

The thermograph for Ibuprofen shows a two-step weight loss, beginning at 40 to about 910°C, showing in the TGA graph at peaks 220 °C and 390 °C, the first and second step, which can be concluded that it may have been the reason for the A and B steps for degradation (Figures 15).

Ibuprofen break down initially started at the extrapolated onset of 200°C then moved to the next step, which began before first degradation step ended. The extrapolated end of the breakdown of Ibuprofen was at 220°C and the total Ibuprofen weight loss on heating from 40910°C was 78.84%. Blank nanoparticles show a two-step weight loss and degradation, the onset at 210-300 °C with total loss of 83.39% of weight, which is attributed to the copolymeric structure degradation. Comparison of IBU-loaded and blank nanoemulsion show a difference of a one-step degradation at 270 – 400°C, which can be attributed to the inclusion of the drug in the formulation. The inclusion of Ibuprofen into the nanoparticulate structure showed enhancement in the thermal stability of Ibuprofen.

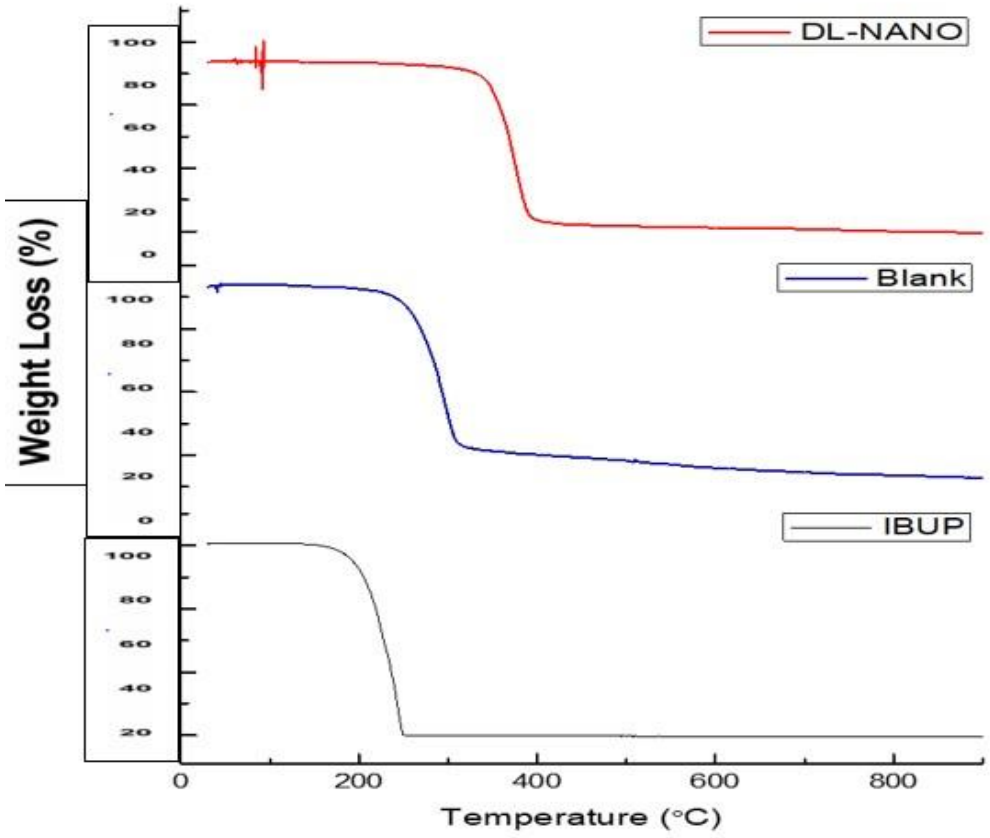


Figure 18: Thermogravimetric curves depicting the stability and thermal degradation of Ibu-PLGA-PVA nanoparticles (Blue curve), Blank nanoparticles (Red curve) and Plain Ibuprofen (Black curve)

3.4 IBU-PLGA drug-loaded Nanoparticles Studies

Drug-loaded Nanoparticles release *in vitro formulation* are shown in Figure 3.16. Ibuprofen release profile from PLGA-PVA nanoparticles at pH 1.2 in 0.1N HCL replicating the stomach gastric fluid that has a pH of 1.2, at a temperature of 37°C indicate that a sodium phosphate at pH of 6.8 to replicating the small intestine conditions. The study was conducted over a 24-hour period. Each point represents mean ± SD (n=3).

Ibuprofen is known to have an adverse effect in the stomach lining causing stomach ulcer and bleeding. The use of an oral ionic PLGA-PVA-IBU loaded nanoparticles formulated nanoemulsion may hinder the drug (Ibuprofen) from breaking down resulting in instability in the stomach acid reduce adverse effect experienced with the use of conventional oral Ibuprofen, improve bioavailability of the drug and potentially provide a controlled release drug delivery system.

The release of drug percentage was analysed up to a day at different pH mediums, representing the stomach environment and intestinal environment. Figure. 3.16 shows release of drug up to 25% after 24 hours for nanoparticles placed at a medium of pH1.2. It can be observed that the nanoparticulate system has protected Ibuprofen from the harsh pH environment. At pH 6.8, percentage drug release was up to 26%. Nanoparticulate formulation released drug faster at elevated alkaline pH, less drug was released at an acid pH.

3.4.1 Evaluation of Cytotoxicity of PLGA-PVA-IBU nanoparticles utilizing HEK 293 cells

Cell viability is ''the number living cells in relation to the sum of cells'' (101). HEK 293 is an immortalized human embryonic kidney cell line. Originally isolated in the 1970s by Alex Van der Eb, a Dutch biologist, postdoc Frank Graham transformed the cell line with sheared adenovirus 5. It was Graham's 293rd research experiment that 293 in HEK cells referred to. This cell line is rapidly intensively growing since its discovery in the 1970s and is mostly used in signalling receptors, for production of proteins in a larger scale and lastly for advancing research in different types of cancer (103)

Table 5: Advantages and Disadvantages of HEK 293 Cell line

Advantages	Disadvantages	Reference
Rapid doubling time and ease of culture.	Possible bacterial Contamination	(103)
General consistent and highly reproducible.	Possible viral Contamination	(101)
Highly efficient at producing large amounts of recombinant proteins	Limited culture Period	(101)
Gene Expression: HEK cells can be used for both transient and stable expression of desired genes.		(99)
Highly amenable to transfection and can be transfected using a variety of chemical and physical methods.		(101)

A cytotoxicity assay was conducted to evaluate the impact of PLGA-PVA-IBU nanoparticles on the viability of HEK-293 cells. A key advantage of this cell line is that results are generally consistent and highly reproducible and these cells were used in this test because HEK-293 cells found to have a low cost in maintaining them and easy to use, and are relatively unfussy in terms of their culturing requirements (103). HEK-293 cells are also reliable growth and propensity for transfection. They are able to produce large amounts of recombinant proteins since their immortalization. Cytotoxicity was evaluated in HEK-293 cells because they are tumorigenic, and they turn to form tumours hence cytotoxicity studies can be evaluated on this cell line. The study will not only evaluate the nanoparticles with the drug but it will evaluate if tumours are formed when the formulation is introduced.

The testing period was 24 and 48 hours. After 48 hours of treatment with Ibuprofen, PLGAPVA-IBU and blank nanoparticles at concentrations of 50, 25 and 12.5 µg/mL, bright field microscopic images were captured (at 10X) and the MTT assay was conducted as fully described in Section 3.2.11.

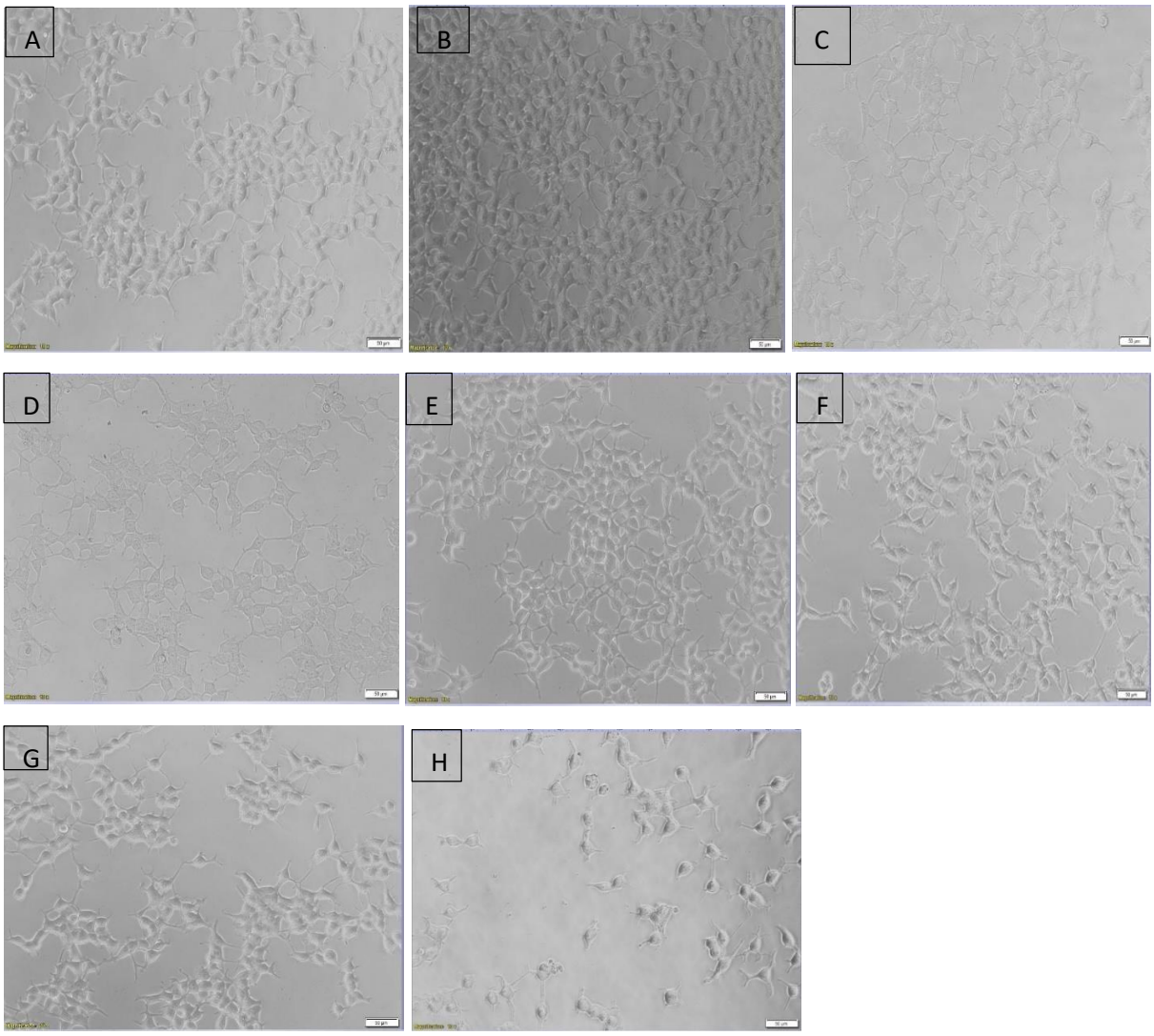


Figure 19: Olympus inverted Microscope Images of HEK 293 cell growth after 24 hours following treatment with (A) Ibu-PLGA-PVA 25 µg/ml, (B) Blank nanoparticles 25 µg/ml, (C) Ibu 25 µg/ml, (D) Ibu-PLGA-PVA 50 µg/ml, (E) Blank nanoparticles 50 µg/ml and (F) Ibu 50 µg/ml,(G) Untreated cells and (H) Positive control 5-Fluorouracil 10 µg/ml.

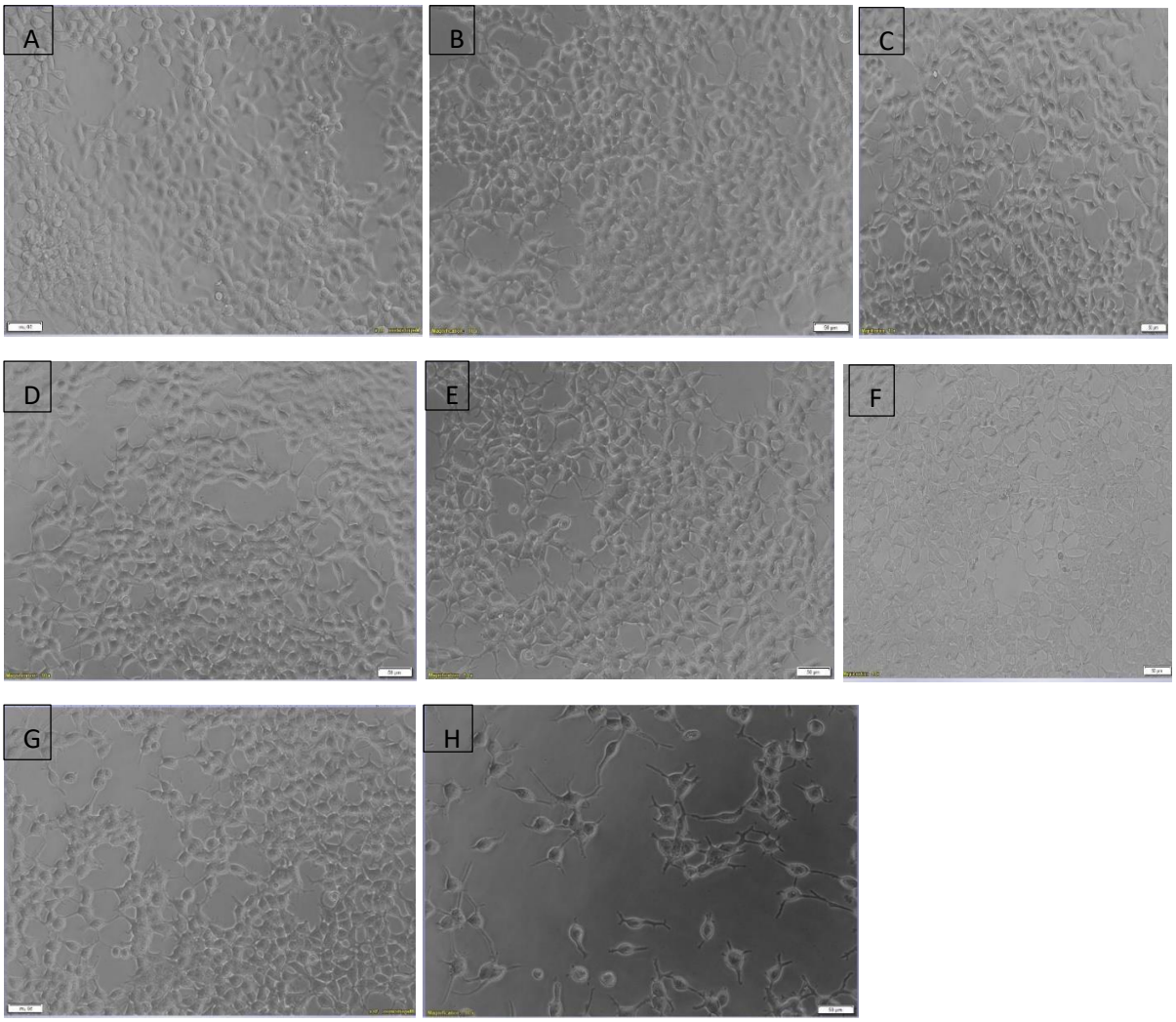


Figure 20: Olympus Inverted Microscope Images of HEK 293 cell growth after 48 hours following treatment with (A) IBU-PLGA-PVA 25 µg/ml, (B) Blank nanoparticles 25 µg/ml, (C) IBU 25 µg/ml, (D)IBU-PLGA-PVA 50 µg/ml, (E) Blank nanoparticles 50 µg/ml and (F) IBU 50 µg/ml, (G) Untreated cells and (H) positive control 5-Fluorouracil 10 µg/ml.

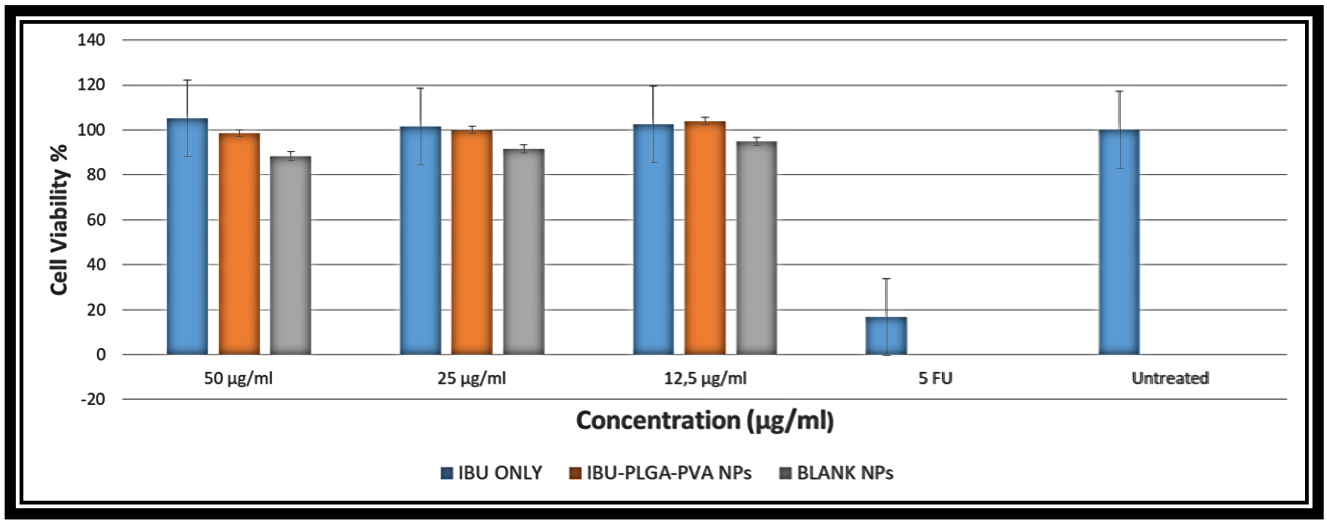


Figure 21: MTT assay for evaluation of the outcome of Ibuprofen-loaded nanoparticles (IBU-PLGA-PVA), blank nanoparticles and Ibuprofen plain drug on percentage viability of HEK 293 cells. The cells treated with various formulations were incubated for 48 hours, prior to evaluation of cell viability. Each percentage cell viability point represents an average ±SD (n=3).

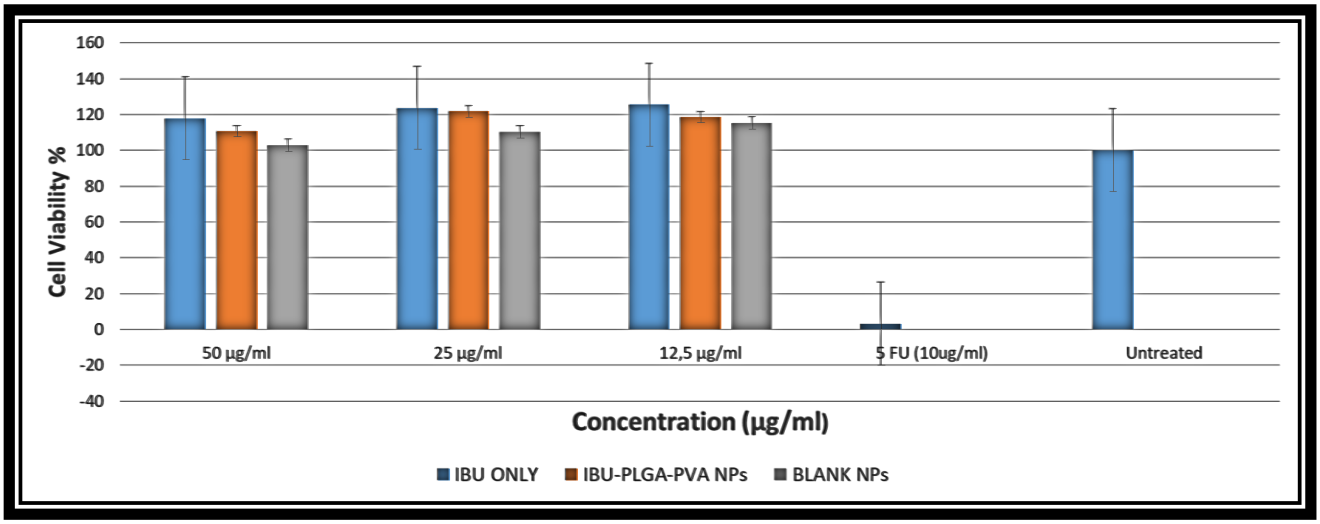


Figure 22: MTT assay for evaluation of the outcome of Ibuprofen-loaded nanoparticles (IBUPLGA-PVA), blank nanoparticles and Ibuprofen plain drug on percentage viability of HEK 293 cells. The cells treated with various formulations were incubated for 48 hours, prior to evaluation of cell viability. Each percentage cell viability point represents an average ±SD (n=3).

Figure 21 Depicts the graph for MTT assay for evaluation of the outcome of Ibuprofen loaded nanoparticles (IBU-PLGA-PVA), blank nanoparticles and Ibuprofen plain drug on percentage viability of HEK-293 cells. The cells were treated with various formulations were incubated for 24 hours, prior to evaluation of cell viability. Figure 19 displays light microscopic images of the HEK-293 cell growth after treatment with after 24 hours. All treated and untreated cells show comparable pattern of cell viability, the positive control cells treated with 5-Fluorouracil 10 µg/ml displayed a high cytotoxicity as expected. On the graph, the plain Ibuprofen-only drug shows a dose-dependent effect, in that at 12.5 µg/mL and 25 µg/mL cell viability was high, the assay was

102.36% and 101.53% respectively compared with untreated cells (set at 100%), and at 50 µg/mL Ibuprofen-only drug concentration, the assay shows a decline at 105.00%. The PLGA-PVA-IBU nanoparticles showed increased cell viability at 50, 25 and 12.5 µg/mL concentrations. The increase in cell viability was found to be dose dependent; at a lower dose, the cells were more viable. The blank nanoparticle treatment slightly inhibited growth compared with untreated cells this may be due to introduction of nanoparticles to the cells however this as a result of less cytotoxicity of the nanoparticles. The different concentrations caused a significant effect on the viability of the cell line.

The PLGA-PVA-IBU nanoparticles depicted an increase in cell viability when compared with plain Ibuprofen. This suggested that the PLGA-PVA nanoparticles are less cytotoxic than the pure drug as reported previously (11). PLGA-PVA-IBU showed less growth inhibition of the HEK 293 cells as compared to drug only (Ibuprofen only), hence suggesting that the nanoparticles are biocompatible. The data suggest that PLGA-PVA nanoparticles can be an effective and safe delivery vehicle for Ibuprofen for migraine headache treatment.

Figure 22 depicts the graph for MTT assay for evaluation of the outcome of Ibuprofen loaded nanoparticles (IBU-PLGA-PVA), blank nanoparticles and Ibuprofen plain drug on percentage viability of HEK-293 cells. The cells were treated with various formulations were incubated for 48 hours, prior to evaluation of cell viability. Figure 20 displays light microscopic images of the HEK-293 cell growth after treatment with after 48 hours. All treated and untreated cells show comparable pattern of cell viability, the positive control cells treated with 5-Fluorouracil 10 µg/ml displayed a high cytotoxicity as expected. On the graph, the plain Ibuprofen-only drug shows a dose-dependent effect, in that at 12.5 µg/mL and 25 µg/mL cell viability was high, the assay was 125.47% and 123.58% respectively compared with untreated cells (set at 100%), and at 50 µg/mL Ibuprofen-only drug concentration, the assay shows a decline at 117.86%. The PLGA-PVA-IBU nanoparticles showed increased cell viability at 50, 25 and 12.5 µg/mL concentrations. The increase in cell viability was found to be dose dependent; at a lower dose, the cells were more viable. The blank nanoparticle treatment slightly inhibited growth compared with untreated cells this may be due to introduction of nanoparticles to the cells however this as a result of less cytotoxicity of the nanoparticles. The two figures above indicate a high cell viability at 24 hours and a declined in viability percentage at 48 hours.

3.5 Concluding remarks for chapter three

The focus in chapter three was on the design of a drug delivery system for treatment of migraine, the design, materials used and methods employed in the synthesis of an ionic Ibuprofen-loaded nanoparticle were described in detail. The characterization of these nanoparticles, which were assessed by utilizing FTIR, SEM, Zeta sizer and Thermal analysis. The size and poly-dispersity index were found to be favourable. The synthesis of the nanoparticle was confirmed by assisting its morphological structure and thermal analysis study described the stability of the nanoparticle in relation to heat. Lastly, the cell viability study was conducted utilizing MTT assay and biocompatibility was confirmed.

CHAPTER 4
CONCLUSIONS AND RECOMMENDATIONS

4.1 Summary and conclusions

Based on the results obtained, Ibuprofen-loaded nanoparticles exhibit good physiochemical properties. Current therapeutics for migraine have limited effectiveness, with many facing challenges including solubility, absorption, low bioavailability, and decreased patient compliance. The BBB prevents to reach the onset of action in the CNS''. An estimated population of 12% is affected by migraine. The need for the development of new strategies to enhance site-specific targeted, non-toxic, drug delivery systems is vital in improving drug efficacy of migraine treatment. Nanotechnological strategies are of great interest in surmounting the limitations associated with conventional pharmaceutical drugs to the brain. FTIR characterization confirmed the integration of Ibuprofen, PLGA and PVA with assembly of nanoparticles. The nanoparticulate systems for brain delivery are less than 200 nm size to pass through barrier between the blood and the barrier, and this study report size formulated Ibuprofen-loaded nanoparticles was 140.6 ± 73.41nm with drug entrapment efficacy (DEE) value of 72% indicative of potential to improve the effect of the drug in relation to availability.

Ibuprofen is quickly absorbed, achieving peak serum levels one to two hours after administration and is highly protein bound. Ibuprofen nanoparticles presented a sustained release of Ibuprofen in a period of 24 hours as compared to free conventional Ibuprofen, which has mean plasma half-life 2 hours, and these findings can be useful for the treatment of migraine headache (95). Nanoparticulate formulation protected Ibuprofen from low pH environment simulating the stomach acid during the drug release studies, with only 20% of the drug released after 24 hrs. From the MTT assay analysis conducted, the results indicated that the IBU-PLGA-PVA loaded nanoparticles were less toxic to the HEK-293 cells compared to free IBU. Stability thermal studies of IBU-PLGA-PVA nanoparticles showed an improved stability profile of Ibuprofen, compared to plain drug. When combing the experiments and results from the formulation IBU-PLGA-PVA of nanoparticles, it is strongly suggested that these nanoparticles can be used as formulation which is invaluable for oral drug delivery of Ibuprofen.

4.2 Recommendations and future outlook

The solubility and functionality of lipophilic active pharmaceutical ingredient can be improved by use of nanoemulsions as the most promising systems. In recently published papers, promising expectations have been raised (104). Some functional groups such as aldehyde,

ketone, and esters for examples flavours and colouring compounds used in food, are susceptible to oxidative and photolytic degradation and encapsulation within nanoemulsions of these ingredients can prevent these deleterious effects and enhance its shelf life. Further studies are required which may include a wide range of nanoemulsion constituting active compounds and the nanoemulsion is used to elucidate the real benefits of nanoemulsions depending on the kind of lipid nanoparticle. The appropriate design and fabrication of NPs effectively surpass conventional oral therapeutic hurdles like by passing the blood brain barrier and gastric barriers.

Utilization of nanoemulsion in delivery of the oral drug delivery systems provides a well-known promising result by ensuring an enhanced effectiveness of drugs to the target site. The oral drug delivery system can bring about an improved bioavailability of the drug, improved ability to penetrate the cells and target the cells and tissues and last improve imaging and therapeutic functions.

In the pharmaceutical industry, several applications propose the use of Nanoemulsions are put forward for innumerable applications in pharmaceutical industry as a tool for delivery of drugs due to the ability to disperse non-polar active compounds (42). Nanotechnology has become an invaluable tool for targeted delivery because of its advantages including small size facilitating entry into the brain, the ability to load hydrophilic and hydrophobic drugs, improvement of stability profile, and enhancement of bioavailability and potential of decreased side effects.

In this study, Ibuprofen loaded PLGA-PVA nanoparticulate system was formulated for treatment of migraine headache, characterized employing techniques such as TGA, FTIR, SEM, zetasizer, Ultraviolet (UV) spectroscopy and MTT assay. This oral nanoparticulate system has a drug loading capacity which is higher, and the study performed *in vitro* Ibuprofen the profile of the drug was concluded to be appropriate. IBU-PLGA-PVA nanoparticles exhibited increased cell viability when compared with plain Ibuprofen. This suggested that the IBU-PLGA-PVA nanoparticles were less cytotoxic than the pure drug. Additionally, highly invasive conventional methods currently used in the treatment of migraine disorder can be replaced by oral nanoparticulate systems, which is, may not cause harm to healthy cells during treatment and may be employed in substitution of the invasive conventional system. Further studies should be conducted to optimize drug loading to provide uniform dosing and evaluate in-vivo behaviour of the IBU-PLGA-PVA nanoemulsion in preclinical studies and immunocompromised human cells to investigate immunocompatability. Further investigations such as conducting stability study placing the formulation in stability chamber with different environment conditions could be conducted to assess the long-term stability of the nanoformulation.

4.3 Next generation in Nanoemulsions

Nanoemulsions constitute one of the most promising systems to improve the solubility and functionality of lipophilic active food ingredients (37). However, the promising expectations arising from the recent publication data are based on few research papers. There is a need for further studies including a wide range of active compounds loaded in nanoemulsions to elucidate the real benefits of nanoemulsions depending on the kind of lipid nanoparticle (37). Despite lipid nanoparticles showing similar digestibility patterns compared to conventionally emulsions, their toxicological safety cannot be certainly assured. The biological path of lipid nanoparticles, once they enter the human gut, should be described to assess tissue location and possible toxicity (37). The appropriate design and fabrication of NPs effectively surpass conventional neurotherapeutic hurdles like crossing the barrier between the blood and the brain and gastric barriers (52).

The employment of nanoemulsion in oral drug delivery systems is regarded as a way to fully achieve results to bring promising results by ensuring the improvement in effectiveness of drugs to the onsite of action with enhanced drug availability, permeability and cell and tissue targeting (46). The therapeutic goal is to achieve a considerable drug concentration in the brain tissues to obtain desired therapeutic outcomes (53). The barriers, nanotechnology was employed in the field of drug delivery and brain targeting (7). Nanopharmaceuticals are a rapidly emerging sub-branch that deals with the drug-loaded nanocarriers or nanomaterials that have unique physicochemical properties and minute size range for penetrating the CNS (8). Additionally, nanopharmaceuticals can be tailored with functional modalities to achieve active targeting to the brain tissues. The magic behind their therapeutic success is the reduced amount of dose and lesser toxicity, whereby localizing the therapeutic agent to the specific site (9).

To overcome toxicity profiles of nanotechnological entities should be incorporated in future studies when consideration is made for upscaling and commercial development. Additional studies into nano-formulation long-term effects on the brain is a critical consideration and should be included in future developments. The above-mentioned will required diagnostic measures of safety and toxicity over an extended period of time and useful benefits that investing.

Chapter four provided a summary, conclusion and future recommendations in which the study can be improved. The formulated nanoparticles loaded with Ibuprofen were proved efficacious, with significantly positive results from the physiochemical characterization, dissolution analysis and *in vitro* studies. Cellular viability studies were proved low in toxicity and showed a potential biocompatibility to physiological tissues. The behaviour of the nanoparticles loaded with Ibuprofen should be studied in *in vivo* and its stability over time.

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