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COLLEGE OF HEALTH SCIENCE**

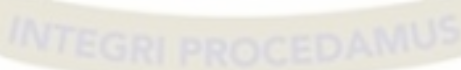


**PHARMACOKINETIC EVALUATION OF CHITOSAN COATED
HYDROXYPROPYLMETHYL CELLULOSE (HPMC) MICROPARTICLES OF
LEVODOPA AND CARBIDOPA**

BY

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DECLARATION

I, Benedicta Obenewaa Dankyi, hereby declare that this project, aside other cited works, is the outcome of my research carried out under the supervision of Dr. Seth Kwabena Amponsah and Prof. Henry Nettey. This work has not in part or whole been submitted and accepted elsewhere for the award of a degree.

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ABSTRACT

Background: Levodopa, a prodrug of dopamine, remains the gold standard in the treatment of Parkinson's disease. Current levodopa drugs are formulated in combination with an aromatic amino acid decarboxylase inhibitor, carbidopa, to prevent peripheral metabolism of levodopa. However, chronic use of levodopa is associated with potentially disabling motor complications and side effects, which arise from variable plasma concentration of the drug. Several attempts have been made to improve the formulation and drug delivery of levodopa with the aim of providing constant plasma levels in order to improve the drug's efficacy. Microparticulate drug delivery systems have shown potentiality to deliver drugs at their target sites over a long of period of time while maintaining constant plasma concentrations. The aim of this study was to formulate and evaluate the pharmacokinetics of chitosan coated hydroxypropylmethyl cellulose (HPMC) microparticles of levodopa and carbidopa using *in vitro* and *in vivo* models.

Methodology: Microparticles were formulated by encapsulating levodopa/carbidopa powders in HPMC using the spray-drying method. The levodopa microparticles were evaluated for size, drug content, percentage drug loading capacity, encapsulation efficiency and *in vitro* release profile. For pharmacokinetic evaluation, Sprague Dawley rats were administered either levodopa/carbidopa powder, levodopa/carbidopa microparticles or Sinemet CR (a controlled release formulation of levodopa/carbidopa). Blood samples were collected after predetermined times after the third dose. Plasma was obtained from blood and levodopa levels determined by high performance liquid chromatography. Pharmacokinetic parameters; maximum plasma concentration (C_{max}), the time it takes to achieve this peak (T_{max}), area under the curve (AUC) and half-life ($t_{1/2}$) of were estimated from concentration-time curves.

Results: The particle size obtained ranged between 0.04 μm to 6 μm with a mean size of 0.2 μm . Of the expected 20% drug loading, the actual drug loading capacity of the microparticles

was found to be 19.1%, giving an encapsulation efficiency of 95.6%. The *in vitro* release kinetics showed a controlled and sustained drug release profile of levodopa microparticles with 80% drug release occurring at 12 h. *In vivo* pharmacokinetic studies showed a kinetic profile of levodopa/carbidopa microparticles as compared to the conventional control release formulation. The AUC (704.5 ± 85.37), and $C_{\max}$ ($262.4 \mu\text{g/mL}$) of levodopa/carbidopa microparticles were relatively higher than Sinemet CR (AUC 252.7 ± 33.88 and $C_{\max}$ $128.8 \mu\text{g/mL}$).

Conclusion: Findings from the study suggest that levodopa/carbidopa microparticles may give adequate levels of levodopa plasma concentration over a period of time.

DEDICATION

This work is dedicated to my family for their encouragement and support throughout this study.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AUC	Area Under the Curve
BBB	Blood Brain Barrier
CD	Carbidopa
C _{max}	Maximum Plasma Drug Concentration
CNS	Central Nervous System
COMT	Catechol-O-Methyl Transferase
CR	Controlled Release
EDTA	Ethylenediaminetetraacetic Acid
GPI	Internal Globus Pallidus
HCL	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
HPMC	Hydroxypropylmethyl Cellulose
K _e	Elimination Rate Constant
LBs	Lewy Bodies
LD	Levodopa
LRRK2	Leucine-rich Repeat Kinase 2 gene

MAO-B	Monoamine Oxidase-B
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MP	Microparticles
PD	Parkinson's Disease
PRKN	Parkin gene
PINK1	PTEN-Induced Putative Kinase 1 gene
PBS	Phosphate Buffered Saline
SD	Sprague-Dawley
SNCA	Alpha Synuclein
SNpr	Substantia Nigra pars reticulata
STDEV	Standard Deviation
T _{max}	Time to achieve maximum plasma drug concentration

CHAPTER ONE

1.0 BACKGROUND

Parkinson's disease (PD) as defined by Khor and Hsu, (2007) is a progressive neurodegenerative disorder of the extrapyramidal nervous system, which affects the individual's mobility and ability to control the skeletal muscular system. The disease mostly presents with tremor (shaking), rigidity, and bradykinesia (slowed movements), with postural instability appearing in some patients as the disease progresses (Kouli *et al.*, 2018).

It was first medically described by the English scientist, James Parkinson in 1817. In a paper he wrote referring to the disease as the Shaking Palsy, he described it as "Involuntary tremulous motion, with lessened muscular power, in parts not in action and even when supported; with a propensity to bend the trunk forward and to pass from a walking to a running pace: the senses and intellects being uninjured" (Parkinson, 1817). Many researchers and clinicians from the 19th century to the present day have attempted to further characterize and decipher the nature of the disease, yielding phenomenal discoveries in therapeutics (Goldman and Goetz, 2007).

After Alzheimer's disease, PD is considered the most common neurodegenerative disorder which usually affects the elderly and the middle aged (Cole and Murphy, 2002). The disease prevalence ranges from 100 - 200 per 100,000 people, and the annual incidence is estimated to be 15 per 100,000 according to Tysnes and Storstein, (2017). A survey done by Chen *et al.*, in 2001 projects that, with an ageing population, both the prevalence and incidence of Parkinson's disease are expected to rise by more than 30% by the year 2030 which will increase the economic and social burden.

PD is pathologically characterized by the loss of dopamine producing neurons in the basal ganglia of the brain. The low dopamine levels in the brain leads to fundamental motor

symptoms such as: bradykinesia (slowed movements), distal tremor (shaking) and muscle rigidity (stiffness). Although, neurodegeneration is not limited to only the nigral dopaminergic neurons but also involves cells located in other regions of the neural network. This often leads to the non-motor features associated with PD and is in many cases evident in PD patients even years or decades before the motor symptoms set in (Kouli *et al.*, 2018). Comorbidities known to occur with PD include sleep disturbances, depression, dementia, falls and fractures and impulse control disorders as reported in several articles in literature (Suzuki *et al.*, 2011; Dissanayaka *et al.*, 2011; Aarsland and Kurz, 2010; Duncan *et al.*, 2012 and Djamshidan *et al.*, 2011).

The manner of the causation of the disease is generally unknown, however, it is postulated that both genetic and environmental factors may be involved (Nord, 2017). With age being the biggest risk factor, the median age for the onset of PD is reported to be 60 years (Lees *et al.*, 2009) with higher prevalence recorded in Europe, North America, and South America compared with African, Asian and Arabic countries (Kalia and Lang, 2015). Environmental influences such as smoking, caffeine consumption, and pesticide exposure have been hypothesized to alter the risk of Parkinson's disease development, however the role of these factors still remains unclear (Kouli *et al.*, 2018).

Due to the idiopathic nature of the disease, treatment of PD focuses on reducing symptoms while improving the overall quality of life of the patient (Horstink *et al.*, 2006). Treatment is symptomatic and this may be achieved through drugs that are metabolized to dopamine, that activate the dopamine receptor, or that prevent the breakdown of endogenous dopamine (Zahoor *et al.*, 2018). Drugs used in the management of PD include Catechol-O-Methyl transferase (COMT) inhibitors, example entacapone; dopamine agonists, example ropinirole; monoamine oxidase B inhibitors, example selegiline; and dopamine precursor, levodopa

(Korczyn, 2004). Currently, the most effective treatment, and also considered gold standard in PD therapy is levodopa-based preparations (Gunay *et al.*, 2016).

Levodopa, a precursor of dopamine, is able to cross the blood brain barrier (BBB) as opposed to dopamine itself which is unable to cross the BBB. After absorption and transport across the BBB, levodopa is converted into the neurotransmitter dopamine by dopa decarboxylase thus restoring the level of dopamine in the depleted striatum (Zahoor *et al.*, 2018; Kouli *et al.*, 2018).

The challenge with the use of levodopa is its peripheral (gut wall, liver and kidney) decarboxylation to dopamine (Waller and Sampson, 2018) that is, the conversion of levodopa to dopamine outside the CNS by dopa decarboxylase.

This often leads to untoward effects such as nausea, vomiting, cardiac arrhythmias, and hypotension. Additionally, the amount of plasma levodopa decreases (as a result of the short half-life of levodopa), and an attempt to increase drug dose often leads to an increase in peripheral adverse effects (Brasnjevic *et al.*, 2009). For this reason, levodopa is co-administered with a peripheral dopa decarboxylase inhibitor, Carbidopa. Carbidopa prevents the peripheral conversion of levodopa to dopamine and as such, controls levodopa-induced peripheral adverse reactions, and increases levels of levodopa in the brain (Afroz and Bach, 2014). Carbidopa does not cross the blood brain barrier (BBB) hence selectively prevents the formation of dopamine in peripheral tissues.

The current and most frequently prescribed combination drugs available on the market for the management of PD is levodopa/carbidopa with the trade name SINEMET®. The compound is available in several formulations, including immediate release preparations, modified release preparations, which is useful for controlling symptoms overnight and limiting early morning

symptoms, as well as suspensions, which can be useful for patients that have swallowing difficulties (Oertel, 2017; Koller and Pahwa, 1994; MacMahon *et al.*, 1990).

Although the combination therapy of levodopa/carbidopa seems effective, studies show that there are a few drawbacks with its prolonged use and can result in significant motor complications such as dyskinesias (abnormal involuntary hyperkinetic movements) and severe on-off motor fluctuations (Salat and Tolosa, 2013). These potentially disabling motor complications can be associated with variable drug absorption and transit across the BBB, and fluctuating plasma concentrations of levodopa due to its short half-life (Senek *et al.*, 2017).

Recent studies have tried novel ways to maintain a near constant plasma concentration of levodopa in order to maximize therapeutic effect. Some of these new approaches include controlled release formulations and improved drug delivery systems of levodopa (Goole *et al.*, 2007; Di Stefano *et al.*, 2009; Ngwuluka *et al.*, 2010; LeWitt, 2015). Studies have reported some success with these approaches that seek to improve the clinical efficacy and side effect profiles of levodopa.

One such approach is formulating levodopa using microparticles, characteristically free flowing powders consisting of proteins or biodegradable polymers, which deliver the drug to target site with specificity and in a controlled manner (Sahil *et al.*, 2010). Microparticles have gained increasing popularity in medical technology due to such advantages as the ability to target the release of the encapsulated material, improve bioavailability of the drug, provide controlled and sustained release profiles, improved efficiency in treatment and better patient compliance (Nagpal *et al.*, 2016).

Microparticles can be made of polymers, which act as carriers in forming efficient drug delivery systems. The polymers are responsible for microparticle stability, drug loading

capacity and tunable properties (Saikia *et al.*, 2015). Chitosan, a natural cationic polymer is a hydrophilic biodegradable polysaccharide which has received increasing attention in research mainly due to its low toxicity, biodegradable and target specific delivery properties (Saikia *et al.*, 2015). The current study, therefore, seeks to formulate and evaluate the pharmacokinetics of chitosan microparticles of levodopa and carbidopa.

1.1 PROBLEM STATEMENT

Parkinson's disease (PD) is the second most common neurodegenerative disorder, being a notable cause of neurological disability (Goole and Amighi, 2009).

Unfortunately, PD has no cure and this may be due to its idiopathic nature. As such, the current therapies in use can neither slowdown nor stop the progression of the disease (Singh *et al.*, 2007). Treatment is therefore aimed at relieving symptoms (while minimizing adverse effects of drugs) and improving quality of life of patients. Of all the drugs available for the management of PD, none surpasses the clinical efficacy of dopamine's biological precursor, levodopa (LeWitt, 2008).

Although levodopa is regarded as the gold standard, the motor complications and side effects that comes with its prolonged use is potentially disabling (Gunay et al, 2016). Patients initially respond well to treatment; however, over time, the beneficial effects are associated with complications such as motor fluctuations and levodopa-induced dyskinesias (Muller, 2012).

Several attempts have been made to improve the clinical efficacy of levodopa. Dopamine agonists may slowdown the occurrence of motor complications by allowing the use of lower doses of levodopa, and ensuring long-lasting receptor stimulation. However, dopamine agonists may even worsen the adverse effects compared to using levodopa alone (Stowe *et al.*, 2008). Also, with the combination of levodopa/carbidopa with COMT inhibitors and/or MAO-

B inhibitors, although there is improved levodopa bioavailability, plasma concentrations still fluctuate (Stocchi and Olanow, 2004; Pfeiffer, 2006).

Other levodopa treatment strategies to minimize motor complications have been developed several years ago, which included intravenous infusion of levodopa. However, it was found that levodopa was irritating to the veins and therefore could not be administered continuously for more than 7-10 days via the intravenous route (Shoulson *et al.*, 1975; Hardie *et al.*, 1984).

Intraduodenal levodopa infusion has also been proven to be a successful therapeutic approach for PD (Kurlan *et al.*, 1988). However, the most frequently reported complications include dislocation of the distal part of the tube from the duodenum into the stomach which leads to sudden failure of clinical response with recurring motor fluctuations (Nyholm *et al.*, 2005).

It is therefore apparent that oral levodopa/carbidopa formulations provide safer profiles as compared to the other treatment strategies. The development of oral controlled release formulations aims to minimize levodopa-associated dyskinesias and motor fluctuations (caused by pulsatile stimulation of dopaminergic receptors which occurs as a result of the short acting levodopa), by providing more continuous dopaminergic stimulation (Olanow *et al.*, 2006).

The most current and effective drug treatment for PD is Sinemet CR, an oral formulation of controlled release levodopa/carbidopa. Pharmacokinetic studies show that steady state levodopa plasma levels do not fluctuate as much with Sinemet CR compared to the immediate release, Sinemet. However, the bioavailability of Sinemet CR is about 30% less than Sinemet (Yeh *et al.*, 1989). Additionally, Sinemet CR is absorbed slowly, and although the absorption occurs over an extended period, there is a late onset of drug action. Clinically, it is difficult to use Sinemet CR in patients with advanced PD since the late onset to clinical benefit could worsen their motor complications (Contin *et al.*, 1996). These drawbacks of Sinemet CR have

been suggested to be a result of delayed gastric emptying and erratic absorption which occurs across the intestinal lumen (Salat and Tolosa, 2013).

Clearly, patients are still left with unmet clinical needs and new oral levodopa formulations are needed to overcome the irregular absorption and variable plasma concentrations of levodopa.

1.2 JUSTIFICATION

The oral route is undoubtedly the most preferred route of drug administration. However, extensive first pass metabolism and narrow therapeutic window associated with the oral route limits the therapeutic benefits of many drugs. Therefore, current research has tried to explore new strategies to improve oral drug delivery. An intelligent approach to improve the bioavailability and pharmacokinetic properties of drugs is through the use of microparticles as carriers for controlled release drug delivery (Kumar *et al.*, 2011).

It has been established in literature that microparticles, because of their distinctive properties such as small particle size, drug loading capacity, entrapment efficiency and controlled release profiles are useful drug delivery systems that are successful in encapsulating both water insoluble and sparingly-soluble agents to improve their efficacy (Bale *et al.*, 2016).

Polymer based delivery systems have been shown to improve the pharmacokinetics of drugs, decrease side effects and increase efficacy (Saikia *et al.*, 2015). One unique polymer for oral drug delivery: pH sensitive and a reactive primary amine group, is chitosan (Sahil *et al.*, 2010). Chitosan, a natural polymer, has remarkable physicochemical (cationic polyamine, easily modified chemically and reactive side groups) and biological (biocompatible, non-toxic and biodegradable) properties (Sandford, 1990). This makes chitosan a promising candidate for drug delivery in the gastrointestinal tract (Saikia *et al.*, 2015). Additionally, chitosan consists of several positively charged groups which readily interact with the negatively charged mucous

membrane, thereby increasing adhesion to the mucosal surface, and thus improves contact time for drug absorption (Saikia *et al.*, 2015). With the current increase in the use of polymer based microparticulate delivery systems, there is the need for extensive studies into chitosan as a way of improving oral drug delivery of levodopa.

Levodopa has a short half-life (Goole *et al.*, 2009), hence the need to develop controlled release formulation in order to reduce fluctuations and improve clinical efficacy. Carbidopa on its own has little pharmacological activity, but it is combined with levodopa as a peripheral decarboxylase inhibitor to inhibit the breakdown of levodopa in the periphery. This increases the levels of levodopa reaching the brain and reduces its peripheral side effects (Safavi *et al.*, 2007). There is currently no known study that has evaluated the pharmacokinetics of microparticles of levodopa for oral drug delivery. If pharmacokinetic studies in the current study show improved bioavailability and constant plasma concentration with levodopa microparticles, this would be of great clinical relevance, as it will address one shortfall in PD therapy.

Furthermore, this novel therapeutic approach, if this shows stable plasma levodopa levels, could be incorporated into standard treatment guidelines for PD. Finally, for patient care, this novel approach may be one that will improve the quality of life of PD patients.

Therefore, the current study seeks to employ the use of chitosan microparticles for controlled release formulation of levodopa and carbidopa in a mucoadhesive oral drug delivery approach.

1.3 AIM

To formulate and evaluate the pharmacokinetics and biodistribution of chitosan microparticles of levodopa and carbidopa using *in vitro* and *in vivo* models.

1.4 SPECIFIC OBJECTIVES

1. To formulate microparticles of levodopa and carbidopa by encapsulating drugs in hydroxypropylmethyl cellulose (HPMC), coating with chitosan and evaluating microparticles for size, drug content and encapsulation efficiency and *in vitro* release characteristics.
2. To estimate the pharmacokinetic profile of the formulated levodopa microparticles in an animal model.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 PARKINSON'S DISEASE

2.1.1 Background and History

Parkinson's disease (PD) is a neurodegenerative disorder characterized by four fundamental motor features: resting tremor, bradykinesia, rigidity and postural instability (Hughes *et al.*, 1992). PD affects a notable percentage of the adult population (Wickremaratchi *et al.*, 2009; McCrone *et al.*, 2011). It was first medically described by the English Scientist, James Parkinson in 1817. In a paper he wrote referring to the disease as the Shaking Palsy, he described the clinical signs and symptoms as "Involuntary tremulous motion, with lessened muscular power, in parts not in action and even when supported; with a propensity to bend the trunk forward and to pass from a walking to a running pace: the senses and intellects being uninjured" (Parkinson, 1817). Parkinson in his paper (An Essay on the Shaking Palsy) perceptively describes the features of the disease as "insidious onset and slow progression, presence of rest tremor, flexed posture and festinating gait".

He observed that some patients had more advanced states with increased immobility and dependence, as well as sleep disturbances and altered bodily functions such as with the bowels, slowed speech and difficulty swallowing. Although Parkinson found that the symptoms presented with the disease had similar features with other neurological diseases published in medical literature, he truly believed that the 'shaking palsy' was unique and warranted further study.

A seminal figure in the history of Parkinson's disease was the French physician, Jean-Martin Charcot (1825-1893). Charcot acknowledged that Parkinson would always be recognized for

the first description of this condition but noting errors in Parkinson's generality, he sought to add his own contributions (Goetz, 1986, 2002). His elaboration of motor and non-motor features of the disorder, distinction of the disease from other similar disorders, advocacy of clinicopathological studies and of course, coining the eponym Parkinson's Disease remain his legacy. Treatment used in Charcot's time did not reveal significant advances in efficacy. Although medications such as strychnine, belladonna and opium were tried with limited benefit, hyoscyamine, an anticholinergic agent, provided some palliative action. Charcot's contribution to PD include not only his studies but also that of his pupils. Pupils of Charcot such as Ordenstein wrote his medical thesis on Parkinson's disease (1867) and introduced belladonna as a treatment. Eduard Brissaud (1852-1909) described midbrain lesions in Parkinson's disease which set the stage for future investigations of phenotypes and clinicopathological correlates of neurological diseases.

Although it has been proposed that PD emerged as a result of the industrial revolution, there is some evidence that a disease known as "kampavata," consisting of shaking (kampa) and lack of muscular movement (vata), existed in the ancient Indian medical system, Ayurveda, as long as 4500 years ago (Manyam, 1990). The *Mucuna Puriens* plant was used in ancient times to treat symptoms, and was later discovered to contain levodopa (Katzenschlager *et al.*, 2004).

Many researchers and clinicians from the 19th century to the present day have attempted to further characterize and decipher the nature of the disease, and have yielded incredible discoveries and advances in medical and surgical therapeutics (Goldman and Goetz, 2007).

2.1.2 Epidemiology

2.1.2.1 Global prevalence of Parkinson's disease

Parkinson's disease, is the second most common neurodegenerative disease and affects over 1% of the population above age 55 and nearly 3 % of the population over age 70 (Samii *et al.*, 2004). In Africa, prevalence rate ranges from 7/100,000 in Ethiopia to 67/100,000 in Nigeria, according to studies done by Williams *et al.*, (2018). The low prevalence rate in Africa is reported to be attributed to the youthfulness of the African population, paucity of published reports and cultural perception of neurologic disorders (Akinyemi and Okubadejo, 2010).

The mean age at which PD usually presents is 60 years, with about 80 % of individuals developing the disorder between the ages of 40 and 70 years. Such cases where individuals develop symptom onset before age 40, is classified as young onset PD and this occurs in about 5 % of the PD cases (Yao *et al.*, 2013).

2.1.2.2 Socio-demographic factors

It has been reported that PD affects more men than women with about 50% cases occurring more in men, however the reasons for this disparity remains unclear (Hirtz *et al.*, 2007). Although PD affects people worldwide, studies have shown that incidence rate is higher in the developed countries. Increase in the use of pesticides has also been found to be associated with the increased risk of acquiring PD among people living in the rural areas. However, these perceivable risks are not fully characterized and age continues to be the major risk factor for PD (Yao *et al.*, 2013).

2.1.3 Comorbidity

2.1.3.1 Depression

PD patients usually experience frustration, demoralisation, grief, and embarrassment as normal psychological reactions to the disease. Depression in PD is as common as the motor symptoms and involves more prevalent mood changes that generally resemble idiopathic forms of depression (Jankovic, 2008). There is convincing evidence that depression in PD is an inherent part of the disorder and not just a reaction to disability. Drugs used to treat psychologic disorders and other anti-Parkinsonian agents may aggravate depression in PD (Jankovic, 2008; Murray *et al.*, 2012).

2.1.3.2 Anxiety

About 40 % of patients with PD usually experience anxiety disorders in the form of agitation, panic and social phobia. This may occur in isolation or together with depression or cognitive impairment (Jankovic, 2008). Drug induced motor fluctuations can aggravate the problem by instigating anxiety during the off periods which in extreme cases may resemble anger attacks. In general, the anxiety syndromes in Parkinson's disease mimic those in idiopathic conditions and frequently co-occur with depression (Jankovic, 2008).

2.1.3.3 Cognitive impairment

Nearly all PD patients experience some level of cognitive impairment. This usually occurs in the advanced stages of the disease and often presents as a result of the dysfunction of the frontal lobe. Common cognitive disorders include difficulty with long-term planning, memorizing or retrieving new information, mental flexibility and word fluency. However, language and simple mathematical skills are relatively spared, unlike in patients with Alzheimer's disease (Ayano, 2016).

2.1.3.4 Psychosis

About 20% of PD patients receiving long term treatment with dopaminergic agents such as bromocriptine, levodopa and pramipexole, usually experience psychotic symptoms. More often than not, the psychotic symptoms present as chronic hallucinations and delusions. Up to about 50% of patients experience these psychotic symptoms over the course of their illness. Psychosis most commonly occurs in patients who have corresponding cognitive impairment (Shergill *et al.*, 1998; Friedman, 2010).

2.1.4 Etiology

Parkinson's disease is dependent on a number of factors, with both genetic and environmental factors playing a major role. Age remains the biggest risk factor for PD, with a mean onset age of 60 years (Lees *et al.*, 2009).

2.1.4.1 Pesticides, herbicides and heavy metals

It was discovered in 1983, that several people after the intravenous administration of drugs contaminated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), exhibited typical signs of PD. Following this, there were findings that MPTP selectively damages dopaminergic cells in the substantia nigra (Langston *et al.*, 1983) and this led to the hypothesis that exposure to environmental toxins might be associated to the risk of PD. MPTP as well as certain herbicides and pesticides have been found to selectively inhibit Complex-I and induce depletion of dopamine in animal studies (Betarbet *et al.*, 2000).

In a study done by Greenamyre and Hastings, (2004) complex I defects in the substantia nigra was found in patients with irregular PD. It has been hypothesized that welding and exposure to heavy metals such as iron, manganese, copper, lead, aluminium or zinc leads to accumulation of these heavy metals in the substantia nigra and increasing oxidative stress which in turn leads

to the degeneration of cells (Lai *et al.*, 2002). However, some investigations have been done and the report suggests that the relation between metal exposure and risk of PD is uncertain (Lai *et al.*, 2002; Jankovic, 2005).

2.1.4.2 Cigarette Smoking

Out of the many risk factors studied for PD, cigarette smoking is one of the few for which very consistent results have been obtained. Interestingly, epidemiological studies have shown a reduced risk of PD among cigarette smokers. The reasons underlying this associated reduced risk are not fully understood. In order to explain this possible neuroprotective effect of smoking, several mechanisms have been propounded. Activation of nicotinic acetylcholine receptors on dopaminergic neurons by nicotine or selective agonists has been shown to be neuroprotective in experimental models of PD (Bordia *et al.*, 2015; Srinivasan *et al.*, 2016).

Nevertheless, nicotine can also stimulate the release of dopamine, which is involved in the reward mechanisms; it is therefore difficult to confirm whether smoking prevents PD or whether PD helps prevent the habitual use of cigarettes. As a result of the reduction in dopamine in PD, patients may be immune to addictive behaviours, and thus less likely to smoke. This hypothesis is supported by the fact that patients with prodromal PD were able to give up smoking much easier than controls, suggesting this association could be due to the diminished responsiveness to nicotine (Ritz *et al.*, 2014).

2.1.4.3 Caffeine

Caffeine as a risk factor for PD has also been studied widely and it has been reported that coffee drinkers have a diminished risk of developing the disease. Caffeine is an adenosine A_{2A} receptor antagonist, which is believed to be protective in PD (Ross *et al.*, 2000) and has been shown to be neuroprotective in a mouse model of PD (Chen *et al.*, 2001). Studies have already

reported that there is a 25% risk reduction in developing PD among coffee drinkers (Noyce *et al.*, 2012). Several other studies also investigated the risk of developing PD among coffee drinkers and non-coffee drinkers and published that coffee drinkers showed a reduced risk of developing PD (Ross *et al.*, 2000; Ascherio *et al.*, 2001; Benedetti *et al.*, 2000).

2.1.4.4 Genetics

Evidences identified that about 15% of individuals living with PD have a very close relative who also has the disease (Samii *et al.*, 2004). Not less than 5% of people have also been shown to have developed the disease as a result of gene mutation (Lesage and Brice, 2009). Mutations in specific genes have been identified by scientists to have undeniably caused PD. The specific genes that have been implicated code for such proteins as leucine-rich repeat kinase 2 (LRRK2 or dardarin), DJ-1, PTEN-induced putative kinase 1 (PINK1), alpha-synuclein (SNCA), and parkin (PRKN) (Davie, 2008; Lesage and Brice, 2009). More often than not, people having these mutations end up developing PD. Mutations in the gene that code for leucine-rich repeat kinase 2 (LRRK2) is however the most common cause of familial and sporadic PD. It is still inconclusive, the mechanism by which LRRK2 is implicated in the pathogenesis of PD but it is somewhat proposed that mutations in this gene leads to an over activation of its kinase activity by which the pathogenesis of the disease might be associated (Greggio *et al.*, 2006).

2.1.5 Pathophysiology

Until the early 20th century, the pathology of PD remained incompletely understood. It was the German pathologist Frederick Lewy who in 1912, reported “neuronal cytoplasmic inclusions in a variety of brain regions”. Another scientist, Tretiakoff, in 1919, found out that the loss of neurons in the substantia nigra pars compacta (SNc) of the brain was the most critical abnormality in PD (a diagram of the brain and parts affected by PD is shown in figure 2.1 below). The importance of dopamine and its depletion from the basal ganglia was discovered

by investigators in the late 1950s to be the key to deciphering the pathophysiology of PD (Hornykiewicz, 2006). Even though the cause of the disease is still unclear, striking advances have been made to possibly explain the underlying mechanisms (Jankovic and Sherer, 2014). The presence of Lewy bodies (LBs) and the loss of dopamine producing neurons have been hypothesized to be the neuropathological hallmarks of PD (Braak and Braak, 2000; Kovari *et al.*, 2009).

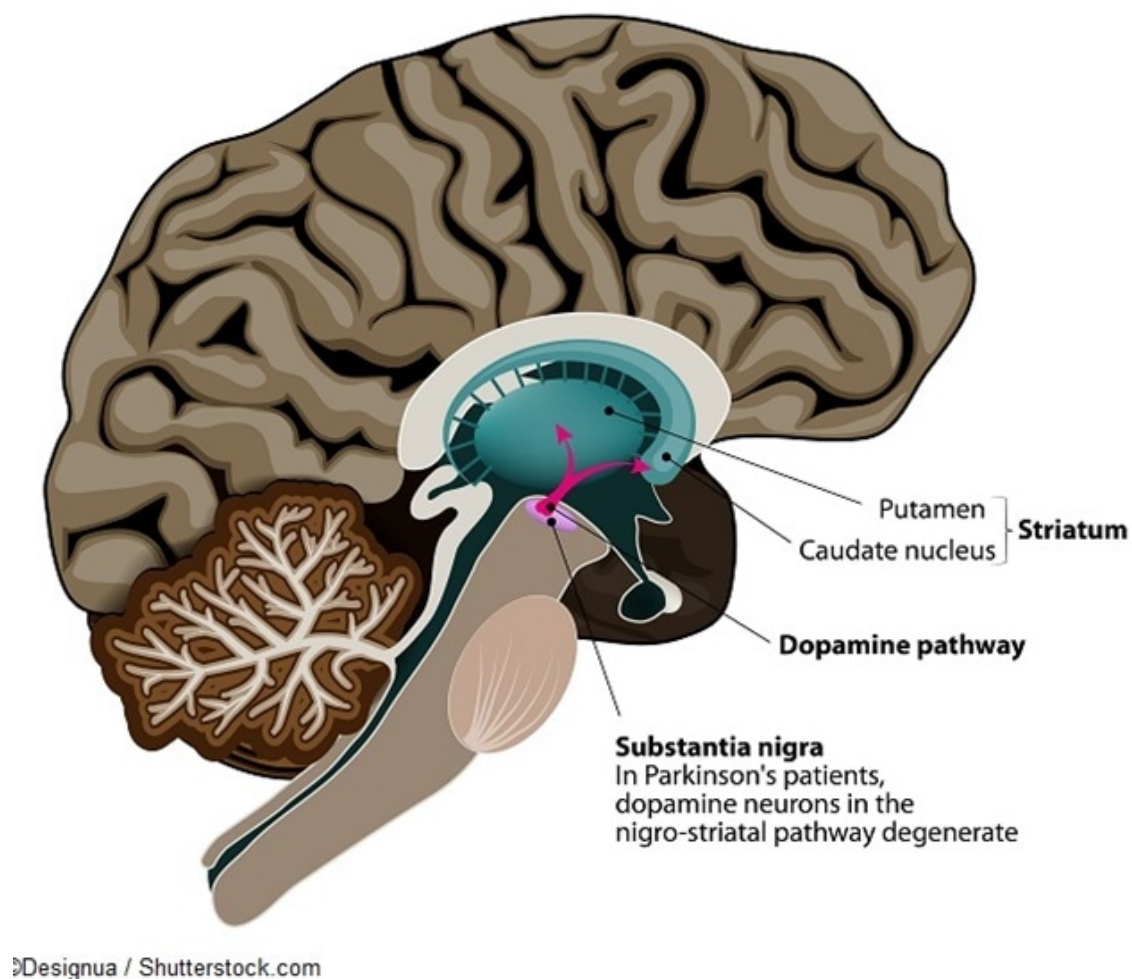


Figure 2.1 : A diagram of the brain showing the parts affected by Parkinson's Disease (Source: Designua/ Shutterstock.com)

The loss of dopaminergic function in PD patients is suggested to result from the progressive degeneration of dopamine producing neurons in the SNc which project to the striatum via the nigrostriatal dopamine pathway. Motor activity in the extrapyramidal system is influenced by two types of dopamine receptors, D1 and D2 which are responsible for excitatory and inhibitory reactions respectively. The extrapyramidal system includes the pars reticulata portion of the substantia nigra (SNpr), and the basal ganglia which involves the internal globus pallidum segment (GPi) of the ventral striatum. The loss of striatal dopamine eventually results in increased activity in the GPi/SNpr circuits which leads to the dysfunction of gamma aminobutyric acid (GABA). The inhibition of GABA subsequently inhibits the thalamus and causes a reduction in the ability of the thalamus to activate the frontal cortex which gives rise to decreased motor activity as presented by PD (DeMaagd and Philip, 2015).

The other hallmark of the disease is the presence of LBs seen in the histopathological studies of PD brain. These LBs are described as intracellular cytoplasmic aggregates composed of proteins, lipids, and other materials (Braak *et al.*, 2003; Del and Braak, 2012). Excessive production of misfolded forms of ubiquitin proteins which are involved in protein recycling has been found to be the precursor for the formation of these LBs. The formation of the LBs causes lesion patterns in the brain and is believed to be the cause of neurodegeneration in such parts of the brain. Lesions in the dorsal nucleus, medulla, and pons are claimed to be the cause of the early olfactory and rapid eye movement features of PD. The common motor features of PD are also thought to be caused by lesions in the nigrostriatal region during later stages of the disease (Braak *et al.*, 2004).

2.1.6 Clinical Presentation

In up to about 90% of patients, PD may begin in an insidious manner such as difficulty in getting out of a chair. Sometimes diagnosis is delayed as the non-motor symptoms preceding

the disease may be misinterpreted as related to normal ageing. The non-motor features (autonomic dysfunction, sleep disorders, olfactory dysfunction, fatigue) usually sets in at the early phase of the disease and can last for roughly 4 to 6 years on average (Meissner, 2012; Postuma *et al.*, 2012). Thermoregulatory dysfunction, neuropsychiatric symptoms and other clinical signs may occur as the disease progresses. For some patients, neuropathic and nociceptive pain may occur at either the early or later stages of the disease (Postuma *et al.*, 2012; Maetzler and Hausdorff, 2012)

The pre-motor or prodromal phase of PD may start as early as 12-14 years before diagnosis (Postuma *et al.*, 2012). There is however a great deal of evidence supporting the fact that the disease may begin in the peripheral autonomic nervous system and/or the olfactory bulb with the pathology then spreading through the CNS affecting the lower brainstem structures before involving the substantia nigra (Katzenschlager *et al.*, 2008). This may thus explain the presence of non-motor symptoms in PD patients before motor symptoms begin. These non-motor symptoms contribute significantly to disability and poor quality of life and thus predict admission to care homes (Noyce *et al.*, 2012).

The clinical features historically associated with PD are the triad of motor symptoms, namely tremor, rigidity and bradykinesia, with postural instability often appearing as the disease progresses (Kouli *et al.*, 2018). Some relationship has been shown between the frequency of motor presentations and the onset of the disease, with tremor specifically, being as twice as common in patients above 64 years than in patients younger than the age of 45 (Suchowersky *et al.*, 2006).

2.1.6.1 Tremor

In about two-thirds of PD patients, tremor occurs as the initial symptom. Deuschi *et al.*, defined tremor as “rhythmical, involuntary, oscillatory movement of a body part produced by

alternating or synchronous contractions of antagonist muscles” (Deuschi *et al.*, 1998). Tremor normally begins in a mild and recurrent fashion and usually occurs even at rest. By the position that underlines the tremor, it can be classified as either rest tremor, that is, tremor occurring in a body part while there is no voluntarily activated muscle contraction, or action tremor, thus involving voluntary muscle contractions and occurring during movements (Deuschi *et al.*, 1998; Bhidayasiri, 2005).

2.1.6.2 Bradykinesia

Bradykinesia, a cardinal motor feature of PD, has been defined by Grabli *et al.*, as “a reduction in the speed, gait and amplitude of a repetitive action involving voluntary movements” (Grabli *et al.*, 2012). Difficulty getting started or initiating movements together with a slow, shuffling gait are common clinical presentations associated with bradykinesia. Patients experiencing bradykinesia may try to overcome it by increasing their walking speed with small, rapid steps in an effort to “catch up” with their displaced centre of gravity (Garcia *et al.*, 2011; Hallett, 2012; Xia and Mao, 2012). It is often difficult for such patients with bradykinesia to turn or enter through a narrow door when confronted with the need to, because of the immobility that is associated with bradykinesia (Mendoca, 2008). Typically, in the advanced states, some patients also experience episodes of “freezing” as an acute manifestation of PD (Xia and Mao, 2012).

2.1.6.3 Rigidity

The third most common feature of PD is rigidity, which presents as worsened muscle tone or heightened resistance to a dormant range of motion. This is best described as tension in the muscle, which displays small jerks or a ratchet-like quality when moved passively. Besides the limbs, rigidity of PD can affect other body parts, such as the face, which can display a “masked” look (hypomimia) (Jankovic, 2008; Reichmann, 2010; Xia and Mao, 2012).

2.1.6.4 Postural instability

As the PD advances, a fourth clinical feature known as postural instability can manifest. This symptom has a many different underlying causes related to other motor symptoms, such as rigidity and neurodegeneration in the hypothalamic brainstem or peripheral nervous system. Because of the associated loss of balance and the risk of falls, postural instability is considered a seriously disabling feature of PD (Kang *et al.*, 2012; Doherty *et al.*, 2011).

2.1.7 Diagnosis

There are no definitive tests to confirm the diagnosis of PD. The cardinal motor features of PD- described as the “classical triad”- include a 4-Hz to 6-Hz resting tremor, “cogwheel” rigidity, and bradykinesia are often reported as the first clinical findings of the disease. A fourth feature, postural instability, occurs usually within five years of diagnosis and it affects approximately 50% of PD patients (Baumann,2012; Berardelli *et al.*, 2013; Reichmann, 2010)

The fact that clinical motor features may not present until approximately 50 to 80% of dopaminergic neurons are lost poses a great challenge in PD diagnosis and quite unfortunately, at this point, significant disease progression may have already started (Berg, 2012; Postuma *et al.*, 2012). There is therefore the need to identify subtle motor features that can easily go unrecognised, such as diminished ability to swing the arms or the jerking motions (Lee *et al.*, 2013; Tolosa *et al.*, 2009).

The presence of non-motor comorbidities, including depression, anxiety, fatigue, constipation, anosmia and sleep disorders, which the clinician may not recognise as being associated with PD, further complicates early diagnosis (Schrag *et al.*, 2015, Kang *et al.*, 2012; Irano *et al.*, 2007). Early identification of these symptoms and their association with PD may enable an

earlier diagnosis. Investigators however, continue to search for biomarkers that may allow a more expeditious diagnosis since the onset of motor features is the point at which PD is usually diagnosed and treatment is initiated (Chahine *et al.*, 2014; Sharma *et al.*, 2013). Patients may have a life expectancy similar to that of unaffected individuals once the diagnosis of PD has been confirmed (Grosset *et al.*, 2010; Suchowersky *et al.*, 2006).

2.1.8 Treatment

The successful management and treatment of PD remains still remains elusive despite the fact that was discovered almost two centuries ago (Ngwuluka *et al.*, 2010). The principal aim in PD management is to treat the motor and non-motor symptoms with the goal of enhancing the quality of life of patients (George and Ashok, 2015).

2.1.8.1 History of the treatment of PD

The first drugs to have been used in the symptomatic therapy of PD were anticholinergics (Brocks, 1999; Schapira, 2005). The discovery of the loss of dopamine in specific areas of the brain in the 1960s, opened the door to modern treatment of PD (Korczyn, 2004). Later on, in 1961, it was discovered that dopamine is unable to cross the blood-brain barrier and this led to the trial studies of its precursor, levodopa (Birkmayer and Hornykiewicz, 1961). Although the modern history of levodopa unfolds from the 19th century, it would be interesting to note that ancient Indian Ayurvedic physicians used the seeds of *Mucuna Pruriens*, which later proved to contain 4 to 6% of levodopa, to treat symptoms of PD as early as 300 BC (Gourie-Devi *et al.*, 1991; Owallath and Deepa, 2013).

Several drugs have been used for the treatment of PD and these include: levodopa, Catechol-O-Methyl transferase (COMT) inhibitors (example entacapone), dopamine agonists (example

apomorphine), Monoamine Oxidase-B inhibitors (example selegiline) (Korczyn, 2004). The most effective of these agents is levodopa, although the oldest, and is considered the gold standard for treatment of PD.

2.2 LEVODOPA

2.2.1 Introduction

Levodopa is chemically known as L-3,4-di-hydroxyphenyl-alanine, a neutral, large amino acid that is synthesized from L-tyrosine by hydroxylation. The schematic representation of the biosynthesis of levodopa is shown in figure 2.2 below. Levodopa undergoes decarboxylation by L-aromatic amino acid decarboxylase (nonspecific enzyme widely distributed in liver, kidney, gut, lungs and brain) to dopamine, a neurotransmitter that cannot pass through the BBB (Holtz, 1993). The active metabolite of levodopa, dopamine, is responsible for the control of symptoms of Parkinson's disease; however, it is not able to traverse the formidable brain barrier. Levodopa therefore acts as a prodrug for dopamine since it is able cross the blood brain barrier (via large neutral amino acid transporters) (Khor and Hsu, 2007).

Levodopa is required by all PD patients because of its better tolerability, low cost and ease of administration. However, the therapeutic efficacy of levodopa was found to be significantly reduced because of its extensive metabolism which lead to a reduction in the bioavailability of the drug (Fahn, 2008; Muzzi *et al.*, 2008). The active metabolite of levodopa (dopamine) was observed to generate adverse effects such as cardiac arrhythmias, vomiting and nausea (Muzzi *et al.*, 2008). In general, approximately 95% of levodopa is metabolized in peripheral tissues (GIT, liver and plasma) whereas only 1% of the ingested dose penetrates the CNS for the treatment of PD (Muzzi *et al.*, 2008; Jankovic, 2002; Kostoff and Briggs, 2008). Metabolism of levodopa both in the periphery and the central nervous system is shown in figure 2.3.

It has been observed that levodopa absorption is highly affected by irregular gastric emptying time, metabolism and competition with aromatic amino acids for absorption and transport. Also, its half-life is relatively short, just about 50 minutes and the time to reach its maximum peak in plasma is also about 1.4 hours (Seeberger and Hauser, 2007). Owing to poor bioavailability of levodopa (33%), further attempts were made to improve the efficacy of oral formulations by increasing levodopa dosage and number of times the dose is given (Nutt *et al.*, 2000; LeWitt and Nyholm, 2004). Unfortunately, these did not reduce the side effects emanating from the extensive metabolism of levodopa but rather exacerbated it (Stocchi, 2003).

Administration of a dopa decarboxylase inhibitor together with levodopa was found to enhance the availability of levodopa in the brain by a ten-fold (Cedarbaum, 1987). However, these dopa decarboxylase inhibitors (benserazide, carbidopa) are not able to cross the blood-brain barrier so their effect is mainly in the peripheral tissues thereby reducing the extensive metabolism of levodopa and subsequently slowing the rate at which levodopa is cleared from plasma. Also, since the peripheral conversion of levodopa to dopamine is reduced, the adverse effects caused by dopamine in the periphery (nausea, vomiting, anorexia) is also ameliorated. Furthermore, administration of levodopa with dopa decarboxylase inhibitors prolongs the half-life of levodopa to approximately 90 minutes and reduces the required dosage by 60 to 80% (Janckovic, 2002). In 1975, the combination of levodopa with either of the dopa decarboxylase inhibitors (carbidopa, benserazide) was made commercially available (Tolosa *et al.*, 1998) and till date, the approved drug by the FDA for the treatment of PD is a combination of levodopa and carbidopa.

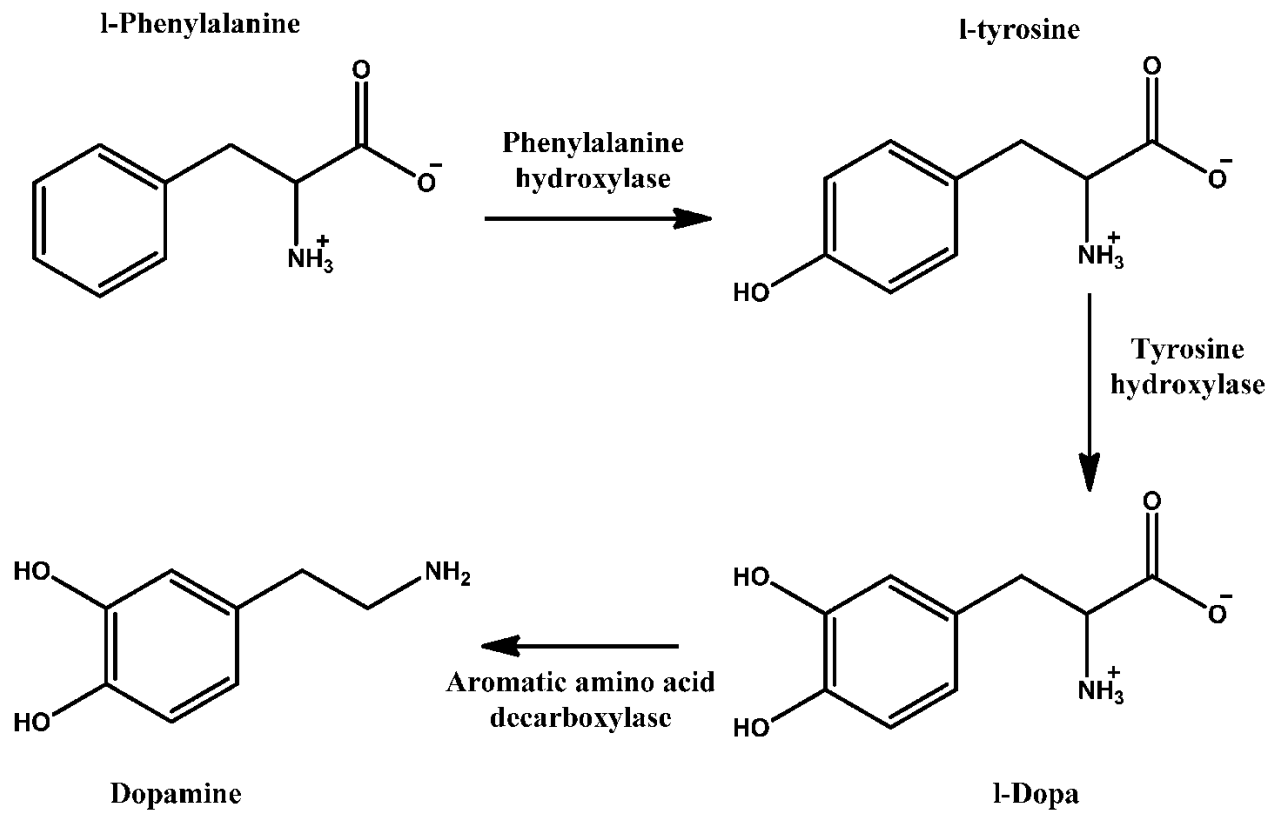


Figure 2.2: Schematic representation of Levodopa and dopamine synthesis pathway (Hadad et al., 2018).

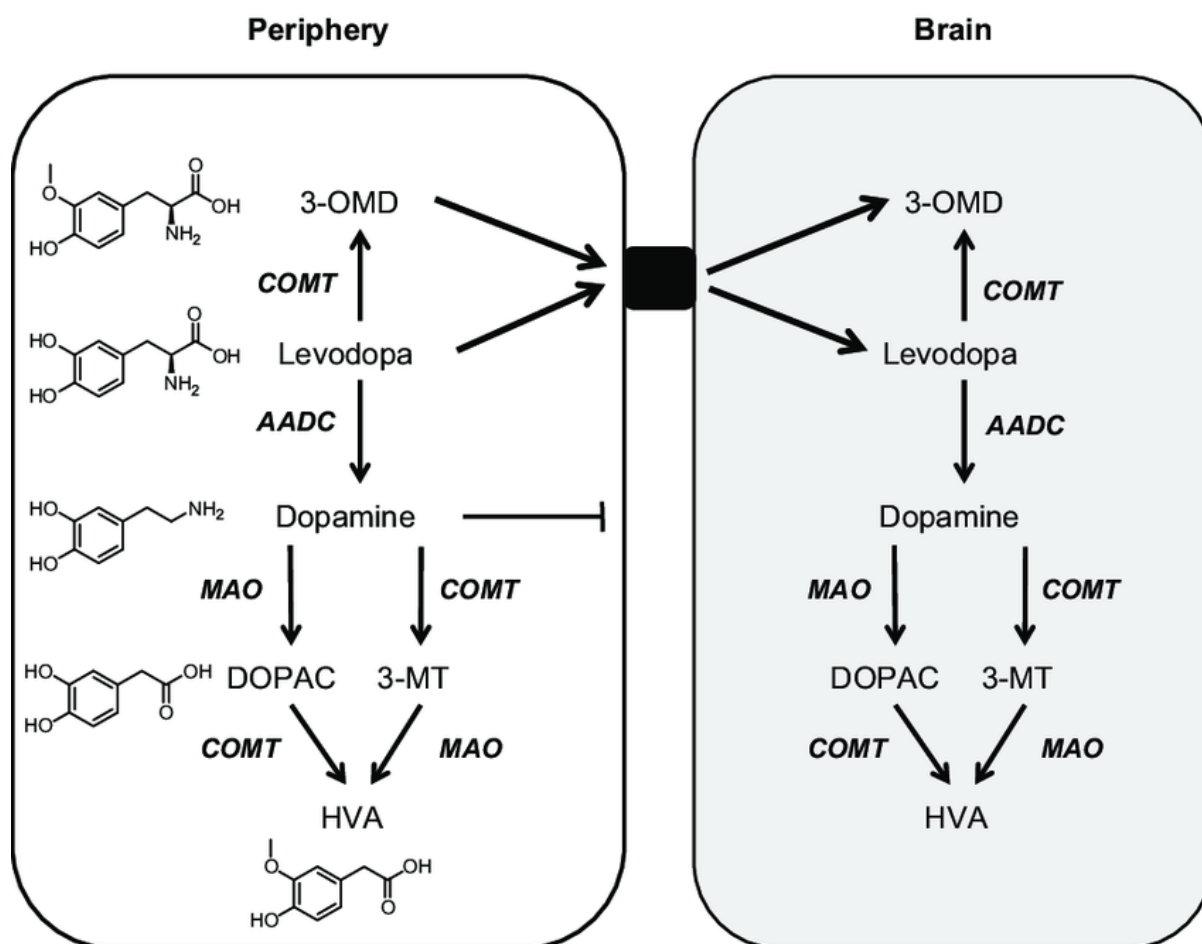


Figure 2.3 : Levodopa metabolism in the periphery and the CNS (Palma *et al.*, 2013).

2.2.2 Pharmacokinetics of levodopa/carbidopa

2.2.2.1 Levodopa

Levodopa is absorbed via L-neutral amino acid transport system. With the administration of levodopa orally, it is practically completely absorbed, with just about 2% of the drug appearing in faeces (Morgan *et al.*, 1971). In spite of this, when levodopa is administered without carbidopa, only about 30% of the initially administered dose reaches systemic circulation, but with the concomitant administration of dopa decarboxylase inhibitors, the bioavailability increases by two to three-fold (Nutt, 1984). At steady states, levodopa has a high volume of distribution and low plasma protein binding (Hinterberger and Andrews, 1972).

Metabolism of levodopa is via four pathways (decarboxylation by aromatic amino acid decarboxylase, 3-O-methylation by Catechol-O-methyltransferase, transamination by tyrosine aminotransferases, and oxidation by tyrosinase). The decarboxylation pathway is the major metabolic pathway for levodopa (Khor and Hsu, 2007). Dopamine, which is the first decarboxylation product may be further metabolised to form 3,4-dihydroxyphenyl acetic acid, homovanillic acid, and to a lesser extent, norepinephrine and vanillinemandelic acid (Khor and Hsu, 2007). Levodopa is mainly excreted via urine. When given alone, levodopa has a plasma half-life of about 50 minutes but this may be increased to 90 minutes when co-administered with carbidopa.

2.2.2.2 Carbidopa

About 40 to 70 % of the dose of orally administered carbidopa is absorbed (Vickers *et al.*, 1974). Carbidopa is absorbed quite slowly than levodopa, and takes approximately 2 hours (for an immediate release formulation) or close to 2.8 hours (for a controlled release formulation) to peak in plasma. The bioavailability of Carbidopa is not affected by the co-administration of Levodopa (Vickers *et al.*, 1974). However, carbidopa is peripherally acting and therefore not able to cross the blood-brain barrier even at high doses (Lotti and Porter, 1970; Porter, 1973). Also Carbidopa is not highly protein bound. The percentage plasma protein binding is $36 \pm 1.6\%$.

Carbidopa undergoes metabolism to form four metabolites, 2-methyl-3-methoxy-4-hydroxy-phenylpropionic acid, 2-methyl-3,4-dihydroxy-phenylpropionic acid, 3-hydroxy- α -methyl-phenylpropionic acid, and 3,4-dihydroxy-phenylacetone. There has not yet been any published information on the potential pharmacological activities of these metabolites (Khor and Hsu, 2007). Carbidopa is mainly excreted through urine and has a half-life of about 2 hours when administered with Levodopa. A similar half-life of 2.08 hours was observed when carbidopa

is given alone (Vickers *et al.*, 1974).

2.2.3 Pharmacodynamical Changes in the body with the Progression of PD

Studies have shown that although PD is initiated by loss of dopaminergic nigral neurons, there are still sufficient neurons to maintain constant striatal dopamine concentrations and continuous activation of the striatal dopamine receptors for normal or close to normal basal ganglia function in the early stage of the disease (Pfeiffer, 2005; Olanow *et al.*, 2006). The dopaminergic neurons convert the administered levodopa to dopamine and can still store, release, re-uptake, recycle or auto regulate large amounts of dopamine to maintain constant dopamine concentrations. Furthermore, it has been proposed that dopaminergic neurons are able to buffer the fluctuation of the plasma levels by masking the pulsatile stimulation of the striatal receptors (Olanow, 2004). However, the pulsatile stimulation becomes magnified as the loss of dopaminergic neurons progresses because the buffering and autoregulation capacity lessens, leading to motor complications such as dyskinesia and motor fluctuations (Olanow *et al.*, 2006).

The conversion of administered levodopa then takes place at the non-dopaminergic sites such as glial cells, serotonergic neurons and non-aminergic interneurons (Melamed *et al.*, 2000). These sites do not store dopamine, but rather convert levodopa to dopamine and release it to the striatum. Lack of storage capacity forces the striatal dopamine receptors to depend almost entirely on the peripheral availability of levodopa, which in turn is limited by its short half-life (Olanow, 2004).

Hence constant striatal dopamine concentration and continuous activation of the striatal dopamine receptors are impaired and replaced by pulsatile stimulation determined by the pharmacokinetics of levodopa, leading to motor complications (dyskinesia and motor fluctuations) experienced by PD patients. Motor fluctuations consist of 'on' periods in which

therapeutic response and good antiparkinsonian effect are experienced, and ‘off’ periods during which patients experience crippling Parkinsonism resulting from the absence of or low levodopa concentrations in the plasma (Olanow, 2004).

Therefore, while combination therapy seemed to solve most of the difficulties initially facing LD therapy, the major drawbacks of levodopa therapy as the disease progresses still persists. The development of drug related fluctuations emphasizes the need for improving drug delivery (LeWitt, 2015).

2.2.4 Levodopa drug delivery systems

2.2.4.1 Immediate release oral drug delivery

The first immediate drug release systems for levodopa were tablets composed of levodopa in combination with carbidopa and registered under the trade name Sinemet® (Koller *et al.*, 1999). Administration of levodopa with carbidopa controlled the concentration of dopamine at appropriate levels with reduced side effects (Sagar and Symth, 2000). This was proposedly achieved through the diminished conversion of levodopa to dopamine in the peripheral tissues, which also permitted lower effective doses of levodopa for the treatment of PD (Sagar and Symth, 2000). The combination, Sinemet, could reduce the peripheral metabolism of levodopa and adverse effects (nausea and vomiting) but was ineffective in controlling dyskinesia and motor fluctuations which accompanied long term use of levodopa (Olanow, 2004).

Generally, Sinemet and Madopar (combination of levodopa with benserazide) produce fluctuations of levodopa plasma levels that either exceed safe therapeutic concentrations or fall below the minimum effective concentrations (Sagar and Symth, 2000). However, the plasma fluctuations depend not only on levodopa’s biological half-life but also on frequency of administration and release rate from the drug delivery systems (Sagar and Symth, 2000). In

order to block the second metabolic pathway of levodopa and improve its half-life, a COMT inhibitor, entacapone was added to make a triple combination and was sold under the name Stalevo.

Even though entacapone increases the plasma level of levodopa by 35% and the half-life from 1.5 (with carbidopa) to 2.4 hours (Hsu and Han, 2006), entacapone has also been observed to increase dopaminergic side effects such as dyskinesias, therefore necessitating levodopa dose reduction (Hauser, 2004). In another study, it was shown that Stalevo could not improve dyskinesias and motor fluctuations mainly because of its inability to provide constant therapeutic plasma concentrations (Olanow and Stocchi, 2004).

With the aim of providing continuous activation of striatal dopamine receptors so as to reduce the incidence of dyskinesias, Smith and co-workers in their study suggested the frequency of dosing of Stalevo be increased to four times daily (Smith *et al.*, 2005). However, frequency of dosing could certainly instigate patient non-compliance, which in return would not achieve the aim of increased dosing. Although certain combinations can enhance the pharmacokinetic profile of immediate release levodopa systems, plasma fluctuations and dyskinesia remain a major set-back along with decreased patient compliance which comes increasing frequency of dosing.

2.2.4.2 Levodopa as liquid formulation

In order to enable rapid onset of action, liquid levodopa formulations were introduced. However, their effects were found to be short acting. Patients were observed to benefit from liquid levodopa formulation within 5 min, with the duration of the effect persisting for 1 to 2 hours (Stacy, 2000). Levodopa liquid formulations are therefore administered to minimize delay in the 'on' effect, which has been proven to be increased by controlled release formulations (Adler, 2002).

Unlike conventional formulations, the pharmacokinetic profiles of liquid LD formulations are not affected by the gastric emptying rate. Liquid formulations allow for a rapid gastric transit as the gastric emptying interval does not affect their absorption (Woitalla *et al.*, 2006). Thus, levodopa liquid formulations may allow more precise dose titration throughout the day and may therefore prove to be useful particularly in patients with prolonged gastric transit time, difficulties in swallowing, advanced motor fluctuations and also during the ‘off’ effect period (Metman, 2002; Weintraub *et al.*, 2008).

It has been observed however, that, despite liquid levodopa formulations being independent to gastric emptying rate, there is still pulsatile drug delivery and the desired constant delivery is still not achieved (Obering *et al.*, 2006). Furthermore, liquid formulations are cumbersome as their therapeutic effects are short-lived, thus requiring patients to take an hourly or bihourly dose, which makes them considerably prone to non-compliance. Thus, patients cannot rely entirely on liquid levodopa because the frequent dosing does not consistently reduce motor fluctuations (Obering *et al.*, 2006).

2.2.4.4 Delivery of levodopa by infusions

Infusions for the delivery of levodopa were introduced with the intention of achieving constant plasma concentrations, which would in turn produce continuous dopaminergic stimulation of the dopamine receptors. Duodopa was developed as levodopa/carbidopa enteral gel with a portable pump and intestinal tube (LeWitt and Nyholm, 2004; Nyholm *et al.*, 2005_a).

In a study that compared intraduodenal infusion as monotherapy with individual combinations of conventional pharmacotherapy, there was significantly improved motor performance with the infusion (Nyholm *et al.*, 2005_a). Nyholm *et al* in another study stated that in addition to improvement of motor performance, a 24 hour intraduodenal infusion improved sleep in advanced PD patients without clinically relevant tolerance or side effects. Hence patients were

found to have had improved quality of life when placed on intraduodenal infusion (Nyholm *et al.*, 2005_a; Mouradian, 2005).

Thus far, levodopa duodenal infusion has been found to produce continuous plasma concentrations of levodopa, and reduced dyskinesias and ‘off time’ in clinical studies (Antonini, 2007).

However, the method is invasive and adverse events associated with the use of the small tube and/or surgical procedure limits the use of intraduodenal levodopa infusion. The most frequently reported complications include dislocation of the distal part of the tube from the duodenum into the stomach which can lead to sudden deterioration of treatment response with recurring motor fluctuations (Nyholm *et al.*, 2005).

2.2.4.5 Nasal delivery of levodopa

The nasal route has been explored for drug delivery because of its large surface area enhanced by the presence of microvilli that cover the highly vascularized epithelial cells (Brime *et al.*, 2000). Additionally, it is easily accessible and avoids first-pass metabolism. Brime *et al.*, (2000) fabricated levodopa-loaded gelatin microspheres by emulsification solvent extraction technique for delivery by means of the trans nasal route. The bioadhesive properties of gelatin facilitated prolongation of the contact between the microspheres and the nasal mucosa, thereby enhancing absorption. However, nasal drug delivery system is an immediate release system and thus would not provide constant and sustained delivery of levodopa for the achievement of continuous dopaminergic stimulation (Ngwuluka *et al.*, 2010).

2.2.4.6 Controlled release drug delivery

The main aim of the development of controlled release delivery systems was to address the difficulties accompanied with traditional methods of administration. This drug delivery system

employs devices such as polymer-based disks, rods, pellets or microparticles that encapsulate drug and release it at controlled rates for reasonably long periods of time. The controlled release system offers several potential advantages over traditional methods of administration (Kim and Pack, 2006).

First, drug release rates can be modified to the needs of a specific application; for example, providing a constant rate of delivery or pulsatile release. Second, provision of protection of drugs that are otherwise rapidly destroyed by the body (example proteins) and finally, improve patient comfort and compliance by replacing frequent doses with infrequent ones. For example, replacing daily doses of injection with once per month injection or replacing four times daily oral doses with once daily dose (Kim and Pack, 2006).

While a variety of devices have been used for controlled release drug delivery, biodegradable polymer microspheres have proven to be the most common types with several advantages (Kim and Pack, 2006).

One of the approaches used to solve the problem of motor fluctuations encountered with levodopa was to reduce the interval between levodopa doses through the administration of controlled release formulations (Barone, 2003). Thus, controlled release levodopa formulations were developed with the hope that they would further prolong the half-life of levodopa and stabilize plasma levels, thereby decreasing the development of motor complications (LeWitt *et al.*, 1989; Hammerstad *et al.*, 1994)

2.2.4.7 Biodegradable microspheres as a drug delivery system for levodopa

Microspheres are controlled release drug delivery systems which have been applied in many instances: hormone therapy, chemotherapy, cardiovascular diseases, neurological disorders, ocular drug delivery, and protein and vaccine deliveries (Benoit *et al.*, 2000; Hickey *et al.*,

2002). Microspheres are known to modulate the release profile of drugs and their absorption characteristics (Vasir *et al.*, 2003). It has been shown that with the use of microspheres as a drug delivery system for levodopa, the dosage size, frequency of administration, systemic side effects and dose-dumping decreased, while the drug could be released continuously (Hickey *et al.*, 2002).

This ultimately enhances patient compliance. Microspheres have been fabricated from a variety of biodegradable polymers, which include gelatin, albumin, polyanhydrides, polyorthoesters, polyesters and polysaccharides (Herrero-Vanrell and Refojo, 2001).

The use of microspheres as drug delivery agents for levodopa and carbidopa is another approach for improving the bioavailability and subsequent clinical response of levodopa (Arica *et al.*, 2005). Arica and co-workers prepared levodopa and carbidopa microspheres by a solvent-evaporation technique using biodegradable polymers, namely poly (DL-lactide) and poly(DL-lactide-co-glycolide). Both *in vitro* and *in vivo* studies were performed. Reduced rotational behaviour was observed in the rats, indicating that levodopa/carbidopa-loaded microspheres exerted functional effects on the striatal dopamine receptors (Arica *et al.*, 2005). The bioavailability of levodopa was improved because the levodopa/carbidopa microparticles released the drug directly on the striatal dopamine receptors, thereby reducing wide distribution of side effects (Arica *et al.*, 2005).

2.3 MICROPARTICLES

2.3.1 Introduction

Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers having a particle size ranging from 1-1000µm. They are sometimes referred to as microparticles (Sahil *et al.*, 2010). Undoubtedly, oral route is the most preferred for taking

medications. However, the short half-life and limited absorption via a specific segment of intestine restricts the therapeutic potential of many drugs. In order to improve the bioavailability and pharmacokinetics profile, it is only rational to release the drug in a controlled and site specific manner. Such is by using microparticles as drug carriers. Microparticulate drug delivery system has been shown to be a reliable means to deliver drugs to the target site with specificity, if modified, and to maintain the desired concentration at the site of interest (Sahil *et al.*, 2010). Hence they play a key role at aiming at improving the bioavailability of conventional drugs and minimizing their adverse effects (Gattani, 2010). There are several opportunities to control characteristics of drug administration and improve the therapeutic efficacy of many drugs due to the variety of techniques for the preparation of microparticles (Sahil *et al.*, 2010).

2.3.2 Techniques of Microparticle preparation

The method by which drugs are entrapped in a polymeric matrix or shell is known as microencapsulation (Kumar *et al.*, 2011). In order to select a method for microencapsulation, the factors to consider are: high yield and drug encapsulation efficiency, integrity of the stability and biological activity of the drug during the microencapsulation process, and microparticles should not exhibit aggregation or adherence. Microencapsulation methods include: hot-melt encapsulation, phase/wet inversion, coacervation, solvent removal, polymerization, solvent evaporation, and spray drying (Subedi *et al.*, 2016). Among these, spray drying method is the commonest and most feasible technique which is extensively studied for the preparation of microparticles.

2.3.2.1 Spray Drying Method

Spray drying is a famous method used in the production of agglomerates, granules or powders from the mixture of drug and excipient solutions as well as suspensions (Agnihotri *et al.*, 2004).

The method is based on drying of atomized droplets in a stream of hot air. In this method, the polymer is first dissolved in aqueous solution, drug is then dissolved or dispersed in the solution and then a suitable cross-linking agent is added. This solution is then atomized into a hot stream of air (He *et al.*, 1999). Atomization leads to the formation of small droplets, from which solvent evaporates instantaneously leading to the formation of free flowing particles as shown in Figure 2.4. The instrument settings such as inlet temperature, rate of feed flow, spray air flow and aspirator flow can together influence the product parameters such as particle size, yield, temperature load and content of residual solvents (Giunchedi, 1995). Significant advantages of using this technique include high encapsulation efficiencies and no residual surfactant.

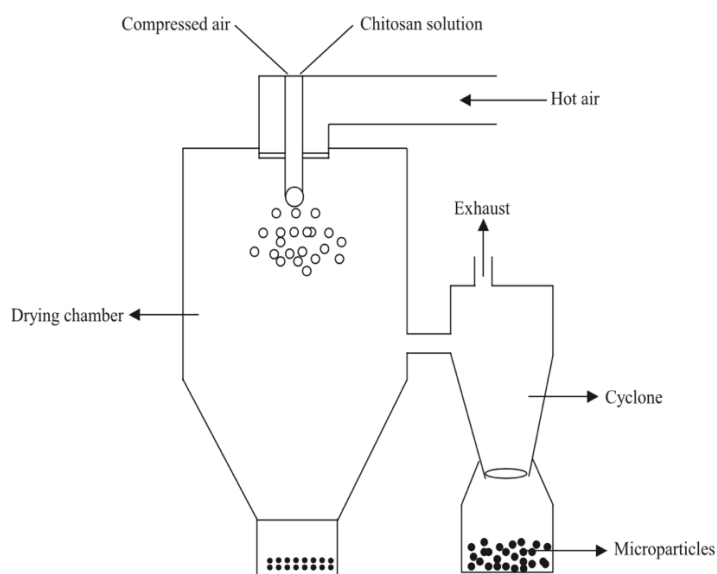


Figure 2.4: Schematic representation of preparation of microparticles by spray drying method (Agnihotri *et al.*, 2004).

2.3.3 Materials used in the Preparation of Levodopa Microparticles

There is diversity of agents that can be used in the preparation of microparticles. Below are the examples of raw materials that have been used in the preparation of levodopa microparticles.

They are: levodopa powder, glutaraldehyde, sodium bisulphite, chitosan, and hydroxypropyl methyl cellulose (HPMC).

2.3.3.1 *Glutaraldehyde*

Glutaraldehyde is a clear, colorless to pale straw-coloured, pungent oily liquid that is soluble in water, alcohol, as well as inorganic solvents. Glutaraldehyde has had great success because of its commercial availability and low cost in addition to its high reactivity. It reacts rapidly with amine groups at around neutral pH (Okuda *et al.*, 1991) and is more efficient than other aldehydes in generating thermally and chemically stable crosslinks (Nimni *et al.*, 1987). Glutaraldehyde acts as a crosslinking agent in the formulation of microparticles.

Acting as a crosslinking agent, glutaraldehyde controls diffusion of drugs from the polymer matrix. Drug release in general is affected by the molecular weight and concentration of the polymer, the crosslinking agent and its concentration, variables like stirring speed, additives, and drug-polymer ratio. Amount of glutaraldehyde used in microparticle formulation affects the release of the drug from the microparticle, the encapsulation efficiency and particle size. The higher the amount of crosslinking agent used, the slower the release rate of the drug which is essential in controlled release formulations (Thanoo *et al.*, 1992).

2.3.3.2 *Hydroxypropyl methyl cellulose (HPMC)*

Hypromellose is the simple name for hydroxypropyl methyl cellulose which is used as a polymer in a controlled-delivery drug in oral-medicaments (deSilver and Oliver, 2005). It is an odorless, tasteless granular or fibrous powder which is creamy white or white in colour and soluble in cold water. HPMC is a semi-synthetic, viscoelastic, non-ionic hydrophilic polymer which is used extensively in various formulations of controlled release dosage form. It exhibits pH independent drug release profile (Rell *et al.*, 2009).

Extensive use of HPMC in controlled drug delivery system has been realized because they offer unique properties so far not been attained by any other polymers. The rate of the drug release from a matrix product depends on the relaxation of the polymer chains, which overall displays sustained release characteristics (Williams *et al.*, 2011). When HPMC is used as a polymeric matrix for the controlled released dosage form, the release of the drug takes place at a steady state rather than the controlled released dosage form which has been coated with normal coating ingredient. This results in uniform drug release with very lower chances of adverse effects like dose dumping (Zajic and Buckton, 1990).

HPMC is used as a coating polymer as well as a matrix former in various drug deliveries. Due to its gel forming nature, it can be well accepted in controlled release drug delivery (Tamasree *et al.*, 2016).

2.3.3.3 Chitosan

Chitosan is a hydrophilic, biocompatible and biodegradable polymer. It is commercially available in a range of molecular weights, degrees of deacetylation and types of salts (such as glutamate, hydrochloride and lactate). Chitosan has –OH and –NH₂ groups that allow hydrogen bonding. Due to its linear molecule, it expresses a sufficient chain flexibility, the conformation of which is highly dependent on ionic strength. These properties are considered essential for mucoadhesion (Peppas and Buri, 1985; Robinson and Mlynek, 1995).

Mucoadhesion can be defined as the state in which two materials, at least one of which is biological in nature, are held together for a prolonged time period by means of interfacial forces (Kaurav *et al.*, 2012). Mucoadhesive microparticles made from the naturally occurring biodegradable polymers like chitosan have attracted considerable attention for several years in sustained drug delivery (Huang *et al.*, 2003). They have advantages such as absorption and enhanced bioavailability of drugs as a result of a high surface to volume ratio, a much more

intimate contact with mucus layer, and specific targeting of drugs to the absorption site (Raval *et al.*, 2010).

Furthermore, the cationic polyelectrolyte nature of chitosan provides a strong electrostatic interaction with mucus or a negatively charged mucosal surface. The importance of this mucoadhesive property of chitosan has been demonstrated in earlier work by Lehr *et al.*, (1992), Illum *et al.*, (1994), Lueßen *et al.*, (1994) Fiebrig *et al.*, (1995); Aspden *et al.*, (1996). More specifically, chitosan has been used as a delivery vehicle for nasal and oral delivery of peptide drugs, in order to improve drug absorption (Illum *et al.*, 1994; Lueßen *et al.*, 1996, 1997). Mucoadhesive tablets containing chitosan, have been developed by Takayama *et al.*, (1990), Miyazaki *et al.*, (1994) and Nakayama *et al.*, (1994). These tablets have both adhesive and sustained release characters. Reports suggest that coating of liposomes with chitosan improved their adsorption to mucosal surfaces (Takeuchi *et al.*, 1996). Below is an image of chitosan microparticles prepared by the spray-drying method (figure 2.5).

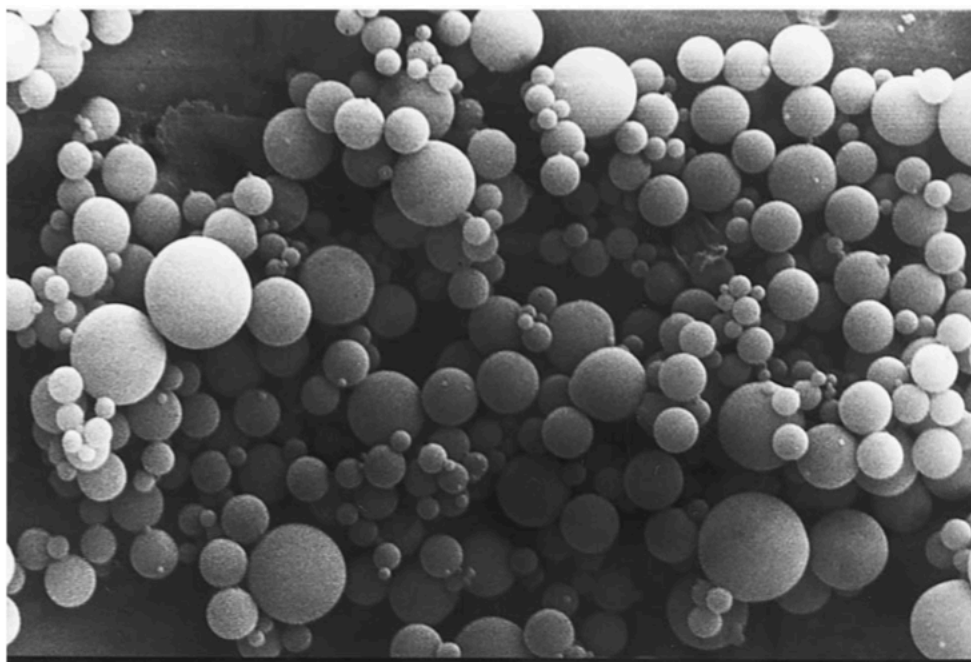


Figure 2.5: Chitosan microparticles prepared by spray drying and crosslinked with glutaraldehyde (He *et al.*, 1999)

2.3.4 Evaluation/Characterization of Microparticles

The essential characterization parameters for microparticles are as follows:

2.3.4.1 Particle size

The mean particle size and the width of particle size distribution are important characterisation parameters as they govern the saturation solubility, dissolution velocity, physical stability and even biological performance of microparticles. Particle size can influence the biopharmaceutical properties of microparticles, their biodistribution and the particle content uptake (Shah *et al.*, 2014). The most common method used to measure particle size of microparticles is dynamic light scattering. The principle of dynamic light scattering is based on the theory that particle size can be determined by measuring the random changes in the intensity of light scattered from a suspension or solution. Small particles in suspension undergo random thermal motion known as Brownian motion (Burgess *et al.*, 2004). This random motion is measured to calculate particles size. The Malvern Nano Zetasizer is an instrument used to measure particle size.

2.3.4.2 Encapsulation efficiency

Encapsulation efficiency is the percentage of drug that is successfully entrapped into the microparticle. Encapsulation efficiency is calculated as a ratio of the actual drug content to the theoretical drug content expressed as a percentage (El-Say, 2016). It has been observed and widely accepted that an increase in the polymer concentration increases the entrapment of the drug inside the microparticle (Subedi *et al.*, 2016). Also, Banerjee *et al.* observed that incorporation of optimized amount of cross-linking agents like glutaraldehyde in the formulation process can improve the entrapment efficiency (Banerjee *et al.*, 2010). A high

entrapment efficiency shows better control of drug release from the matrix system (Ranjha *et al.*, 2010).

2.3.4.3 Drug loading capacity

The drug loading capacity is the amount of drug loaded per unit weight of the microparticle, indicating the percentage of mass of the microparticle that is due to the encapsulated drug. Drug loading can be done by two methods, that is, during the preparation of microparticles (incorporation) and after the formation of particles (incubation) (Padalkar *et al.*, 2011). In these systems, drug is physically embedded into the matrix in the case of incorporation method or adsorbed onto the surface, in the incubation method. Maximum drug loading can be achieved by incorporating the drug during the formation of microparticles (Agnihotri *et al.*, 2004). Drug loading serves as a complementary characteristic to encapsulation efficiency hence a high drug loading capacity is indicative of a high encapsulation efficiency (Vyslouzil *et al.*, 2014).

2.3.4.4 In Vitro release studies

The *in vitro* release studies aid in understanding the behaviour of the delivery system in terms of drug release and their efficacy. Since microsphere is a heterogeneous system, the drug release from the polymer takes place through a diffusion process, in an *in vitro* environment. The release of the drug is determined by the extent of degradation of polymeric microsphere. There are several apparatus of varying designs that have been used to study the *in vitro* release characteristics of microparticles (Jain, 2004).

The various methods include Beaker method which involves the adhesion of dosage form of the microparticles at the bottom of a beaker containing the medium and stirring uniformly using the overhead stirrer, Interface diffusion system, which uses four compartments (each representing the oral cavity, buccal membrane, body fluids and protein binding respectively)

containing different medium (Venkatesh, 1989).

There is also the Modified Keshiary Chien Cell apparatus which contains distilled water as the dissolution medium and uses a trans membrane drug delivery system placed in a glass tube and fitted at the bottom of the medium (Save and Venkatachalam, 1994).

Lastly, the Dissolution apparatus method, which is the method used in the current study for investigating the release profile of the prepared microparticles. In this method, a weighed quantity of the microsphere is placed in a dissolution basket, which is immersed in a larger volume of continuous phase acceptor fluid (phosphate buffered saline). The compartment is stirred and the drug which diffuses out of the microspheres into the continuous phase is periodically sampled and assayed (Lopez *et al.*, 1998).

CHAPTER THREE

3.0 MATERIALS AND METHODS

Levodopa and carbidopa powders were purchased from Sigma-Aldrich (USA). Hydroxypropylmethyl cellulose (HPMC) was a gift from Ernest Chemist Limited (Ghana), glutaraldehyde was purchased from Amresco, Inc. VWR International (USA), sodium bisulfite and all other reagents used for experiments were of analytical grade and purchased from approved suppliers.

3.1 STUDY DESIGN

The study was an experimental design using *in vitro* assays and animal models.

3.1.1 Study Location

The formulation aspect of the study (preparation and characterization of microparticles) was done at the Pharmaceutics Research Laboratory of the School of Pharmacy, University of Ghana. The Pharmacokinetic aspect of this study was done at the Animal Experimentation Unit of the School of Biomedical and Allied Health Sciences, University of Ghana, Korle-Bu, and drug levels in plasma was done at the Mycotoxin and Food Analysis Laboratory, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

3.2 PREPARATION AND CHARACTERIZATION OF LEVODOPA MICROPARTICLES

The preparation and characterization of levodopa microparticles was done according to methods described by Nettey *et al.*, (2017) with minor modifications. Carbidopa microparticles were also prepared to be administered in combination with levodopa microparticles for the pharmacokinetic studies.

3.2.1 Preparation of microparticles

Microparticles of levodopa/carbidopa were prepared using the spray drying technique. In order to make a 20% drug loading capacity of the drug-polymer solution, a 1- part drug solution was added to 4 parts of the polymer solution. The polymer (HPMC) solution was prepared by weighing 10 g of the HPMC powder into 200 mL of distilled water. A 2.5 g of levodopa/carbidopa powder was weighed into 100 mL of distilled water, followed by the dropwise addition of 32% hydrochloric acid (HCl) until the drug was completely dissolved in the solution. The levodopa/carbidopa solution was slowly added to the HPMC solution with stirring. This was followed by the addition of distilled water to make a final volume of 400 mL in order to dilute the viscous solution. The homogenous mixture obtained was stirred on the magnetic stir plate for 1 h, after which 200 mL glutaraldehyde (50%) solution was added to cross-link the HPMC molecules. This was left to stir for another 1 h. In order to quench the cross-linking, 2 mL of a 1% sodium bisulfite solution was added to the mixture and stirring continued for 1 h. After, a 75 mg of chitosan powder was weighed and dissolved in 50 mL of distilled water with the dropwise addition of HCl. The chitosan solution was then added to the drug-HPMC solution in order to further coat the drug and the polymer. The solution was left to stir on the magnetic stir plate for 1 h. The resulting solution was fed into the Bilon-6000Y laboratory spray-dryer and levodopa/carbidopa microparticles obtained.

3.2.2 Particle Size Determination

One milligram (1 mg) of the levodopa microparticles was weighed and suspended in 20 mL of distilled water. The suspension was further diluted by adding more of the distilled water and transferring the suspension into a cuvette. The sample in the cuvette was placed into the cell holder of the Malvern Nano Zetasizer, where it was illuminated with a laser. The intensity

fluctuations in the scattered light was analysed and used to calculate the size of microparticles by an inbuilt digital correlator.

3.2.3 Content Analysis

In order to determine the actual amount of drug contained in the microparticles, 25 mg of levodopa microparticles (weighed in triplicate) was crushed in a mortar, and suspended in 10 mL of distilled water. The suspension was transferred into Eppendorf tubes, and centrifuged at 3000 rpm for 10 min. A portion of the supernatant was pipetted into new Eppendorf tubes and 20 µL was injected into a High Performance Liquid Chromatography (HPLC) system as described subsequently for the analysis of the drug content. The samples were analysed against a standard plot to calculate the drug content of the microparticles.

3.2.4 Encapsulation Efficiency

Encapsulation efficiency of microparticles was measured to determine how effective the HPMC polymer was able to encapsulate the drug. From the actual drug content determined initially, the encapsulation efficiency was calculated as the ratio of actual drug concentration to the expected drug concentration. Mathematically this can be represented as:

$$\% \text{Encapsulation Efficiency} = \frac{\text{Actual drug concentration}}{\text{Expected drug concentration}} \times 100$$

The concentration of levodopa was determined using HPLC as described subsequently.

3.2.5 *In Vitro* Drug Release Test

Samples (75 mg) of levodopa microparticles were weighed and transferred into empty size 0 gelatin capsule shells. The filled capsules were placed in baskets of USP Dissolution Apparatus 1, and each of the outer flasks were filled with 500 mL of phosphate buffered saline (PBS), of pH 7.4. The baskets were allowed to rotate at 100 rpm (and at a temperature of 37°C). At 0,

0.25, 0.5, 1, 2, 4, 8, 12 and 24 h, 5 mL of the samples were drawn from the flask and replaced with an equal amount of PBS. The amount of drug released was determined using HPLC as described below. The release experiment was performed in triplicate.

3.3 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) FOR LEVODOPA LEVELS

For content analysis and release studies, samples were analysed using Agilent Technologies system (Jos. Hansen, Hamburg/Germany) with the following chromatographic conditions: Mobile Phase comprised of 0.01 M Phosphate buffer (adjusted to a pH of 2.68 with hydrochloric acid) and Methanol (20:80 v/v). An injection volume of 20 μ L, a flow rate of 1.0 ml/min for 10 min at wavelength of 280 nm were used. A Vertex Plus C18, 150 X 4 mm column was used for all analysis.

3.3.1 Preparation of Stock and Working Solutions.

A stock standard solution (1 mg/mL) of levodopa was prepared by accurately weighing 50 mg of levodopa and transferring into a 50 mL volumetric flask. To that, 30 mL of deionised water was added followed by dropwise addition of HCl to dissolve the drug completely. Then the volume was made up to the 50 mL mark with more deionised water. Further working standard solution of levodopa was prepared by making two-fold serial dilutions with concentrations 1.25, 5, 12.5, 25 and 50 μ g/mL. Twenty microliters (20 μ L) of the standard working solutions were injected and the retention time and peak area were determined. Standard plots were made from the HPLC values and the equation of the calibration curve obtained. The sample solutions were also analysed by injecting 20 μ L of the sample solution into the HPLC system and peak area determined for each sample. The amount of levodopa in microparticles was calculated by substituting the peak areas determined from the HPLC analysis into the equation of the line obtained from the calibration curve ($y = mx + c$) as shown in Appendix B.

3.4 *IN VIVO* PHARMACOKINETIC EVALUATION OF LEVODOPA MICROPARTICLES

3.4.1 Animal Acquisition and Housing

Male *Sprague-Dawley* (SD) rats (Hsd; SD strain), weighing 150-200 g and 6-8 weeks old, were obtained from the Department of Animal Experimentation, School of Biomedical and Allied Health Sciences, University of Ghana, Korle-Bu. The animals were housed in stainless steel cages. Each rat occupied a minimum space of 2 cubic feet (61 cm x 31 cm) with soft wood shavings as bedding for their comfort. They were fed with normal pellet diet (AGRIMAT Kumasi), given water *ad libitum*, and maintained under optimal laboratory conditions (temperature $25 \pm 1^{\circ}\text{C}$, relative humidity 60-70%, and 12-hour light-dark cycle). All feeding and water troughs were cleaned regularly to prevent contamination. The animals were acclimatized to this environment for two weeks before experimentation. All animal procedures and techniques used in this study were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (N.R.C., 2010).

3.4.2 Animal Groupings for Pharmacokinetic Study

By simple random sampling, SD rats were put into 3 groups consisting of 5 rats each. Group 1 received levodopa/carbidopa (Sinemet CR), Group 2 received levodopa/carbidopa powder and animals in Group 3 were given microparticles of levodopa/carbidopa. Levodopa and carbidopa microparticles were combined in a 1:4 ratio of carbidopa to levodopa.

3.4.3 Drug Administration and Sample Collection

For pharmacokinetic studies, carbidopa and levodopa microparticles were combined (in a 1:4 ratio respectively before administration). Animals were fasted overnight prior to administration of treatments. A dose of (5/20 mg/kg) of carbidopa/levodopa of the respective formulations

was given via the oral route to rats in each group. Treatments were administered every 12 h. After administration of the dose on the second day, rat tails were snipped and blood samples collected into Ethylenediaminetetraacetic acid (EDTA) tubes at time intervals of 0.25, 0.5, 1, 2, 4, and 12 h. A solution of 25% sodium metabisulfite in water was made and added in a ratio of 1: 10 (v/v) to rat blood samples collected to minimize oxidation of the levodopa in blood. Blood samples were centrifuged at 10,000 rpm for 5 min to separate plasma and stored at -20°C until analysis.

3.4.4 Determination of Plasma Levodopa Levels

Levodopa in plasma was analysed using HPLC as described by Elbarbry *et al.*, (2018) with minor modifications.

3.4.4.1 Chromatographic Conditions

The Cecil Adept HPLC system (Cecil Instrumentation Services Ltd., Cambridge, UK) consisting of an auto-sampler, a binary pump and a model Diode-array detector was used. The chromatographic analysis was performed using the Zorbax C₁₈ column (4.6 mm x 300 mm) with a particle size of 5 µm and column temperature of 40 °C. The mobile phase consisted of 20 mM phosphate buffer (pH 2.5) and methanol HPLC grade (95:5, v/v). This was run at a flow rate of 1 mL/min for 10 min. Levodopa's absorbance was detected at 230 nm.

3.4.4.2 Preparation of Stock and Working Solutions

Stock solution of levodopa was prepared fresh everyday by dissolving the analytical standard in 1 M perchloric acid at a concentration of 4.6 mg/mL. Working solutions were obtained by serial dilution of stock solution with methanol containing 0.05% v/v perchloric acid at concentrations of 46, 460, 920, and 1840 µg/mL. A stock of the internal standard (IS) solution was prepared by dissolving 20 mg of methyl dopa in 15 mL of water to obtain a concentration

of 1.3 mg/mL. Working internal standard solution was prepared by diluting 1 μ L of the methyl dopa standard in 99 μ L of methanol containing 0.05% perchloric acid to obtain a final volume of 100 μ L. All stock and working standards were stored at -20°C until analysis using HPLC. Standard plots were made by correlating peak ratios of the analytical and internal standards to the known standard concentrations and a calibration equation obtained. Calibration curve showing equation of the line and the coefficient of correlation is shown in Appendix D.

3.4.4.3 Sample Preparation

Levodopa in plasma was extracted using protein precipitation method with perchloric acid as the precipitating agent. To 50 μ L of rat plasma, 100 μ L of the working internal standard solution and 25 μ L of 0.5 M perchloric acid were added. The mixture was vortexed for 2 min. After, centrifugation was done at 10,000 rpm and the supernatant transferred into an auto-sampler vial. An injection of 20 μ L of the supernatant was made directly into the analytical column for immediate HPLC analysis. Quantitation of the unknowns (levodopa levels in plasma) was by interpolation from the weighted linear regression line of the ratios of the peak areas of the analytical and internal standard.

The study protocol was approved by the Ethics and Protocol Review Committee for College of Health Sciences, University of Ghana, with a Protocol Identification Number: *CHS-Et/M.8 - 5.14/2018 – 2019*.

3.5 DATA ANALYSIS

Statistical test of significance was taken as $p < 0.05$ and performed on all continuous data using unpaired t-test for two independent sample means, and one-way analysis of variance (ANOVA) for comparison of more than two independent sample means. All tests were expressed as mean and standard deviation from mean (STDEV).

Pharmacokinetic parameters for levodopa in the 3 groups were determined by non-compartmental analysis. The maximum plasma drug concentration ($C_{\max}$) and the time to achieve this peak ($T_{\max}$) of levodopa were extrapolated from concentration-time curves. The elimination rate constant (K_e) was determined by linear regression analysis of the terminal-linear part of the log plasma concentration-time curves. Area under the concentration-time curve (AUC) was calculated by the linear trapezoidal rule.

CHAPTER FOUR

4.0 RESULTS

4.1 CHARACTERIZATION OF LEVODOPA MICROPARTICLES

4.1.1 Particle Size Distribution

The particle size of the prepared levodopa microparticles was determined using Malvern Nano Zetasizer. The size distribution of levodopa microparticles ranged between 0.04 μm to 6 μm with a mean size of 0.2 μm as shown in Figure 4.1.

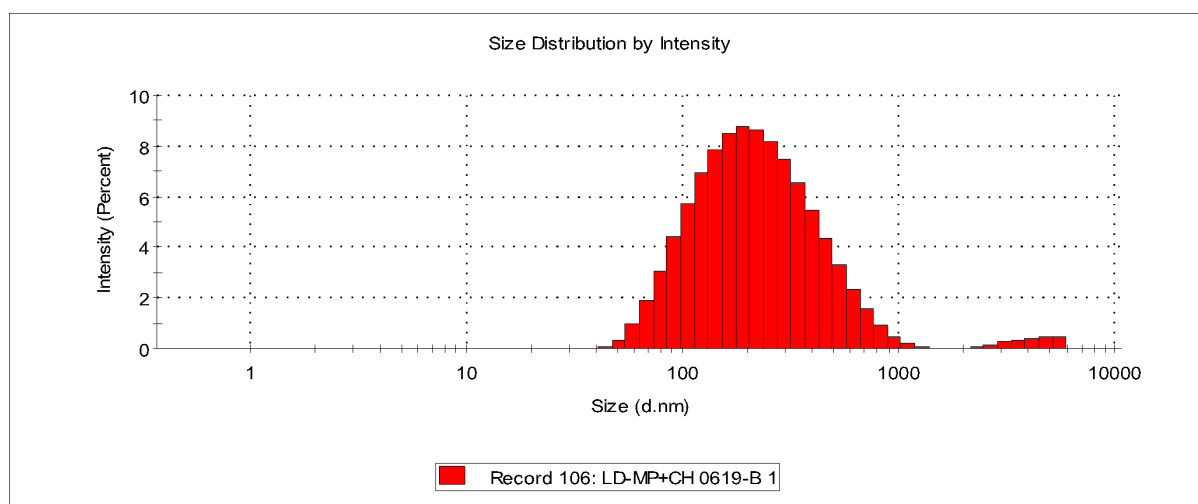


Figure 4.1: Results showing the particle size distribution of the formulated levodopa microparticles

4.1.2 Drug Content Analysis

Content analysis was done to determine the actual amount of levodopa in the microparticles. HPLC was used to analyse the levodopa standard solutions prepared. The peak areas generated from the chromatogram were plotted against the various concentrations of the standard solutions and equation of the straight line ($y = mx + c$) obtained, this is shown in Figure 4.2.

For the content analysis test, peak areas that were integrated from the HPLC analysis were substituted into the equation of the straight line obtained from the standard curve. This was used to calculate for levodopa concentrations (shown in Table 4.1).

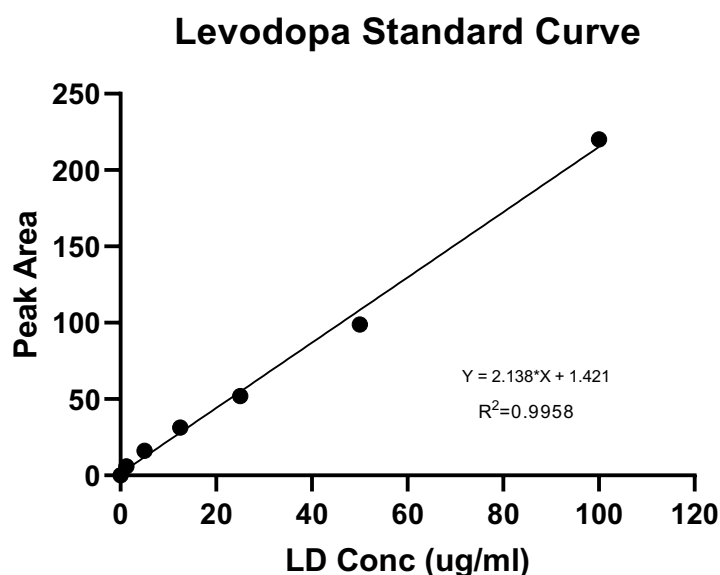


Figure 4.2: Levodopa standard curve showing the equation of the line ($y = 2.1383x + 1.4208$) and the correlation coefficient ($R^2 = 0.9958$)

Table 4.1: Concentrations obtained from the content analysis of levodopa

Levodopa microparticles	Peak Area	Concentration ($\mu\text{g/mL}$)	Average Concentration ($\mu\text{g/mL}$) $\pm$ STDEV
Run 1	1082	505.34	478.84 $\pm$ 29.03
Run 2	959	447.82	
Run 3	1035	483.36	

Tests were done in triplicate giving an average levodopa concentration of 478.84 $\mu\text{g/mL}$.

4.1.3 Percent Drug Loading

Results obtained from the drug content analysis translated to 478.84 µg mass of the drug in every mL of solution. Therefore, for the 10 mL of distilled water that was used for content analysis, the mass of microparticle contained in that was 4788.4 µg. In order to calculate for the actual drug loading, the actual mass of the drug was compared to the total drug-polymer weight of 25 mg. All microgram units were converted to milligrams and the values substituted into the equation below:

$$\begin{aligned}\% \text{Drug Loading} &= \frac{\text{actual mass of drug}}{\text{total drug-polymer weight}} \times 100 \\ &= \frac{4.788 \text{ mg}}{25 \text{ mg}} \times 100 \\ &= 19.15 \%\end{aligned}$$

Therefore, out of the expected 20% drug loading, the actual drug loading was 19.15%.

4.1.4 Encapsulation Efficiency

From the drug content analysis, the encapsulation efficiency was determined by the percentage of the ratio of the actual levodopa concentration to the expected levodopa concentration. This was calculated as follows:

$$\% \text{ Encapsulation efficiency} = \frac{\text{actual drug concentration}}{\text{theoretical drug concentration}} \times 100$$

Amount of microparticles weighed for content analysis = 25 mg

With the expected 20% drug loading, expected amount of levodopa contained in the 25 mg =

$$\frac{20}{100} \times 25 \text{ mg} = 5 \text{ mg}$$

Volume of water used to dissolve the microparticles = 10 mL

$$\text{Expected concentration of levodopa} = \frac{\text{amount of drug}}{\text{volume of water dissolved in}} = \frac{5 \text{ mg}}{10 \text{ mL}} = 0.5 \text{ mg/mL}$$

$$= 500 \text{ } \mu\text{g/mL}$$

$$\% \text{ Encapsulation Efficiency} = \frac{478.84}{500} \times 100 = 95.6\%$$

Therefore, for an expected concentration of 500 $\mu\text{g/mL}$, the actual concentration of levodopa in the microparticles was 478.84 $\mu\text{g/mL}$, indicating an encapsulation efficiency of 95.6% for the prepared levodopa microparticles.

4.2 *IN VITRO* RELEASE OF LEVODOPA FROM MICROPARTICLES

In vitro release studies of levodopa microparticles was performed in phosphate buffered saline (pH 6.8) at 37°C using the Dissolution Apparatus 1 equipment. The dissolution studies revealed that *in vitro* drug release of the levodopa microparticles was sustained with maximum release occurring at 12 h followed by a decrease in release-time points as shown in Figure 4.3. Results of the *in vitro* drug release profile of levodopa microparticles is summarised in Table 4.2.

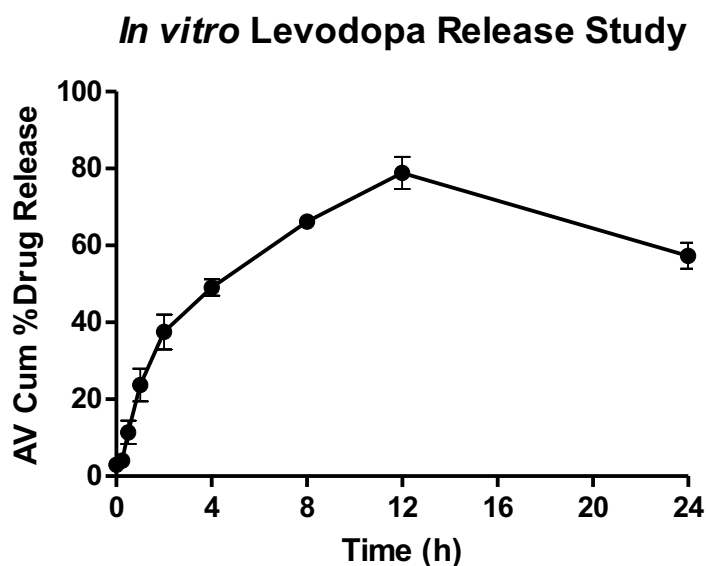


Figure 4.3: The average cumulative percentage release of levodopa against time

Table 4.2: *In vitro* release of levodopa from the formulated microparticles

Time (h)	Average Cumulative % Drug Release $\pm$ STDEV
0	2.98 $\pm$ 0.94
0.25	4.07 $\pm$ 1.18
0.5	11.4 $\pm$ 3.04
1	23.71 $\pm$ 4.24
2	37.51 $\pm$ 4.52
4	49.07 $\pm$ 2.10
8	66.22 $\pm$ 1.62
12	78.87 $\pm$ 4.15
24	57.33 $\pm$ 3.37

4.3 *IN VIVO* PHARMACOKINETIC EVALUATION OF LEVODOPA MICROPARTICLES

4.3.1 Concentration-time curves for 3 treatment groups of *Sprague-Dawley* (SD) rats

The concentration-time curves for SD rats in the 3 groups; Group 1 administered Sinemet CR, Group 2 administered levodopa/carbidopa powder and Group 3 administered microparticles of levodopa/carbidopa are shown in Figure 4.4. From the graph, it can be observed that the concentration-time curve of rats administered microparticles of levodopa/carbidopa had the highest peak.

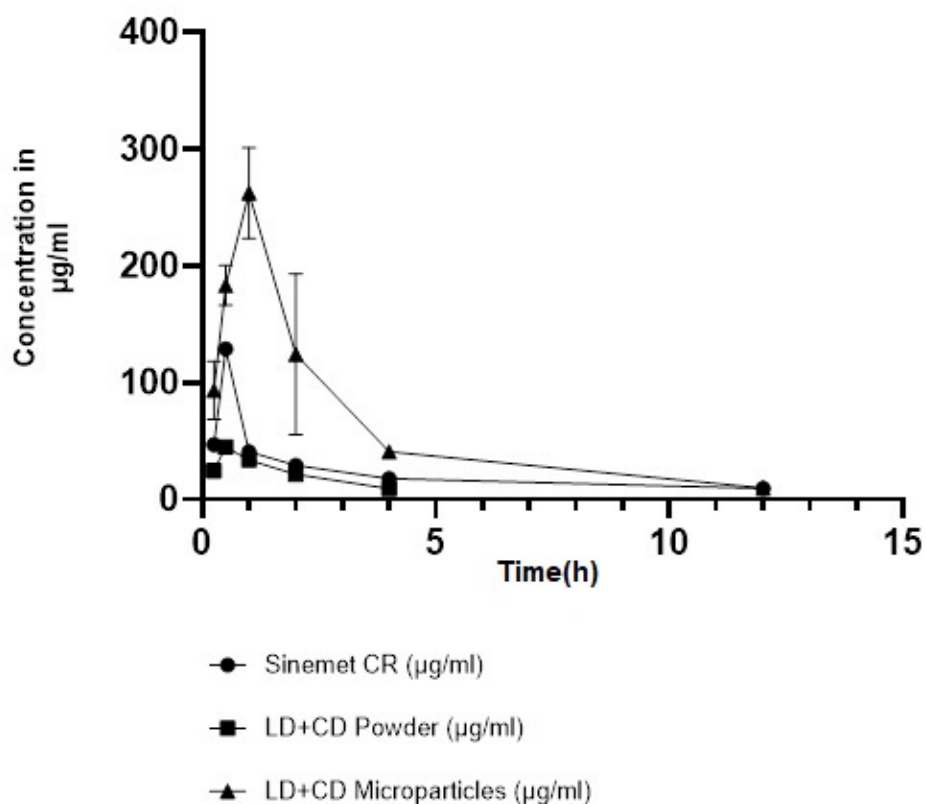


Figure 4.4: Concentration-time curves for the 3 treatment groups of SD rats

4.3.2 Pharmacokinetic parameters of rats in the 3 treatment groups

4.3.2.1 One-way ANOVA among 3 treatment groups

The pharmacokinetic parameters of SD rats in the 3 treatment groups obtained from the concentration-time curve are summarized in Table 4.3. Analysis of pharmacokinetic parameters with one-way ANOVA showed that T_{max} , C_{max} , $AUC_{0 \rightarrow \infty}$, K_e and $t_{1/2}$ varied significantly ($p < 0.05$) between the 3 treatment groups.

Table 4.3: Pharmacokinetic parameters of SD rats in the 3 treatment groups

PK parameter	Treatment Group [Mean (STDEV)]			p-value
	Sinemet CR	LD+CD powder	LD+CD MP	
$T_{\max}$	0.5	0.67 (0.17)	1	0.027
$C_{\max}$	128.8 (45.38)	46.0 (6.57)	262.35 (22.43)	0.0058
$AUC_{0 \rightarrow \infty}$	355 (36.28)	107.85 (4.75)	749.77 (70.8)	0.0002
K_e	0.11 (0.05)	0.44 (0.08)	0.20 (0.05)	0.016
$t_{1/2}$	6.3 (1.97)	1.66 (0.27)	4.0 (1.07)	0.0304

LD+CD powder = levodopa plus carbidopa powder; LD+CD MP = levodopa plus carbidopa microparticles

4.3.2.2 Post hoc analysis with Tukey's multiple comparison

Time to reach peak concentration ($T_{\max}$)

A comparison between Sinemet CR and levodopa/carbidopa powder showed that the time to reach peak concentration after administration ($T_{\max}$) did not differ significantly ($p = 0.48$). A comparison of $T_{\max}$ between levodopa/carbidopa powder and levodopa/carbidopa microparticles also showed no statistically significant difference ($p = 0.10$). However, $T_{\max}$ was found to differ significantly ($p = 0.024$) between levodopa/carbidopa microparticles and Sinemet CR.

Peak levodopa concentration (C_{max})

A comparison between Sinemet CR and levodopa/carbidopa powder showed that the peak concentration after administration (C_{max}) did not differ significantly ($p = 0.196$). However, a comparison of C_{max} between levodopa/carbidopa microparticles and levodopa/carbidopa powder showed a statistically significant difference ($p = 0.005$) between these two. Also, C_{max} was found to differ significantly ($p = 0.04$) between levodopa/carbidopa microparticles and Sinemet CR.

Area under the concentration-time curve (AUC)

A comparison between Sinemet CR and levodopa/carbidopa powder showed a statistically significant difference ($p = 0.0208$) in the area under the concentration time curves (AUC) obtained after administration. A comparison of AUC between levodopa/carbidopa microparticles and Sinemet CR also showed a statistically significant difference ($p = 0.0022$) between these two. The difference between the AUC values obtained for levodopa/carbidopa microparticles and levodopa/carbidopa powder was also statistically significant ($p = 0.0002$).

Elimination half-life ($t_{1/2}$)

A comparison between the elimination half-lives of Sinemet CR and levodopa/carbidopa powder was found to differ significantly ($p = 0.0448$). However, a comparison between the half-life of Sinemet CR and levodopa/carbidopa microparticles showed no significant difference ($p = 0.224$). Also, half-life of levodopa/carbidopa microparticles did not differ found significantly ($p = 0.4600$) from the that of the levodopa/carbidopa powder.

The Tukey's multiple comparison table has been shown in Appendix E.

CHAPTER FIVE

5.0 DISCUSSION

Particle size influences many properties of particulate materials and is a valuable indicator of quality and performance. In the pharmaceutical industry, the size of active ingredients influences some vital drug properties including content uniformity, dissolution and absorption rates (PSA Guidebook, 2016). For these reasons, it is important to measure and control the particle size distribution of drugs (microparticulate in some instances) in drug delivery. The size of the microparticles influences the biodistribution and particle content uptake of the drug and thus influences its pharmacokinetic properties in the body. In the current study, microparticles were measured for their average particle size using dynamic light scattering.

Results from dynamic light scattering are typically reported as an intensity distribution which quantifies distribution width. From the results obtained, the size of the prepared microparticles ranged between 0.04 μm to 6 μm with a mean size of 0.2 μm . The small size of the microparticles could be accounted for by the process of fabrication and also the formulation parameters. The spray drying method has traditionally been used to produce particles with a narrow size distribution usually ranging from 0.1 to 10 μm . It is known that, the manufacturing parameters (nozzle used, pump rate and compressed spray air flow) affects the particle size of the resultant microspheres (Kumar et al., 2011). Smaller nozzle diameter as well as increased air flow rate produces microparticles with smaller sizes. A small size of microparticles confers the extra advantage of a large surface area to volume ratio. Thus, for a given rate of drug diffusion through the microparticle, the rate of flux of drug out of the microsphere, per mass of formulation, will increase with decreasing particle size. In addition to a large surface area to volume ratio, water penetration into smaller particles may also be quicker (due to shorter

distance from the surface to the centre of the particle) hence ensuring a more efficient drug release (Kim and Pack, 2006).

The percentage drug loading of the levodopa microparticles was measured to determine the percentage of the actual mass of active ingredient. With a 19.1% drug loading, the actual drug content of the microparticles was 478.84 µg/mL out of the expected 500 µg/mL, giving an encapsulation efficiency of 95.6%. The very high drug content and encapsulation efficiency calculated gives an indication of how effective the HPMC polymer was in entrapping the drug which leads to prolonged release. These findings are consistent with work done by Choudhary *et al.*, (2010) in which preparative variables for floating microspheres of levodopa/carbidopa were evaluated and optimized as a gastro retentive drug delivery approach. Results from the study clearly indicate that a high drug load (resulting from increasing encapsulation efficiency) exhibited prolonged drug release for about 10 hours and remained buoyant for more than 12 hours (Choudhary *et al.*, 2010). Arica *et al.*, also prepared and evaluated injectable biodegradable carbidopa/levodopa microspheres for the intracerebral treatment of Parkinson's disease. Likewise, optimization of formulation variables was done and increasing entrapment efficiency increased drug yield leading to an extended duration of drug release (Arica *et al.*, 2005).

The *in vitro* release study was performed in phosphate buffer, pH 6.8 and at 37°C to mimic physiologic conditions. The release of the drug from the levodopa microparticles was controlled, showing a steady increase in drug release with increasing time for the first 12 h followed by decreasing release with time. The increase in drug release was sustained over a long period with 78% release of levodopa occurring at 12 h. The release profile showed better sustained release characteristics rather than the usual initial burst effect associated with smaller microparticles (Bozdag *et al.*, 2001). This could be as a result of the 'incorporation' technique

(combining both the drug and the polymer before spraying into microparticles) used in drug loading, which yielded a high drug loading capacity and therefore was able to entrap most of the drug in the polymer. The high entrapment effect prevented the adsorption of drug to the surface of microparticles thus prevented a burst release and ensured a more controlled drug release from the microparticles. However, the decrease in release after 12 h could result from the degradation of levodopa after its release into the dissolution medium. This result is similar to some others from literature. Pappert *et al.*, (1996) investigated the environmental factors that promote levodopa stability in solution and confirmed that levodopa in solution is an unstable compound and degrades naturally over time. In a plot of levodopa levels against time, the graph showed decreasing levels of levodopa with increasing time when the solution was kept at room temperature. In the current study, the samples were kept at room temperature during the experiment before refrigerating overnight prior to analysis. Therefore, the degradation process could have already started in the dissolution medium at room temperature before refrigeration.

Autoxidative reaction of levodopa in solution is catalysed by oxygen, a reaction which is faster at higher pH (Umek *et al.*, 2018). A study done by Di Stefano *et al.*, (2006) supports the claim that levodopa is unstable and undergoes chemical hydrolysis at higher pH while it has good stability at low pH. D'Aurizio *et al.*, in 2011 performed an *in vitro* release study of microparticles of a prodrug of levodopa in a dissolution medium at a low pH and found that the stability of the prodrug was ensured after its release into the dissolution medium. The *in vitro* release kinetics from the investigation showed increasing percentage levodopa release with increasing time. The pH of the phosphate buffer saline which was used as the dissolution medium in the present study was (6.8) which is quite high, and this could have also promoted the degradation of levodopa microparticles in the dissolution medium. The degradation lead to the decrease in the percentage release of the levodopa with time hence the descending release-time points observed in the *in vitro* release graph.

Furthermore, the drawbacks with the conventional oral controlled release formulation are erratic absorption and latency to onset of action which was attributed to the irregular gastric emptying of the gastrointestinal tract (Salat and Tolosa, 2013). The present study used a gastro-retentive drug delivery approach (mucoadhesion by coating microparticles with chitosan) in the formulation of oral controlled release levodopa microparticles. As part of the study, the pharmacokinetic characteristics of the formulated microparticles in SD rats was evaluated.

From the concentration-time curves, it was evident that the highest plasma levodopa levels ($C_{\max}$) was achieved with the microparticles, compared to the conventional controlled release form (Sinemet CR) and the pure levodopa powder. These results are similar to other results published in literature on the comparison of microparticles with traditional dosage forms (Nettey *et al.*, 2017; LeWitt, 2015). The very small size of the levodopa microparticles enhance substantially the extent of absorption from the site of administration into circulation due to the large surface area to volume ratio (Nettey *et al.*, 2017). Coating microparticles with chitosan may have also enabled the microparticles to adhere to the intestinal mucosal surface thereby enhancing contact time for absorption and resulting in a higher plasma concentration-time profile for the levodopa/carbidopa microparticles (Raval *et al.*, 2010). The peak plasma concentration ($C_{\max}$) of a drug is often related to the intensity of pharmacological response and should ideally be above minimum effective concentration but less than what would lead to adverse drug reactions (Brahmankar *et al.*, 2009). Since efficacy studies have not yet been done to ascertain the minimum and maximum effective concentrations of the levodopa/carbidopa microparticles, the high $C_{\max}$ may be desirable and cause improvement in the pharmacological benefit of levodopa or disadvantageous and may lead to adverse effects like dyskinesia (Nyholm *et al.*, 2012).

The AUC of a plasma drug concentration versus time plot reflects the total drug exposure after administration (DiPiro *et al.*, 2005). The AUC was found to be highest with the levodopa/carbidopa microparticles followed by Sinemet CR and levodopa/carbidopa powder. There was a significant difference between the AUCs of the microparticles and the conventional formulation. Also, the microparticles had, a two-fold increase in $AUC_{0 \rightarrow \infty}$ compared to Sinemet CR. This high AUC of the microparticles could be attributed to the small particle size of the microparticles and the mucoadhesive property conferred by the chitosan which resulted in enhanced and efficient absorption across the intestinal lumen. Furthermore, because bioavailability describes the extent of drug eventually reaching the systemic circulation, comparison of the AUCs of the various formulations may give an indication of bioavailabilities (DiPiro *et al.*, 2005). Thus, it can be inferred from the results that levodopa/carbidopa microparticles attained greatest bioavailability than the levodopa/carbidopa powder and conventional controlled release product on the market (Sinemet CR).

The time to achieve peak concentration in plasma after drug administration (T_{max}) was also evaluated. This parameter is of particular importance in assessing the time to reach peak concentration in circulation. A comparison between Sinemet CR and levodopa/carbidopa powder showed no significant difference in the time to reach peak concentration after administration. However, there was a significant difference between the T_{max} of levodopa/carbidopa microparticles and Sinemet CR. The mean C_{max} for Sinemet CR, was reached at 0.5 h (T_{max}), whereas for levodopa/carbidopa microparticles, a mean C_{max} was reached after 1 h. From the concentration-time graph, it could be observed that at time 0.5 h, the plasma levels of microparticles of levodopa/carbidopa was higher than that of both Sinemet CR and levodopa/carbidopa powders. This means that although levodopa/carbidopa microparticles had a longer T_{max} , the plasma concentrations at the various time points before

$C_{\max}$ was attained were relatively high. This initial rapid absorption and high plasma levels of levodopa/carbidopa microparticles could be advantageous in achieving an early onset of drug action even before peak plasma concentration is reached.

The elimination half-lives of the various formulations were also assessed. This is the time it takes for the drug concentration in the blood to decrease to half the original concentration (Anderson, 2005). This parameter gives an indication of how the drug is cleared from the plasma. It also suggests whether accumulation of the drug will occur under a multiple dosage regimen, and it is an essential parameter when it comes to deciding on the appropriate dosing interval (Buclin *et al.*, 2009). A comparison of the ($t_{1/2}$) of Sinemet CR and levodopa/carbidopa powder showed a significant difference. This difference could be due to the fact that Sinemet CR has been designed to release levodopa/carbidopa in plasma over a period of time (controlled release) whereas levodopa/carbidopa powder has not undergone any modifications to retard its stay in plasma. This resulted in Sinemet CR having a longer half-life than levodopa/carbidopa powder. Comparing the half-life of Sinemet CR and levodopa/carbidopa microparticles, it was observed that the half-life of Sinemet CR was longer than levodopa/carbidopa microparticles. Sinemet CR, which is an already established drug, most likely has undergone many formulation modifications in order to prolong its release as compared to the experimental levodopa/carbidopa microparticles, and this may be the reason for Sinemet CR having a longer half-life than the levodopa/carbidopa microparticles. Again, although levodopa/carbidopa microparticles had a larger AUC than Sinemet CR, the decline in the concentration-time curves was sharper, with levodopa/carbidopa microparticles. This means that levodopa/carbidopa microparticles were eliminated faster than the Sinemet CR and resulted in Sinemet CR having a longer half-life than the levodopa/carbidopa microparticles. However, the difference in half-life between these two formulations is not statistically significant.

5.1 CONCLUSION

Comparing AUC and the $C_{\max}$, the formulated levodopa/carbidopa microparticles had better profiles compared to levodopa/carbidopa powder Sinemet CR (the current controlled release product on the market). The mucoadhesive drug delivery approach used in the formulation of the levodopa/carbidopa microparticles may have aided in overcoming the irregular gastric emptying associated with oral administration as this is seen in the enhanced absorption of levodopa/carbidopa microparticles in the current study.

5.2 RECOMMENDATIONS

Based on half-life of levodopa/carbidopa microparticles, additional studies should be done to further sustain the release of levodopa/carbidopa microparticles by optimizing the formulation variables in order to prolong the half-life.

Also, further studies should be done to ascertain the biodistribution of levodopa/carbidopa microparticles in the brain. This will be essential in knowing the levels of levodopa reaching the brain.

Efficacy studies could also be performed, using animal models, in order to establish suitable dosing concentrations for possible clinical utilization of oral levodopa microparticles in the treatment of Parkinson's disease.

REFERENCES

- Aarsland D. and Kurtz M. W. (2010) a. The epidemiology of dementia associated with Parkinson's disease. *J. Neurol. Sci.* 289: 18-22.
- Adler C.H. (2002). Relevance of motor complications in Parkinson's disease. *Neurol*; 58:51S-56.
- Afroz F. and Bach C. (2014). Improvement in drug delivery for Parkinson's disease. ASEE 2014 vbZone I Conference, April 3-5, University of Bridgeport :1-4.
- Agnihotri S.A., Aminabhabi T.M., and Malikarjuna N.N. (2004). Recent advances on chitosan-based micro and nano particles in drug delivery. *J Control Release*; 100:5-28.
- Ahtila S., Kaakkola S., Gordin A., *et al.* (1995). Effect of entacapone, a COMT inhibitor, on the pharmacokinetics and metabolism of levodopa after administration of controlled release levodopa-carbidopa in volunteers. *Clin Neuropharmacol*; 18: 46-57.
- Anderson P. L. (2005). The ABCs of Pharmacokinetics. *The Body: HIV/AIDS Resource. Posit Aware* ;19: 4-7.
- Antonini A. (2007). Continuous dopaminergic stimulation-from theory to clinical practice. *Parkinson Relat Disord* ;13: S24-8.
- Antonini A., Chaudhuri R.K., Martinez-Martin P., and Odin P. (2010). Oral and infusion Levodopa-Based Strategies for Managing Motor Complications in Patients with Parkinson's Disease. *CNS Drugs* 24(2): 119-129.

- Arica B., Ka S., Moghdam A. *et al.* (2005). Carbidopa/L-dopa-loaded biodegradable microspheres: in vivo evaluation on experimental parkinsonism in rats. *J. Control Release* 102: 689-97.
- Aspden, T.J., Illum, L., Skaugrud, O., 1996. Chitosan as a nasal delivery system-evaluation of insulin absorption enhancement and effect on nasal membrane integrity using rat models. *Eur. J. Pharm. Sci.* 4, 23–31.
- Aspden T.J., Illum L., Skaugrud O. (1996). Chitosan as a nasal delivery system. Evaluation of insulin absorption enhancement and effect on nasal membrane integrity using rat models. *Eur J. Pharm Sci* ;4:23-31.
- Ascherio A, Zhang SM, Hernán MA, Kawachi I, Colditz GA, Speizer FE, *et al.* (2001). Prospective study of caffeine consumption and risk of Parkinson's disease in men and women. *Ann Neurol.* Jul;50(1):56–63.
- Bale S., Khurana A., Reddy A.S., Singh M., and Godugu C. (2016). Overview on Therapeutic Applications of Microparticulate Drug Delivery Systems. *Crit Rev Ther Drug Carrier Syst.* 33(4): 309-361.
- Banerjee S., Gaurav C., Dilipkumar P., Ashoke G., Animesh G., and Santanu K. (2010). Investigation on crosslinking density for development of novel interpenetrating polymer network (IPN) based formulation. *J Sci Indust Res* ;10:777-84.
- Barone P. (2003). Clinical strategies to prevent and delay motor complications. *Neurol* ;61: S12-6.
- Baumann C.R. (2012). Epidemiology, diagnosis and differential diagnosis in Parkinson's disease tremor. *Parkinsonism Relat Disord*; 18(suppl 1): S90-S92.

- Benoit J, Faisant N, Venier-Julienne M, Menei P. (2000). Development of microspheres for neurological disorders: from basics to clinical applications. *J Control Release*; 65:285-96.
- Benedetti M.D, Bower J.H, Maraganore D.M, McDonnell S.K, Peterson B.J, Ahlskog J.E, *et al.* (2000). Smoking, alcohol, and coffee consumption preceding Parkinson's disease: A case-control study. *Neurology*; 14;55(9):1350–8.
- Berardelli A., Wenning G.K., and Antonini A. (2013). EFNS/MDS-ES recommendations for the diagnosis of Parkinson's disease. *Eur J Neurology*; 20:16-34.
- Berg D. (2012). Is pre-motor diagnosis possible? The European experience. *Parkinsonism Relat Disord*; 18(suppl 1): S195-S198.
- Betarbet R., Sherer T.B., MacKenzie G., Garcia-Osuna M., Panov A.V., Greenamyre J.T. (2000). Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat Neurosci*; 3(12):1301–6.
- Bhidayasiri R. (2005). Differential diagnosis of common tremors syndromes. *Postgraduate Medical Journal* ;81(962): 756-762.
- Birkmayer W. and Hornykiewicz O. (1961). Der 1-3,4-dioxyphenylalanin (=DOPA)-Effekt bei parkinson-akinese. *Wein Klin Wochenschr*; 73:787-8.
- Bordia T., McGregor M., Papke R.L., Decker M.W., McIntosh J.M., Quik M. (2015). The $\alpha 7$ nicotinic receptor agonist ABT-107 protects against nigrostriatal damage in rats with unilateral 6-hydroxydopamine lesions. *Exp Neurol*. Jan ;263:277–84

- Bozdag S.S., Calis S., Kas H.S., Ercan M.T., Peksoy I., and Kincal A.A. (2001). *In vitro* evaluation and intraarticular administration of biodegradable microspheres containing naproxen sodium. *J. Microencap.*, 18(4):443–456.
- Braak H. and Braak E. (2000). Pathoanatomy of Parkinson's disease. *J Neurol.* 247 Suppl 2:113-10.
- Braak H., Ghebremedhin E., Rub U. (2004). Stages in the development of Parkinson's disease-related pathology cell tissue. *Res* 318: 121-134.
- Braak H., Del Tedrici K., de Vos R.A., Jansen S. E., Braak E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging.* 24(2): 197-211
- Brahmankar D. M. Treatise A., Prakashan V. and Jaiswal S. B. (2009). *Pharmacokinetics: Basic Considerations in Biopharmaceutics and Pharmacokinetics.* Second edition, pages 235-39.
- Brasnjevic, I., *et al.*, (2009). Delivery of peptide and protein drugs over the blood–brain barrier. *Progress in Neurobiology.* 87(4): 212-251
- Bredberg E., Hans L. and Paalzow L. (1994). Pharmacokinetics of Levodopa and Carbidopa in Rats Following Different Routes of Administration. *Pharmaceutical Research*; 11(4).
- Brock D.R. (1999). Anticholinergic drugs used in Parkinson's disease: an overlooked class of drugs from a pharmacokinetic perspective. *J Pharm Pharmaceut Sci*; 2:39-46
- Brooks J, Ding J, Simon-Sanchez J, Paisan-Ruiz C, Singleton AB, *et al.* (2009) Parkin and PINK1 mutations in early-onset Parkinson's disease: comprehensive screening in publicly available cases and control. *J Med Genet* 46: 375-381.

- Buclin T., Arab-Alameddine M., Dilulio J., Rotger M., Lubeminov R., Cavassini M. Fayet A., Decosterd L.A. (2009). Swiss HIV Cohort Study. Pharmacogenetics-based population pharmacokinetics analysis of effavirenz in HIV-1 infected individuals.
- Burgess D., Dutty E., Etzler F., and Hickey A.J. (2004). Particle size analysis: AAPS workshop report, cosponsored by the Food and Drug Administration and the United States Pharmacopeia. *AAPS J* ;6(3) :20.
- Caballol N., Marti M.J., and Tolosa E. (2013). Cognitive dysfunction and dementia in Parkinson disease. *Mov Dissord* 22 Suppl 17: S358-366.
- Cao, L., F. van Rantwijk, and R.A. Sheldon. (2000). Cross-linked enzyme aggregates: a simple and effective method for the immobilization of penicillin acylase. *Org. Lett.* 2:1361-1364.
- Cedarbaum J.M., Breck L., and Kutt H. (1987). Controlled-release levodopa/carbidopa. II. Sinemet CR4 treatment of response fluctuations in Parkinson's disease. *Neurology* ;37:1607-12.
- Chahine L.M., Stern M.B., and Chen-Plotkin A. (2014). Blood-based biomarkers for Parkinson's disease. *Parkinsonism Relat Disord* ;20(suppl 1) :S99-S103.
- Chan K. Y. Kwee P. N. and Debra S. M. S. (2015). *Pharmacological Basis of Acute Care*. Chapter 1 pages 9-17. Springer International Publishing, Switzerland
- Chase TN. (1998) Levodopa therapy: consequences of the nonphysiologic replacement of dopamine. *Neurology*; 50: S17-25.

- Chase T. N, Baronti F., Fabbrini G., Heuser I.J, Juncos J.L, Mouradian M.M. (1989). Rationale for continuous dopaminomimetic therapy of Parkinson's disease. *Neurology*; 39(suppl 2):7–10.
- Choudhary H., Agrawal A. K., Malviya R., Yadav S. K., Jaliwala Y. A. and Patil U. K. (2010). Evaluation and Optimization of preparative variables for controlled-release floating microspheres of levodopa/carbidopa. *Pharmazie* 65: 194–198.
- Chen R.C, Chang S.F, Su C.L, Chen T.H, Yen M.F, Wu H.M, *et al.*, (2001). Prevalence, incidence, and mortality of PD: A door-to-door survey in Ilan county, Taiwan. *Neurology*. 13;57(9):1679–86.
- Chen C., Cowles E. V., Sweeney M., Stolyarov I. D. and Illarioshkin S. N. (2012). Pharmacokinetics of Levodopa/Carbidopa Delivered from Gastric–Retentive Extended–Release Formulations in Patients with Parkinson's Disease. *Journal of Clinical Pharmacology*. 52: 1069–1077
- Cole N. B., and Murphy D. D. (2002). The cell biology of alpha-synuclein: a sticky problem? *Neuromol. Med* 1:95–109
- Contin M., Riva R., Albani F. and Baruzzi A. (1996). Pharmacokinetic optimization in the treatment of Parkinson's disease. *Clin Pharmacokinet* 30(6); 463-481.
- Dallapiazza R.F., Philippe D.V., Anton F., Darrin J.L., Clement H., Renato M.P., Hodaie M., Lozano M.A., Alfonso F., and Kalia S.K. (2018). Considerations for patient and target selection in deep brain stimulation surgery for Parkinson's disease. Chapter 8. Pages 145-156. Codon Publications, Brisbane, Australia.
- Davie C.A. (2008). A review of Parkinson's disease. *Br. Med. Bull.* 86: 109-127.

- D'Aurizio E., Sozio P., Cerasa L. S., Vacca M., Brunetti L., Orlando G., Chivaroli A. Hennink W. E. and Di Stefano A. (2011). Biodegradable Microspheres Loaded with an Anti-Parkinson Prodrug: An in Vivo Pharmacokinetic Study. *Mol. Pharmaceutics*; 2408-2415
- DeLong M. R. and Wichmann T. (2007). Circuits and circuit disorders of the basal ganglia. *Arch. Neurol.* 64(1), 20–24.
- DeLau LM, Breteler MM (2006) Epidemiology of Parkinson's disease. *Lancet Neurol* 5(6):525–535
- DeMaagd G. and Philip A. (2015). Parkinson's Disease and Its Management Part 1: Disease Entity, Risk Factors, Pathophysiology, Clinical Presentation and Diagnosis; 40(8): 504-508.
- DeRijk MC, Breteler MM, Graveland GA, Ott A, Grobbee DE, van der Meché FG, *et al.*, (1995). Prevalence of Parkinson's disease in the elderly: The Rotterdam Study. *Neurology*; 45(12):2143–6.
- DeSilver D.J. and Oliver J.M. (2005). Hydroxypropylmethyl cellulose (HPMC) lubricant facilitates insertion of porous spherical orbital implants. *Opthal Plast Reconstr Surg* ;21(4): 301-2
- Deuschl G., Bain P., Brin M., and Adhoc Scientific Committee. (1998). Consensus statement of the movement disorder society on tremor. *Mov Disord* ;13(suppl 3): 2-23.
- Diem-Zangerl, A., K. Seppi, G. K. Wenning, E. Trinkla, G. Ransmayr, W. Obergerner, *et al.* (2009). Mortality in Parkinson's disease: a 20-year follow-up study. *Mov. Disord.* 24:819–825.

- DiPiro J., Spruill W., Wade W., and Blouin R. (2005). Concepts in clinical pharmacokinetics. 4th Edition. Lesson 7 Pages 107-108.
- DiStefano A., Sozio P., Iannitelli A. and Laura Cerasa S. (2009). New drug delivery strategies for improved Parkinson's disease therapy. *Expert Opin. Drug Deliv.* 6(4): 389-404.
- DiStefano A., Sozio P., and Alessandra C. (2006). L-Dopa and Dopamine-R-A-Lipoic Acid Conjugates as Multifunctional Codrugs with Antioxidant Properties. *J Med Chem* ;49(4): 11486-93.
- Dissanayaka, N. N., A. Sellbach, P. A. Silburn, J. D. O'Sullivan, R. Marsh, and G. D. Mellick. (2011). Factors associated with depression in Parkinson's disease. *J. Affect. Disord* 132:82– 88
- Duncan, R. P., A. L. Leddy, J. T. Cavanaugh, L. E. Dibble, T. D. Ellis, M. P. Ford, *et al.* (2012). Accuracy of fall prediction in Parkinson disease: six-month and 12-month prospective analyses. *Parkinsons Dis.* 2012:237673
- Djamshidian, A., B. B. Averbeck, A. J. Lees, and S. S. O'Sullivan. (2011). Clinical aspects of impulsive compulsive behaviours in Parkinson's disease. *J. Neurol. Sci.* 310:183– 188.
- Doherty K.M., van de Warrenburg B.P., and Peralta M.C. (2011). Postural deformities in Parkinson's disease. *Lancet Neurol* 10:538-549.
- El-Say K.M. (2016). Maximizing the encapsulation efficiency and the bioavailability of controlled-release cetirizine microspheres using Draper-Lin small composite design. *DrugDesign, Development and Therapy* ;10:825-839.

- Elbarbry F., Nguyen V., Mrika A., Zwickey H., and Rosenbaum R. (2018). A new validated HPLC method for the determination of levodopa: Application to study the impact of ketogenic diet on the pharmacokinetics of levodopa in Parkinson's participants. Pages 1-27.
- Fahn S. (2008). The history of dopamine and levodopa in the treatment of Parkinson's disease. *Mov Disord.* 23(suppl 3): S497-508.
- Fiebrig, I., Harding, S.E., Rowe, A.J., Hyman, S.C., Davis, S.S., (1995). Transmission electron-microscopy studies on pig gastric mucin and its interactions with chitosan. *Carbohydrate polymers* 28, 239–244.
- Friedman J. H. (2010). Parkinson's disease psychosis 2010: a review article. *Parkinsonism Relat Disord* 16: 553-560.
- Garcia R.P., Catalan M.J., and Fernandez C.J. (2011). Initial motor symptoms of Parkinson's disease. *Neurologist* 17(suppl 1): S18-S20.
- Gattani Ys. (2010). Floating Multiparticulate Drug Delivery Systems: An Overview *International Journal of Pharmaceutical and Biosciences*, 6(2): 35-40.
- Gauthier S. and Amyot D. (1992). Sustained release antiparkinson agents: Controlled release levodopa. *Can J Neurol Sci*, 19(1); 153-155.
- George D.M. and Ashock P. (2015). Parkinson's disease and its management. Part 1: Disease entity, risk factors, pathophysiology, clinical presentation and diagnosis. Vol. 40 Number 8.

- Giunchedi, P., (1995). Spray-Drying as a Preparation Method of Microparticulate Drug Delivery Systems: An Overview. *S.T.P. Pharma Sciences*, 5(4): 276.
- Goldman J. G. and Goetz G.C. (2007). History of Parkinson's disease. *Handbook of Clinical Neurology*; 83(5) :109-127. W.C. Koller, E. Melamed Editors.
- Goole J, and Amighi K. (2009). Levodopa delivery systems for the treatment of Parkinson's disease: An overview. *Int J Pharm* 380: 1–15.
- Goole J., Vanderbist F., and Amighi K. (2007). Development and Evaluation of New Multiple-Unit Levodopa Sustained-Release Floating Dosage Forms. *Int J Pharm* 334: 35-41.
- Gourie-Devi M., Ramu M.G., and Venkataram B.S. (1991). Treatment of Parkinson's disease in Aryurveda (ancient Indian system of medicine): Discussion paper. *J R Soc Med*;84: 491-2.
- Grabli D., Karachi C., Welter M.L. (2012). Normal and pathological gait: what we learn from Parkinson's disease. *J Neurol Neurosurg Psychiatry* 12: 697-985
- Graham AG. (1978). Oxidative pathways for Catecholamines in the Genesis of Neuromelanin and cytotoxic quinones. *Molecular Pharmacology*; 14: 633 – 643.
- Greenamyre J.T, Hastings T.G. (2004). Biomedicine: Parkinson's—divergent causes, convergent mechanisms. *Science*; 304: 1120–22.
- Greggio E., Jain S., Kingsbury A., Bandopadhyay R., Lewis P. (2006). Kinase activity is required for the toxic effects of mutant LRRK2/ dardarin. *Neurobiol Dis* 23: 329-341.
- Grosset D.G., Macphee G.J.A., and Nairin M. (2010). Diagnosis and pharmacological management of Parkinson's disease: summary of SIGN guidelines. *BMJ*; 340:B5614.

- Gunay M.S., Ozer A.Y. and Chalon S. (2016). Drug Delivery Systems for Imaging and Therapy of Parkinson's Disease. *Curr Neuropharmacol.* 14 (4): 376-391
- Guttman, M., P. M. Slaughter, M. E. Theriault, D. P. DeBoer, and C. D. Naylor. (2001). Parkinsonism in Ontario: increased mortality compared with controls in a large cohort study. *Neurology* 57:2278–2282.
- Hadad F., Sawalha M., Kwhawaja Y., Najjar A., and Rafik K. (2018). Dopamine and levodopa prodrugs for the treatment of Parkinson's disease. *Molecules* ;23(1): 40.
- Hallett M. (2012). Parkinson's disease tremor: pathophysiology. *Parkinsonism Relat Disord.* 18(suppl 1): S85-S86.
- Hammerstad J.P., Woodward W.R., Nutt J.G., Ganchar S.T., Block G.A., and Cyhan G. (1994). Controlled release levodopa/carbidopa 25/100 (Sinemet CR/100): Pharmacokinetics and clinical efficacy in untreated parkinsonism patients. *Clin Neuropharmacol*; 17:429-434.
- Hardie RJ, Lees AJ, Stern GM. (1984). On-off fluctuations in Parkinson's disease: a clinical and neuropharmacological study. *Brain*; 107: 487-506
- Hauser R.A. (2004). Levodopa/carbidopa/entacapone (stalevo). *Neurology*; 62:564-71.
- He P., Davis S. S., and Illum L. (1999). Chitosan microspheres prepared by spray drying. *International Journal of Pharmaceutics*; 187: 53-65.
- Herrero-Vanrell R., and Refojo M.F. (2001). Biodegradable microspheres for vitreoretinal drug delivery. *Adv Drug Deliv Rev*; 52:5-16.

- Hirtz D, Thurman DJ, Gwinn-Hardy K, Mohamed M, Chaudhuri AR, *et al.* (2007) How common are the "common" neurologic disorders? *Neurology* 68: 326-337.
- Hickey T, Kreutzer D, Burgess DJ, Moussy F. (2002). Dexamethasone/PLGA microspheres for continuous delivery of an anti-inflammatory drug for implantable medical devices. *Biomaterials*; 23:1649-56.
- Hinterberger H., and Andrews C.J. (1972). Catecholamine metabolism during oral administration of levodopa. *Arch Neurol* ;26: 245-52.
- Holtz P. (1993). Dopadecarboxylase. *Naturwissenschaften*; 64: 216-23.
- Hornykiewicz O. (1998). Biochemical aspects of Parkinson's disease. *Neurology* 51: S2-S9.
- Horstink M., Tolosa E., Bonuccelli U., Deuschl G., Friedman A., Kanovsky P., Larsen J.P., Lees A., Oertel W., Poewe W., Rascol O., Sampaio C. (2006). European Federation of Neurological Societies; Movement Disorder Society-European Section. Review of the therapeutic management of Parkinson's disease. Report of a joint task force of the European Federation of Neurological Societies and the Movement Disorder Society-European Section. Part I: early (uncomplicated) Parkinson's disease. *Eur. J. Neurol.*13:1170–1185
- Hou, W.M., Miyazaki, S., Takada, M., (1994). Preparation and evaluation of ion-exchange cross-linked chitosan microspheres. *Yakuzaigaku* 54, 10–18.
- Hsu A.F., and Han C.H. (2006). Oral disintegrating dosage forms. WO2006073729.
- Hughes AJ, Daniel SE, Kilford L, Lees AJ. (1992). Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinicopathological study of 100 cases. *J Neurol Neurosurg Psychiatry*; 55:181-184.

- Huang Y.C., Chang C.H., and Yeh M.K. (2003). Optimizing formulation factors in preparing chitosan microparticles by spray-drying method. *J Microencapsul* ;20: 247-260.
- Illum, L., Farraj, N.F. and Davis S.S. (1994). Chitosan as a novel nasal delivery system for peptide drugs. *Pharm. Res.* 11, 1186 – 1189.
- Iranzo A., Molinuevo J.L and Santamria J. (2007). Rapid-eye movement sleep behaviour disorder as an early marker for a neurodegenerative disorder: a descriptive study. *Lancet Neurol* ; 5:572-577.
- Jain S.K., Rai G., Saraf D.K., and Agrawal G.P. (2014). The preparation and evaluation of albendazole microspheres for colonic delivery, *Pharmaceutical Technology* ; 66-70.
- Jankovic J. (2005). Motor fluctuations and dyskinesias in Parkinson's disease: clinical manifestations. *Mov Disord*; 20: S11-6.
- Jankovic J. (2008). Parkinson's disease: Clinical features and diagnosis. *J Neurol, Neurosurg & Psychiatr.*;79(4):368–76.
- Jankovic J. and Sherer T. (2014). The future of research in Parkinson's disease. *JAMA Neurol*; 71(11): 1351-1352
- Jimenez-Shahed Joohi (2016). A review of current and novel levodopa formulations for the treatment of Parkinson's disease. *Ther. Deliv.* 7(3); 179-191.
- Kalia LV, Lang AE. (2015). Parkinson's disease. *Lancet*; 386(9996):896–912.
- Kang P., Kloke J., and Jain S. (2012). Olfactory dysfunction and parasympathetic dysautonomia in Parkinson's disease. *Clin Auton Res.* 22:161-166.

- Kasten M, Kertelge L, Brüggemann N, Van der Vegt J, Schmidt A, *et al.* (2010). Non-motor symptoms in genetic Parkinson disease. *Arch Neurol* 67: 670-676.
- Kaur I. P, Bhandari R., Bhandari S. and Kakkar V. (2008). Potential of solid lipid nanoparticles in brain targeting. *J Control Release* 127:97–109.
- Kaurav H., HariKumar S. L. and Kaur A. (2012). “Mucoadhesive Microspheres as carriers in Drug Delivery: A Review”, *Int. J. Drug Dev. & Res.*, 4(2): 21-34
- Katzenschlager R, Manson AJ, Evans A, Watt H, Lees AJ. Low dose quetiapine for drug induced dyskinesias in Parkinson’s disease: a double blind cross over study. *J Neurol Neurosurg Psych* ; 75: 295–297.
- Katzung B. G., Susan B. and Trevor A. J. (2012). *Basic and Clinical Pharmacology*. Twelfth Edition. Chapter 28. Section V pp 484. The McGraw-Hill Companies, Inc. United States.
- Keranen T., Gordin A. and Harjola V. P. (1993). The effect of catechol-O- methyl transferase inhibition by entacapone on the pharmacokinetics and metabolism of levodopa in healthy volunteers. *Clin Neuropharmacol*; 16: 145-56.
- Krewson C.E., Klarman M.L. and Saltzman W.M. (1995). Distribution of nerve growth factor following direct delivery to brain interstitium, *Brain Res.* 680 (1–2) 196–206.
- Khor Soo-Peang and Hsu Ann (2007). The Pharmacokinetics and Pharmacodynamics of Levodopa in the Treatment of Parkinson’s Disease. *Current Clinical Pharmacology*; 2(3):234-243.
- Kim K.K. and Pack D.W. (2006). Microparticles for drug delivery. *BioMEMS and Biomedical Neurotechnology*, 1, 21.

- Korczyn D. A. (2004). Drug treatment of Parkinson's Disease. *Dialogues Clin Neurosci* 6(3): 315-322.
- Koller W. C. and Pahwa R. (1994). Treating motor fluctuations with controlled-release levodopa preparations. *Neurology*.44(7 Suppl 6): S23–8.
- Koller W.C., Hutton J.T., Tolosa E., and Capilldeo R. (1999). Immediate-release and controlled release carbidopa/levodopa in PD: a 5 year randomized multicentre study. *Neurology*; 53: 1012-9.
- Kouli A., Torsney M. K. and Kuan W. (2018). Parkinson's disease: Etiology, Neuropathology, and Pathogenesis. Chapter 1. Pages 3-18. Codon Publications, UK.
- Kostoff R.N. and Briggs M.B. (2008). Literature-Related Discovery (LRD): Potential treatments for Parkinson's disease. *Technol Forecast Soc Change*;75: 226-38.
- Kovari E., Hovarth J., Bouras C. (2009). Neuropathology of Lewy body disorders. *Brain Res Bull* 80:203-210.
- Kumar S.S, Natraj K., Khan A., Kumar K., and Rao J. V. (2011). Development and Validation of RP-HPLC Method for Simultaneous Estimation of Levodopa and Carbidopa in Pharmaceutical Dosage Forms. *J Pharma Res*; 4(11).
- Kurlan R., Rothfield K.P., and Woodward W.R. (1988). Erratic gastric emptying of levodopa may cause random fluctuations of parkinsonism mobility. *Neurology* ;38(3): 419-421.
- Langston J.W., Ballard P., Tetrud J.W, Irwin I. (1983). Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* ;219(4587): 979–80.

- Lai B.C, Marion S.A, Teschke K., Tsui J.K. (2002). Occupational and environmental risk factors for Parkinson's disease. *Parkinsonism Relat Disord*; 8: 297–309.
- Lee C.N., Kim M., and Lee H.M. (2013). The interrelationship between non-motor symptoms in atypical parkinsonism. *J Neurol Sci*; 327:15-21.
- Lees A.J., Hardy J., Revesz T. (2009). Parkinson's disease. *Lancet*; 373(9680):2055–66.
- Lehr, C. M., Bouwstra, J. A., Schacht, E. H., and Junginger, H. E. (1992). *In vitro* evaluation of mucoadhesive properties of chitosan and some other natural polymers. *Int. J. Pharm.* 78, 43–48.
- Lesage S. and Brice A. (2009). Parkinson's disease: From monogenetic forms to genetic susceptibility factors. *Hum Mol Genet.* 18(R1): R48-59
- Lueben H.L., Lehr C.O., Rentel A.B.J., Noah A.G., Boer J.C., Verhoef H.E., and Junginger H.E. (1994). Bioadhesive polymers for the peroral delivery of peptide drugs. *J Control Release* ;29:329-338.
- LeWitt PA. (1992). Clinical studies with and pharmacokinetic considerations of sustained-release levodopa. *Neurology*; 42(Suppl 1): S29–32.
- LeWitt P. A. and Nyholm D. (2004). New developments in levodopa therapy. *Neurology*; 62(suppl 1): S9–S16.
- LeWitt P.A. (2008). Levodopa for the treatment of Parkinson's Disease. *N Engl J Med* 359:2468-76.

- LeWitt P.A., Saint-Hilaire M.H., Grosset D.G., Hauser R., Stocchi F., Freed M.I. (2015). Inhaled Levodopa Provides Rapid Motor Improvements after Administration to Parkinson's Disease Patients when OFF. *Mov Disord.* 30:1996.
- Lopez C.R., Portero A., Vila-Jato J.C., and Alonso M.J. (1998). Design and evaluation of chitosan/ethylcellulose mucoadhesive bilayered devices for buccal drug delivery. *J. Control Rel*; 55:143-52.
- Lotti V.J., and Porter C.C (1970). Potentiation and inhibition of some central actions of Levodopa by decarboxylase inhibitors. *J. Pharmacol Exp Ther*; Apr, 172(2): 406-15.
- Lueßen, H.L., Lehr, C.M., Rental, C.O., Noach, A.B.J., de Boer, A.G., Verhoef, J.C., Junginger, H.E. (1994). Bioadhesive polymers for the peroral delivery of peptide drugs. *J. Contr. Rel.* 29, 329–338.
- MacMahon DG, Sachdev D, Boddie HG, Ellis CJ, Kendal BR, Blackburn NA. (1990). A comparison of the effects of controlled-release levodopa (Madopar CR) with conventional levodopa in late Parkinson's disease. *J Neurol Neurosurg Psychiatry.*53(3):220–3.
- Maetzler W, and Hausdorff JM. (2012) Motor signs in the prodromal phase of Parkinson's disease. *Mov Disord*; 27:627–633.
- Manyam BV. (1990). Paralysis agitans and levodopa in “Ayurveda”: Ancient Indian medical treatise. *Mov Disord* 5: 47–48.
- McCrone, P., C. A. Payan, M. Knapp, A. Ludolph, Y. Agid, P. N. Leigh, (2011). The economic costs of progressive supranuclear palsy and multiple system atrophy in France, Germany and the United Kingdom. *PLoS ONE* 6:e24369.

- McRae-Degueurce A., S. Hjorth, D.L. Dillon, D.W. Mason, T.R. Tice, (1988). Implantable microencapsulated dopamine (DA): a new approach for slow-release DA delivery into brain tissue, *Neurosci. Lett.* 92; 303–309.
- McRae A., S. Hjorth, D.W. Mason, D.L. Dillon, T.R. Tice, (1991). Microencapsulated dopamine (DA)-induced restitution of function in 6-OHDA denervated rat striatum in vivo: comparison between two microsphere excipients, *J. Neural Transpl. Plast.* 2; 165–173.
- Md S., Ahuja A, Khar R.K., Baboota S., Mishra A. K, Ali J. (2011). Gastroretentive drug delivery system of acyclovir-loaded alginate mucoadhesive microspheres. www.informahealthcare.com; 18(4). 255-264.
- Mendoca D.A. (2008). Talks of attention augment rigidity in mild Parkinson's disease. *Can J. Neurol Sci* ;35(4): 501-5
- Meissner W.G. (2012). When does Parkinson's disease begin? From prodromal Parkinson's disease to motor signs. *Rev Neurol* 168: 809-814.
- Melamed E., Offen D., Shirvan A., and Ziv I. (2000). Levodopa- an exotoxin or a therapeutic drug? *J Neurol* ; 247:11135-11139.
- Mittur A., Gupta S. and Modi N. B. (2017). Pharmacokinetics of Rytary, an Extended-Release Capsule Formulation of Carbidopa-Levodopa. *Clin Pharmacokinet* 56: 999-1014
- Miyazaki S., Yamaguchi H., Yokouchi C., Takada M., Hou W.M. (1988). Sustained-release and intragastric-floating granules of indomethacin using chitosan in rabbits, *Chem. Pharm. Bull.* 36; 4033 – 4038.

- Morgan J.P., Bianchine J.R., Spiegel H.E., Rivera-Calmin L., and Hershet R.M. (1971). Metabolism of levodopa in patients with Parkinson's disease. Radioactive and fluorometric assays. *Arch Neurol*; 26(1): 39-44.
- Mouradian M.M. (2005). Should levodopa be infused into the duodenum? *Neurol*; 64:182-3
- Murray E.D., Butter E.A., Price B.H. (2012). Depression and Psychosis in Neurological Practice. In Bradley W.G., Daroff R.B. Feniel G.M., Jankovic J. (eds). *Bradley's Neurology in Clinical Practice: Expert Consult-Online and Print*, 6E. Philadelphia, PA:Elsevier/Saunders 102-103.
- Muzzi C. Bertocci E., and Terzuoli L. (2008). Simultaneous determination of serum concentrations of levodopa, dopamine, 3-O-methyldopa and alpha-methyldopa by HPLC. *Biomed Pharmacother*; 62: 253-8.
- Nagpal M., Kumari S., Geeta A., Puneet U., Jain K., and Sharma P. (2016). Microparticles Drug Delivery System: A Review. *World Journal of Pharmacy and Pharmaceutical Sciences*. Volume 5 Issue 03, 543-566.
- Nettey H., Allotey-Babington G.L, Somuah I., N'guessan B.B., Afrane B., Amponsah S.K., Annor H., Darko H., Hanson K, Aidoo A., Broni M.N., Sasu C., and Nyarko A. (2017). Assessment of formulated amodiaquine microparticles in Leishmania Donovan infected rats. *J Microencapsul* ; 0265-2048: 1-32
- Ngwuluka N., Pilay V., DuToit L.C., Ndesendo V., Choonara Y., Modi G. and Naidoo D. (2010). Levodopa Delivery Systems: advancement in delivery of the gold standard. *Expert Opin. Drug Deliv*. 7(2): 203-224

- Nimni, M.E., Cheung D., Strates B., Kodama M., and Sheikh K. (1987). Chemically modified collagen: a natural biomaterial for tissue replacement. *J. Biomed. Mater. Res.* 21:741-771.
- Nord M. (2017). Levodopa pharmacokinetics –from stomach to brain. A study on patients with Parkinson's disease ;1567 :1-79
- Noyce A.J, Bestwick J.P, Silveira-MoriyamaL, Hawkes C.H, Giovannoni G., Lees A.J, *et al.*, (2012). Meta-analysis of early nonmotor features and risk factors for Parkinson disease. *Ann Neurol*;72(6): 893–901.
- Nutt J.G. Obeso J.A., and Stocchi F. (2000). Continuous dopamine-receptor stimulation in advanced Parkinson's disease. *Trends Neurosci* ;23 :S109-15.
- Nutt J.G., Woodward W.R., Hammerstad J.P., Crter J.H., and Anderson J.L. (1984). The on-off phenomenon in Parkinson's disease. Relation to levodopa absorption and transport. *The New England journal of medicine* ;310(8): 483-8.
- Nyholm D., Nilsson R.A., and Dizdar N. (2005) a. Duodenal levodopa infusion monotherapy vs oral polypharmacy in advanced Parkinson's disease. *Neurology* 64: 216-23.
- Nyholm D., Johansson A., Lennernas H., and Askmark H. (2012). Levodopa infusion combined with entacapone or tolcapone in Parkinson disease: a pilot trial. *Eur. J. Neurol.* 19(6):820-826.
- Obering C.D., Chen J.J., and Swope D.M. (2006). Update on apomorphine for the rapid treatment of hypomobility (off) episodes in Parkinson's disease. *Pharmacother* ;26:840-52.

- Obeso J.A., Rodriguez-Oroz M.C., Chana P., Lera G., Rodriguez M., Olanow C.W. (2000).
The evolution and origin of motor complications in Parkinson's disease. *Neurology*
55: S13-S20.
- Oertel W.H. (2017). Recent advances in treating Parkinson's disease. *F1000Research*.;6:260.
- Olanow C.W, Obeso J.A, Stocchi F. (2006). Continuous dopamine- receptor treatment of
Parkinson's disease: scientific rationale and clinical implications. *Lancet Neurol.* 5(8),
677-687
- Okubadejo N. U., Bower J. H., Rocca W. A. and Maraganore M. D. (2006). Parkinson's
Disease in Africa: A Systemic Review of Epidemiologic and Genetic Studies.
Movement Disorders Vol 21, No. 12, pp 2150-2156.
- Okuda, K., I. Urabe, Y. Yamada, and H. Okada. (1991). Reaction of glutaraldehyde with amino
and thiol compounds. *J. Ferment. Bioeng.* 71:100-105.
- Ovallath S. and Deepa P. (2013). The history of parkinsonism: Descriptions in ancient Indian
medical literature. *Mov Disord* ;28:566-8.'
- Padalkar A.N., Sadhana R.S., and Mahesh W. (2011). Microparticles: An approach for
betterment of drug delivery system. *International Journal of Pharma. Research and
Development- Online (IJPRD)*. ISSN 0974-9446.
- Pappert E. J., Buhrfiend C., Lipton J. W., Carvey P. M., Stebbins G. T. and Goetz C. G. (1996).
Levodopa Stability in Solution: Time Course, Environmental Effects, and Practical
Recommendation for Clinical Use. *Movement Disorders* Volume 11, No.1, pp. 24-26.
- Parkinson J. (1817). *Essay on the shaking palsy*. London: Sheerwood, Neely and Jones.

- Particle Size Analysis Guidebook (2016). Why is particle size important? Horiba Instruments, Inc.
- Palma P.N., Kissi L.E. and Soares-da-Silva P. (2013). Catechol-O-Methyl-Transferase Inhibitors. Present problems and Relevance of the New Ones. Chapter 4 Page 85.
- Peppas N.A., and Korsmeyer R.W. (1987) in: N.A. Peppas (Ed.), *Hydrogels in Medicine and Pharmacy*, vol. 3, CRC Press, Boca Raton, FL, p. 103.
- Periquet M, Latouche M, Lohmann E, Rawal N, De Michele G, *et al.* (2003) Parkin mutations are frequent in patients with isolated early-onset parkinsonism. *Brain* 126: 1271-1278.
- Pezzoli Gianni and Zini Michela (2010). Levodopa in Parkinson's Disease: from the past to the future. *Expert Opin. Pharmacother.* 11(4): 627-635.
- Pfeiffer R.F. (2005). A promising new technology for Parkinson's disease. *Neurol* ; 65:S6-10.
- Porter C.C. (1973). Inhibitions of Aromatic Amino Acid Decarboxylase- Their Biochemistry, *Advances in Neurology*, Raven Press, New York ; Vol 2,pp. 37-58.
- Postuma R.B., Aarsland D, and Barone P. (2012). Identifying prodromal Parkinson's disease: pre-motor disorders in Parkinson's disease. *Mov Disord* ;27:617–626.
- Ranjha N.M., Khan H., and Naseem S. (2010). Encapsulation and characterization of controlled release flurbiprofen loaded microspheres using beeswax as an encapsulating agent. *J Matter Sci Mater Med* ;21(5): 1621-30.
- Raval J. A., Patel J. K. and Patel M. M. (2010). Formulation and *In Vitro* Characterization of Spray-dried microspheres of Amoxicillin. *Acta Pharm.* 60; 455-465.

- Rama D., Shame T.C. and Bhaskar R. K.V. (2009). Microencapsulation Technology and Applications. *Defence Science Journal*; 59(1): 82-83.
- Reichmann H. (2010). Clinical criteria for the diagnosis of Parkinson's disease. *Neurodegenerative Dis.* 7:284-290.
- Rell, M. T., Tiwari, B. S., and Krayz, T. G. (2009). "Formulating Hydrophilic Matrix System." *Drug Deliv. Tech.* 9: 20-4.
- Ritz B, Lee P-C, Lassen CF, and Arah OA. (2014). Parkinson disease and smoking revisited: Ease of quitting is an early sign of the disease. *Neurology*; 83(16):1396–402.
- Robinson J.R. and Mlynek M.G. (1995). Bioadhesive and phase change polymers for ocular drug delivery. *Advanced Drug Delivery Reviews*; Vol 16 Issue 1. Pages 45-50.
- Ross GW, Abbott RD, Petrovitch H, Morens DM, Grandinetti A, Tung KH, *et al.* (2000). Association of coffee and caffeine intake with the risk of Parkinson disease. *JAMA*; 283(20):2674–9.
- Safavi A., Tohidi M. (2007). Simultaneous kinetic determination of levodopa and carbidopa by H-point standard addition method. *J Pharm Biomed Anal* 44:313-318.
- Sagar K.A., and Symth M.R. (2000). Bioavailability studies of oral dosage forms containing levodopa and carbidopa using column-switching chromatography followed by electrochemical detection. *Analyst*; 125: 439-45.
- Sahil Kataria, Middha Akanksha, Sandhu Premjeet, Ajay Bilandi and Bhawana Kapoor (2010). Microsphere: A Review. *International Journal of Research in Pharmacy and Chemistry*; 1(4).

- Saikia Chinmayee, Pankaj Gogol and Tarun K. Majl (2015). Chitosan: A Promising Biopolymer in Drug Delivery Applications. *J. Mol Genet Med* S4
- Salat D. and Tolosa E. (2013). Levodopa in the treatment of Parkinson's disease: current status and new developments. *J. Parkinsons Dis.* 3(3), 255–269
- Samii A, Nutt JG, Ransom BR. Parkinson's disease. *Lancet.* 2004;363(9423):1783–93.
- Sandford P.A. (1990). Chitosan- commercial uses and potential application in chitosan and chitin. Brack G.G., Anthonsen T. and Sandford P. Eds ; pp 51-59. Elsevier, NewYork, NY. USA.
- Sarlesh R., Preeti A., Pathak A., Shrivastava N., Baghel S. S and Baghel R. S. (2015). A review on microspheres: Methods of Preparation and Evaluation. *W J Pharma Pharmaceutical Sci*; 1(1): 422-438.
- Save T. and Venkatechalam P. (1994). Bioadhesive tablets of Nifedipine: Standardization of a novel buccoadhesive erodible carrier. *Drug delivery. Ind. Pharm*; 20(19): 3005-14.
- Schapira A.H.V. (2005). Present and future drug treatment for Parkinson's disease. *J. Neurol Neurosurg Psych*; 76: 1472-8
- Schrag A., Horfall L., and Walters K. (2015). Prediagnostic presentations of Parkinson's disease in primary care: a case-control study. *Lancet Neurol*; 1:57-64.
- Seeberger L.C. and Hauser R.A. (2007). Optimizing bioavailability in the treatment of Parkinson's disease. *Neuropharmacol*; 53:791-800.

- Senek M., Sten-Magnus A., Askmark H., Bergquist F., Radu C., Anders E., Lycke S. Medvedev A., Memedi M., Ojsson F., Spira J., Westin J. and Nyholm D. (2017). Levodopa/carbidopa microtablets in Parkinson's disease: a study of pharmacokinetics and blinded motor assessment. *Eur J Clin Pharmacol* 73: 563-571.
- Shah U., Joshi G., and Sawant K. (2014). Improvement in antihypertensive and antianginal effects of felodopine by enhanced absorption from PLGA nanoparticles optimized by factorial design. *Mater. Sci. Eng. C*; 35:153-163.
- Sharma S., Moon C.S., and Khogli A. (2013). Biomarkers in Parkinson's disease (recent update). *Neurochem Int*; 63:201-229.
- Shergill SS, Walker Z, Le Katona C. (1998). A preliminary investigation of laterality in Parkinson's disease and susceptibility to psychosis. *J Neurol Neurosurg Psychiatry* 65: 610-611.
- Shoulson I, Glaubiger GA, Chase TN. (1975). On-off response: clinical and biochemical correlations during oral and intravenous levodopa administration in parkinsonian patients. *Neurology*; 25: 1144-8
- Singh, N.; Pillay, V.; Choonara, Y.E. (2007) Advances in the treatment of Parkinson's disease. *Prog. Neurobiol*; 81, 29-44.
- Smith L.A., Jackson M.J., and Al-Barghouthy G. (2005). Multiple small doses of levodopa plus entacapone produce continuous dopaminergic stimulation and reduce dyskinesia induction in MPTP-treated drug-naïve primates. *Mov Disord* ;20: 306-14.

- Srinivasan R, Henley BM, Henderson BJ, Indersmitten T, Cohen BN, Kim CH, *et al.* (2016) Smoking-relevant nicotine concentration attenuates the unfolded protein response in Dopaminergic neurons. *J Neurosci*; 36(1):65–79.
- Stacy M. (2000). Pharmacology for advanced Parkinson's disease. *Pharmacother*; 20:8S-16S.
- Stocchi F. (2003). Prevention and treatment of motor fluctuations. *Parkinsonism Relat Disord*; 9:73-81.
- Stowe R.I., Ives N.J., and Clarke C. (2008). Dopamine agonist in early Parkinson's Disease. *Cochrane Database Syst Rev* (2):CD006564.
- Streubel A, Siepmann J, Bodmeier R. (2006). Drug delivery to the upper small intestine window using gastroretentive technologies. *Curr Opin Pharmacol*; 6:501-8.
- Subedi G., Kumar A.S., and Shakya S. (2016). Study of effect of different factors in formulation of micro and nanospheres with solvent evaporation technique. *Open Pharmaceutical Sciences Journal*; 3:182-195.
- Suchowersky O, Reich S, Perlmuter J, *et al.* (2006) Practice parameter: diagnosis and prognosis of new onset Parkinson disease (an evidence-based review): report of the Quality Standards Sub- committee of the American Academy of Neurology. *Neurology*; 66:968–975.
- Suzuki, K., Miyamoto M., Miyamoto T., Iwanami M. and Hirata K. (2011). Sleep disturbances associated with Parkinson's disease. *Parkinsons Dis.*:219056

- Takayama H., Hirata M., Machida Y., Masada T., Sannan T., and Naggai T. (1990). Effect of interpolymer complex formation on bioadhesive property and drug release phenomenon of compressed tablets consisting of chitosan and sodium hyaluronate. *Chem Pharm Bull* ;38: 1993-1997.
- Takahata H., Lavelle E.C., Coombes A.G.A., and Davis S.S. (1999). The distribution of protein associated with poly(DL-lactide-co-glycolide) microparticles and its degradation in simulated body fluids. *Journal of controlled release*, 59, 237-246.
- Takeuchi H., Hiromitsu Y. and Toshiyuki N. (1996). Enteral absorption of insulin in rats from mucoadhesive chitosan-coated liposomes. *Pharmaceutical Research* ;13(6) :896-901.
- Talukder R., Fassihi R. (2004). Gastroretentive delivery systems a mini review. *Drug Dev Ind Phar* 30: 1019–1028.
- Tamasree M. Biswas G.R. and Majee S. B. (2016). Hydroxypropyl Methyl Cellulose: Different Aspects in Drug Delivery. *Journal of Pharmacy and Pharmacology*; 4: 381-385.
- Thanoo, B.C., Sunny, C.M., Jayakrishnan, A., (1992). Crosslinked chitosan microspheres: preparation and evaluation as a matrix for the controlled release of pharmaceuticals. *J. Pharm. Pharmacol.* 44, 283–286.
- Tiarks F, Landfester K. and Antonietti M. (2001). Preparation of Polymeric Nanocapsules by miniemulsion Polymerization. *Langmuir*; 17: 908-18.
- Tolosa E., Gaig C., and Santamaria J. (2009). Diagnosis and the premotor phase of Parkinson's disease. *Neurology*; 72 (suppl) :S12-S20.

- Tolosa E., Marti M.J., Vallderiola F., and Molinuevo J.L. (1998). History of levodopa and dopamine agonists in Parkinson's disease treatment. *Neurology* 50(6 Suppl. 6), S2-S10; discussion S44-S18.
- Tysnes O. and Storstein A. (2017). Epidemiology of Parkinson's Disease. *J Neural Transm* ;124(8) :901-905.
- Umek N., Gersak B., Vintar N., Sostaric M., and Mavri J. (2018). Dopamine autoxidation is controlled by acidic Ph. *Front Mol Neurosci* ;11: 11-467.
- Vasir JK, Tambwekar K, Garg S. (2003). Bioadhesive microspheres as a controlled drug delivery system. *Int J Pharm* ;255:13-32.
- Venkatesh H. (1989). A buccal delivery system of Salbutamol Sulphate. *M Pharm* ;Budrinarayan.
- Vickers S., Stuart E.K., and Bianchine J.R. (1974). Metabolism of carbidopa, an aromatic amino acid decarboxylase inhibitor, in the rat, rhesus monkey, and man. *Drug Metab Dispos* ;2:9-22.
- Vyslouzil J., Dolezel P., Sova M.K., Masek J., Robert L., Kostal V., Vetchy D., and Ackova K.D. (2014). Influence of different formulations and process parameters during the preparation of drug-loaded PLGA microspheres evaluated by multivariate data analysis. *Acta Pharm* ;64:403-417.
- Waller D. G., Sampson A. P. (2018). *Medical Pharmacology and Therapeutics*. Fifth Edition. Chapter 24. Pages 325–336.
- Weintraub D., Comella C.L., and Stacy H. (2008). Part 2: Treatment of motor symptoms. *Am J Manag Care*; 14: S49-58.

- Wickremaratchi N.M., Knipe M.D., Sastry B.S. (2011). The motor phenotype of Parkinson's disease in relation to age of onset. *Mov Disord* 11:457-463.
- William H.D., Ward R., Hardy I.J., and Melia C.D. (2011). Drug release from HPMC Matrices in Milk and Fat-Rich Emulsion. *Journal of Pharmaceutical Sciences* ;100: 4823-35.
- Woitalla D., Goetze O., and Kim J. (2006). Levodopa availability improves with progression of Parkinson's disease. *J Neurol* ;253: 1221-6.
- Xia R., and Mao Z.H (2012). Progression of motor symptoms in Parkinson's disease. *Neurosci Bull* 28: 39-48.
- Yao SC, Hart AD, and Terzella MJ. (2013). An evidence-based osteopathic approach to Parkinson disease. *Osteopathic Family Physician*. 5: 96-101
- Yeh K.C., August T.F., Bush D.F., *et al.* (1989). Pharmacokinetics and bioavailability of Sinemet CR: a summary of human studies. *Neurology* 39: 25-38
- Zahoor I., Shafi A. and Haq E. (2018). Pharmacological Treatment of Parkinson's disease. Chapter 7 pages 129- 132.
- Zajic, L., and Buckton, G. (1990). "The Use of Surface Energy Values to Predict Optimum Binder Selection for Granulation." *Int. J. Pharm.* 59 (2): 155-64.

APPENDICES

APPENDIX A

Calculation of Animal Equivalent Dose (AED) of Levodopa/Carbidopa

Human dose = 50/200 mg b.i.d (carbidopa/levodopa)

Average human weight = 60 kg

Dosage_{carbidopa} = 50 mg/60 kg = 0.83 mg/kg

Dosage_{levodopa} = 200 mg/60 kg = 3.33 mg/kg

AED = Human dose x Km ratio

Km = correction factor = body weight of species/body surface area = 6.2

AED_{carbidopa} = 0.83 mg/kg x 6.2 = 5.146 mg/kg

AED_{levodopa} = 3.33 mg/kg x 6.2 = 20.646 mg/kg

Animal equivalent dose = 5/20 mg/kg

APPENDIX B

Calculation for concentrations obtained from content analysis of levodopa

Interpolation from calibration equation ($y = 2.1383x + 1.4208$)

y-intercept = 1.4208

$$\text{slope} = 2.1383$$

y = peak areas obtained from HPLC

x = concentration of levodopa

$$\text{concentration of levodopa (x)} = (y - 1.4208) / 2.1383$$

From the triplicate test done,

Run 1, Peak area = 1082

$$\text{Concentration of levodopa} = (1082 - 1.4208) / 2.1383 = 505.34 \mu\text{g/mL}$$

Run 2, Peak area = 959

$$\text{Concentration of levodopa} = (959 - 1.4208) / 2.1383 = 447.822 \mu\text{g/mL}$$

Run 3, Peak area = 1035

$$\text{Concentration of levodopa} = (1035 - 1.4208) / 2.1383 = 483.36 \mu\text{g/mL}$$

APPENDIX C

Calculation for average percentage cumulative release of levodopa

% Average Cumulative Release = (Average concentration of levodopa released into the dissolution medium/ expected concentration) X 100

Expected concentration = (actual amount of levodopa in the weighed 75 mg of microparticles used for *in vitro* release test)/ volume of dissolution medium

Expected concentration = $15 \text{ mg} / 500 \text{ mL} = 0.03 \text{ mg/mL} = 30 \text{ } \mu\text{g/mL}$

Average concentration of levodopa released = average concentrations interpolated from the calibration equation

At time 0

% Average cumulative release = $(0.89/30) \times 100 = 2.98 \%$

At time 0.25 h,

% Average cumulative release = $(1.22/ 30) \times 100 = 4.07 \%$

Time 0.5 h,

% Average cumulative release = $(3.42/ 30) \times 100 = 11.40 \%$

Time 1 h,

% Average cumulative release = $(7.11/30) \times 100 = 23.71 \%$

Time 2 h,

% Average cumulative release = $(11.25/30) \times 100 = 37.51 \%$

Time 4 h,

% Average cumulative release = $(14.72/30) \times 100 = 49.07 \%$

Time 8 h,

% Average cumulative release = $(19.86/30) \times 100 = 66.22 \%$

Time 12 h,

$$\% \text{ Average cumulative release} = (23.66/30) \times 100 = 78.87 \%$$

Time 24 h,

$$\% \text{ Average cumulative release} = (17.2/30) \times 100 = 57.33 \%$$

APPENDIX D

Calculation of concentrations of Standard Levodopa for pharmacokinetic analysis

46 mg of levodopa powder in 10 mL of water = 4.6 mg/ mL = 4600 µg/mL stock standard

Add 5 µL of stock standard to 495 µL of methanol containing 0.05 % perchloric acid

$$C_1 V_1 = C_2 V_2$$

$$C_2 = (4600 \times 5 \text{ µL}) / (500 \text{ µL}) = 46 \text{ µg/mL}$$

Add 50 µL of stock standard to 450 µL of methanol containing 0.05 % perchloric acid = 460 µg/mL

Add 100 µL of stock standard to 400 µL of methanol containing 0.05 % perchloric acid = 920 µg/mL

Add 200 µL of stock standard to 300 µL of methanol containing 0.05% perchloric acid = 1840 µg/mL

Preparation of calibration standards for injection into HPLC

Addition of working analytical standards to internal standards:

50 μ L of levodopa standard 1 (46 μ g/mL) + 100 μ L of dilute methyldopa solution

50 μ L of levodopa standard 2 (460 μ g/mL) + 100 μ L of dilute methyldopa solution

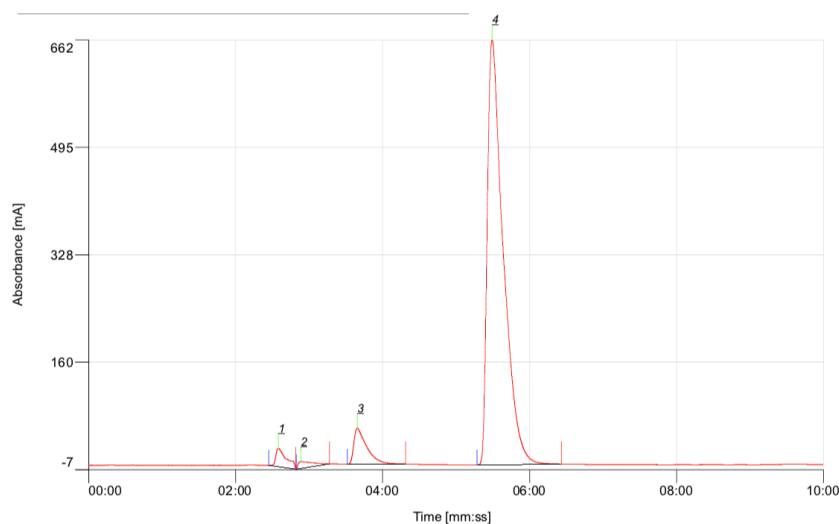
50 μ L of levodopa standard 3 (920 μ g/mL) + 100 μ L of dilute methyldopa solution

50 μ L of levodopa standard 4 (1840 μ g/mL) + 100 μ L of dilute methyldopa solution

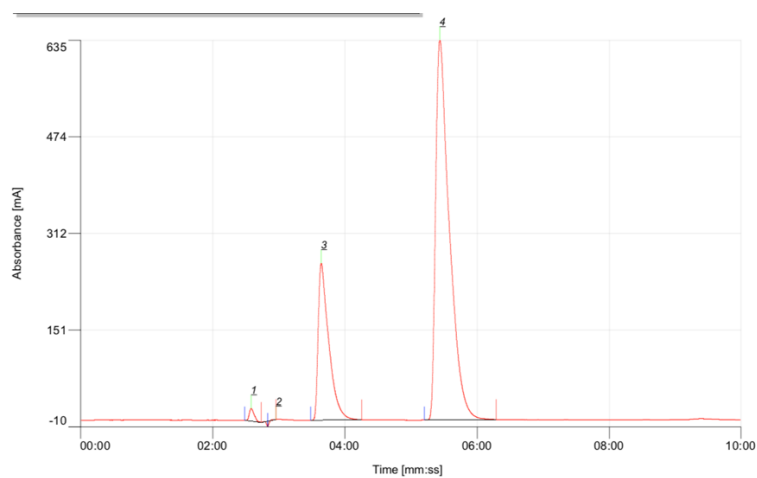
20 μ L each out of the final volumes were injected into HPLC

Chromatograms generated from the HPLC analysis of the calibration standards

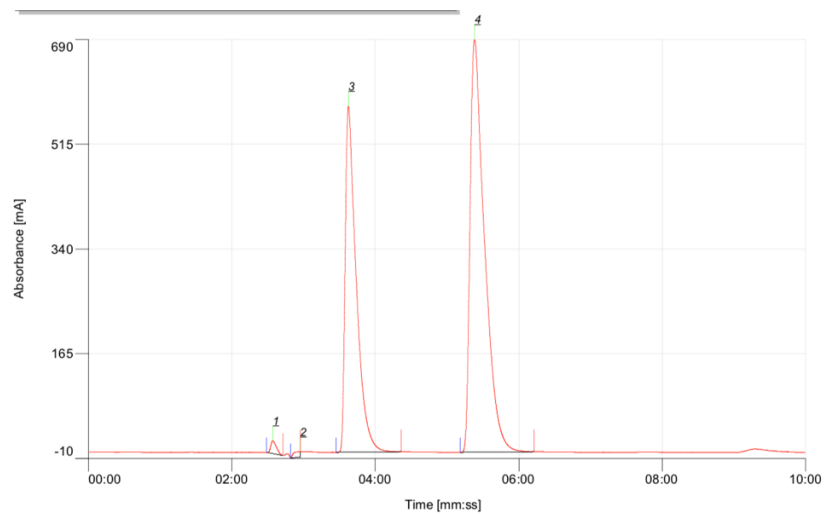
(A)



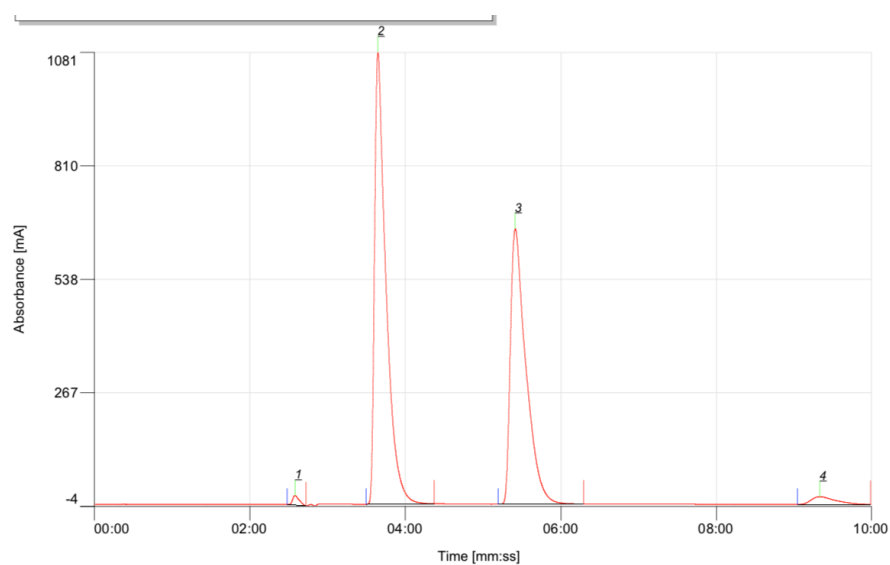
No.	Peak Name	Ret. Time [mm:ss]	Start Time [mm:ss]	End Time [mm:ss]	Area [mAs]	Height [mA]	Qty [μ g/ml]
001	***	02:34.9	02:27.1	02:49.4	291.48	28.61	N/A
002	***	02:53.3	02:49.4	03:16.6	146.69	10.86	N/A
003	L-Dopa	03:39.5	03:31.4	04:18.7	682.34	55.89	0.0000
004	M-Dopa	05:29.6	05:17.1	06:26.1	9947.30	660.99	0.0000

(B)

No.	Peak Name	Ret. Time [mm:ss]	Start Time [mm:ss]	End Time [mm:ss]	Area [mAs]	Height [mA]	Qty [µg/ml]
001	***	02:34.9	02:29.0	02:43.9	107.75	21.06	N/A
002	***	02:57.4	02:49.8	02:57.4	-10.11	0.04	N/A
003	L-Dopa	03:38.6	03:29.1	04:15.4	2907.32	261.66	0.0000
004	M-Dopa	05:26.3	05:12.4	06:17.4	9184.32	634.25	0.0000

(C)

No.	Peak Name	Ret. Time [mm:ss]	Start Time [mm:ss]	End Time [mm:ss]	Area [mAs]	Height [mA]	Qty [µg/ml]
001	***	02:34.4	02:29.3	02:43.0	114.50	21.52	N/A
002	***	02:57.4	02:49.3	02:57.6	59.19	8.54	N/A
003	L-Dopa	03:37.6	03:27.1	04:21.9	6169.50	578.40	0.0000
004	M-Dopa	05:23.1	05:11.2	06:12.9	9660.54	690.22	0.0000

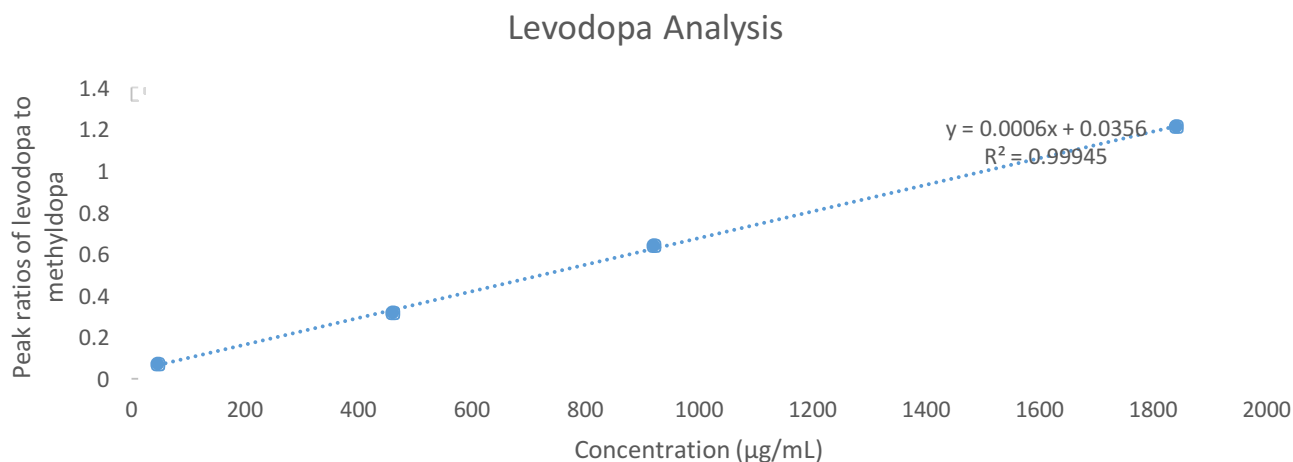
(D)

No.	Peak Name	Ret. Time [mm:ss]	Start Time [mm:ss]	End Time [mm:ss]	Area [mAs]	Height [mA]	Qty [µg/ml]
001	***	02:34.9	02:28.9	02:43.5	131.33	22.49	N/A
002	L-Dopa	03:38.9	03:29.8	04:22.5	11017.87	1080.22	0.0000
003	M-Dopa	05:24.9	05:11.6	06:17.5	9098.32	658.39	0.0000
004	***	09:20.3	09:03.1	09:59.4	393.64	18.40	N/A

Chromatograms showing the peak areas of (a) calibration standard 1, (b) standard 2, (c) standard 3 and (d) standard 4.

Calibration curve for levodopa levels determination

□

**APPENDIX E*****Post Hoc* Analysis with Tukey's Multiple Comparison***Time to reach peak concentration (T_{max})*

ANOVA summary

F	7.0
P value	0.0270
P value summary	*
Significant diff. among means ($P < 0.05$)?	Yes
R square	0.70

Tukey's multiple comparisons test

	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Sinemet CR ($\mu\text{g/ml}$) vs. LD+CD Powder ($\mu\text{g/ml}$)	-0.17	-0.58 to 0.25	No	ns	0.4827
Sinemet CR ($\mu\text{g/ml}$) vs. LD+CD Microparticles ($\mu\text{g/ml}$)	-0.50	-0.92 to -0.082	Yes	*	0.0242
LD+CD Powder ($\mu\text{g/ml}$) vs. LD+CD Microparticles ($\mu\text{g/ml}$)	-0.33	-0.75 to 0.084	No	ns	0.1089

Peak levodopa concentration (C_{max})

ANOVA summary

F	14
P value	0.0058
P value summary	**
Significant diff. among means (P < 0.05)?	Yes
R square	0.82

Tukey's multiple comparisons test

	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Sinemet CR (µg/ml) vs. LD+CD Powder (µg/ml)	83	-45 to 211	No	ns	0.1964
Sinemet CR (µg/ml) vs. LD+CD Microparticles (µg/ml)	-134	-261 to -5.7	Yes	*	0.0423
LD+CD Powder (µg/ml) vs. LD+CD Microparticles (µg/ml)	-216	-344 to -88	Yes	**	0.0049

Area under the concentration-time curve (AUC)

ANOVA summary

F	50
P value	0.0002
P value summary	***
Significant diff. among means (P < 0.05)?	Yes
R square	0.94

Tukey's multiple comparisons test

	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Sinemet CR (µg/ml) vs. LD+CD Powder (µg/ml)	248	48 to 447	Yes	*	0.0208
Sinemet CR (µg/ml) vs. LD+CD Microparticles (µg/ml)	-394	-594 to -195	Yes	**	0.0022
LD+CD Powder (µg/ml) vs. LD+CD Microparticles (µg/ml)	-642	-842 to -442	Yes	***	0.0002

Elimination half-life ($t_{1/2}$)

ANOVA summary

F	5.0
P value	0.0518
P value summary	ns
Significant diff. among means ($P < 0.05$)?	No
R square	0.63

Tukey's multiple comparisons test

	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Sinemet CR ($\mu\text{g/ml}$) vs. LD+CD Powder ($\mu\text{g/ml}$)	5.8	0.16 to 11	Yes	*	0.0448
Sinemet CR ($\mu\text{g/ml}$) vs. LD+CD Microparticles ($\mu\text{g/ml}$)	3.5	-2.2 to 9.1	No	ns	0.2224
LD+CD Powder ($\mu\text{g/ml}$) vs. LD+CD Microparticles ($\mu\text{g/ml}$)	-2.3	-8.0 to 3.3	No	ns	0.4600