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Research paper

Influence of layer position on in vitro and in vivo release of levodopa methyl ester and carbidopa from three-layer matrix tablets

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Abstract

A versatile oral controlled release system for the simultaneous delivery of levodopa methyl ester and carbidopa, consisting of a three-layer matrix tablet, has been studied and developed. Each individual layer of the matrix exhibited a different release mechanism, i.e. the first layer was swellable (S), the second one was erodible (E) and the third one was disintegrating (D). The three layers have been assembled in the monolithic matrix in different relative positions. It was found that in the monolith the three layers could interact, producing in vitro release profiles depending on their relative position. The monoliths having the configurations DSE and SDE were administered to human volunteers in order to determine the plasma profiles. The pharmacokinetic data showed a significant difference between the early time plasma curves: the monolith DSE, having the fast release profile, gave rise to a rapid appearance of a high levodopa plasma level, whereas the slower releasing monolith SDE produced a smoothed plasma concentration profile.

Keywords: Three-layer matrix; Parkinson's disease therapy; In vitro release; In vivo release; Release mechanism interaction

1. Introduction

Levodopa, as a dopamine precursor, is prescribed for the treatment of Parkinson's disease. For the prevention of the dopamine peripheral side effects, carbidopa, a decarboxylase inhibitor not crossing the blood–brain barrier, is co-administered. Levodopa methyl ester hydrochloride (LDME) is a pro-drug of levodopa, with higher water solubility [1,2].

Parkinsonian patients require a tailored levodopa dosage and controlled drug release as an effective method for modulating levodopa plasma levels. However, prolonged release preparations could give rise to a long latency, because of the flat plasma curve without an early peak of levodopa. Clinical data have demonstrated that the appropriate dose regimen is a balance between rapid onset and extended duration of action. In particular, the first levodopa intake of the day must provide prompt plasma levels of levodopa in order to reduce the morning blockage [3]. This therapeutic problem is often addressed by concomitant administration of immediate and controlled release dosage forms of levodopa [4]. Therefore, the development of a single dosage form able to give flexible delivery would be of value for simplifying levodopa administration.

Multi-layer matrices have been used for oral delivery of drugs [5,6]. In these systems the control of the overall release kinetics is primarily determined by the composition of each layer [7,8]. When there are more than two layers, an effect on the release rate of the relative position of the individual layers could be envisaged.

The aim of this work was to study and develop an oral controlled release system for the delivery of LDME and carbidopa in the upper part of the gastrointestinal (GI) tract. In order to introduce versatility in release kinetics, the system was designed as a three-layer matrix tablet. Each individual layer of the matrix exhibited a different release mechanism, i.e. one layer was swellable (S), the second was erodible (E) and the third was disintegrating (D). The three layers could be differently located in the monolithic matrix, giving rise to three monoliths differing for the relative layer position. It was expected that by changing the relative position of each layer in the three-layer

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monolith, the release kinetics would be affected by the mutual interactions of the closest layers. In such a way it could be possible to have three delivery systems having the same composition but different kinetic performance, simply by changing the relative position of the layers.

The in vitro release kinetics of LDME and carbidopa from monolithic three-layer tablets, differing for the relative position of the individual layers, have been studied and compared with in vivo behaviour.

2. Materials and methods

LDME (water solubility at 20 °C: ≈500 mg/ml) (Chiesi Farmaceutici, S.p.A., Parma, Italy) and carbidopa monohydrate (water solubility at 20 °C: ≈2.5 mg/ml) (Chiesi Farmaceutici, S.p.A., Parma, Italy) were used as received. All the excipients used were of pharmacopoeial grade.

Three granulates, each one corresponding to a layer, were prepared in the same conditions by wet granulating with povidone solution 10% w/v in ethanol; the ingredients are reported in Table 1. The composition of the layers was formulated in order to obtain a swellable layer (coded S), an erodible layer (coded E) and a disintegrating or immediate release layer (coded D). Magnesium stearate and talc in usual amounts were used as lubricant and glidant for tabletting.

Three-layer monolithic tablets were manufactured in a single punch tabletting machine (EKO, Korsh, Berlin, Germany), by layering in the die (diameter 12 mm) the amount of each granulate, according to the active principle doses reported in Table 1, and compressing with concave cylindrical punches. The monoliths were compressed to a thickness of 6.0 ± 0.3 mm. The weight of each monolith was 709 mg. All monoliths contained 376.8 mg of LDME, corresponding to 300 mg of levodopa, and 81 mg of carbidopa monohydrate, corresponding to 75 mg of carbidopa. They were identified as high-strength monoliths.

Table 1
Composition of swellable, erodible and disintegrating layers

	Layer composition (mg)
Swellable (S)	
LDME HCl	201
HPMC (Methocel K15M)	56.6
PVP (in 10% w/v ethanol solution)	11.3
Talc	11.3
Magnesium stearate	2.8
Total weight	*283*
Erodible (E)	
LDME HCl	50.2
Carbidopa H_2O	54
Glyceryl palmitostearate	11.7
Lactose	100.6
Potassium methabisulfite	1.1
PVP (in 10% w/v ethanol solution)	4.7
Talc	9.4
Magnesium stearate	2.3
Total weight	*234*
Disintegrating (D)	
LDME HCl	125.6
Carbidopa H_2O	27
Croscarmellose sodium	7.8
Potassium methabisulfite	1
PVP (in 10% w/v ethanol solution)	1.9
Microcrystalline cellulose	19
Talc	7.8
Magnesium stearate	1.9
Total weight	*192*

For comparison purposes single layer tablets of each granulate were prepared as well.

In addition, a reduced-strength monolith, in configuration DSE, weighing 474 mg (two-thirds of the high-strength monolith) and containing 251.2 mg of LDME, corresponding to 200 mg of levodopa, and 54 mg of carbidopa monohydrate, corresponding to 50 mg of carbidopa, was prepared by compressing the layers to a thickness of 5.6 ± 0.06 mm using a punch set of 10 mm diameter.

In vitro release experiments were carried out at 37 °C in the USP dissolution Apparatus II (Esadissolver, Advanced Products, Milan, Italy) with paddle rotating at 100 rev./min. As dissolution medium in the first hour, 500 ml of artificial gastric fluid at pH 1.2 without enzymes (USP 24) was used. Throughout the following 4 h, 500 ml of phosphate buffer at pH 5.5 was employed. Nitrogen was bubbled in the medium during dissolution in order to avoid the oxidative degradation of carbidopa. The amount of drug released was determined by HPLC (integrator C-R6A, Shimadzu, Japan; pump LC-10AS Shimadzu, Japan; injection valve Rheodyne, Cotati, CA; detector UV-VIS at 280 nm, SPD-10A, Shimadzu, Japan; column NovaPack C18, 3.9 × 150 mm, Waters, Milford, MA; injection loop 20 μl; phosphate buffer at pH 3.3 as mobile phase).

An open randomized, single dose, three-way crossover pilot bioavailability study in six healthy volunteers was carried out on the two monoliths SDE and DSE of the high-strength dosage form. As reference treatment, one tablet of the controlled release Sinemet® CR (200 mg of levodopa and 50 mg of carbidopa, DuPont Pharma, Firenze, Italy) and half a tablet of the immediate release Sinemet® (corresponding to 125 mg of levodopa and 12.5 mg of carbidopa) were concomitantly administered. Thus, the total dose administered was 325 mg of levodopa and 62.5 mg of carbidopa.

A second open single dose one-way pilot study in the other six healthy volunteers was subsequently carried out with the DSE reduced-strength monolith.

The blood samples collected at fixed times were maintained in ice in order to avoid the hydrolysis of LDME. Plasma was separated by centrifugation at 4 °C for 10 min at 1500 × *g*. An aliquot of 0.8 ml of plasma was thoroughly mixed in a vial containing 20 μl of a 20% solution of sodium methabisulfite and 40 μl of a 70% solution of perchloric acid. The obtained samples were kept in ice for 15 min, then centrifuged at 4 °C for 15 min at 1250 × *g*. Two aliquots of 200 μl each of the supernatant were withdrawn

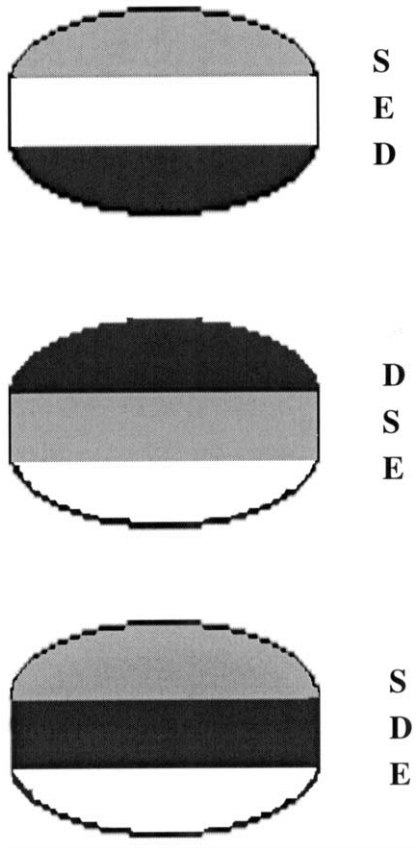

Fig. 1. Scheme and codes of the three monolithic tablets.

and analyzed by HPLC according to the method described by Rondelli et al. [9]. The results were expressed as levodopa and carbidopa concentrations.

The two clinical protocols were approved by the ethical committees of the San Raffaele Hospital, Milan, Italy and AAI/LAB, Germany.

The pharmacokinetic and statistical analysis was performed using the SAS® 6.08 software (SAS Institute, Cary, NC).

3. Results and discussion

The drug delivery system studied was a three-layer monolithic matrix in which each layer included different amount of drugs and exhibited different release kinetics. The three granulates composing each monolith layer were formulated in order to parcel-out the delivery of the drug dose by means of different release control mechanisms. Therefore, one layer (code D), designed to promptly deliver one-third of the total dose of LDME and carbidopa, was formulated for immediate disintegration. A second layer (code E), containing the remaining part of the carbidopa dose and a small fraction (13% w/w) of LDME dose, designed for a quasi-constant release rate, was formulated as an erodible matrix. The third layer (code S), containing the remaining part of the LDME dose, was formulated as a swellable matrix to prolong drug release under swelling and diffusion control. Carbidopa was not included in this slow release layer due to its long half-life. Changing the position of the swellable, erodible and disintegrating layers, three different three-layer monoliths were produced and coded as SDE, DSE and SED, respectively (Fig. 1).

To accomplish the drug delivery from the three-layer monolith in the first part of the GI tract [10], layer compositions were adjusted in order to have more than 85% of drug delivered in vitro in less than 5 h [11].

Preliminarily, each granulate was compressed as individual tablets in order to study the peculiar release kinetics of each layer. In Fig. 2, LDME (Fig. 2a) and carbidopa (Fig. 2b) release curves from the tablets prepared with each individual layer granulate are reported. From the tablet having the composition of a disintegrating layer, the two drugs were promptly and completely released in 10 min. The tablet having the composition of an erosive layer showed a quick delivery of both drugs in 1 h. More than 80% of LDME was released in 2 h from the tablet prepared with the swellable layer granulate, with the kinetics characterized by an initial fast release rate progressively slowing down with time. When the three individual layers were dissolved

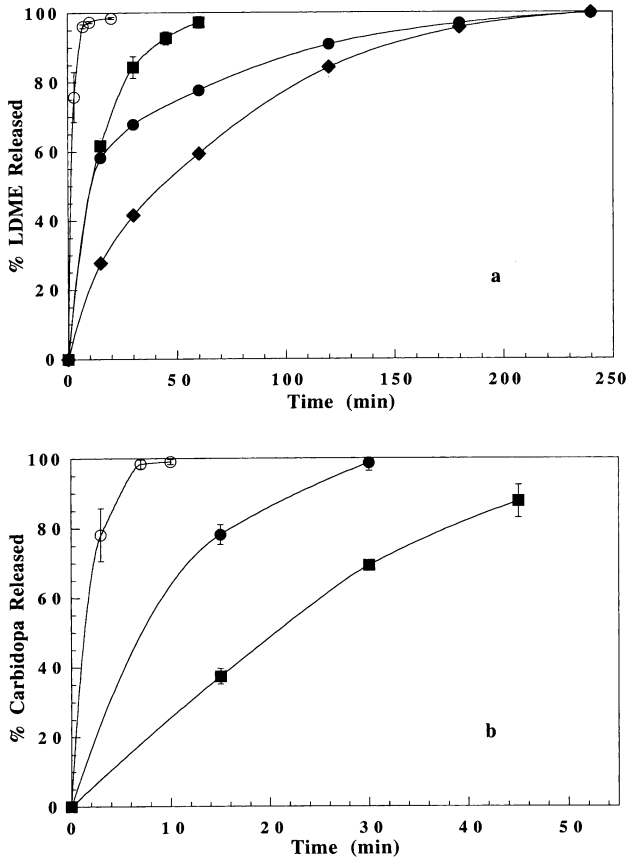

Fig. 2. LDME (a) and carbidopa (b) in vitro release from individual layers: S (diamond), D (empty circle), E (solid square), all layers together (solid circle). Mean values ± standard deviation are shown ($n = 3$)

together in the same vessel, any interaction between the individual layers was expected. In fact, the resulting drug release profile corresponded to that obtained cumulating the amount individually released from each layer.

The preparation of a three-layer tablet gave rise to three monoliths, differing for the middle layer position. The in vitro release experiments performed on the high-strength three-layer monoliths (Fig. 3) showed that the relative position of the layer could determine different release profiles. In the monoliths where the disintegrating layer was in one of the external positions (DSE and SED), the drug release profiles obtained were not significantly different to each other. When the disintegrating layer, D, was compressed between the swellable and erodible layers, as in the SDE monolith, the release curves were significantly different than with the previous two configurations. In this case a more linear profile and lower release rate were observed.

In all cases, LDME and carbidopa (data not shown) were released from each monolith with the same kinetics.

The release curves of the three-layer monoliths were substantially different from the simple sum of the individual layer release, as can be easily seen by comparing the amount of drugs released from each individual layer (see Fig. 2).

The explanation of this behaviour was the different area exposed to dissolution medium by each layer, dependent on its position in the monolith. In fact, the area of release of each layer was restricted in the monolith configuration and the extent of area exposed to the dissolution medium depended on the behaviour of the adjacent layer. For example, when the disintegrating layer was located in one of the two possible external positions (DSE or SED), its prompt disintegration quickly exposed to dissolution medium the swellable or the erodible layers in the middle position. As a consequence, equivalent two-layer monoliths were generated in both cases, justifying their non-significantly different release kinetics.

An unexpected result was obtained with the SDE monolith, in which the disintegrating layer, compressed between

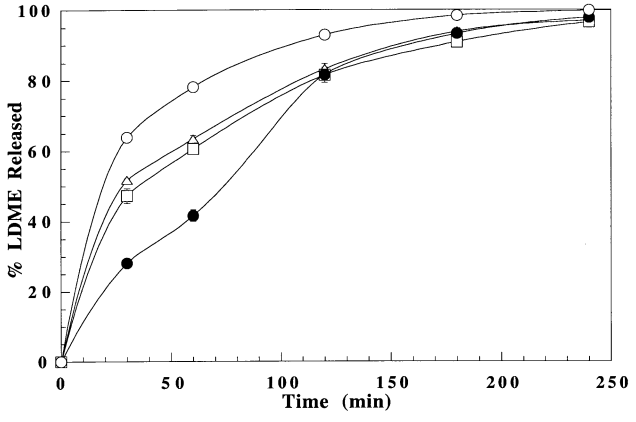

Fig. 3. LDME in vitro release from Sinemet® CR + 1/2 Sinemet® (empty circle) and three-layer monoliths: SDE (solid circle), DSE (square), and SED (triangle). Mean values ± standard deviation are shown ($n = 3$).

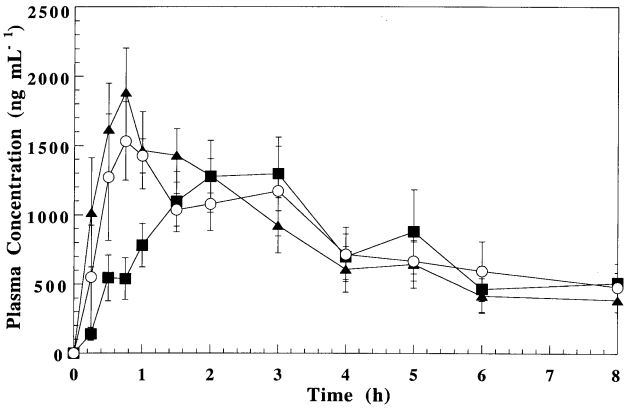

Fig. 4. Levodopa plasma profiles after single administration of high-strength SDE (square), DSE (circle) and reference formulations (triangle) in volunteers. Mean values ± standard deviation are shown ($n = 6$).

the other layers, exposed to the medium a small surface area. It could have been predicted in this case that the disintegration of the middle layer would have split the monolith in two pieces, i.e. the swellable and the erodible layers. On the contrary, it was visually observed that the important swelling of the overlaid layer, S, provided quite a coating on the middle layer, D, slowing down its disintegration and delaying for more than 1 h the splitting of the monolith in two halves. Therefore, in the dissolution condition adopted, the location of the disintegrating layer in the middle or in the external position of this three-layer monolith determined an important difference in in vitro release behaviour. As a consequence of the change in the layers' relative position, a combination of the release mechanisms in the monolith occurred, thus giving rise to release kinetics that could not be simply anticipated on the basis of the single layer behaviour. This phenomenon, related to the disintegrating and swellable layer proximity, could be considered quite an interaction, affecting the release rate of the three-layer monolith having the disintegrating layer in the middle position.

Since the monoliths DSE and SDE, despite the same composition, delivered the two drugs with different kinetics, they could be used alternatively in Parkinson treatment for satisfying the need of a fast or slow delivery.

In order to verify if the behaviour observed in vitro could be found also in vivo, pilot pharmacokinetic studies were carried out on SDE and DSE systems. The levodopa plasma levels obtained from the two high-strength monoliths are reported in Fig. 4. Plasma concentrations of levodopa methyl ester were always under the limit of quantification (<30 ng/ml), confirming its rapid pre-systemic hydrolysis to levodopa. As reference treatment, a classically prescribed combination of two different dosage forms, i.e. a controlled release and an immediate release form of Sinemet®, was used. The in vitro release kinetics of the Sinemet® CR + 1/2 Sinemet® is reported in Fig. 3.

The DSE monolith gave rise to a levodopa plasma profile significantly different from the SDE monolith, in terms of the rate of absorption (Fig. 4). With the DSE monolith having the

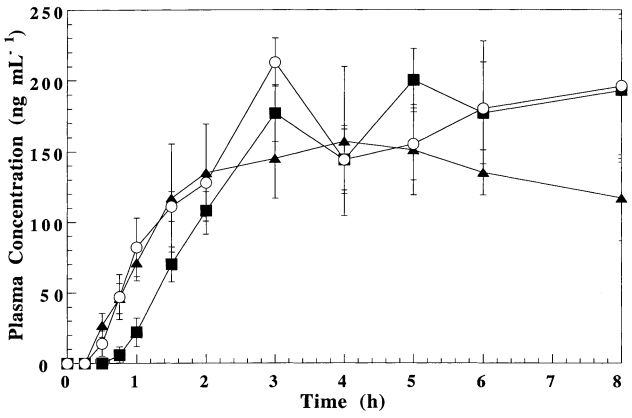

Fig. 5. Carbidopa plasma profiles after single administration of high-strength SDE (square), DSE (circle) and reference formulations (triangle) in volunteers. Mean values ± standard deviation are shown ($n = 6$).

disintegrating layer externally, a levodopa peak, followed by a prolonged drug plasma level, was observed. On the contrary, from the SDE monolith with the disintegrating layer in the middle the peak of levodopa was delayed, giving rise to a plateau of drug plasma concentration.

The reference formulation, combining the immediate and delayed release dosage forms, was significantly mimicked by the DSE monolith, both exhibiting a levodopa peak followed by a sustained release. Therefore, this monolith behaved both as a prompt and delayed delivery system of levodopa.

Fig. 5 shows the corresponding carbidopa plasma concentration profiles. Both monoliths sustained the plasma level of carbidopa for a prolonged period of time, mainly due to the long half-life of carbidopa. The DSE system showed a carbidopa plasma level not different from the reference treatment, while with the SDE monolith a delayed onset was observed.

Pharmacokinetic parameters for levodopa and carbidopa are reported in Tables 2 and 3, respectively. The $AUC_{0-8\ h}$ normalized by dose for levodopa and carbidopa of the two monoliths and the reference formulation were non-significantly different, suggesting equivalence in blood concentration profiles between the tested and reference formulations.

However, $AUC_{0-1\ h}$/dose from SDE, both for levodopa and carbidopa, was significantly lower ($P < 0.05$) than DSE and reference treatment, thus indicating a non-equivalent rate of absorption.

We also tested a lower-strength DSE monolith that could be of advantage for tailoring the therapy for parkinsonian patients requiring a lower dosage. This low dose system possessed the same configuration of the high-strength dosage form, but a different size. The in vitro release curve exactly overlapped that of the corresponding higher-strength monolith (data not shown). The levodopa plasma profiles were similar in shape to that of the higher-strength DSE. T_{max} (0.83 ± 0.54 h), C_{max}/dose (9.76 ± 1.46 ng/mg ml) as well as AUC normalized by dose (AUC_{0-1h}/dose = 5.79 ± 1.32 ng h/mg ml; AUC_{0-8h}/dose = 26.43 ± 5.52 ng h/mg ml) were not significantly different from the corresponding values of the high-strength monolith (see Table 2). Similar considerations could be made for carbidopa (AUC_{0-1h}/dose = 0.58 ± 0.31 ng h/mg ml; AUC_{0-8h}/dose = 11.38 ± 4.9 ng h/mg ml; C_{max}/dose = 2.36 ± 0.73 ng/ml; T_{max} = 3.5 ± 0.55 h).

Results obtained indicated that in vivo data reflected quite well the in vitro behaviour of the two monoliths tested. In fact, the monolith that presented the fastest dissolution profile, i.e. DSE, gave rise in vivo to a rapid appearance of levodopa plasma levels, whereas the slower dissolving monolith, i.e. SDE, exhibited a smoothed plasma concentration profile.

The data confirmed the relevance of the middle position for the behaviour of these three-layer monoliths. The drug release from monoliths DSE and SED was scarcely influenced by the disintegrating layer that, in both cases, was in the external position. On the contrary, the location of the disintegrating layer in the middle position, due to the described interaction with the swellable layer, resulted in a prolonged delivery of levodopa and delayed delivery of the carbidopa dose.

4. Conclusion

A new versatile delivery system for oral administration of LDME and carbidopa has been prepared. The system consists of a three-layer matrix tablet, where each layer is characterized by its own delivery rate and kinetics. In the three-layer monolith, the drug release kinetics depends on the composition of layers and their relative position in the monolith. Interactions between layers, dependent on their relative position, have been observed in vitro. These were

Table 2
Pharmacokinetics parameters for levodopa (mean ± standard deviation) obtained after administration of the three formulations

	Sinemet® CR + 1/2 Sinemet®[a]	SDE	DSE
C_{max}/dose (ng/mg ml)	6.62 ± 1.71	6.16 ± 141	7.25 ± 1.4
T_{max} (h)	1.17 ± 0.97	2.75 ± 1.25	1.88 ± 2.25
$AUC_{0-1\ h}$/dose (ng h/mg ml)	4.04 ± 1.86	1.34 ± 0.83[b]	3.39 ± 1.82
$AUC_{0-8\ h}$/dose (ng h/mg ml)	20.10 ± 3.69	20.60 ± 3.07	22.08 ± 1.48

[a] Reference treatment.
[b] Difference statistically significant with respect to reference treatment and DSE, $P < 0.05$.

Table 3
Pharmacokinetics parameters for carbidopa (mean ± standard deviation) obtained after administration of the three formulations

	Sinemet® CR + 1/2 Sinemet®[a]	SDE	DSE
C_{max}/dose (ng/mg ml)	3.48 ± 1.9	3.17 ± 1.3	3.46 ± 1.04
T_{max} (h)	4.92 ± 2.25	6.17 ± 1.60	5.67 ± 2.25
$AUC_{0-1\ h}$/dose (ng h/mg ml)	0.43 ± 0.22	0.05 ± 0.08[b]	0.34 ± 0.25
$AUC_{0-8\ h}$/dose (ng h/mg ml)	15.63 ± 6.37	14.74 ± 3.36	15.54 ± 4.60

[a] Reference treatment.
[b] Difference statistically significant with respect to reference treatment and DSE, $P < 0.05$.

relevant when the disintegrating layer occupied the middle position, since the proximity of the swellable layer affected its disintegration.

The differences between the in vitro release kinetics of the three-layer monoliths were confirmed in vivo. Therefore, two differently performing systems in vivo have been obtained by simply changing the relative positions of the layers in a monolithic three-layer tablet, without modifying the composition.

For therapeutic use, the DSE monolith, having the swellable layer in the middle, can be useful for the reduction of the morning on–off fluctuation, because of the early levodopa plasma peak. The SDE monolith, showing prolonged release properties, may be useful for the afternoon administration, in order to avoid end-of-dose deterioration. They can be combined in a product having a morning and afternoon administration schedule, in order to tailor better the levodopa Parkinson therapy to individual patient requirements.

Acknowledgements

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References

[1] C. Marrel, G. Boss, H. van de Watterbemb, D. Cooper, P. Jener, C.D. Marsden, B. Testa, L-DOPA esters as potential prodrugs, Eur. J. Med. Chem.-Chim. Ther. 20 (1985) 459–465.

[2] H.D. Kao, A. Trabouisi, S. Itoh, L. Dittert, A. Hussain, Enhancement of the systemic and CNS specific delivery of L-DOPA by the nasal administration of its water soluble prodrugs, Pharm. Res. 17 (8) (2000) 978–984.

[3] F. Stocchi, N.P. Quinn, L. Barbato, P.N. Patsalos, M.T. O'Connel, S. Ruggeri, C.D. Marsden, Comparison between a fast- and a slow release preparation of levodopa and a combination of the two: a clinical a pharmacokinetic study, Clin. Neuropharmacol. 17 (1994) 1–7.

[4] J.M. Cedarbaum, H. Kutt, F.H. McDowell, A pharmacokinetic and pharmacodynamic comparison of Sinemet CR (50/200) and standard Sinemet (25/100), Neurology 39 (1989) 38–44.

[5] U. Conte, L. Maggi, Multi-layer tablets as drug delivery devices, Pharm. Tech. Eur. February (1998) 18–25.

[6] R.A. Fassihi, W.A. Ritschel, Multiple-layer, direct-compression, controlled-release system: in vitro and in vivo evaluation, J. Pharm. Sci. 82 (1993) 750–754.

[7] P. Colombo, P.L. Catellani, N.A. Peppas, L. Maggi, U. Conte, Swelling characteristics of hydrophilic matrices for controlled release: new dimensionless number to describe the swelling and release behaviour, Int. J. Pharm. 88 (1992) 99–109.

[8] R. Bettini, P. Colombo, G. Massimo, P.L. Catellani, T. Vitali, Swelling and drug release in hydrogel matrices: polymer viscosity and matrix porosity effects, Eur. J. Pharm. Sci. 2 (1994) 213–219.

[9] I. Rondelli, D. Acerbi, F. Mariotti, P. Ventura, Simultaneous determination of levodopa methyl ester, levodopa, 3-O-methyldopa and dopamine in plasma by high-performance liquid chromatography with electrochemical detection, J. Chromatogr. B 653 (1994) 17–23.

[10] A.J. Moës, Gastroretentive dosage forms, Crit. Rev. Ther. Drug Carrier Syst. 10 (1993) 143–195.

[11] P. Chiesi, P. Ventura, D. Acerbi, R. Musa, R. Bettini, G. Caponetti, P.L. Catellani, Int. Patent Appl. # PCT/EP/06228 (Int. Publ. # WO99/17745).