

# Improved bioavailability of levodopa using floatable spray-coated microcapsules for the management of Parkinson's disease

Baek, Jong-Suep; Tee, Jie Kai; Pang, Yi Yun; Tan, Ern Yu; Lim, Kah Leong; Ho, Han Kiat; Loo, Joachim Say Chye

2018

Baek, J.-S., Tee, J. K., Pang, Y. Y., Tan, E. Y., Lim, K. L., Ho, H. K., & Loo, J. S. C. (2018). Improved bioavailability of levodopa using floatable spray-coated microcapsules for the management of Parkinson's disease. *NeuroMolecular Medicine*, 20(2), 262-270.  
doi:10.1007/s12017-018-8491-0

<https://hdl.handle.net/10356/92269>

<https://doi.org/10.1007/s12017-018-8491-0>

---

© 2018 Springer Science+Business Media US. All rights reserved. This is a post-peer-review, pre-copyedit version of an article published in *NeuroMolecular Medicine*. The final authenticated version is available online at: <http://dx.doi.org/10.1007/s12017-018-8491-0>

*Downloaded on 07 Aug 2024 18:17:24 SGT*

[Click here to view linked References](#)

## **Improved Bioavailability of Levodopa using Floatable Spray Coated-Microcapsules for the Management of Parkinson's Disease**

Jong-Suep BAEK<sup>a</sup>, Jie Kai TEE<sup>b</sup>, Yi Yun PANG<sup>b</sup>, Ern Yu TAN<sup>c</sup>, Kah Leong LIM<sup>d</sup>, Han Kiat HO<sup>b</sup>, Say Chye Joachim LOO<sup>a,e\*</sup>

*<sup>a</sup>School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue, 639798, Singapore*

*<sup>b</sup>Department of Pharmacy, Faculty of Science, National University of Singapore, 18 Science Drive 4, Singapore 117543, Singapore*

*<sup>c</sup>General Surgery Clinic, Tan Tock Seng Hospital, 11 Jalan Tan Tock Seng, Singapore 308433, Singapore*

*<sup>d</sup>National Neuroscience Institute (NNI), 11 Jalan Tan Tock Seng, Singapore 308433, Singapore*

*<sup>e</sup>Singapore Centre on Environmental Life Sciences Engineering (SCELSE), Nanyang Technological University, 637551, Singapore*

**Keywords:** Levodopa-induced dyskinesia; Controlled release; Floating drug delivery system; Pharmacokinetics; Brain concentration

\*Corresponding author:  
Say Chye Joachim Loo  
Phone: +65 6790-4603  
Fax: +65 6790-9081  
Email: joachimloo@ntu.edu.sg

## **Abstract**

Oral administration of levodopa (LD) is the gold standard in managing Parkinson's disease (PD). Though LD is the most effective drug in treating PD, chronic administration of LD induces levodopa-induced dyskinesia. A continuous and sustained provision of LD to the brain could, therefore, reduce peak-dose dyskinesia. In commercial oral formulations, LD is co-administrated with an AADC inhibitor (carbidopa) and a COMT inhibitor (entacapone) to enhance its bioavailability. Nevertheless, patients are known to take up to five tablets a day because of poor sustained releasing capabilities that lead to fluctuations in plasma concentrations. To achieve a prolonged release of LD with the aim of improving its bioavailability, floatable spray-coated microcapsules containing all three PD drugs were developed. This gastro-retentive delivery system showed sustained release of all PD drugs, at similar release kinetics. Pharmacokinetics study was conducted and this newly-developed formulation showed a more plateaued delivery of LD that is void of the plasma concentration fluctuations observed for the control (commercial formulation). At the same time, measurements of LD and dopamine of mice administered with this formulation showed enhanced bioavailability of LD. This study highlights a floatable, sustained-releasing delivery system in achieving improved pharmacokinetics data compared to a commercial formulation.

## **Introduction**

Parkinson's disease (PD) is the gradual degeneration of the nigrostriatal pathway that leads to diminished concentrations of the neurotransmitter dopamine (Dauer et al., 2003; Duvoisin, 1987; Tanner, 1992; Gibb, 1992; Blandini and Greenamvre, 1999). It is clinically characterized by severe motor impairments, such as hypokinesia, rigidity and resting tremors. Although many drug candidates have been recently formulated for Parkinson's disease treatment (Wollmer E and Klein S, 2017; Trapani A et al., 2017; Lin Q et al., 2017), oral administration of levodopa (LD), the metabolic precursor of dopamine, remains the simplest, yet most effective pharmacological approach in managing PD. LD is predominantly absorbed in the upper small intestinal mucosa and is converted to dopamine by the aromatic L-amino acid decarboxylase (AADC) enzyme, where it is metabolized to 3-O-methyldopa (3-OMD) by the catechol-O-methyltransferase (COMT) after crossing the blood-brain barrier. Unfortunately, LD also undergoes extensive decarboxylation in the peripheral system, with only a minute amount (~1 %) eventually reaching the brain (Mathers et al., 1988). For this reason, AADC and COMT inhibitors, such as carbidopa (CD) or entacapone (ENT) respectively, or a combination of these, are co-administered to increase the bioavailability of LD (Ciesielska et al., 2015; Boiki et al., 2008). Studies have shown that the co-administration of CD and ENT reduces dosing frequency, and aids in maintaining appropriate plasma levels of LD within the therapeutic window (Brooks, 2008; Hauser et al., 2013).

Though LD is the most effective drug for treating PD, chronic administration of LD causes levodopa-induced dyskinesia (LID). LID occurs at peak LD plasma concentrations during intermittent or pulsatile LD stimulation (Schapira et al., 2009; Ren et al., 2011). A continuous and sustained provision of LD to the brain could, therefore, reduce peak-dose dyskinesia or delay the emergence of LID (Hsu et al., 2015). As such, different pharmacological strategies,

including intestinal infusion, have been explored to provide a continuous delivery of LD in the bid to reduce dyskinesia (Schaeffer et al., 2014). Other advanced oral formulations have also been developed to provide controlled-release functionalities (i.e. immediate-release, extended-release, etc.), with some formulations shown to be superior to others (Hsu et al., 2015). Sinemet<sup>®</sup> CR, a controlled-release formulation, is known to be absorbed over 4–6 hours, but is associated with an erratic absorption and variable LD plasma concentrations (Pahwa et al., 1996). Promising pharmacokinetics results were shown from the recently approved extended-release (ER) carbidopa–levodopa formulation (IPX066 – Rytary<sup>®</sup> in the USA) (Waters et al., 2015; Greig and McKeage, 2016). With this new ER formulation, LD reaches an initial peak at 1 hour and achieved a maximum concentration at a mean time of ~4.5 hours. LD concentrations subsequently decrease, with 10 % of peak LD concentration at 10 hours. As such, regular dosing is still required to maintain adequate plasma concentration of LD to mitigate LID. Unfortunately, non-sustained-releasing formulations will lead to fluctuations of plasma LD concentrations resulting in “wearing-off” symptoms.

To further reduce or mitigate dyskinesia, a prolonged (>10 hours) and continuous provision of LD to the brain would thus provide greater benefits. Herein, we hypothesize that a “once-a-day” prolonged, sustained-releasing formulation could potentially overcome the “wearing-off”, “on-off” phenomena and dyskinetic movements associated with pharmacological fluctuations. With this, we developed a floatable, spray-coated microcapsule for the delivery of three different PD drugs, using United States (US) Food and Drug Administration (FDA)-approved polymers (Lee et al., 2013; Baek et al., 2016). This microencapsulation technology is a simple, economical, scalable method that allows for the controlled, sustained release of multiple drugs from this formulation, while avoiding possible drug-drug interactions. Since the main site for absorption of all PD drugs is in the upper gastrointestinal tract, designing a floatable system was a key consideration to increase the absorption of LD. For instance, El Nabarawi MA et al., reported that their floating system

showed significantly enhanced bioavailability of Mebeverine HCl in beagle dogs compared to commercial tablets (Duspatalin®) (Nabarawi et al., 2017). Similarly, Chai X et al., reported that floating tablets containing domperidone exhibited a significant improvement in its oral bioavailability (222 %) (Chai et al., 2017). From *in vitro* studies, this microcapsule formulation is shown to exhibit excellent buoyancy in fed-state simulated gastric fluid (FeSSGF) with a sustained release of the PD drugs up to 24 hours.

In this present work, the objective was to investigate the pharmacokinetics of this advanced sustained-releasing formulation against a commercial formulation, and to determine the oral bioavailability of LD and its subsequent conversion to dopamine in the brain of mice.

## **Materials and methods**

### **Materials**

Poly-L-lactide (PLLA) (IV: 2.4, Purac), Polycaprolactone (PCL) (molecular weight 10 kDa, Sigma-Aldrich), and Polyvinyl alcohol (PVA) (molecular weight 30 – 70 kDa, Sigma-Aldrich) were used without further purification. LD, CD, ENT, Tween 20, HCl solution (37% v/v Fuming), acetic acid and ethyl acetate (ETA) were purchased from Sigma-Aldrich (Steinheim, Switzerland). Olive oil (Pietro Coricelli) was used. All other chemicals and reagents used were of analytical grade.

### **Preparation of spray-coated hollow microcapsules and characterization**

The microcapsules were prepared by the double emulsion technique, as previously reported (Baek et al., 2016). To evaluate the buoyancy of the microcapsules, the samples were dispersed into FeSSGF (10 mL) with stirring at 200 rpm, at 37 °C. At predetermined time points, the number of floating microcapsules were counted.

### **In vitro release study**

Release study was carried out in FeSSGF and FeSSIF for 24 h. The composition of FeSSGF (pH 5.0) was sodium chloride (240 mM), Acetic acid (17 mM) and sodium acetate (30 mM) in a mixture of milk and acetate buffer (1:1). FeSSIF (pH 6.5) consists of sodium taurocholate (10 mM), lecithin (2 mM), glyceryl monooleate (5 mM), sodium oleate (0.8 mM), maleic acid (55.02 mM), sodium chloride (125.5 mM) in distilled water. The microcapsules (20 mg) were dispersed in 20 mL of FeSSGF in a rotating incubator set at 37 °C. At the predetermined time points, the release medium (10 mL) was replenished with fresh medium (10 mL).

#### In vivo study

##### Animal

C57BL6 female mice, 12 to 14 weeks of age (18-25 g), were obtained from InVivos (Singapore). Animals were housed in plastic cages (5 animals/cage) under standard laboratory conditions with 12 hr light-dark cycle. Food and water were available ad libitum. The animals were handled in accordance with approved NUS Institutional Animal Care and Use Committee (IACUC) protocol R15-0486.

##### Pharmacokinetics

The experimental mice were divided into three groups (control, MC x1 and MC x 3), each comprising of three animals. Drug pellets were prepared fresh each experimental day in 0.6% methyl cellulose diluted with saline solution. Mice were subsequently administered with 200ul (control) or 300 µl (pellet) single dose of drug solution at LD:CD:ENT = 10:2.5:20 mg.kg<sup>-1</sup> (Group 1, conventional formulation), LD:CD:ENT = 10:2.5:20 mg.kg<sup>-1</sup> (Group 2, MC x1) and LD:CD:ENT = 30:7.5:60 mg.kg<sup>-1</sup> (Group 3, MC x3) via oral gavage using a feeding syringe. At stipulated time points of 0.25, 0.5, 1, 2, 4, 8, 12, 24 hr, the mice were euthanized and blood was collected via cardiac puncture with ethylenediaminetetraacetic acid

(EDTA) as the anticoagulant. The blood samples were then centrifuged (4,500 rpm) for 10 min at 25°C to obtain the plasma. In addition, the brain was harvested and flash freeze in liquid nitrogen. Samples were stored in -80 °C before further analysis via LC/MS. Analysis of drugs in plasma and brain was conducted using LC/MS (Ribeiro et al., 2015). An Agilent 1290 HPLC system with an Agilent 6120 Quadrupole Mass Spectrometer was used to measure plasma and brain concentrations of drugs. The mobile phase consisted of a gradient of (A) 0.1 % (v/v) formic acid (FA) and a mixture of ACN:MeOH (90:10, v/v) containing 0.1 % (v/v) FA. The gradient elution is tabulated in **Table 1**. XBridge C8 column (150 × 4.6 mm; particle size 5 µm) was used at 30°C. The injection volume of samples was 20 µl. Plasma and brain extracted solution were mixed with internal standard and extracted by solid-phase extraction. The calibration curves were linear over the range of 2 to 2000 ng/mL for LD, 2 to 400 ng/mL for CD and 5 to 3000 ng/mL for ENT.

**Table 1.** The gradient profile of (A) 0.1 % (v/v) formic acid and (B) ACN:MeOH (9:1, v/v).

Time (min)	A (%)	B (%)	Flow rate (mL/min)
0	100	0	1
2	98	2	1
2.1	10	90	1
3.5	10	90	1
3.6	98	2	1
8.0	98	2	1

## Results and Discussion



This sustain-releasing formulation composing of floatable, spray-coated microcapsules containing three PD drugs (i.e. LD, CD and ENT) were prepared using an established double-emulsion solvent evaporation method (Baek et al., 2016). The same amount of LD, CD and ENT were loaded into these microcapsules at a ratio of 4:1:8 (LD:CD:ENT), similar to that of a commercial formulation (e.g. Stalevo<sup>®</sup>-100). Through a rapid evaporation of organic solvent using a rotary-evaporator, drug-loaded hollow microcapsules were obtained. To achieve similar release rates for all three PD drugs, the more hydrophobic ENT was spray-coated together with PCL onto the hollow microcapsules, while the hydrophilic LD and CD were encapsulated within the microcapsule. **Fig. 1** shows the scanning electron microscopy (SEM) images of the spray-coated spherical microcapsules with a mean diameter of ~600  $\mu\text{m}$ , with its cross-section revealing a hollow structure.

Before pharmacokinetics studies, *in vitro* evaluation was conducted on these microcapsules. In order to mimic *in vivo* conditions, the buoyancy of the microcapsules was determined in FeSSGF for 24 h at 37 °C (**Fig. 2**), with 92 % of the microcapsules remaining afloat up to 24 hr. Its floatability is attributed to the olive oil-filled hollow cavity that reduces its overall density. In addition, the use of hydrophobic polymers inhibits water absorption, thus providing good buoyancy to these microcapsules. *In vitro* drug release study was next investigated in FeSSGF and fed-state simulated intestinal fluid (FeSSIF), at 37 °C, under two conditions: 1. Drug release study in FeSSGF for 24 hr; 2. Drug release study in FeSSGF for 5 h, followed by FeSSIF for 24 h (**Fig. 3**); whereby the latter is to simulate the physiological transition of the microcapsules through the gastrointestinal tract. Commercial PD formulation was used as control (control). Control in FeSSGF showed a complete release of all three drugs within 4 hr, with a slightly retarded release of lipophilic ENT ( $\log P = 2.0$  (Erkki, 2010)) compared to hydrophilic LD ( $\log P = -2.39$  (Sangster, 1993)) and CD ( $\log P = -2.8$  (Sangster, 1993)). The newly developed advanced sustained-release microcapsules formulation, on the other hand, exhibited prolonged release and with all three drugs. Similar rate constants (K)

were calculated from Higuchi's equation (i.e. LD  $\sim 16.5 \text{ h}^{-1/2}$ , CD  $\sim 16.1 \text{ h}^{-1/2}$ , ENT  $16.4 \text{ h}^{-1/2}$ ) with good linear-regression coefficients ( $r^2$ ) (i.e. LD: 0.9214, CD: 0.9345, ENT: 0.8942). The drug delivery design was optimized to achieve similar release kinetics for all drugs, to maximize on drug synergy.

The optimized formulation was next evaluated for its pharmacokinetics in mice, and the profiles of the three PD drugs are shown in **Fig. 4**, after a single oral administration. The corresponding estimated pharmacokinetic parameters (calculated with WinNonlin<sup>TM</sup> 6.4) of the drugs are tabulated in **Table 2**.

**Table 2.** Pharmacokinetics parameters of LD, CD and ENT from drug solution (LD:CD:ENT = 10:2.5:20 mg.kg<sup>-1</sup>), MC x 1 (LD:CD:ENT = 10:2.5:20 mg.kg<sup>-1</sup>), MC x 3 (LD:CD:ENT = 30:7.5:60 mg.kg<sup>-1</sup>) after oral administration to mice. Results are expressed as the mean  $\pm$  SD (n=3).

	Levodopa			Carbidopa			Entacapone		
	Control	MC x 1	MC x 3	Control	MC x 1	MC x 3	Control	MC x 1	MC x 3
<b>C<sub>max</sub> (ng/ml)</b>	2,979.96	2,174.44	5,038.20	315.86	253.79	629.11	3289.26	2819.52	7869.79
<b>t<sub>max</sub> (h)</b>	0.5	4	4	2	8	4	0.25	2	2
<b>t<sub>1/2</sub> (h)</b>	1.98	3.44	5.40	2.12	4.13	4.58	0.54	4.10	4.48
<b>AUC<sub>0-∞</sub> (h·g/ml)</b>	7,984.88	2,7201.75	7,0492.05	1,371.52	3,355.92	8,899.61	2,473.32	29,220.16	76,829.53
<b>CL (ml/h/kg)</b>	1,252.37	367.62	425.58	1,822.80	744.95	842.73	8086.28	684.46	780.95
<b>MRT (h)</b>	2.57	10.15	10.15	3.62	8.71	9.44	0.72	7.55	7.61

**C<sub>max</sub>**: maximum concentration

**AUC<sub>0-∞</sub>**: area under the curve from zero to infinity

**t<sub>max</sub>**: time to reach C<sub>max</sub>

**t<sub>1/2</sub>**: elimination half-time

**CL**: extracellular fluid clearance

**MRT:** mean residential time

From Table 2, the pharmacokinetic data of all three PD drugs from the microcapsules exhibited a prolonged delivery that was well-correlated to our preliminary *in vitro* release. On the other hand, PD drugs (control) exhibited rapid absorption achieving fast peak plasma concentrations (i.e. LD ~ 0.5 h, CD ~2 h, ENT ~ 0.25 h). The LD plasma concentration, however, decreased rapidly, reaching 10 % of peak at 8 h. CD and ENT similarly exhibited rapid elimination, reaching its 10 % of peak at 8 and 12 h, respectively. For the microcapsules, they exhibited a slower maximum absorption peak but with longer absorption duration compared to control. The  $t_{max}$  of LD, CD and ENT released from the microcapsules was 4, 8, and 2 hr respectively, which were all longer than the control. The  $t_{1/2}$  and MRT of LD of the microcapsules formulation were 3.44 and 10.15 h, respectively, which were again longer than the control. The  $t_{1/2}$  and MRT can be used in a comparative way to explain the pharmacokinetics performance of this advanced sustained-releasing formulation. The prolonged  $t_{1/2}$  and MRT of the microcapsules suggests that the drug can be retained longer in the systemic circulation (Robinson and Lee, 1987).

In addition, it was observed that the microcapsules exhibited higher area under the curve ( $AUC_{(0-\infty)}$ ) (27,201.75 (h·g/ml)) of LD than the control group (7,984.88 (h·g/ml)). Besides, the microcapsules also exhibited significantly higher  $AUC_{(0-\infty)}$  of CD (3,355.92 (h·g/ml)) and ENT (29,220.16 (h·g/ml)) compared to the control group (CD; 1,371.52 (h·g/ml), ENT; 2,473.32 (h·g/ml)). AUC is an indicator of drug bioavailability (Rescigno, 2000). The microcapsules showed almost 3-fold, 2.5-fold and 10-fold increments in AUC of LD, CD and ENT as compared to the control. The enhanced oral bioavailability of LD can be attributed to the several reasons. First of all, the buoyancy of the microparticles lengthens the gastric retention time of these particles. This reduces the possibility of the microparticles from transiting too rapidly into the intestine. With this, the stomach acts as a reservoir for the drugs

to be released in the upper gastrointestinal tract where absorption occurs. While conventional tablets generally stay in the stomach for only 2 hr (Hong and Park, 2011), this new formulation allows for the drugs to be retained for a longer period of time because its intrinsic buoyancy acts against gastric emptying within a filled stomach, thus providing some prolonged effects in terms of drug bioavailability. The second explanation for its improved bioavailability lies in the controlled-release capabilities of this encapsulation process. By understanding drug release profiles and kinetics from *in vitro* studies, drug microcapsules can be designed to elicit a desirable release profile that is optimal for the treatment regimen. The use of a spray-coated layer allows for the lipophilic drug (i.e. ENT) to be released in a faster manner, while the other hydrophilic drugs are encapsulated to provide a slower diffusion release profile. The design of this delivery system is therefore optimized to release all three drugs at similar rates to mitigate any premature decarboxylation of LD in the peripheral system. Encapsulation also protects the highly sensitive LD until it is required for absorption. The third reason lies in the ability in co-encapsulating both the AADC and COMT inhibitor drugs, and achieving similarly release profiles and rates of the drugs. As highlighted earlier, these drugs act in synergy to increase the bioavailability of LD in the brain. These reasons represent the hallmark of encapsulation in providing controlled release while protecting chemically-labile substances against the harsh *in vivo* environment.

In order to confirm the successful delivery of LD to the brain and its subsequent conversion to dopamine (DA), the brain tissue of mice was harvested and determined by LC-MS. The mean ( $\pm$ SD) LD and DA concentrations in the brain are shown in **Fig. 5**.

While the LD concentration in the brain for the mice with the control formulation showed a spike at the first hour, the LD concentration profile was more plateaued for the advanced controlled-release microcapsules formulation (MC x 1), with a similar profile observed for MC x 3. In other words, a continuous, prolonged provision of LD was observed for the advanced formulation. LD-induced dyskinesia (LID) are abnormal movements due to chronic

LD therapy that occurs when LD concentration in the brain is the highest (Cart et al., 2006). Conventional formulations have huge burst release of LD that is often associated with high concentrations of LD in plasma and brain (Fahn et al., 2004; Kishore and Popa, 2014). As such, the therapeutic duration becomes progressively shorter until it reaches the half-life of the drug – the “wearing-off” effect (Pahwa and Lyons, 2009; Müller and Russ, 2006). To avoid these, it is critical to maintain a consistent LD concentration in the brain for prolonged periods. This was shown to be achievable through this advanced sustained-releasing delivery system. These microcapsules could therefore potentially enhance therapeutic benefits, while minimizing motor complications. For DA concentrations in the brain, the microcapsules (MC x 1) exhibited a gradual increase in DA concentration, and was maintained above the initial brain DA concentration throughout the study period (24 hr). The control, on the other hand, achieved a DA peak concentration quickly (<1 hr), but eventually reverting to its baseline concentration within 4 hr. Mounting reports in the literature state that high dosage and the non-continuous delivery of LD to the brain are the major reasons of LID (Nutt and Fellman, 1984; Fahn, 2005; Chapuis et al., 2005; Rascol et al., 2000). Here, the co-administration of LD/CD/ENT through an advanced sustained-releasing formulation provided for a more consistent plasma LD levels and improved bioavailability of LD.

## Conclusions

In summary, the present study describes for the first time a single oral administration of three PD drugs delivered through floatable spray-coated microcapsules for the management of PD. The results showed enhanced bioavailability of LD through a gastro-retaining delivery system that simultaneously provides a controlled, sustained release of three synergistic PD drugs. When compared to a commercial formulation, a more consistent LD concentration in the brain of healthy mice was observed. Similarly, a higher than baseline brain DA

concentration was observed for mice administered with this advanced formulation, for up to 24 hr. This sustained LD concentration with reduced fluctuation achieved may allow for a consolidation of “on” periods with a reduction of the incidence of dyskinesia. Future work will involve pharmacokinetics and pharmacodynamics study in larger animals, i.e. marmosets, as a bridge to clinical trials.

### Acknowledgements

The authors would like to acknowledge the financial support from the Singapore Centre for Environmental Life Sciences Engineering (SCELSE) (M4330001.C70), the School of Materials Science and Engineering (M020070110), the NTU-National Healthcare Group (NTU-NHG) grant (ARG/14012), the Ministry of Education Tier 1 grant (RG11/16), and the SPARK programme.

### References

- Baek, J.S., Choo, C.C., Qian, C., Tan, N. S., Shen, Z., & Loo, S. C. (2016). Multi-Drug-Loaded Microcapsules with Controlled Release for Management of Parkinson's Disease. *Small*, 12, 3712-3722.
- Blandini, F., & Greenamyre, J.T. (1999). Protective and symptomatic strategies for therapy of Parkinson's disease. *Drugs of Today*. 35, 473-483.
- Boiko, A.N., Batysheva, T.T., Minaeva, N.G., Badina, L.A., Vdovichenko, T.V., Zhuravelva, E.Y., et al., (2008). Use of the new levodopa agent Stalevo (levodopa/carbidopa/entacapone) in the treatment of Parkinson's disease in out-patient clinical practice (the START-M open trial). *Neuroscience and Behavioral Physiology*, 38, 933-936.
- Brooks, D.J. (2008). Optimizing levodopa therapy for Parkinson's disease with

- levodopa/carbidopa/entacapone: implications from a clinical and patient perspective. *Neuropsychiatric Disease and Treatment*, 4, 39-47.
- Carta, M., Lindgren, H.S., Lundblad, M., Stancampiano, R., Fadda, F., & Cenci, M. A. (2006). Role of striatal L-DOPA in the production of dyskinesia in 6-hydroxydopamine lesioned rats. *Journal of Neurochemistry*, 96, 1718-1727.
- Chai, X., Chai, H., Wang, X., Yang, J., Li, J., Zhao, Y., et al., (2017). Fused Deposition Modeling (FDM) 3D Printed Tablets for Intra-gastric Floating Delivery of Domperidone. *Scientific Reports*, 7, 2829.
- Chapuis, S., Ouchchane, L., Metz, O., Gerbaud, L., & Durif, F. (2005). Impact of the motor complications of Parkinson's disease on the quality of life. *Movement Disorders*, 20, 224-230.
- Ciesielska, A., Sharma, N., Beyer, J., Forsayeth, J., & Bankiewicz, K. (2015). Carbidopa-based modulation of the functional effect of the AAV2-hAADC gene therapy in 6-OHDA lesioned rats. *PLoS One*, 10, e0122708.
- Dauer, W., & Przedborski, S. (2003). Parkinson's disease: mechanisms and models. *Neuron*, 39, 889-909.
- Duvoisin, R. (1987). History of parkinsonism. *Pharmacology & Therapeutics*, 32, 1-17.
- El Nabarawi, M.A., Teaima, M.H., Abd El-Monem., El Nabarawy, N.A., Gaber, D.A. (2017). Formulation, release characteristics, and bioavailability study of gastroretentive floating matrix tablet and floating raft system of Mebeverine HCl. *Drug Design, Development and Therapy*, 11, 1081-1093.
- Erkki, N. (2010). Basic Aspects of Catechol-O-methyltransferase and the Clinical Applications of Its Inhibitors. Elsevier.
- Fahn, S., Oakes, D., Shoulson, I., Kieburtz, K., Rudolph, A., Lang, A., et al., (2004). Parkinson Study Group, Levodopa and the progression of Parkinson's disease. *The New England Journal of Medicine*, 351, 2498-2508.
- Fahn S. (2005). Parkinson Study Group, Does levodopa slow or hasten the rate of progression

- of Parkinson's disease?. *Journal of Neurology*, 252, IV37-IV42.
- Gibb WRG. (1992). Neuropathology of Parkinson's disease and related syndromes. *Neurologic Clinics*, 10, 361-376.
- Greig, S.L., & McKeage, K. (2016). Carbidopa/Levodopa ER Capsules (Rytary®), Numient™): A Review in Parkinson's Disease. *CNS Drugs*, 30, 79-90.
- Hauser, R.A., Hsu, A., Kell, S., Espay, A.J., Sethi, K., Stacy, M., et al., (2013) Extended-release carbidopa-levodopa (IPX066) compared with immediate-release carbidopa-levodopa in patients with Parkinson's disease and motor fluctuations: a phase 3 randomised, double-blind trial. *The Lancet Neurology*, 12, 346-356.
- Hong, W., & Park, K.N. (2011). Oral Controlled Release Formulation Design and Drug Delivery: Theory to Practice. John Wiley & Sons.
- Hsu, A., Yao, H.M., Gupta, S., Modi, N.B. (2015). Comparison of the pharmacokinetics of an oral extended-release capsule formulation of carbidopa-levodopa (IPX066) with immediate-release carbidopa-levodopa (Sinemet®), sustained-release carbidopa-levodopa (Sinemet® CR), and carbidopa-levodopa-entacapone (Stalevo®). *The Journal of Clinical Pharmacology*, 55, 995-1003.
- Kishore, A., & Popa, T. (2014). Cerebellum in levodopa-induced dyskinesias: the unusual suspect in the motor network. *Frontiers in Neurology*, 5, 157.
- Lee, W.L., Wee, P., Nugraha, C., Loo, S.C. (2013). Gastric-floating microcapsules provide controlled and sustained release of multiple cardiovascular drugs. *Journal of Materials Chemistry B*, 1, 1090-095.
- Lin, Q., Wong, H.L., Tian, F.R., Huang, Y.D., Xu, J., Yang, J.J., et al., (2017). Enhanced neuroprotection with decellularized brain extracellular matrix containing bFGF after intracerebral transplantation in Parkinson's disease rat model. *International Journal of Pharmaceutics*, 517, 583-394.
- Mathers, S.E., Kempster, P.A., Swash, M., & Lees, A.J. (1988). Constipation and paradoxical



puborectalis contraction in anismus and Parkinson's disease: a dystonic phenomenon?  
*Journal of Neurology, Neurosurgery, and Psychiatry*, 51,1503-1507.

Müller, T., & Russ, H. (2006). Levodopa, motor fluctuations and dyskinesia in Parkinson's disease, *Expert Opinion on Pharmacotherapy*, 7,1715-1730.

Nutt, J.G., & Fellman, J.H. (1984). Pharmacokinetics of levodopa. *Clinical Neuropharmacology*, 7, 35-49.

Pahwa, R., Lyons, K., McGuire, D., Dubinsky, R., Hubble, J. P., & Koller, W. C. (1996). Early morning akinesia in Parkinson's disease: effect of standard carbidopa/levodopa and sustained-release carbidopa/levodopa. *Neurology*, 46, 1059-1062.

Pahwa, R., & Lyons, K.E. (2009). Levodopa-related wearing-off in Parkinson's disease: identification and management. *Current Medical Research and Opinion*, 25, 841-849.

Rascol, O., Brooks, D.J., Korczyn, A.D., De Deyn, P. P., Clarke, C. E., & Lang, A. E. (2000). A five-year study of the incidence of dyskinesia in patients with early Parkinson's disease who were treated with ropinirole or levodopa. *The New England Journal of Medicine*, 342, 1484-1491.

Ren, T., Yang, X., Wu, N., Cai, Y., Liu, Z., & Yuan, W. (2011). Sustained-release formulation of levodopa methyl ester/benserazide for prolonged suppressing dyskinesia expression in 6-OHDA-leisoned rats. *Neuroscience letters*, 502, 117-122.

Rescigno A. (2000). Area under the curve and bioavailability. *Pharmacological Research*, 42, 539-540.

Ribeiro, R.P., Gasparetto, J.C., de Oliveira Vilhena, R., Guimarães de Francisco, T.M., Martins, C.A., Cardoso, M.A., et al., (2015). Simultaneous determination of levodopa, carbidopa, entacapone, tolcapone, 3-O-methyldopa and dopamine in human plasma by an HPLC-MS/MS method. *Bioanalysis*, 7, 207-220.

Robinson, J.R., & Lee, V.H.L. (1987). *Controlled Drug Delivery: Drugs and the Pharmaceutical Sciences*. New York: Marcel Dekker.

Sangster, J. (1993). LOGKOW DATABANK. Sangster Research Laboratories, Montreal, QC, Canada

Schapira, A.H., Emre, M., Jenner, P., & Poewe, W. (2009). Levodopa in the treatment of Parkinson's disease. *European Journal of Neurology*, 16, 982-989.

Schaeffer, E., Pilotto, A., & Berg, D. (2014). Pharmacological strategies for the management of levodopa-induced dyskinesia in patients with Parkinson's disease. *CNS Drugs*, 28, 1155-1184.

Tanner, C.M. (1992). Epidemiology of Parkinson's disease. *Neurologic Clinics*, 10, 317-329.

Trapani, A., Tricarico, D., Mele, A., Maqoud, F., Mandracchia, D., Vitale, P., et al., (2017). A novel injectable formulation of 6-fluoro-1-DOPA imaging agent for diagnosis of neuroendocrine tumors and Parkinson's disease. *International Journal of Pharmaceutics*, 519, 304-313.

Waters, C.H., Nausieda, P., Dzyak, L., Spiegel, J., Rudzinska, M., Silver, D.E., et al., (2015). Long-Term Treatment with Extended-Release Carbidopa-Levodopa (IPX066) in Early and Advanced Parkinson's Disease: A 9-Month Open-Label Extension Trial. *CNS Drugs*, 29, 341-350.

Wollmer, E., & Klein, S. (2017). A review of patient-specific gastrointestinal parameters as a platform for developing in vitro models for predicting the in vivo performance of oral dosage forms in patients with Parkinson's disease. *International Journal of Pharmaceutics*, 533, 298-314.

Figure legend.

**Fig. 1.** SEM image of (a) uncross-sectioned and (b) cross-sectioned spray-coated hollow microcapsules loaded LD, CD and ENT.

**Fig. 2.** Buoyancy (%) of three PD drugs-loaded microcapsules in FeSSGF at 37 °C for 24 h (n=3).

**Fig. 3.** *In vitro* release profiles of LD, CD and ENT from (a) commercial tablets (CPDP) and (b) the microcapsules in FeSSGF at 37 °C for 24 h. (c) *In vitro* release profiles of LD, CD and ENT from the microcapsules in FeSSGF for 5 h, followed by FeSSIF for 24 h at 37 °C (n=3).

**Fig. 4.** Plasma concentration-time profile of (a) LD, (b) CD and (c) ENT from free drug solution (LD:CD:ENT = 10:2.5:20 mg.kg<sup>-1</sup>), MC x 1 (LD:CD:ENT = 10:2.5:20 mg.kg<sup>-1</sup>), MC x 3 (LD:CD:ENT = 30:7.5:60 mg.kg<sup>-1</sup>). Results are expressed as the mean ± SD (n=3).

**Fig. 5.** Normalized brain concentration of (a) LD and (b) DA after oral administration of control (LD:CD:ENT = 10:2.5:20 mg.kg<sup>-1</sup>), MC x 1, and MC x 3. Results are expressed as the mean ± SD (n=3).









