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Pharmacoscintigraphic and pharmacokinetic evaluation on healthy human volunteers of sustained-release floating minitablets containing levodopa and carbidopa

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ABSTRACT

In this study, scintigraphic and pharmacokinetic studies were conducted on 10 healthy, fed volunteers. Two concepts of sustained-release floating minitablets – Levo-Form 1 (matrix) and 2 (coated) – were evaluated and compared to the marketed product Prolopa® HBS 125. All the floating forms were radiolabelled with ^{111}In in order to evaluate their gastric residence time using γ-scintigraphy. It was shown that the three formulations offered almost the same mean gastric residence time, which was about 240 min. Prolopa® HBS 125 and Levo-Form 2 presented intragastric disintegration, which can lead to a more pronounced "peak & valley" effect on the plasma concentration–time profile of levodopa. In contrast, the plasma concentration–time profile of levodopa following the administration of Levo-Form 1 was more evenly distributed. Moreover, Levo-Form 1 provided the lowest variations between men and women in terms of AUC and C_{max} values. Finally, when the same amount of inhibitors of extracerebral dopa decarboxylase – carbidopa and benserazide – had been administrated, the mean AUC, C_{max} and T_{max} values obtained for benserazide were lower than those obtained for carbidopa.

1. Introduction

Parkinson's disease is a common progressive neurological disorder caused by the degeneration of dopaminergic neurones within the *substantia nigra* (Monville et al., 2005). The loss of nigral dopamine neurons, combined with the decline of striatal dopamine activity in the nerve cells, causes a loss of control with both physical and mental dysfunctions (Forte et al., 2005). Trials with oral dopamine have always failed because dopamine cannot cross the blood–brain barrier (Kordower and Goetz, 1999). This discovery led to the use of levodopa in order to enhance synaptic dopamine transmission (Monville et al., 2005). The aminoacid, levodopa, is a precursor of the neurotransmitter dopamine, which easily enters the central nervous system, where it is converted into dopamine by an aromatic amino acid decarboxylase (Zhang et al., 2001). A conventional oral dopa medication controls the evolution of Parkinson's disease adequately for about 5 years (Mardsen and Parkes, 1977). Unfortunately, this therapy, when conducted over a longer period of time leads, in 70% of treated patients, to the development of adverse fluctuations in motor response, called dyskinesia (Duvoisin, 1974). The neural mechanisms underlying these symptoms have notably been associated with pulsatile stimulation of dopamine receptors (Bezard et al., 2001). Indeed, due to the relatively short elimination plasma half-life time of levodopa ($t_{1/2}$ = 1 h) (Clarke, 1986), its plasma concentrations rapidly rise and fall after drug intake leading to an oscillating clinical response (Stocchi et al., 1994). Co-administration of levodopa with inhibitors of extracerebral dopa decarboylase (IEDD) – such as carbidopa and benserazide – allows a marked reduction in levodopa dosage without compromising the therapeutic effect. Indeed, the IEDD diminish the optimum dose of levodopa by about 70–80%, decreasing the oscillating extent of its plasma concentrations. This kind of combination also reduces the time to onset of the therapeutic benefit due to an increase in the biovailability of levodopa and to a decrease of

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the incidence and the severity of the side-effects (Pinder et al., 1976). However, with awareness of a possible relationship between plasma concentrations, clinical effects of levodopa and development of fluctuations in the therapeutic response, improvement of clinical efficacy may be achieved by the development of slow-release formulations. These preparations are characterized by a more sustained pharmacokinetic profile of the drug than those obtained with immediate-release dosage forms, making them more suitable for stabilizing the plasma levels of levodopa (Crevoisier et al., 1987; Grahnén et al., 1992). Moreover, as levodopa is characterized by a narrow absorption window in the upper part of the gastrointestinal tract, the development of a sustained-release dosage form with prolonged gastric residence time (GRT) would be the optimal delivery system for this kind of drug (Rouge et al., 1996). After oral administration, a gastroretentive dosage form would be retained in the stomach and would release the drug in a controlled and extended manner. This mode of administration would thus provide a continuous supply of the drug to its absorption site, yielding sustained and prolonged levodopa input to the systemic blood circulation (Hoffman and Stepensky, 1999).

In previous works, two concepts of sustained-release floating minitablets (FMT) were developed and evaluated *in vitro* (Goole et al., 2007, 2008a). These minitablets were composed of granulates made by melt granulation (Hamdani et al., 2002) and containing at least one active drug, a meltable lipidic binder and gas-generating agents. The first floating system developed contained Methocel® K15M as a swellable polymer both to trap the generated carbon dioxide and to sustain the release of the active drug (Goole et al., 2007). For the second floating system developed, Methocel® K15M was completely removed and a coating step was introduced in the manufacturing process in order to provide a coating layer capable of maintaining the generated carbon dioxide inside the dosage form for a prolonged period of time (Goole et al., 2008a).

The new floating minitablets, containing a combination of levodopa and carbidopa, were investigated for research purposes in order to evaluate their potential for improving the performances of classical marked product used in the treatment of Parkinson's disease.

The aim of the present investigation was to assess the pharmacokinetic profiles of levodopa and the associated IEDD after the administration of the commercial Hydrodynamically Balanced System (HBS™) Prolopa® HBS 125, and to compare them with those following the administration of the new sustained-release FMT. Moreover, a pharmacoscintigraphic study was performed to evaluate the GRT of the sustained-release FMT and of the marketed Prolopa® HBS 125 floating capsule in fed condition. The use of non-invasive technology such as γ-scintigraphy allows the investigation of gastric emptying. This external imaging procedure requires the formulations to be radiolabelled with gamma-emitting radioisotope (Kedzierewicz et al., 1999). Indium-111, a radioisotope with a relatively long half-life, was selected to visualize the floating forms in the stomach for a prolonged period of time.

2. Materials and methods

2.1. Minitablet formulations

Levodopa (Newsmart, Nantong, China) and carbidopa (Teva, Petah Tiqva, Israel) were used in the evaluated uncoated (Levo-Form 1) and coated (Levo-Form 2) FMT as the model drug and the IEDD, respectively. Glyceryl palmitostearate (Precirol® ATO 5 = Gelucire® 52/02) was used as a meltable binder (Gattefosse, Saint-Priest, France). Tartaric acid (Federa, Brussels, Belgium), sodium bicarbonate (Merck, Darmstadt, Germany) and calcium carbonate (Welphar, Brussels, Belgium) were employed as carbon dioxide-generating agents. Lactose 450 mesh (DMV Int., Veghel, The Netherlands) was used as an hydrophilic diluent. The uncoated FMT (Levo-Form 1) contained high viscosity grade HPMC—Methocel® K15M, used as a gel-forming polymer (Colorcon, Kent, England). The insoluble polymer used to make the gas-trapping membrane of the coated FMT (Levo-Form 2) was Eudragit® RL 30D (Rhöm Pharma, Darmstadt, Germany). Citroflex A2® (acetyl triethylcitrate—ATEC), used as a plasticizer, was supplied by Reilly (Hautrage, Belgium). Talc with a mean particle size of approximately 10 μm (Aldrich Chemical Co. Ltd., Gillingham, England) and antifoam emulsion (silicone emulsion, Vel. S.A., Seneffe, Belgium) were used as received. The commercialized floating capsule Prolopa® HBS 125 (Roche, Brussels, Belgium), containing 100 mg of levodopa and 25 mg of benserazide, was used as a comparator.

Table 1
Compositions of the investigated granules (all quantities are given as percentages w/w) and corresponding minitablet properties

	Uncoated FMT	Coated FMT
Levodopa	25.0	25.0
Carbidopa	6.25	6.25
Precirol® ATO 5	12.0	12.0
Methocel® K15M	25.0	–
$CaCO_3$	10.0	10.0
$NaHCO_3$	4.0	4.0
Tartaric acid	3.0	15.0
Lactose 450 mesh	14.75	27.75
Diameter (mm)	3	
Weight (mg)	20	
Compression force (N)	50–100	
Hardness (N) ($n = 10$)	5 ± 1	

Radioactive indium oxinate (^{111}In) solution (1 mCi/ml, i.e. 37 MBq/ml at the calibration date), obtained from Mallinckrodt (Petten, The Netherlands), was used to radiolabel the floating forms. The elution of technetium-99m as sodium pertechnetate (^{99m}Tc) was obtained from a ^{99}Mo–^{99m}Tc generator, from the same manufacturer, to image the stomach. Amberlite® IR-120, a cation-exchange insoluble resin, was used as ^{111}In-binding carrier excipient (Sigma–Aldrich® Chemic GmbH, Munich, Germany).

2.2. Methods

2.2.1. Preparation of minitablets

2.2.1.1. Granulate manufacture. Granulates were made in a small vertical laboratory-scale high-shear mixer, Mi-Pro® (Pro-C-EpT, Zelzate, Belgium), equipped with a transparent bowl and a heating jacket (Hamdani et al., 2002). The granulate compositions are listed in Table 1.

All experiments were started at an impeller speed (IS) of 1800 rpm and a chopper speed (CS) of 130 rpm, while the temperature of the heating jacket was set at 60 °C. In order to avoid any further product temperature increase, the IS was reduced to 600 rpm after the granule formation step, while the CS was increased to 1000 rpm to break any possible agglomerates. The massing time was kept constant at 5 min. The length of the whole granulate manufacturing process was around 20 min. At the end of the process, the granules were cooled at ambient temperature.

The mean particle size of granulates, represented by the equivalent volume diameter $D[4,3]$, should be around 150 μm to provide good flow properties (Scirocco 2000, Malvern Instruments, Worcestershire, UK).

2.2.1.2. Minitablet preparation. Minitablets (MT) were prepared by direct compression. Granulates were fed manually into the die of an instrumented single-punch tableting machine (Korch, Germany) to

Table 2
Formulation used for the coating of the coated FMT

Formulation	F1
Eudragit® RL30D (g) (dry basis)	200
ATEC (g)	40
Talc (g)	50
Lactose (g)	20
Antifoam (g)	2
Water (g)	842
Solid content (%, w/w)	25.6
Coating level (%, w/w)	20

produce MT using concave-faced multiple – (eight) – punches and dies, 3 mm in diameter each. The compression force, the weight and the hardness of the MT are summarized in Table 1. The hardness was measured with a hardness tester (Computest, Kreamer GmbH, EL Ektronik, Darmstadt, Germany). The friability of the MT was 0.13 ± 0.03%. This indicated that they were able to withstand the mechanical stress of the subsequent coating process.

2.2.1.3. Preparation of the coating dispersion. The aqueous dispersion used for the coating of the MT is given in Table 2. Talc was previously dispersed in water in the presence of an antifoam agent and mixed with the possible water-soluble additive using a T45 Ultra-Turrax® (Janke & Kunkel GmbH, Staufen, Germany). The dispersion containing Eudragit® RL30D requires the addition of 20% (w/w) (relative to film former content) of plasticizing agent. The plasticizer was added to the polymer aqueous dispersion under gentle stirring. All the components of the coating dispersion were then mixed under magnetic stirring for at least one hour before starting the coating process.

2.2.1.4. Preparation of coated MT. Minitablets were transferred into a fluidized bed coating apparatus Uni-Glatt® (Glatt GmbH, Binzen, Germany) equipped with a bottom-spray coating process in a Würster column and coated with the coating dispersions until the desired film weight was deposited. During the coating operation, the aqueous dispersion was stirred continuously to prevent sedimentation of insoluble particles. The conditions for layering were shown to be as follows: preheating temperature, 40 ± 2 °C; preheating time, 10 min; inlet and outlet temperatures, 40 ± 2 and 35 ± 2 °C, respectively; flow rate, 6 g/min; pneumatic air pressure, 1 bar. After coating, the coated MT were further fluidized for 10 min and subsequently cured at 60 °C for 8 h.

2.2.2. Study design

The study design was an open single-dose, three-treatment, three-period cross-over study with a wash-out of at least 6 days between the three phases of the study for each volunteer. An equal number of subjects were randomly assigned to each of the three possible dosing sequences. All volunteers received the drug treatment as one capsule filled with 20 minitablets containing 100 mg of levodopa and 25 mg of carbidopa (Levo-Form 1 and 2) or one Prolopa® HBS 125 floating capsule containing 100 mg of levodopa and 25 mg of benserazide, in fed condition (Table 3). Following administration, the volunteers were instructed to sit or remain standing for the duration of the study to avoid the possibility of posture affecting the gastric emptying of the floating forms (Stops et al., 2006). The three products were radiolabelled with up to 18.5 MBq of ^{111}In. Ten minutes before the first scintigraphic acquisition, the volunteers also received 111 MBq of ^{99m}Tc intravenously for gastric imaging. The administered dose of radionuclide was almost equal to the dose used for a typical thyroidal clinical exam. No pharmacological impact or side effect was observed for these radionuclides.

2.2.3. Volunteers

Ten healthy volunteers (four men and six women, 25 ± 5 years, body mass index between 19 and 30 kg/m^2) were included in the study on the basis of inclusion and exclusion criteria and medical examination. The volunteers were non-smokers and were free from clinically significant disorders. They also refrained from alcohol during the 3 weeks of the study, and from drugs having a systemic effect for at least 3 days before the study. Before starting the study, the nature of the clinical trial was explained and written consent was obtained from all volunteers. The study was conducted at the Erasme Hospital (Brussels, Belgium), in accordance with the principles stated in the Declaration of Helsinki, and approval was obtained from the Ethics Committee of Erasme Hospital (Ref.: P2007/086) and the Belgian Minister of Social Affairs and Public Health (Ref.: EudraCT No. 2007-000708-33).

2.2.4. Safety assessment

Each subject underwent a physical examination, routine clinical chemistry, haematology and urinalysis at the beginning and at the end of the study. No adverse events were noted for any dosage form in any subject.

2.2.5. The radiolabelling method

The radioactive ^{111}In solution, containing the required activity, was exactly calibrated using a radioisotope calibrator (Capintec, NJ, USA). A fixed amount (5% (w/w) relative to the granule content) of ion-binding carrier excipient was added to the radiopharmaceutical solution. The volume (3 ml) of the resultant suspension was adjusted to 2 ml by evaporation. The suspension was stirred for 5 min at 30 rpm and then allowed to stand until complete sedimentation (Labinco B.V., Breda, The Netherlands). The supernatant was thoroughly withdrawn to remove free radiolabel. The carrier excipient was then transferred to a watch-glass and desiccated to dryness in a stove (80 °C, 10 min). The final activity dose of the radiolabelled carrier was determined by means of a probe counter (Ortec®, AC Joure, The Netherlands) comparatively to an equivalent dose of known activity of the same radionuclide in solution (Timmermans et al., 1989). The distance between the probe and the sample was adjusted prior to measurement for optimal counting efficiency. The dosage forms to be labelled were extemporaneously prepared one by one as follows. The required exact weight amount of the radiolabelled carrier excipient, obtained according to the described technique, was added as a dry residue to the total amount of formulation powder for the three dosage forms. The granules and the radiolabelled resin were then mixed together in a rotational stirring apparatus (Labinco B.V., Breda, The Netherlands) for 5 min at 30 rpm before the compression step. The minitablets were filled into white-coloured hard HPMC capsules 0 (Quali-V®, Qualicaps Europe, Madrid, Spain) to reach 100 and 25 mg of levodopa and carbidopa in each, respectively. The blend corresponding to the HBS formulation was refilled into its initial capsule without further modification. For each period, the dosage forms were prepared the day before the study.

2.2.6. Assessment of radiolabelled dosage forms

Before initiation of the clinical phase study, *in vitro* validation experiments were conducted to demonstrate that alteration of the dissolution and floating properties of the evaluated dosage forms did not occur during the labelling process, and that the floating forms were also able to sustain the release of the radioactivity. For each dosage form, the dissolution profiles of the drugs and the floating properties of the dosage forms were determined and compared against those obtained from the dosage forms containing the radiolabelled resin.

Table 3
Time schedule and feeding regimens used throughout the *in vivo* study

Sample no.	Theorical time (h:min)	Remarks	Blood sample	Scintigraphic imaging
P0	−01:00	Predose sample	Y	
	−00:30	Standardized breakfast (167 Kcal)		
		200 ml of orange juice		
		3 slices of bread		
		10 g of margarine		
		20 g of jam		
		50 g of low fat white cheese		
	−00:10	111 MBq of ^{99m}Tc by intra-venous		
	00:00	Administration of one capsule of reference or dosage forms with 200 ml of tap water		Y
P1	00:30		Y	Y
P2	01:00		Y	Y
P3	01:30		Y	Y
P4	02:00	Standardized snack (230 kcal)	Y	Y
		75 g of apple pie		
		150 ml of coffee or tea		
		+200 ml of tap water		
	02:30			Y
P5	03:00		Y	Y
	03:30			Y
P6	04:00		Y	Y
	04:30	Standardized lunch (499 kcal)		Y
		200 ml of green soup		
		150 g of roasted chicken		
		100 g of potatoes cooked in water		
		200 ml of mineral water		
P7	05:00		Y	Y
	05:30			Y
P8	06:00	200 ml of tap water	Y	Y
	06:30			Y
	07:00			Y
	07:30			Y
P9	08:00	200 ml of tap water	Y	Y
	08:30	Standardized snack (230 kcal)		
		75 g of apple pie		
		150 ml of coffee or tea		
P10	10:00		Y	
	10:30	Standardized dinner (718 kcal)		
		400 g of Tagliatelli ham cheese		
		55 g of bread stick		
		10 g of butter		
		120 g of banana		
		200 ml of mineral water		
P11	12:00		Y	

The dissolution studies were performed on a Disteck 2100C USP 29 dissolution apparatus (Distek Inc., North Brunswick, NJ, USA), Type II (paddle method). The rotational speed employed was 50 rpm. Release testing was carried out in 900 ml of phosphate buffer solutions (0.05 M) containing 0.05% (w/v) Polysorbate 20 at pH 3.0 to simulate gastric pH in fed condition. The temperature of the dissolution media was maintained at 37.0 ± 0.2 °C. Dissolutions were carried out on an equivalent of 100 mg of levodopa and 25 mg of IEDD. The percentages of drug release were measured by the validated HPLC method described below at preselected time intervals and averaged (n = 5). The HPLC system consisted of a High Performance Liquid Chromatography System (HP 1090 series II, Agilent Technologies, Brussels, Belgium), equipped with a binary pump, an autosampler, and a diode-array detector (DAD) set at 282, 280 and 220 nm for levodopa, carbidopa and benserazide, respectively. The separation system was a 150 mm × 4.6 mm (5 μm particle size) reversed-phase C18 column Luna® (Phenomenex, CA, USA). Samples of 20 μl volume were injected. The mobile phase was composed of a KH_2PO_4 solution (0.035 M) adjusted to pH 2.0 with H_3PO_4 and 100% acetonitril HPLC grade in a ratio of 98:2 (v/v), respectively. The solutions were mixed and passed through a 0.2 μm filter in order to be degassed prior to use. The flow rate was 0.8 ml/min. The analyses were performed at 30 °C. The percentages of radioactivity released were determined by gamma counting using a Cobra auto gamma counter 5003 (Packard bioscience, Minnesota, USA). Using these conditions, the retention time of levodopa, carbidopa and benserazide was 4, 12 and 6 min, respectively.

To determine the buoyancy capabilities of the FMT and Prolopa® HBS 125, a resultant-weight apparatus designed for dynamic measurement of the total force acting vertically on an immersed object was also used (Timmermans and Moës, 1990). By convention, a positive RW signifies that the object is able to float, whereas a negative RW means that the object sinks. The floating forms were placed in a specially designed basket sample holder, which was immersed in 1200 ml of preheated 0.1 N HCl solution containing 0.05% (w/v) Polysorbate 20 (pH 1.2, 37 °C). The RW was measured every minute for 13 h.

2.2.7. Gamma scintigraphy analysis

In vivo evaluation of the gastric retention of the evaluated floating dosage forms was achieved by γ-scintigraphic imaging (Griffiths et al., 1966). ^{111}In was used as a γ-emitter for indirect radiolabelling of the oral dosage forms. The γ-ray of ^{111}In had sufficient energy (171 and 245 keV) to penetrate body tissues, and when it reached the γ-camera detector, it was absorbed and converted into

light photons, thus allowing γ-camera imaging (Timmermans et al., 1989). The half-life of ^{111}In is 67.4 h, which is long enough for handling and imaging a gastro-retentive sustained-release oral dosage form. Moreover, due to the intra-venous injection and despite its short half-life (6 h) (Wilding et al., 2001), the intravenous injection of ^{99m}Tc was sufficiently sustained to visualise the gastric wall during the whole period of the study.

The γ-camera was a double-headed camera (DHD, Sopha Medical, CT, USA) equipped with 40 cm parallel hole medium energy collimators. The acquisition time was fixed at 120 s. Two images were simultaneously acquired by each camera head: one centred on the energy windows of ^{111}In photons (A), the second with a peak energy detection set on 140 keV ± 20%, the energy of ^{99m}Tc photon (B). The camera was connected to an image computer processing system (Vision, Sopha Medical, CT, USA) for data analysis, image treatment and representation. Image B permitted a good visualisation of the gastric wall despite the detection of scattered ^{111}In photons. Regions of interest were drawn around the stomach on this image, then reported on image A. Image A contained only counts due to ^{111}In activity and was used to follow the floating dosage forms. Radiation from ingested isotopes is scattered or absorbed by intervening tissues and bone before reaching the γ-camera, causing the radiation registered to be attenuated (Tothill et al., 1978). Therefore, both anterior and posterior images were acquired at each time interval as a decrease of the radiation from the stomach could indicate the gastric emptying of the floating form. However, at the study start, the radioactivity was located in a small area, smaller than two folds the imaging system resolution, then later, radioactivity was dispersed in much larger regions. In such situation, a reliable quantitative analysis revealed to be impossible because of the non-linearity of counting due to partial volume effect in small foci of activity and because of the non-homogenous attenuation by anterior and posterior tissues.

2.2.8. Pharmacokinetic analysis

Venous blood samples (7 ml) were collected at pre-dose and at 30 min, 1, 1.5, 2, 3, 4, 5, 6, 8, 10 and 12 h post-dose (Table 3) in order to quantify plasma levels of levodopa, carbidopa and benserazide. After centrifugation (3000 rpm, 10 min, 4 °C), the plasma samples were decanted and divided into two approximately equal portions of not less than 2 ml per tube (aliquot) and rapidly stored at −80 °C, in upright position. Each aliquot tube contained 0.1 ml of metabisulfite solution as anti-oxidant.

The extraction of levodopa, carbidopa and benserazide was performed by passing the plasma samples through an anionic exchange cartridge SPE HAX (Biotage, Uppsala, Sweden) placed in a Gilson XL4 system (Gilson, Middeltown, USA). The cartridge was first conditioned with 1 ml of methanol and 1 ml of diammonium hydrogenophosphate buffer (20 mM, pH 10), respectively. Then, 400 μl of sample (205 μl of plasma + 5 μl solution of hydrazine 20% (w/w) + 21 μl of internal standard + 168 μl of diammonium hydrogenophosphate buffer 20 mM, pH 10) were loaded, washed with 0.8 ml and then eluted with 0.8 ml of formic acid 3% (v/v). 50 μl of ascorbic acid 2.0% (w/v) was finally added to the eluted solution. An aliquot was injected into the validated LC/MS/MS method described below (Belveal et al., 2007).

An HPLC system (HP 1100 series, Agilent Technologies, CA, USA), coupled to an API365 quadrupole mass spectrometer (Applied Biosystems/MDS Sciex, Concord, Canada), was used to measure levodopa, carbidopa and benserazide in plasma samples. The separation system was a 150 mm × 2 mm Polar-RP Synergi column (Phenomenex, CA, USA). The mobile phase was composed of methanol and a 0.2% formic acid solution in a ratio of 20:80, respectively. The flow rate was 150 μl/min. The analyses were performed at 30 °C. Samples of 50 μl were injected. The desolvation temperature was kept at 400 °C. The limit of quantification of the method was 10 ng/ml and 2 ng/ml for levodopa and each IEDD, respectively.

The calculation of pharmacokinetic parameters was as follows:

Maximal plasma concentration (C_{max}) and time to maximal plasma concentration (T_{max}) were taken directly from the plasma concentration–time profile. The area under curve (AUC) was calculated by the trapezoidal rule from measured data points from the time of administration until the time of the last quantifiable concentration.

2.2.9. Statistical evaluation

As recommended in the FDA's Guidances for Industry, the similarity factor f_2 was used as a determination for assessing the similarity of dissolution profiles (FDA, 1997; Shah et al., 1998). The compared dissolution profiles were obtained under the same test conditions and their dissolution time points were the same, e.g. for controlled release products, they were 1, 3, 5 and 8 h. As indicated by Shah et al. (1998), the similarity factor f_2 value has to be higher than 50 in order to assess the similarity between two dissolution profiles.

The repeated-measures ANOVA test was used to compare the pharmacokinetic data obtained with the three formulations. The Student's test was used to compare the gastric retention time of the tested products. For all tests, the significance level was set at $p = 0.05$.

3. Results and discussion

3.1. Assessment of labelled dosage forms

The sustained-release floating dosage forms were labelled with a γ-emitting radionuclide (^{111}In) by the incorporation of 5% (w/w) of labelled ion-exchange resin. First of all, it was demonstrated that the presence of resin did not alter the sustained-release and floating properties of the floating forms and that the release of levodopa – the only drug incorporated in the three evaluated dosage forms – was correlated with the release of the radiolabel.

A sustained release of levodopa and of the IEDD occurred immediately after immersion with no burst effect, regardless of the floating form tested (data not shown). The entire dose of levodopa and carbidopa were released after 12 and 24 h from Levo-Form 1 and Levo-Form 2, respectively. Levodopa and carbidopa being characterized by a similar solubility in an acidic media (Pinder et al., 1976), the dissolution profiles obtained from Levo-Form 1 ($f_2 = 87$) and Levo-Form 2 ($f_2 = 64$) remained statistically similar until the complete release of these drugs. In contrast, benserazide was released faster than levodopa from Prolopa® HBS 125 (entire dose released after 12 h vs. 24 h). Indeed, after 12 and 24 h, the dissolution profiles of benserazide and levodopa were not statistically similar ($f_2 = 46$). The standard deviations noticed with Prolopa® HBS 125 increased, both for levodopa and benserazide, after 5 h due to the fragmentation of the jelly mass.

The incorporation of the ion-exchange resin in the formulations did not alter the dissolution profiles of the drugs (Table 4), regardless of the floating dosage form evaluated. The sustained-release

Table 4
Similarity factors from dissolution profiles of levodopa, carbidopa and benserazide, obtained with the three formulations – Levo-Form 1 (uncoated), Levo-Form 2 (coated) and Prolopa® HBS 125 – with and without ion-exchange resin

	Levodopa	Carbidopa	Benserazide
Uncoated	98	90	–
Coated	76	76	–
Prolopa® HBS	96	–	80.6

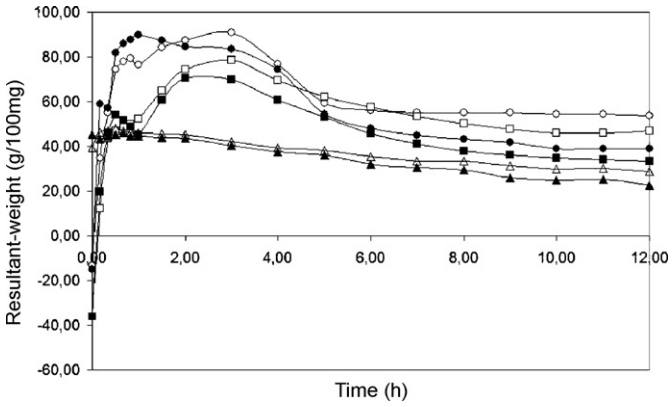

Fig. 1. Resultant-weight profiles obtained from Levo-Form 1 (○) without and (●) with resin; Levo-Form 2 (□) without and (■) with resin; Prolopa® HBS 125 (△) without and (▲) with resin ($n = 3$).

properties were preserved and the drugs were released within the same periods of time as those obtained when the floating forms were free of resin. Moreover, the floating properties of the FMT and Prolopa® HBS 125 were also preserved (Fig. 1). Levo-Form 1 and Levo-Form 2 floated within 10 min and remained buoyant for more than 13 h, regardless of whether resin was incorporated. As observed for formulations without resin, the HBS capsule presented no floating lag time, but its floating strength decreased after 1 h as a result of the development of its hydrodynamic equilibrium (Goole et al., 2007). Regardless of whether resin was incorporated, the maximal resultant-weight values remained similar and were about 80, 75 and 45 mg/100 mg for the Levo-Form 1, Levo-Form 2 and Prolopa® HBS 125, respectively.

The dissolution profile of levodopa and the release profile of the radioactivity were then compared for each formulation (Fig. 2). The dissolution properties were not similar from one floating dosage form to another. Indeed, the underlying mechanisms of drug (or radioactivity) release from water-swellable matrix systems are mainly diffusion and erosion (Kiil and Dam-Johansen, 2003), whereas diffusion is the only important mechanism characterizing the release of a drug from coated systems (Siepmann et al., 1999). As ^{111}In is exchanged with protons at the surface of the ion-exchange resin, the release of the radioactivity was not similar for the three formulations. The radioactivity was mainly released after erosion of the matrix from Levo-Form 1 and Prolopa® HBS 125, according to the corresponding area of contact, which depends on the shape of the dosage form. From Levo-Form 2, the radioactivity was released after the diffusion of the aqueous media inside the dosage form. However, the dissolution profile of levodopa remained statistically similar to the release profile of the radioactivity for at least 8 h. Indeed, the similarity factors obtained with Levo-Form 1, Levo-Form 2 and Prolopa® HBS 125 were 57, 73 and 59, respectively. So, the radioactivity remained within the device long enough for the position of the floating forms *in vivo* to be observed.

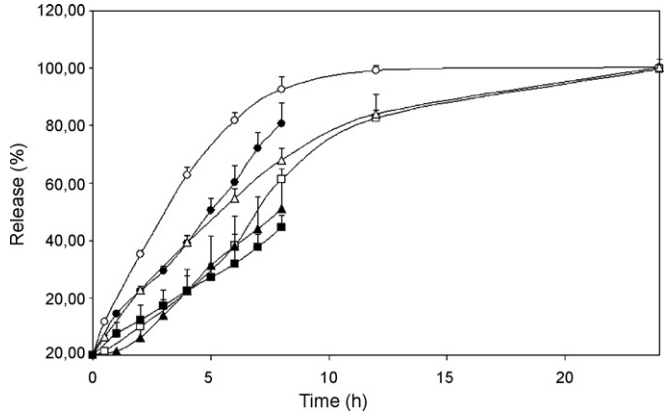

Fig. 2. Dissolution profiles of levodopa obtained from (○) Levo-Form 1, (□) Levo-Form 2, (△) Prolopa® HBS 125, and the ^{111}In release profiles obtained from (●) Levo-Form 1, (■) Levo-Form 2, (▲) Prolopa® HBS 125 ($n = 5$).

3.2. In vivo study

The combination of scintigraphy with pharmacokinetic studies has now become an important means of providing information about the GRT of floating dosage forms and subsequent drug absorption (Wilding et al., 2001). The GRT was evaluated as the time of the image preceding the first image that showed clear evidence of the total gastric emptying of the pharmaceutical dosage form (Moës, 1993). As a radiolabelled resin was incorporated inside the floating forms, it was possible to follow their movement in the stomach using a gamma camera. Ten volunteers took part to the present study and the results obtained in all subjects showed almost the same trend for the movement of the radiolabelled FMT – Levo-Form 1 and Levo-Form 2 – in the stomach. The movement of Prolopa® HBS 125 in the stomach appeared to be specific. Typical acquisitions obtained from volunteer 1, after administration of one capsule containing Levo-Form 1, are shown in Fig. 3.

At $t = 0$ min, immediately after ingestion, the capsule containing the FMT was located on the surface of the gastric fluid. At that time, as all the minitablets were confined in the capsule, the radioactivity could be visualised as a single hotspot (Fig. 3a). At $t = 30$ min, the capsule opened and the FMT began to be dispersed at the bottom of the stomach (Fig. 3b). At $t = 60$ min, the FMT were localized at the upper part of the stomach (Fig. 3c). The strength of the floating force was sufficient for the FMT to move upwards through the ingested meal to its surface, rather than be pushed down by the weight of food. Moreover, the floating lag time of the FMT did not increase with the presence of food. Indeed, as observed *in vitro*, the FMT floated within less than 30 min after dispersion (Goole et al., 2007, 2008b). At $t = 150$ min, two distinct hotspots could be visualized. One group of FMT began to sink to the gastric *antrum*, while the main mass still remained buoyant at the upper part of the stomach (Fig. 3d). At $t = 210$ min, the FMT can be seen to start emptying into the small intestine, since a hotspot could be visualized out of the region of interest (Fig. 3e). All the FMT were emptied from the stomach after 300 min (Fig. 3f).

When Levo-Form 2 was administrated, a dispersion of the radioactivity was sometimes visualized throughout the stomach at different acquisition times (Fig. 4a). When cracks appear on the surface of the film, the integrity of the coating is not maintained and the core of the minitablets is thus dispersed. This phenomenon was already observed *in vitro* (Goole et al., 2008a).

As observed *in vitro*, the Prolopa® HBS 125 presented no floating lag time due to its very low density (Goole et al., 2007). It floated on the surface of the gastric fluid immediately after ingestion. A small hotspot of radioactivity, similar to that observed in Fig. 3a, could be visualized. However, at the acquisition time preceding the gastric emptying, the size of the hotspot increased, probably due to fragmentation of the jelly mass (Fig. 4b). A pharmacoscintigraphic study conducted by Seth and Tossounian on a HBS system containing chlordiazepoxide has already shown an intra-gastric disintegration of the jelly mass 4 h following the ingestion of the dosage form (Seth and Tossounian, 1984).

Table 5 presents complete data on the three dosage forms, showing the GRT of the formulations, as well as the mean AUC, C_{max} and T_{max} of levodopa, carbidopa and benserazide.

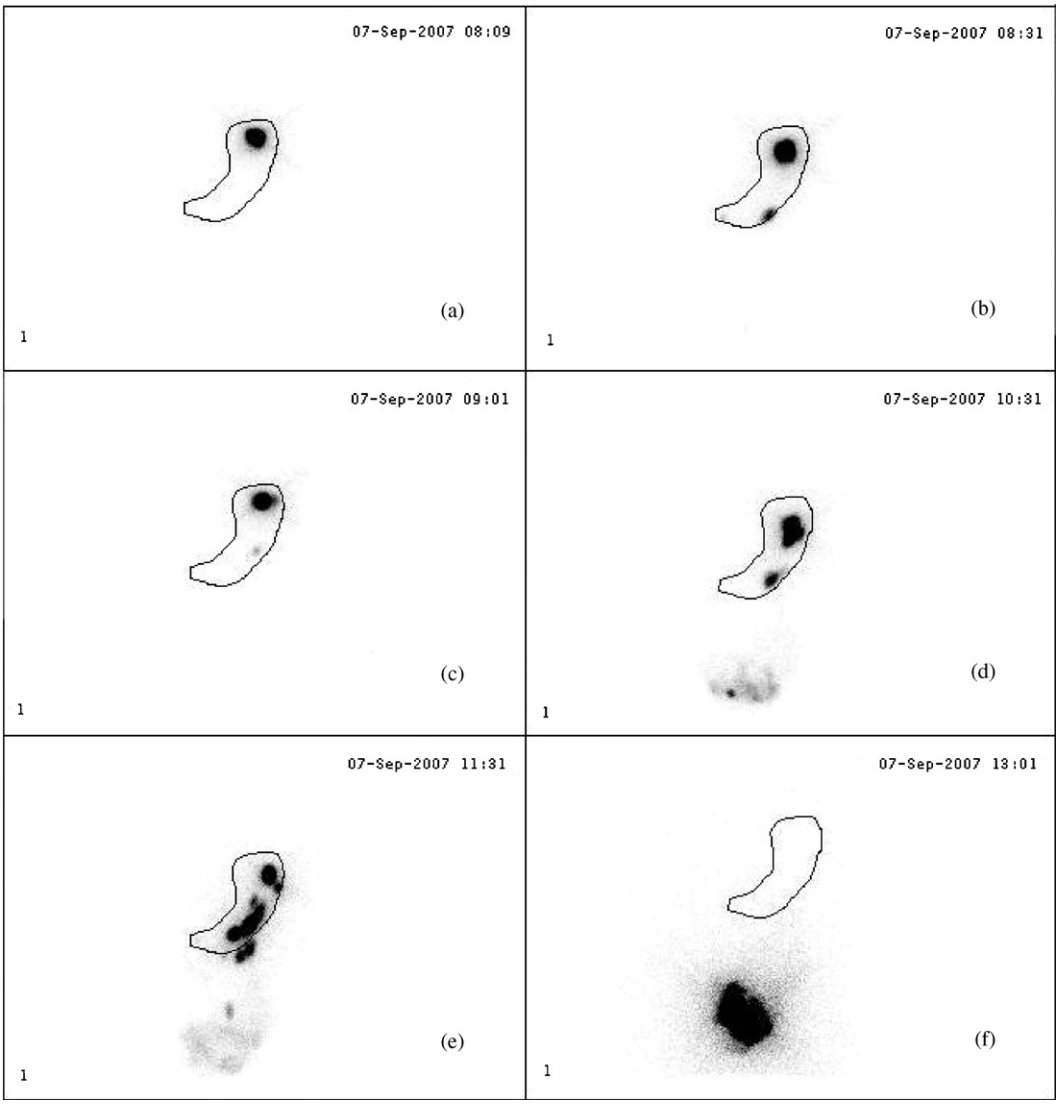

Fig. 3. Typical scintigraphic images obtained from volunteer 1 after administration of one capsule containing Levo-Form 1 showing the behaviour of the floating minitablets in fed stomach. Panels A–F demonstrate the radiolabelled Levo-Form 1 after oral administration and at 30, 60, 150, 210 and 300 min, respectively.

The three formulations offered almost the same mean GRT, which was 228 ± 121, 294 ± 121 and 261 ± 93 min for Prolopa® HBS 125, Levo-Form 1 and Levo-Form 2, respectively. The GRT observed for Prolopa® HBS 125 in this study corroborates the scintigraphic results described in the literature (Seth and Tossounian, 1984). It can be noticed that men seem to present a lower GRT of the floating forms than women. This is a general observation, already described in the literature for every oral dosage forms (Moës, 1993). The T_{max} of levodopa, carbidopa and benserazide were also similar from one system to another, regardless of gender. The mean T_{max} value of

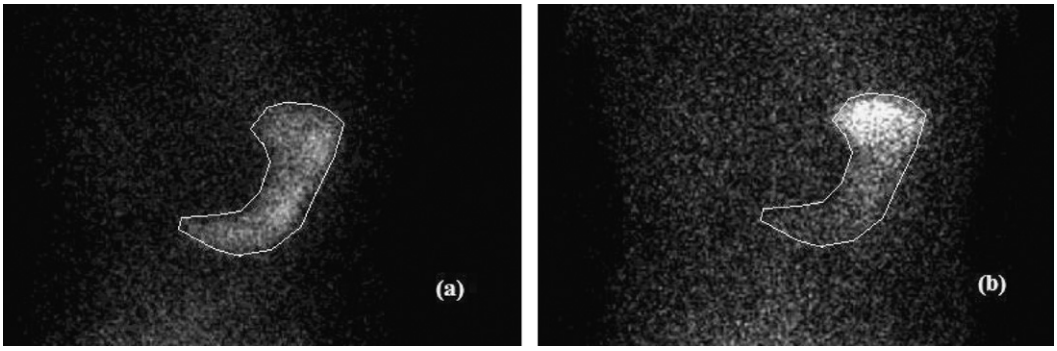

Fig. 4. Scintigraphic images, obtained from volunteer 3, showing the dispersion of the radioactivity throughout the stomach after administration of (a) Levo-Form 2 (t = 180 min) and (b) Prolopa® HBS 125 (t = 210 min).

Table 5
Gastric residence time of the formulations with AUC, C_{max} and T_{max} (mean ± S.D.) of levodopa, carbidopa and benserazide

		Prolopa® HBS	Levo-Form 1	Levo-Form 2
GRT (min)	Women (n = 6)	245 ± 58	345 ± 131	300 ± 104
	Men (n = 4)	203 ± 57	218 ± 45	203 ± 15
	Mean (n = 10)	228 ± 59	294 ± 121	261 ± 93
Levodopa				
AUC (ng h/ml)	Women	1554 ± 771	926 ± 428	1709 ± 897
	Men	799 ± 179	821 ± 319	379 ± 120
	Mean	1252 ± 702	884 ± 373	1177 ± 961
C_{max} (ng/ml)	Women	594 ± 339	248 ± 107	577 ± 302
	Men	258 ± 47	243 ± 72	119 ± 43
	Mean	459 ± 308	246 ± 90	394 ± 327
T_{max} (min)	Women	270 ± 33	290 ± 25	280 ± 31
	Men	240 ± 85	210 ± 35	240 ± 85
	Mean	258 ± 57	258 ± 49	264 ± 58
		Benserazide Prolopa® HBS	Carbidopa Levo-Form 1	Carbidopa Levo-Form 2
AUC (ng h/ml)	Women	55 ± 39	81 ± 41	99 ± 68
	Men	6 ± 8	87 ± 30	68 ± 34
	Mean	36 ± 39	84 ± 35	87 ± 56
C_{max} (ng/ml)	Women	15 ± 9	21 ± 10	24 ± 15
	Men	4 ± 3	19 ± 3	22 ± 13
	Mean	11 ± 9	20 ± 8	23 ± 14
T_{max} (min)	Women	210 ± 99	310 ± 25	315 ± 59
	Men	128 ± 126	330 ± 180	330 ± 199
	Mean	177 ± 112	318 ± 106	321 ± 124

levodopa obtained after administration of the three floating forms was higher than the one obtained after a single administration of a conventional slow-release formulation, regardless of the incorporated amount of levodopa (Cedarbaum et al., 1989; Gasser et al., 1998). Due to the high variability and to the similarity of the GRT values, no statistical correlation could be done between the GRT of the dosage forms and the T_{max} of the drugs.

Fig. 5a shows the mean plasma levels of levodopa, the only drug incorporated in the three dosage forms, obtained from Prolopa® HBS 125, Levo-Form 1 and Levo-Form 2 as a function of time. There was no rapid initial absorption phase and, instead, a gradual build up in the absorption profile occurred. Due to the large inter-subject variability and the small number of subjects, the AUC, C_{max} or T_{max} values obtained for levodopa did not show a significant difference between the three evaluated floating forms ($p > 0.05$). However, the plasma concentration–time profile obtained for Prolopa® HBS 125 and Levo-Form 2 were quite similar. In both cases, the plasma level of levodopa sharply increased after 3 h to reach a mean C_{max} value of 459 and 394 ng/ml after 5 h for Prolopa® HBS 125 and Levo-Form 2, respectively. This phenomenon could be explained by the intra-gastric disintegration of the dosage forms observed by scintigraphy. The small agglomerates, created after disintegration, offered a higher surface of contact between the solid form and the gastric fluid, increasing the dissolution rate of the drug and its subsequent absorption. In comparison, the mean C_{max} value following single administration of an immediate-release formulation containing 100 mg levodopa to 10 volunteers reached 750 ng/ml in 2 h (Pinder et al., 1976). Moreover, Prolopa® HBS 125 and Levo-Form 2 presented a similar mean AUC that was 1252 and 1117 ng h/ml, respectively. In contrast, the plasma concentration–time profile of levodopa following the administration of Levo-Form 1 was more evenly distributed than those obtained following the administration of Prolopa® HBS 125 and Levo-Form 2, decreasing the possibility of pulsatile stimulation of dopamine receptors. The mean AUC and the mean C_{max} values decreased compared to those observed for Prolopa® HBS 125

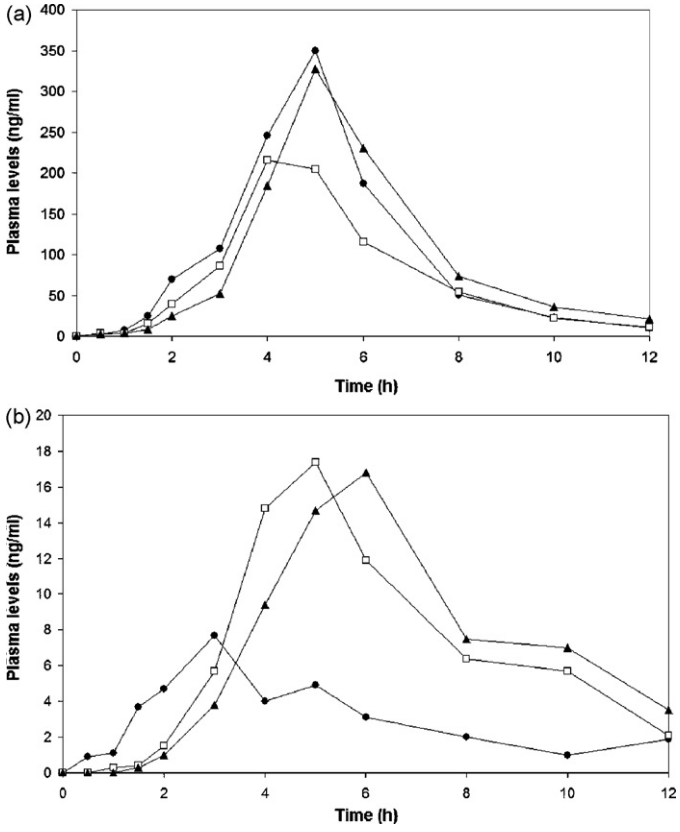

Fig. 5. Mean plasma levels of (a) levodopa following administration of (●) Prolopa® HBS 125, (□) Levo-Form 1 and (▲) Levo-Form 2 and (b) IEDD—(●) benserazide, (□) carbidopa Levo-Form 1 and (▲) carbidopa Levo-Form 2 (n = 10).

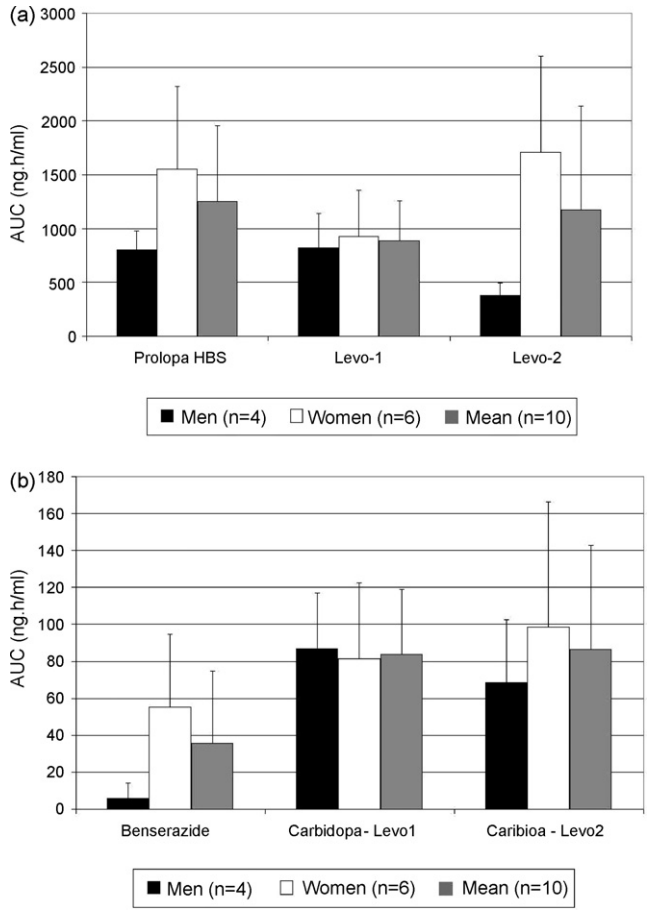

Fig. 6. Comparative mean AUC of (a) levodopa and (b) IEDD form Prolopa® HBS 125, Levo-Form 1 and Levo-Form 2.

and Levo-Form 2 and were 884 ng h/ml and 246 ng/ml, respectively. As no disintegration occurred with Levo-Form 1, the release rate did not increase. If the complete release of levodopa did not occur before the FMT had passed the absorption window, the dose available for absorption could be reduced, decreasing the bioavailability of the drug (C_{max} and AUC). So, in the future, it could be useful to decrease the amount of swelling agent in order to accelerate the release of levodopa or to increase the level of gas-generating agents in order to improve the floating strength which could increase the GRT of the FMT. One or more minitablets providing an immediate release of levodopa could be also associated to the sustained-release FMT in the same capsule in order to increase the plasma levels of levodopa as fast as possible after ingestion.

Even if Levo-Form 1 offered the lowest mean AUC value for levodopa, this floating form provided the lowest variations between men and women in term of AUC (Fig. 6a and b) and C_{max} values for both levodopa and carbidopa (Table 5). The absence of disintegration observed with Levo-Form 1 showed a stronger resistance to the more important contractile activity of the stomach observed in men than in women (Kamba et al., 2000). This allows a lower variability in the drug release and in its subsequent absorption between men and women. As Parkinson's disease affects both men and women without significant difference (Sharstry, 2001), a gastroretentive sustained-release dosage form, capable of providing a similar plasma concentration–time profile in both genders, could result in a more reproducible therapeutic effect. Additionally, Levo-Form 1 presented the lowest total inter-subject variability for AUC and C_{max} for levodopa and carbidopa.

The same amount of IEDD having been administrated (25 mg), the mean AUC, C_{max} and T_{max} values obtained for benserazide (Prolopa® HBS 125) were lower than those obtained for carbidopa (Levo-Form 1 and 2) (Fig. 5b). For instance, the AUC of benserazide was more than two times lower than that obtained with carbidopa, for both Levo-Form 1 and Levo-Form 2 (Table 5). An incomplete absorption occurred with benserazide due to rapid oxidation to quinine-like molecules at the pH of intestinal fluid (Pinder et al., 1976). Moreover, the AUC value of benserazide seems to be very dependant on gender as it reached 55 ng h/ml for women and fell down to 6 ng h/ml for men (Fig. 6b). So, carbidopa seems to be the most effective IEDD for the treatment of Parkinson's disease in association with levodopa. However, this study did not allow the evaluation of the therapeutic efficacy obtained with a combination of levodopa and carbidopa rather than with a combination of levodopa and benserazide.

4. Conclusion

In conclusion, this study has shown that the new sustained-release FMT were able to float on the surface of gastric fluid for more than 4 h. They also provided a sustained pharmacokinetic profile of levodopa and carbidopa. The coated FMT (Levo-Form 2) provided values of levodopa for AUC and C_{max} that were almost similar to those of Prolopa® HBS 125, but benserazide offered a lower bioavailability, which seems to be very dependent on gender, than carbidopa. Even if the lowest AUC value of levodopa was obtained following the ingestion of the uncoated FMT (Levo-Form 1), this floating form did not show intragastric disintegration and, so, offered the lowest variability. Levo-Form 1 also presented the most evenly distribution of the plasma level values of levodopa, decreasing a possible pulsatile stimulation of dopamine receptors. Despite these encouraging results, no significant statistical difference has been found in the GRT, AUC, C_{max} and T_{max} values obtained from the FMT and Prolopa® HBS 125. In the near future, in order to increase the AUC value of levodopa obtained after the ingestion of Levo-From 1, it could be useful to decrease the amount of swelling agent for accelerating the release of levodopa or to increase the level of gas-generating agents in order to improve the floating capabilities which could increase the GRT of the FMT. An association of sustained-release and immediate-release formulation could be also investigate. Moreover, other pharmacokinetic studies should be conducted on a larger number of subjects.

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