IJBPAS, May, 2018, 7(5): 748-773

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

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LEVODOPA/CARBIDOPA BI-LAYER TABLET

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Received 1st Jan. 2018; Revised 4th Feb. 2018; Accepted 17th March 2018; Available online 1st May 2018

DOI: https://doi.org/10.31032/IJBPAS/2018/7.5.4435

ABSTRACT

The presence of tremor at rest, rigidity, bradykinesia, and postural instability are considered the hallmark motor features of idiopathic Parkinson's disease (IPD). These clinical features of IPD were adeptly described in 1817 by James Parkinson. The prevalence of IPD increases with age, affecting 1% of people older than 65 years of age and 2.5% of those older than 80 years.

The compression of the multilayer tablet was achieved by using a single punch tablet machine. for the optimized bi-layer tablet, both levodopa and carbidopa concentrations were determined using the UV-Visible Spectrophotometry method to measure the amount of released dug. The data obtained was confirmed by HPLC method.

The amount of guar gum polymer in the formulation was found to affect the levodopa and carbidopa release rate significantly. A complete release of levodopa and carbidopa occurred from the bilayer tablet (A5) within 10 hours, so it was considered the optimized bilayer tablet containing the optimized amount of guar gum.

Keywords: Parkinson's, Bradykinesia, Bilayer tablet, Levodopa, Carbidopa

INTRODUCTION

Background

The presence of tremor at rest, rigidity, bradykinesia, and postural instability are considered the hallmark motor features of idiopathic Parkinson's disease (IPD). These clinical features of IPD were adeptly described in 1817 by James Parkinson (Parkinson 2002). The prevalence of IPD increases with age, affecting 1% of people older than 65 years of age and 2.5% of those older than 80 years (Stephen, *et al.* 2003; Twelves, *et al.* 2003). The true etiology of IPD is unknown, but factors such as genetic constitution and toxin (intrinsic or extrinsic) exposure most likely play a role. In IPD, a key histopathologic feature is the degeneration of dopaminergic neurons in the substantia nigra that project to the striatum (i.e., the nigrostriatal pathway) (Moore, *et al.* 2005). In the substantia nigra pars compacta (SNc), the two hallmark histopathologic features of IPD are depigmentation of dopamine-producing neurons (i.e., loss of SNc neurons) and presence of Lewy bodies (neuronal cytoplasmic filamentous aggregates composed of the presynaptic protein α-synuclein) in the remaining SNc neurons (Braak, *et al.* 2003). Pathologic findings reveal a correlation between the extent of nigrostriatal dopamine loss and the severity of certain IPD motor features (e.g. Bradykinesia). The threshold for the onset of clinically detectable IPD appears to be the loss of 70% to 80% of SNc neurons (Bernheimer, *et al.* 1973). L-Dopa is the immediate precursor of dopamine and, in combination with a peripherally acting L-amino acid decarboxylase inhibitor (carbidopa or benserazide), remains the most effective drug for the symptomatic treatment of IPD (Miyasaki 2002). L-Dopa crosses the blood brain barrier, whereas dopamine, carbidopa, and benserazide do not. The combination of L-dopa with carbidopa or benserazide, reduces the unwanted peripheral conversion of L-dopa to dopamine. As a result, increased amounts of L-dopa are transported into the brain, and peripheral adverse effects of dopamine, such as nausea, are reduced. In the SNc, L-dopa is converted, via decarboxylation, to dopamine by the enzyme L-amino acid decarboxylase (Joseph 2008).

Justification

An initial maintenance L-dopa regimen of 300 mg/day (in divided doses and in combination with carbidopa or benserazide) often is adequate. With regards to carbidopa, about 75 mg/day is required to sufficiently inhibit the peripheral activity of L-amino acid decarboxylase, but some patients require more. Therefore, the usual initial dose of carbidopa/L-dopa regimen is 25/100 mg three times daily. As IPD progresses to more severe symptoms, use of higher dosages is required. There is no maximum allowable total

daily L-dopa dose; however, the usual maximal dose needed by patients, even those with severe IPD, is 800 to 1,000 mg/day (Joseph*, et al.* 2008). Unfortunately, long-term L-dopa therapy is associated with a variety of motor complications, of which end-of-dose "wearing off" (motor fluctuations) and L-dopa peak-dose dyskinesias are the two most commonly encountered. In other words, these complications are known as "On-Off" effects (Hauser, *et al.* 2006).

The "On effect" refers to dyskinesia that is characterized by involuntary choreiform movements involving usually the neck, trunk, and lower/upper extremities. These are often associated with peak striatal dopamine levels (peak dose dyskinesia). The "Off effect" refers to dystonias (sustained muscle contractions) which occur more commonly in a distal lower extremity (e.g., the foot). Dystonias often occur in the early morning hours and improve with the first L-dopa dose of the day. In literature, some clinical trials proved the concept of control release over the conventional release on improving side effects of levodopa-carbidopa; but some others could not prove it (García, *et al.* 1997; Pahwa, *et al*. 1997). At the beginning of the treatment, patients of Parkinson disease usually depend on both the remaining endogenous dopamine that is released from the intact neural cells and the exogenous dopamine from the dosage form. With time, however, some of the neural cells die and so the endogenous dopamine gradually decreases followed by the "wearing off" effect. That is why sustained release dosage forms also fail over long term treatment (Hauser, *et al*. 2006). Therefore, titration of the dose and frequency of dosing for each patient of Parkinson disease over the entire period of treatment will enhance and improve the side effects whether immediate release or sustained release was used (Block, *et al.* 1997). However, it is not practically acceptable for the patient to take an immediate release dosage form more than three times per day because of poor compliance. For this reason, sustained release dosage form is preferable. Nevertheless, there remains a significant flaw in the therapeutic application of controlled release carbidopa-levodopa; that is, the considerable delay in onset of action. Mean time to peak concentration in healthy elderly subjects was found to be two hours for controlled release carbidopa-levodopa and only 0.5 hours for the conventional form (LeWitt PA, *et al*. 1989). Therefore, a controlled release dosage form that could also provide rapid onset of action, at least equivalent to that of conventional carbidopa-levodopa, would have an obvious clinical advantage. A multilayer tablet that contains a layer of an immediate release and another layer that can provide a slow release option is thought

to solve the problem (Stocchi, *et al*. 1994; LeWitt, *et al*. 1992).

OBJECTIVE

General Objectives

The strategy proposed in the present research is to formulate a bilayer tablet containing both immediate and controlled release layers of carbidopa-levodopa. Next is to determine the difference between the in-vitro profile release of this bilayer tablet and that of the immediate/controlled release tablets currently available in the market.

Specific Objectives

The specific objectives were to:

1. formulate the immediate release layer using wet granulation method;
2. sustain the controlled release layer using Guar gum as a matrix polymer;
3. determine the optimized drug: polymer ratio for adequate drug release;
4. characterize the optimized Bilayer tablet using general physical tests and study the drug release kinetics.

LEVODOPA

Dihydroxyphenylalanine; L-Dopa; 3-Hydroxy-L-tyrosine; Laevodopa; Lévodopa; Levodopum. (−)-3-(3,4-Dihydroxyphenyl)-Lalanine.

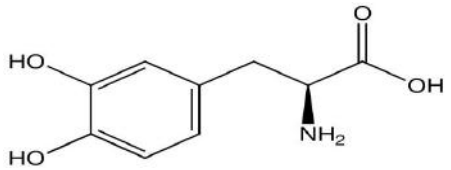

Fig 1.1 Levodopa chemical structure

Pharmacokinetic

Levodopa is rapidly absorbed from the gastrointestinal tract by an active transport system and most absorption takes place in the small intestine; absorption is very limited from the stomach, and since decarboxylation may take place in the stomach wall, delays in gastric emptying may reduce the amount of levodopa available for absorption. Peak plasma concentrations are achieved within 2 hours of oral doses. Levodopa is about 10 to 30% bound to plasma proteins (Nutt and Fellman, 1984).

Levodopa is rapidly decarboxylated by the enzyme aromatic L-amino acid decarboxylase, mostly in the gut, liver, and kidney, to dopamine, which is metabolized in turn, principally to dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Other routes of metabolism include O-methylation, transamination, and oxidation, producing a variety of minor metabolites including noradrenaline and 3-O-methyldopa; the latter may accumulate in the CNS due to its relatively long half-life. The elimination half-life of levodopa itself is reported to be about 30 to 60 minutes.

Levodopa is actively transported across the blood-brain barrier, but because of the extent of peripheral decarboxylation very little is available to enter the CNS unless it is given with a

peripheral Dopa-decarboxylase inhibitor (Cedarbaum, 1987).

Major Adverse Effects

Adverse effects of levodopa are dyskinesia in 75% of patients, psychiatric disturbances in 25%, nausea and vomiting in 40 to 50% with gradual regress and hypotension in 25 to 30% which is generally asymptomatic (Calne, 1972).

CARBIDOPA

Carbidopum; Carbidopum Monohydricum; Karbidopa; Karbidopa monohydrát; α-Methyldopa Hydrazine; MK-486. (+)-2- (3,4-Dihydroxybenzyl)-2- hydrazinopropionic acid monohydrate; (−)-L-α-Hydrazino-3,4-dihydroxy-α-methylhydrocinnamic acid monohydrate.

GENERAL DESCRIPTION

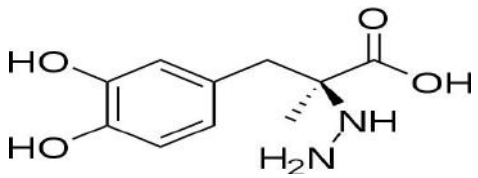

Fig 1.2 Carbidopa chemical structure

Ph. Eur. 6.2 (Carbidopa).

Carbidopa is a white or yellowish-white powder. It is slightly soluble in water, very slightly soluble in alcohol, practically insoluble in dichloromethane and dissolves in dilute solutions of mineral acids. Carbidopa should be protected from light.

PHARMACOKINETIC

Carbidopa is rapidly but incompletely absorbed from the gastrointestinal tract. It is rapidly excreted in the urine both unchanged and in the form of metabolites. It does not cross the blood-brain barrier. In rats, carbidopa has been reported to cross the placenta and to be distributed into breast milk (Joseph *et al* 2008).

COMBINATION OF LEVODOPA AND CARBIDOPA

Pinder *et al* (1976) stated that carbidopa is a peripheral dopa-decarboxylase inhibitor with little or no pharmacological activity when given alone in usual doses. It inhibits the peripheral decarboxylation of levodopa to dopamine and as it does not cross the blood brain barrier, unlike levodopa, effective brain concentrations of dopamine are produced with lower doses of levodopa. At the same time reduced peripheral formation of dopamine reduces peripheral adverse effects, notably nausea and vomiting, and cardiac arrhythmias; although the dyskinesias and adverse mental effects associated with levodopa therapy tend to develop earlier.

GUAR GUM AS A POLYMER IN THE CONTROLLED RELEASE LAYER

Guar gum is a non-ionic polysaccharide derived from the seeds of *Cyamopsis tetragonolobus*, family Leguminosae. It consists of linear chains of "(1->4)-β-D-mannopyranosyl units with a-D-galactopyranosyl units attached by (1----6)

linkages. In pharmaceuticals, guar gum is used in solid-dosage forms as a binder and disintegrant; in oral and topical products as a suspending, thickening, and stabilizing agent; and also, as a controlled-release carrier. Guar-gum-based three-layer matrix tablets have been used experimentally in oral controlled-release formulations.

MATERIAL & METHODOLOGY

MATERIAL

Levodopa (BP 2007)

It was a gift by Alpha Chem Advanced Pharmaceutical Industries Co. / ACAPI/ Badr City/ Cairo/ Egypt

Carbidopa (USP 31)

It was a gift by Alpha Chem Advanced Pharmaceutical Industries Co. / ACAPI/ Badr City/ Cairo/ Egypt

Guar Gum

CASR No. (9000-30-0), Lab Line Co. /Khartoum/Sudan

Microcrystalline Cellulose (Avicel)USP / NF, Ph.Eur, JP

JRS PHARMA GMBH & CO. Kg D-73494 Rosenberg (Germany)

Starch Powder (Corn)

Laboratory BURGOYNE REAGENTS URBIDGES & CO (India) MUMBAI

Laboratory BURGOYNE REAGENTS URBIDGES & CO (India) MUMBAI

COLOURING AGENTS

Carmine

E Number: E120; CAS NO: [1260-17-9]

Brilliant blue FCF

E Number: E133; CAS NO: [2650-18-2]

Dosage forms

SINEMET® CR

APIs	200 mg Levodopa 50 mg Carbidopa
Source	Healthy-U Pharmacy / 59 Lustrells Vale, Saltdean, Brighton, BN2 8FA
Manufacturer	Bristol-Myers Squibb Pharmaceuticals Ltd. Lot V5859

DOPAL - M

APIs	100 mg Levodopa 10 mg Carbidopa
Source	Abdalla Khaleel pharmacy / Omdurman / Khrtoum / Sudan
Manufacturer	IBN HAYAN PHARMACEUTICALS R. FAYSAL & CO. HOMS-SYRIA
Batch NO	221

EQUIPMENT

Laboratory Sieve (mesh # 10 & 12)

Measuring cylinders

Volumetric Flasks

Mortar and Pestle

Plastic Funnels

Glass Beakers

Glass Pipettes

Filter papers

Test Tubes

Spatulas

Sensitive Electronic Balance

Max 62 g D = 0.1 mg

Manufacturer OHAUS, Switzerland

Fluid Bed drier

Manufacturer; Sherwood Scientific, Serial No 144753 KW; 18.5 Kg

Single Punch Tablet Machine

Type No SSF3

Serial No 190/Z/05-06

Size of Punch 12 mm

Manufacturer Cadmach Machinery CO. PVT. LTD

3.2.2.4 High performance Liquid Chromatography (HPLC)

Source	Available at the University of Medical Sciences & Technology
System	High pressure gradient UV detection system
Serial No	5102149
Pump	2 LC - 10 ADvp Pumps
Auto sampler	SIL – 10 ADvp
Column Oven	CTO – 10 A(c) vp
Detector	SPD – 10 A (v) vp
Column	Shim – pack VP – ODS (150mm x 4.6mm i.d.5um)
Manufacturer	SHIMADZU CORPORATION ANALYTICAL & MEASURING INSTRUMENTS DIVISION

UV-Visible Spectrophotometer

Source	Available at the University of Medical Sciences & Technology
Model Name	UV – 1800
CAT No	206-25400-38
Serial No	A11454682301
Manufacturer	SHIMADZU CORPORATION ANALYTICAL & MEASURING INSTRUMENTS DIVISION

The product complies with the requirement of EMC Directive 2004/108/EC and low voltage Directive 2006/95/EC.

Tablet Dissolution Tester

Product Model	Dis 6000
Serial No	13010
Type	NE4 – COP
Supplied by	COPLEY SCIENTIFIC, Nottingham, UK, NG42JY

Friability Tester

Type	FR 1000
Serial No	12945
Supplied by	COPLEY SCIENTIFIC, Nottingham, UK, NG42JY

Tablet Hardness Tester

Type	TH 3 (Basic force GAUGE)
BFG	500 N Serial No 08-0206-09
Supplied by	COPLEY SCIENTIFIC, Nottingham, UK, NG42JY

FORMULATION OF LEVODOPA-CARBIDOPA "BILAYER" TABLET

Formula of IR layer

Table 1: Percent & amount of Pharmaceutical Ingredients in the Immediate release layer

Ingredients	(w/w) % per Tablet	Amount (mg) / Tablet
Levodopa	34.55	100.1
Carbidopa	9.29	26.9
Microcrystalline Cellulose	27.4	79.3
Starch	27.4	79.3
Mg+ Stearate	1.31	1.3
Total	100	289.4

Formula of (controlled release) CR

Table 2: Compositions of Various Trial Formulations for the CR Layer (All Quantities Given in mg)

	Formulation Code				
Ingredients	A1	A2	A3	A4	A5
Levodopa	200.10	200.10	200.10	200.10	200.10
Carbidopa	53.80	53.80	53.8	53.8	53.8
Starch				16.48	
Guar gum	110.39	85.77	64.25	56.05	45.33
Coloring agent*	0.1	0.1	0.1	0.1	0.1
Mg+ Stearate	3.67	3.43	3.21	3.29	3.021
Total	368.06	334.20	321.46	329.82	302.35

*Either Carmine or Brilliant color

Table 3: Percentage of Guar gum in the various trial formulations of the CR layer

	Formulation Code				
Ingredient	A1	A2	A3	A4	A5
Guar gum	30	25	20	17	15

IMMEDIATE RELEASE LAYER

Levodopa, carbidopa, microcrystalline cellulose and starch were mixed together in the planetary mixer for 5 minutes. A sufficient quantity of pure water was added while kneading until a wet agglomerate was formed which then passed through lab mesh sieve (mesh # 10) to form wet granules. These wet granules were transferred into the fluid bed drier to be dried at 50^{o}C for 15 minutes. Then the dried granules were passed through sieve (mesh # 12). Finally, the dried granules were mixed with magnesium stearate to be ready for compression.

Controlled Release layer

Levodopa, carbidopa, guar gum and magnesium stearate (used as internal lubricant [Herbert 1989]) were mixed together in the planetary mixer for 5 minutes. A sufficient quantity of pure water containing the coloring agent was added while kneading until a wet agglomerate was formed which then passed through lab (mesh #10) to form wet granules. These wet granules were transferred into the fluid bed drier and dried at 50^{o}C for 30 minutes. The dried granules were then transferred into the ball miller to reduce the granules size mesh # 12.

COMPRESSION

The compression of the multilayer tablet was achieved by using a single punch tablet machine. Although such machine does not usually produce such type of tablets, it can be achieved manually via double compression technique by moving the upper punch reversibly after slight compression of the 1st layer and before the ejection of the lower punch. This allows the second layer to be added, then compressed and ejected as a whole multilayer tablet. To perform such procedure in that type of tablet machine, 289.4 mg of IR layer and 302.52 mg of CR layer were weighed separately and fed up manually into the single punch tablet machine to be compressed by such double compression technique. This process was repeated until desired number of tablets was prepared.

TABLET CHARACTERIZATION

Hardness Test

The tablet was placed in the hardness tester and pressure was applied manually until the tablet was crushed down in the middle. Then the pressure was recorded.

Friability Test

Ten tablets were weighed, then inserted into the Friability Tester adjusted at 25 rpm for 4 minutes (i.e. 100 revolutions) and allowed to work. After it had stopped the tablets were reweighed and the difference in weight was calculated as a percentage.

Weight Variation Test

The weight variation of the tablets was evaluated for 10 tablets using sensitive electronic balance. The weight of each tablet was recorded and then average weight was calculated. The difference between the average weight and the individual weights were calculated as percentages.

DRUG CONTENT STUDY

Twenty tablets were taken and crushed to powder with a mortar and pestle. Quantity of the powder equivalent to 0.25 g of levodopa was taken and shaken with 60 ml of 0.1 M HCL for 15 minutes and then sufficient 0.1M hydrochloric acid was added to produce 100 ml of solution. The solution was filtered through 0.45-µm filter paper and 10 ml of the clear filtrate was diluted to 50 ml with 0.1M hydrochloric acid. The total amount of drug within the tablets was analyzed using the HPLC and UV-Visible spectroscopy methods against the reference solution of levodopa and carbidopa reference standards, prepared in the same procedure.

Stock solutions of levodopa and carbidopa were prepared in 0.1 M HCL. Then a twenty-four-point standard curve was prepared for each of the analytes after appropriate dilution of stock solutions to obtain final concentrations,

The standard calibration curve was prepared taking either the peak area of the analyte (levodopa or carbidopa) versus the concentration

(% w/v) using HPLC method or the absorbance of the analyte (for levodopa only since there is no BP method for carbidopa) versus the concentration (% w/v) using UV-Visible spectroscopy method. The regression equation of the calibration curve was then used to calculate the drug content and in vitro drug release.

DRUG RELEASE STUDY

Dissolution Test

The dissolution test was carried out for SINEMET® CR, DOPAL – M and the multilayer tablet. It was complied with British Pharmacopoeia 2007. The basket apparatus (USP 1) was used in which 750 ml of 0.1M HCL was used as the medium, $37^{o}C \pm 0.5$ was the range of temperature at a rotation of 50 revolution per minute (rpm). Within specified time interval, 5 ml from a zone midway between the surface of the dissolution medium and the top of the rotating basket was withdrawn and filtered. 2 ml of the filtrate was completed to 10 ml with 0.1M HCL.

The concentration of levodopa in each time interval for each dosage form was then measured by using UV-Visible spectroscopy and high-performance liquid chromatography. Although all the three dosage forms contain both levodopa and carbidopa, the dissolution profile was based only on levodopa concentration.

UV-Visible Spectrophotometer

The absorbance of the serial diluted solutions of only levodopa (shown in TABLE 3) was measured at 280 nm wave length. Then the concentration was plotted against the absorbance to form a calibration curve that was used to determine and calculate the concentration of levodopa at each time interval of the dissolution test using the straight-line equation of the resulted curve.

Straight line equation: Y = mx + b.....(1)

Where, **Y** is the absorbance of levodopa in the standard solution at 280 nm wave length, ***m*** is the slope of the straight line (specific absorbance), ***x*** is the concentration (%w/v) of the levodopa in the standard solution, and ***b*** is the Y-axis intercept.

However, since the samples withdrawn at different time intervals contain both levodopa and carbidopa, there may be interference between their absorbance. Thus, the absorbance (at 280 nm) of serially diluted standard solutions containing both levodopa and carbidopa were compared with the absorbance (at 280 nm) of serial diluted standard solutions containing only levodopa to exclude or include the interference of carbidopa with the absorbance of levodopa at 280 nm.

High Performance Liquid Chromatography (HPLC)

This method was complied with British Pharmacopoeia 2007. The chromatographic

procedure was carried out using a stainless steel column (150 mm × 4.6 mm) packed with stationary phase ODS, 0.1M potassium dihydrogen orthophosphate adjusted to pH 3.0 with 1M orthophosphoric acid as the mobile phase with a flow rate of 1.5 ml per minute and a detection wavelength of 282 nm.

Then from the standard calibration curve of peak area versus the concentration (% w/V), a straight-line equation was also used to determine and calculate the concentration of levodopa at each time interval of the dissolution test.

Straight line equation: Y = mx + b.....(2)

Where, **Y** is the peak area of levodopa in the standard solution, ***m*** is the slope of the straight line, ***x*** is the concentration (%w/v) of the levodopa in the standard solution, and ***b*** is the Y-axis intercept.

RESULTS

Tablet Characteristics

The optimum weight of the IR layer was fixed to 289.4 mg for better release of levodopa and carbidopa as well as to maintain the optimum swallowable oral dosage form. Coloring agent (Carmine / Brilliant blue) was used to differentiate the two layers.

The weight taken for carbidopa was calculated considering the percentage of weight loss on drying as shown in (Table 2). Therefore, the exact amount of carbidopa taken was 26.9 mg and 53.8 mg for IR and SR layer, respectively, instead of the expected weights of 25 mg in IR layer and 50 mg in the SR layer. The tablets of different formulations were subjected to various evaluation tests such as weigh variation, hardness, friability, and drug content. The results of these parameters are given in Table 4.

DRUG RELEASE STUDY

UV-Visible Spectrophotometry

The comparison between the standard calibration curves of levodopa alone and in the presence of carbidopa excluded the interference of carbidopa with the absorbance of levodopa at 280 nm as shown in **figure (3) and Table (9).** Hence UV-Visible Spectrophotometry can be used as a reliable method for measuring and calculating the levodopa concentration at different time intervals during the dissolution study. However, the release profile of the optimized bilayer tablet was reconfirmed using HPLC method.

The value of absorbance of each sample at 280 nm withdrawn in specified interval time was used in the above equation of straight line of the standard calibration curve instead of the value (Y) to obtain the concentration (X) of Levodopa in such sample. These values of absorbance and the calculated concentrations are shown in Table 7.

Table 4: Physical Properties of the Final Bilayer Tablets Containing 300 mg Levodopa and 75 mg Carbidopa

Formulation Code	Weight Variation* (%)	Friability† (%)	Hardness† (kg/cm2)	Drug Content*(%)	
				Levodopa	Carbidopa
A1	0.12 ± 0.01	0.09 ± 0.001	9.8 ± 0.12	97.32 ± 0.08	98.18 ± 0.05
A2	0.14 ± 0.01	0.08 ± 0.002	9.5 ± 0.15	98.41 ± 0.11	98.98 ± 0.14
A3	0.12 ± 0.02	0.09 ± 0.001	9.6 ± 0.17	98.68 ± 0.31	97.98 ± 0.17
A4	0.13 ± 0.03	0.12 ± 0.009	9.8 ± 0.10	97.95 ± 0.08	97.55 ± 0.23
A5	0.10 ± 0.01	0.05 ± 0.001	9.5 ± 0.05	99.01 ± 0.12	99.10 ± 0.23

*All values are expressed as *M* *SE*, *n* = 20.
† All values are expressed as *M* *SE*, *n* = 10

Table 5: Absorbance of serial diluted solutions containing only standard levodopa in 0.1M hydrochloric acid at 280 nm and 0.1 M HCL in the reference cell. The Concentration range of 0.0001-0.05 % W/V was plotted against Absorbance to form a standard calibration curve that was linear only over the range of 0.0001-0.02 % W/V

	Levodopa	
Serial Diluted solutions	Concentration % w/v	Absorbance
1	0.0001	0.032
2	0.0002	0.049
3	0.0003	0.058
4	0.0004	0.066
5	0.0005	0.080
6	0.0006	0.097
7	0.0007	0.111
8	0.0008	0.123
9	0.0009	0.146
10	0.0010	0.152
11	0.0020	0.320
12	0.0030	0.496
13	0.0040	0.642
14	0.0050	0.791
15	0.0060	0.933
16	0.0070	1.081
17	0.0080	1.231
18	0.0090	1.372
19	0.0100	1.517
20	0.0200	2.753
20	0.0200	2.753

Table 6: Absorbance of serial diluted solutions containing standard levodopa and carbidopa (Ratio of 1:10 respectively) in 0.1M hydrochloric acid at 280 nm and 0.1 M HCL in the reference cell. Levodopa concentration range of 0.0001-0.05 % W/V was plotted against its absorbance to form a standard calibration curve that was linear only over the range of 0.0001-0.02 % W/V. No significant difference was observed in the presence of carbidopa

	Levodopa	
Serial Diluted solutions	Concentration % w/v	Absorbance
1	0.0001	0.032
2	0.0002	0.050
3	0.0003	0.057
4	0.0004	0.067
5	0.0005	0.082
6	0.0006	0.099
7	0.0007	0.114
8	0.0008	0.125
9	0.0009	0.146
10	0.0010	0.150
11	0.0020	0.323
12	0.0030	0.498
13	0.0040	0.642
14	0.0050	0.791
15	0.0060	0.935
16	0.0070	1.080
17	0.0080	1.230
18	0.0090	1.374
19	0.0100	1.519
20	0.0200	2.753

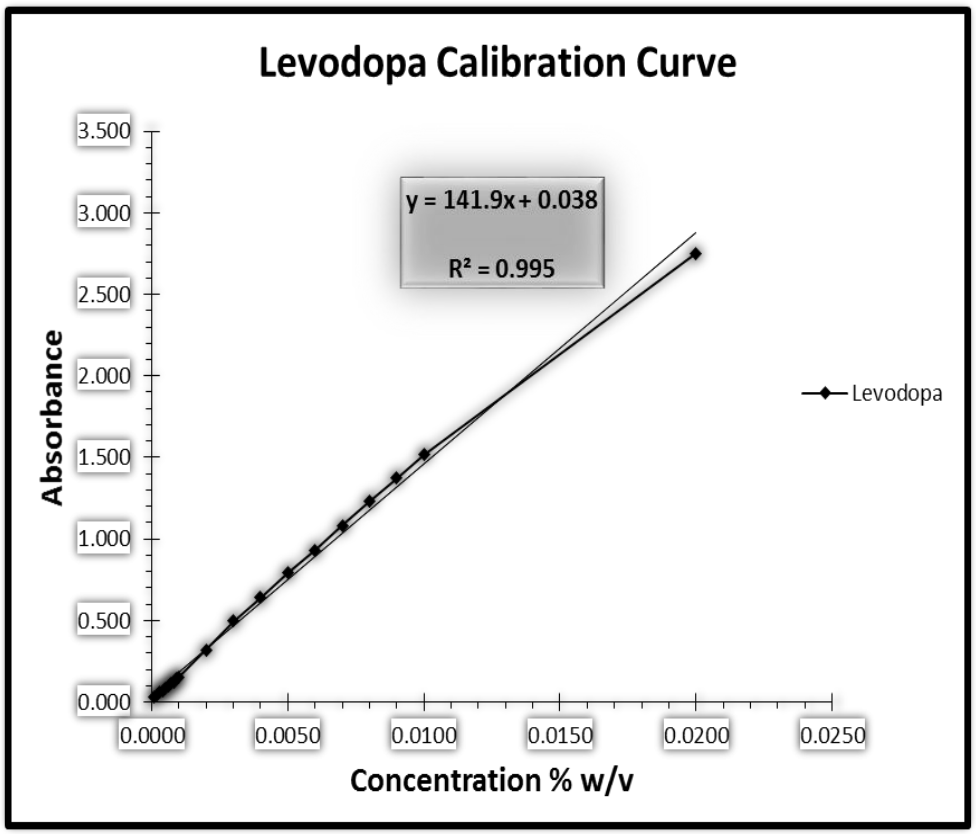

Figure 1: Standard calibration Curve of Levodopa using UV-Visible Spectrophotometry at 280 nm and 0.1 M HCL in the reference cell. The correlation coefficient is 0.995 (y = 141.9x + 0.038)

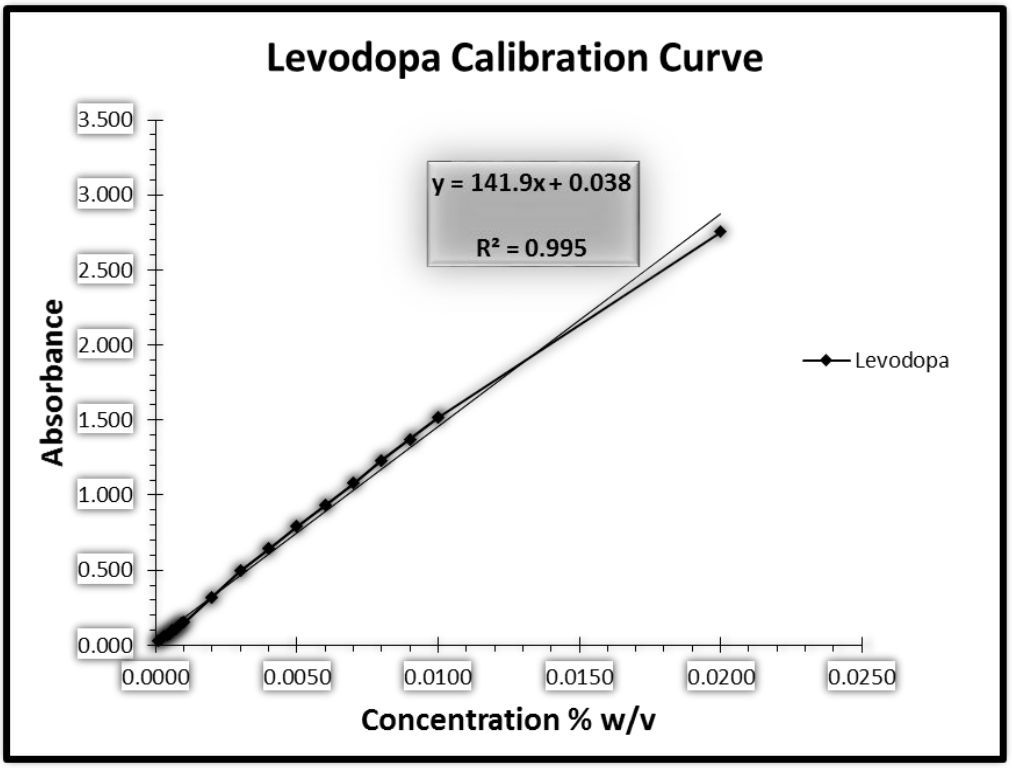

Figure 2: Standard calibration Curve of Levodopa in the presence of Carbidopa using UV-Visible Spectrophotometry at 280 nm and 0.1 M HCL in the reference cell. The correlation coefficient is 0.995 (y = 141.9x + 0.038)

Table 7: Absorbance, concentration and Cumulative drug release of Levodopa in each sample withdrawn in specified time of the dissolution profile of the multilayer tablet with the formula code (A1), using UV-Visible Spectrophotometry at 280 nm and 0.1 M HCL in the reference cell.

		Levodopa	
Time Intervals (hr)	**Absorbance**	**Concentration % w/v**	**Cumulative drug release**
0.083	**0.105**	**0.000465**	**5.8**
0.250	**0.210**	**0.001205**	**15.1**
0.500	**0.321**	**0.001987**	**24.8**
0.750	**0.446**	**0.002868**	**35.9**
1.000	**0.482**	**0.003122**	**39.0**
1.500	**0.497**	**0.003228**	**40.3**
2.000	**0.501**	**0.003256**	**40.7**
2.500	**0.509**	**0.003312**	**41.4**
3.000	**0.513**	**0.003340**	**41.8**
3.500	**0.524**	**0.003418**	**42.7**
4.000	**0.534**	**0.003488**	**43.6**
4.500	**0.549**	**0.003594**	**44.9**
5.000	**0.551**	**0.003608**	**45.1**
5.500	**0.559**	**0.003665**	**45.8**
6.000	**0.568**	**0.003728**	**46.6**
6.500	**0.579**	**0.003805**	**47.6**
7.000	**0.601**	**0.003961**	**49.5**
7.500	**0.607**	**0.004003**	**50.0**
8.000	**0.612**	**0.004038**	**50.5**

Table 8: Absorbance, concentration and Cumulative drug release of Levodopa in each sample withdrawn in specified time of the dissolution profile of the multilayer tablet with the formula code (A2), using UV-Visible Spectrophotometry at 280 nm and 0.1 M HCL in the reference cell.

		Levodopa	
Time Intervals (hr)	Absorbance	Concentration % w/v	Cumulative drug release
0.083	0.105	0.000465	5.8
0.250	0.210	0.001205	15.1
0.500	0.321	0.001987	24.8
0.750	0.446	0.002868	35.9
1.000	0.501	0.003256	40.7
1.500	0.535	0.003495	43.7
2.000	0.575	0.003777	47.2
2.500	0.600	0.003953	49.4
3.000	0.629	0.004158	52.0
3.500	0.640	0.004235	52.9
4.000	0.660	0.004376	54.7
4.500	0.679	0.004510	56.4
5.000	0.691	0.004595	57.4
5.500	0.701	0.004665	58.3
6.000	0.718	0.004785	59.8
6.500	0.739	0.004933	61.7
7.000	0.750	0.005011	62.6
7.500	0.769	0.005144	64.3
8.000	0.780	0.005222	65.3

Table 9: Absorbance, concentration and Cumulative drug release of Levodopa in each sample withdrawn in specified time of the dissolution profile of the multilayer tablet with the formula code (A3), using UV-Visible Spectrophotometry at 280 nm and 0.1 M HCL in the reference cell.

		Levodopa	
Time Intervals (hr)	Absorbance	Concentration % w/v	Cumulative drug release (%)
0.083	0.110	0.000500	6.3
0.250	0.205	0.001170	14.6
0.500	0.331	0.002058	25.7
0.750	0.435	0.002791	34.9
1.000	0.510	0.003319	41.5
1.500	0.559	0.003665	45.8
2.000	0.600	0.003953	49.4
2.500	0.648	0.004292	53.6
3.000	0.665	0.004412	55.1
3.500	0.701	0.004665	58.3
4.000	0.754	0.005039	63.0
4.500	0.779	0.005215	65.2
5.000	0.788	0.005278	66.0
5.500	0.812	0.005447	68.1
6.000	0.841	0.005652	70.6
6.500	0.849	0.005708	71.4
7.000	0.859	0.005779	72.2
7.500	0.881	0.005934	74.2
8.000	0.901	0.006075	75.9

Table 10: Absorbance, concentration and Cumulative drug release of Levodopa in each sample withdrawn in specified time of the dissolution profile of the multilayer tablet with the formula code (A4), using UV-Visible Spectrophotometry at 280 nm and 0.1 M HCL in the reference cell.

		Levodopa	
Time Intervals (hr)	Absorbance	Concentration % w/v	Cumulative drug release (%)
0.083	0.099	0.000423	5.3
0.250	0.180	0.000994	12.4
0.500	0.303	0.001860	23.3
0.750	0.425	0.002720	34.0
1.000	0.510	0.003319	41.5
1.500	0.482	0.003122	39.0
2.000	0.512	0.003333	41.7
2.500	0.574	0.003770	47.1
3.000	0.613	0.004045	50.6
3.500	0.642	0.004249	53.1
4.000	0.690	0.004588	57.3
4.500	0.701	0.004665	58.3
5.000	0.758	0.005067	63.3
5.500	0.820	0.005504	68.8
6.000	0.825	0.005539	69.2
6.500	0.850	0.005715	71.4
7.000	0.903	0.006089	76.1
7.500	0.946	0.006392	79.9
8.000	0.998	0.006758	84.5

Table 11: Absorbance, concentration and Cumulative drug release of Levodopa in each sample withdrawn in specified time of the dissolution profile of the multilayer tablet with the formula code (A5), using UV-Visible Spectrophotometry at 280 nm and 0.1 M HCL in the reference cell.

		Levodopa	
Time Intervals (hr)	Absorbance	Concentration % w/v	Cumulative drug release (%)
0.083	0.101	0.000436	5.40
0.250	0.206	0.001176	14.7
0.500	0.335	0.002085	26.0
0.750	0.446	0.002868	35.8
1.000	0.501	0.003255	40.6
1.500	0.534	0.003488	43.6
2.000	0.563	0.003692	46.1
2.500	0.587	0.003861	48.2
3.000	0.604	0.003981	49.7
3.500	0.656	0.004348	54.3
4.000	0.712	0.004742	59.2
4.500	0.795	0.005327	66.5
5.000	0.851	0.005722	71.5
5.500	0.864	0.005813	72.6
6.000	0.887	0.005976	74.7
6.500	0.891	0.006004	75.0
7.000	0.949	0.006412	80.1
7.500	0.996	0.006744	84.3
8.000	1.075	0.007300	91.2
8.500	1.108	0.007533	94.1
9.000	1.080	0.007336	91.7
9.500	1.091	0.007413	92.6
10.000	1.151	0.007836	97.9

Table 12: Absorbance, concentration and Cumulative drug release of Levodopa in each sample withdrawn in specified time of the dissolution profile of SINEMET® CR tablet, using UV-Visible Spectrophotometry at 280 nm and 0.1 M HCL in the reference cell.

	Levodopa		
Time Intervals (hr)	Absorbance	Concentration % w/v	Cumulative drug release
0.5	0.151	0.000789	14.8
1.0	0.380	0.002403	45.1
1.5	0.506	0.003291	61.7
2.0	0.681	0.004524	84.9
2.5	0.766	0.005123	96.1
3.0	0.724	0.004827	90.6
0.5	0.151	0.000789	14.8
1.0	0.380	0.002403	45.1

Table 13: Absorbance, concentration and Cumulative drug release of Levodopa in each sample withdrawn in specified time of the dissolution profile of DOPAL-M tablet, using UV-Visible Spectrophotometry at 280 nm and 0.1 M HCL in the reference cell.

	Levodopa		
Time Intervals (min)	Absorbance	Concentration % w/v	Cumulative drug release
5	0.271	0.001635	61.3
15	0.376	0.002375	89.1
30	0.375	0.002368	88.8
45	0.356	0.002234	83.8
60	0.410	0.002615	98.0
75	0.387	0.002452	92.0

High Performance Liquid Chromatography (HPLC)

Table 14: Concentration and peak area of serial diluted standard solutions of Levodopa and Carbidopa using HPLC. 750 ml of 0.1M hydrochloric acid was used as a dissolution media and rotating the basket at 50 revolutions per minute.

Serial Diluted solutions	Concentration (% w/v)	Peak Area	Concentration (% w/v)	Peak Area
1	0.0001	10914	0.00001	1146
2	0.0002	16818	0.00002	2391
3	0.0003	25712	0.00003	3137
4	0.0004	39616	0.00004	4582
5	0.0005	52821	0.00005	5828
6	0.0006	60525	0.00006	6573
7	0.0007	72329	0.00007	8219
8	0.0008	79233	0.00008	9364
9	0.0009	99037	0.00009	10010
10	0.0010	110041	0.0001	13455
11	0.0020	198082	0.0002	21911
12	0.0030	297123	0.0003	34366
13	0.0040	396164	0.0004	45822
14	0.0050	500205	0.0005	59277
15	0.0060	604246	0.0006	68732
16	0.0070	699287	0.0007	83188
17	0.0080	772328	0.0008	91643
18	0.0090	891369	0.0009	107099
19	0.0100	1000410	0.001	114554

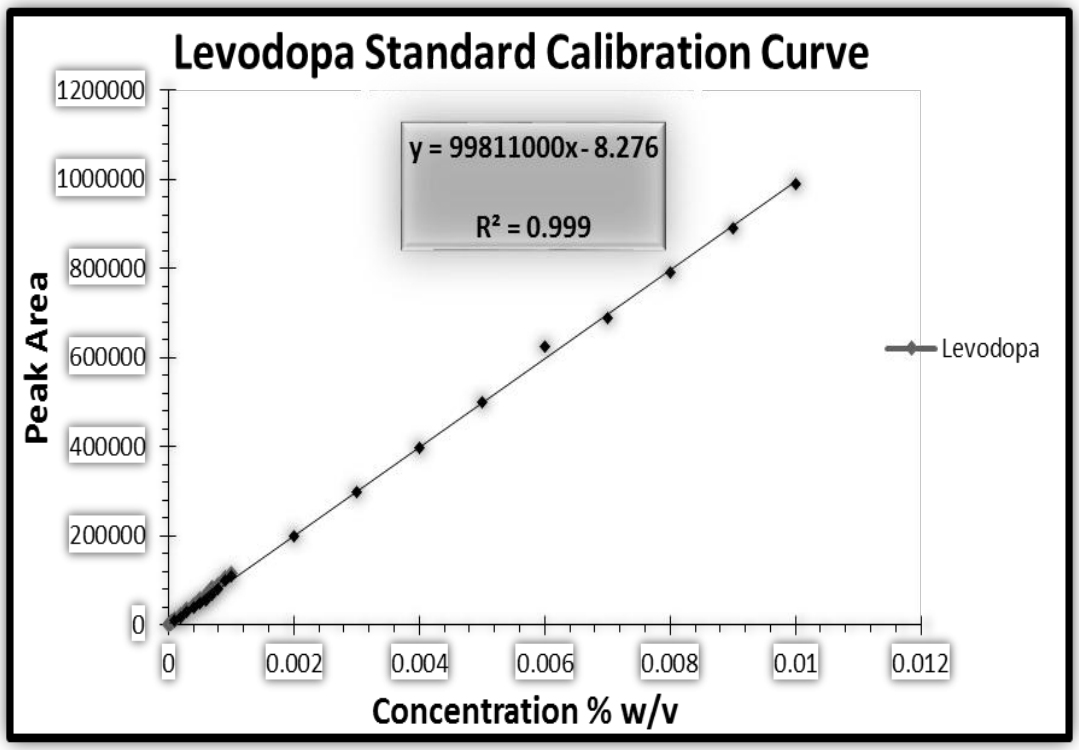

Figure 3: Standard calibration Curve of Levodopa using HPLC. The correlation coefficient is 0.999 (y = 99811000x - 8.276)

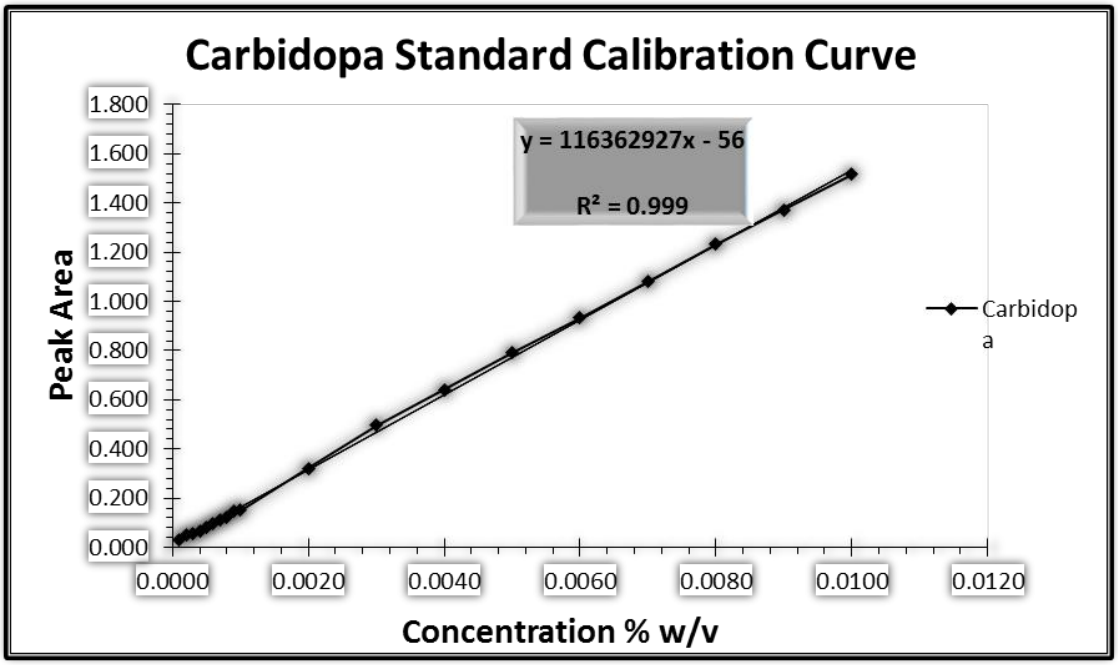

Figure 4: Standard calibration Curve of Carbidopa using HPLC. The correlation coefficient is 0.999 (y = 116362927x – 56)

Table 15: Peak Area, concentration and Cumulative drug release of Levodopa in samples withdrawn in specified time of the dissolution profile of the multilayer tablet with the formula code (A5)

	Levodopa		
Time Intervals (hr)	Peak Area	Concentration % w/v	Cumulative drug release (%)
0.083	044707	0.000448	5.60
0.250	117769	0.001180	14.8
0.500	205602	0.002060	25.8
0.750	256207	0.002567	32.1
1.000	324877	0.003255	40.7
2.000	368494	0.003692	46.2
3.000	364402	0.003651	45.6
4.000	469303	0.004702	58.8
5.000	576001	0.005771	72.1
6.000	596462	0.005976	74.7
7.000	639980	0.006412	80.2
8.000	731007	0.007324	91.6
9.000	733403	0.007348	91.9
10.00	785105	0.007866	98.3

Table 16: Peak Area, concentration and Cumulative drug release of Carbidopa in samples withdrawn in specified time of the dissolution profile of the multilayer tablet with the formula code (A5)

	Carbidopa		
Time Intervals (hr)	Peak Area	Concentration % w/v	Cumulative drug release (%)
0.083	9951	0.000086	04.3
0.250	35202	0.000303	15.2
0.500	54053	0.000465	23.3
0.750	74620	0.000642	32.1
1.000	95333	0.000820	41.0
2.000	107347	0.000923	46.2
3.000	104089	0.000895	44.8
4.000	139056	0.001196	59.8
5.000	169339	0.001456	72.8
6.000	173325	0.001490	74.5
7.000	188801	0.001623	81.1
8.000	214052	0.001840	92.0
9.000	228481	0.001964	98.2
10.00	225223	0.001936	96.8

Release Profile of Levodopa from Bilayer Tablets

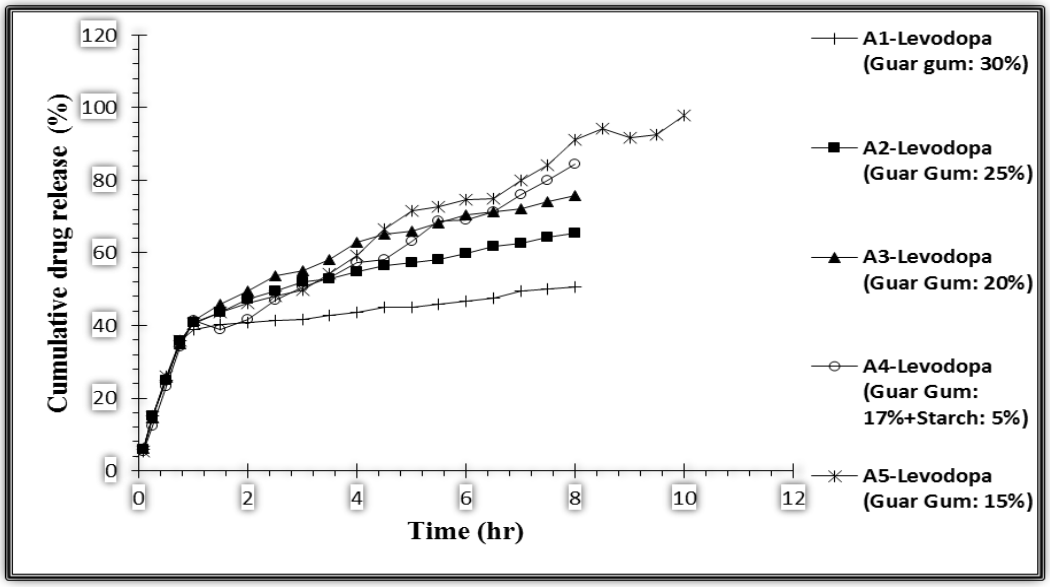

Figure 5: Effect of polymer concentration on release profile of Levodopa from the bilayer matrix tablets (A1, A2, A3, A4 and A5)

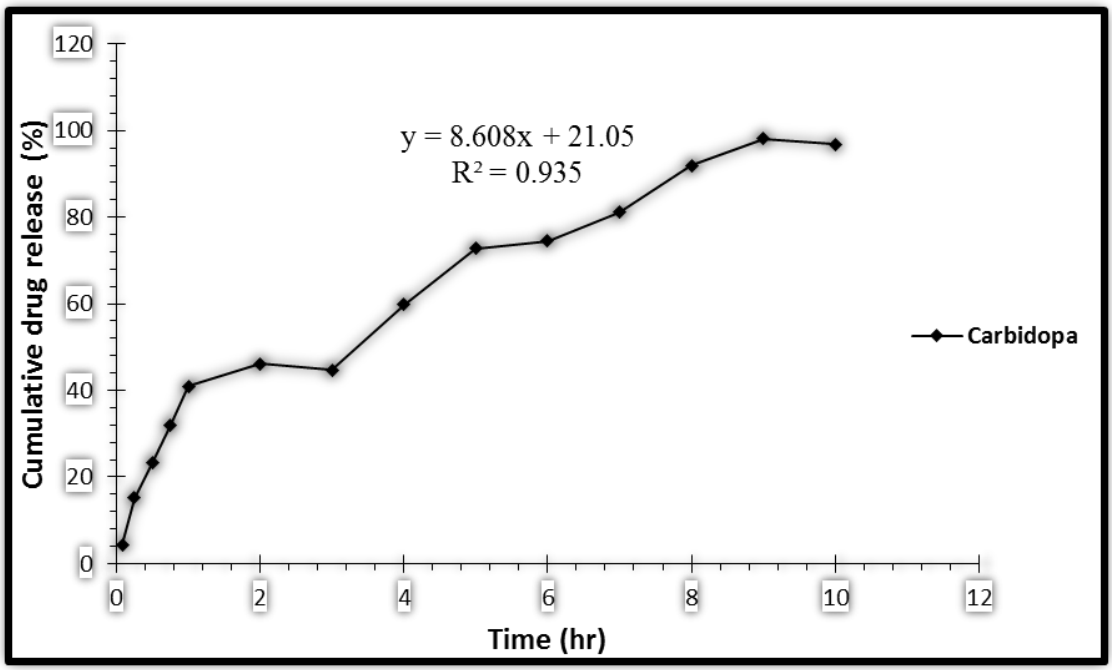

Figure 6: Release profile of Carbidopa from the optimized Bilayer tablet (A5). The correlation coefficient is 0.935 (y = 8.608x + 21.05)

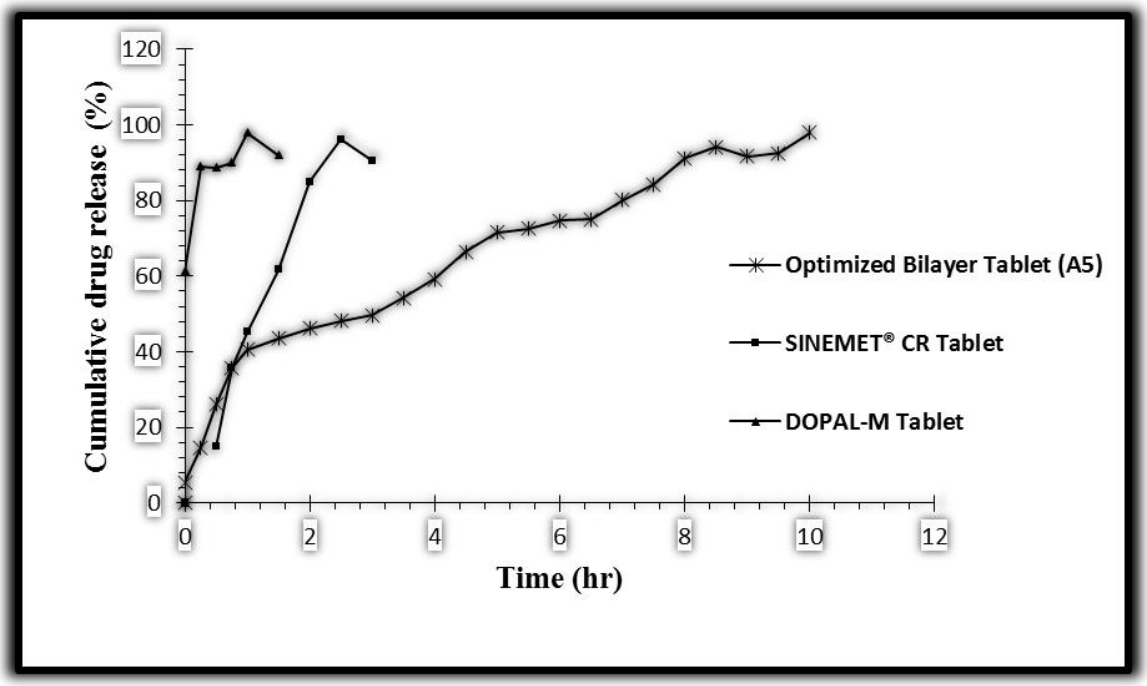

Figure 7: Comparison of Levodopa Release profiles among DOPAL-M Tablet, SINEMET® CR Tablet and Optimized Bilayer Tablet (A5)

CONCLUSION & RECOMMENDATION

Tablet Characteristics

An IR layer of less than 200 mg caused the release of less than 85% of levodopa and carbidopa in over 45 minutes due to sticking of the IR layer with the polymer of the CR layer. Therefore, the optimum weight of the IR layer was fixed to 289.4 mg for better release of levodopa and carbidopa as well as to maintain the optimum swallowable oral dosage form.

In a weight variation test, the pharmacopoeial limit (British Pharmacopea, 2007 and United States Pharmacopeia, 2000) for the percentage deviation for tablets of more than 324 mg is ± 5%. The average percentage deviation of all tablet formulations was found to be within the above limit, and hence all formulations passed the uniformity of weight as per official requirements of the United States Pharmacopeia (2000). Good uniformity in drug content was found among different tablets and the percentage of drug content was more than 95%. Tablet hardness is not an absolute indicator of strength (Banker & Ander, 1987). Another measure of a tablet's strength is friability. Conventional compressed tablets that lose less than 1% of their weight are generally considered acceptable. In the present study, the percentage of friability for all the formulations was below 1%, indicating that the friability was within the prescribed limits (Banker & Ander, 1987).

Drug Release Study

Because levodopa is sparingly soluble in 0.1 M HCL and carbidopa is freely soluble in 3N hydrochloric acid (Ph. Eur. 6.2 and USP 31), 0.1 M HCL was used a dissolution media and as a blank solution in the UV-Visible spectrophotometry.

Comparing the absorbance and correlation coefficients obtained from the standard calibration curve of levodopa in the absence and presence of carbidopa as shown in figure (1) and (2), respectively, indicates no significant difference. Therefore, this validated the UV-Visible spectrophotometry method for use in measuring the absorbance of levodopa during the dissolution time of different tablets containing both levodopa and carbidopa without interference.

However, when the optimized bilayer tablet was determined, both levodopa and carbidopa concentrations were determined using the HPLC method during the dissolution time and therefore HPLC served as a confirmatory method of the UV-Visible spectrophotometry data obtained of the levodopa.

The amount of guar gum polymer in the formulation was found to affect the levodopa and carbidopa release rate significantly. From Figure (5) it is evident that as the polymer content (guar gum) in the formulation increases, the percent of levodopa release decreases, resulting in greater

controlled release. This observation is in good agreement with the literature (Krishnaiah et al, 2002), (Krishnaiah et al 2002) and (Al-Saidan et al 2005)). A complete release of levodopa and carbidopa occurred from the bilayer tablet (A5) within 10 hours, therefore, the tablet was considered as the optimized bilayer tablet containing the optimized amount of guar gum.

CONCLUSION

Modified bilayer matrix tablets containing 200 mg levodopa and 50 mg carbidopa as sustained release from one layer and 100 mg levodopa and 25 mg carbidopa as immediate release from another layer have been successfully prepared by wet granulation method. Fifteen percent (15%) guar gum used as matrix-forming polymer for the sustained release layer enables desired drug release for up to 10 hours through combination mechanisms of diffusion and erosion. This bilayer tablet will be further tested in vivo in an animal model for its pharmacokinetic and pharmacodynamic characteristics as well as to establish optimum drug: polymer conditions leading to desired bioavailability.

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