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Bioavailability studies of oral dosage forms containing levodopa and carbidopa using column-switching chromatography followed by electrochemical detection†

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A reliable multi-dimensional column chromatographic method employing amperometric detection using a carbon fibre microelectrode procedure was used for monitoring the plasma profiles and to evaluate the pharmacokinetics and bioavailability of levodopa (L-dopa) and carbidopa (C-dopa), after ingestion of oral formulations containing these drugs. The peak currents obtained for the different analytes were directly proportional to the analyte over the concentration range 0.02–4 μg ml^{-1}. Using this method, the minimum detectable concentration was estimated to be 5 and 8 ng ml^{-1} for L-dopa and C-dopa, respectively. Recovery studies ranged from 93.83 to 89.76%, with a relative standard deviation of less than 7%. The study was carried out in two separate weeks on five healthy non-patient fasted male/female volunteers in the age range 20–37 years and weighing between 60 kg and 78 kg. The pharmacokinetic profile of two controlled-release products containing both L-dopa and C-dopa (Sinemet CR3 and CR4) was compared on the one hand and Sinemet conventional tablets on the other. The pharmacokinetic parameters, peak concentration (C_{max}), the time taken to obtain this level (T_{max}), elimination half-time $T_{1/2}$, elimination rate constant (K_{el}), plasma level ratio, fluctuation index (FI) and the area under the time–concentration curve ($AUC_{0–8}$), were investigated for each individual formulation. A comparison of the uptake of L-dopa from the conventional formulation showed that L-dopa entered the plasma and achieved peak levels higher than that of the controlled release formulations. However, it showed a much higher fluctuation index and the plasma concentrations were more stable with the controlled release formulations. The data also indicated a very low accumulation of both levodopa and carbidopa following repeated administration of the drugs, which was consistent with their relatively short half-lives (less than 2 h). In contrast, the half-life for the metabolite 3-orthomethyl dopa (3-OMD) is in the order of 13 h. As a result, there was an extensive accumulation of 3-OMD and its levels were significantly higher than those of levodopa or carbidopa upon repeated administration. Urine recoveries of the three analytes over one 8 h dosing interval showed that the majority of the excreted levodopa and carbidopa was recovered during the first 4 h, and there is proportionally greater excretion of the carbidopa dose than the levodopa dose.

Introduction

Carbidopa [C-dopa; L-α-hydrazino-α-methyl-β-(3,4-dihydroxybenzene)-propionic acid monohydrate] is used in conjunction with levodopa [L-dopa; L-α-amino-β-(3,4-dihydroxybenzene)-propionic acid] (Fig. 1) to diminish the conversion of L-dopa to dopamine in the peripheral tissues, thus permitting low doses to be used effectively for the treatment of parkinsonism and reducing the relative incidence of side-effects.[1–3] A change in the concentration of one or both drugs in the body may influence the bioavailability and biopharmaceutic properties of the pharmaceutical preparation and subsequently their magnitude of action.[1,4] In the presence of C-dopa, the primary metabolite of L-dopa in the peripheral circulation is 3-*o*-methyldopa (3-OMD). This compound has been implicated in the dose-related side-effects of chronic L-dopa therapy.[5] The understanding of the relationship between plasma levels of 3-OMD and L-dopa and the therapeutic response to L-dopa in patients has come under increasing scrutiny, since it has been reported that co-administration of 3-OMD reduces the clinical effect of L-dopa in the brain.[6] It is also necessary to measure 3-OMD in plasma in order to assess whether a modification of drug delivery mode would affect the rate of decarboxylation inhibition as manifested in the time profiles of this primary metabolite. Following long-term L-dopa therapy patients may develop clinical fluctuations in motor response, due to the rapid fluctuations in L-dopa plasma levels.[7,8] Such an effect is a major problem in the treatment of Parkinson's disease;[4,9,10] using a properly designed delivery system can achieve a constant plasma concentration of these drugs and often results in an improved clinical response and can reduce the fluctuation phenomenon and stabilise the symptoms.[1,9,10] Conventional oral dosage forms often produce fluctuations of drug plasma levels that either exceed safe therapeutic levels or quickly fall below the minimum effective level; this effect is usually totally dependent on the particular agent's biological half-life, frequency of administration, and release rate. It is now recognised that studying drug bioavailability of the dosage form is clinically useful in Parkinson's disease.[10,11] The study of the bioavailability and the drug delivery system can minimize the factors that can affect absorption of L-dopa and C-dopa.

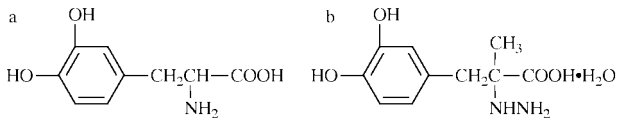

Fig. 1 Basic structural formulae of levodopa (a) and carbidopa (b).

To study the bioavailability and biopharmaceutical properties of these formulations, it became necessary to develop reliable methods for the simultaneous determination of these drugs and their metabolites. One of the most significant advances in quantitation techniques has been the development of electro-

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chemical detectors combined with high-performance liquid chromatography (HPLC-EC) which can offer the capability of selective and sensitive determination of electro-active oxidizable or reducible drug products, with little interference from biological matrix interferences[12,13] and with low limits of detection down to picogram quantities.[14] Pre-column switching has recently become a widespread means of improving the sample throughput, separation efficiency and, if the precolumn is used for concentration purposes, also the loadability of liquid chromatographic systems.[15,16]

In this study we investigated a comparison of drug release and bioavailability from different oral controlled-release and conventional oral formulations containing levodopa and carbidopa. We monitored the plasma profiles and evaluated the pharmacokinetics and availability of these drugs in biological fluids, using reliable multi-dimensional column chromatography employing amperometric detection with a carbon fibre microelectrode procedure.[17]

Experimental

Chemicals and reagents

All reagents and chemicals were ACS Reagent Grade and were used without purification. Sodium dihydrogen orthophosphate was purchased from Fluka (Buchs, Switzerland). Orthophosphoric acid was supplied by BDH (BDH Ltd., Poole, Dorset, UK). All other chemicals including L-dopa and C-dopa used for *in vitro* studies were obtained from Sigma (Pool, Dorset, UK). Water was distilled and then further purified by passing through a Milli-Q water purification system (Millipore, Milford, MA, USA). Plasma samples were obtained from normal human volunteers. Heparinized plastic tubes used for whole blood collection 'LiHeparin Menovette', were supplied by Sarstedt (Numbrecht, Germany). Carbon fibres, 14 μm in diameter, were obtained from AVCO (Lowell, MA, USA). The surface of these fibres has no external coating. Silver epoxy was purchased from RS components (Corby, Northants., UK) and the glassy carbon electrode used in the comparison studies was obtained from EG&G Princeton Applied Research (Princeton, NJ, USA).

HPLC mobile phase

The mobile phase used was a mixture of 16.5 g of sodium dihydrogen orthophosphate, 980 ml of water, 1.0 ml of 0.10 M disodium ethylenediaminetetraacetic acid (EDTA), 20 ml of methanol and 1.2 ml of 0.5 mM sodium heptanesulfonate (HAS) with the pH adjusted to 3.4 with 1 M orthophosphoric acid. The a flow rate was 1 ml min^{-1} at ambient temperature.

The mobile phase was filtered through a pore size 0.45 μm cellulose membrane filter (HVLP, Millipore, Milford, MA, USA), degassed under reduced pressure. The EDTA was used to sequester any oxidisable metal ions.

HPLC columns

An octyl-bonded/reversed phase column (12.5 cm × 4.0 mm id; 5 μm particle size, Lichrosorb RP-8 Hibar; Jones Chromatography, Lakewood, CO, USA) was used for the purpose of the purification of deproteinized plasma and urine samples as well as for preconcentration of the different analytes. The second column, 25.0 cm × 4.0 mm id, 5 μm reversed-phase column (Ultrasphere), was used for the separation of the compounds eluted from the concentration column. A 2 μm pre-column filter (Rheodyne, Cotati, CA, USA) was also used. The column was normally operated at a back-pressure of 93 bar. The analytical column was primed by recycling the mobile phase overnight at a flow-rate of 0.3 ml min^{-1}. At the end of a use period, the column was washed free of mobile phase components with 400–500 ml of water and was then returned by means of a gradient to 70% methanol for storage. The column in use retained good efficiency and resolution for over six months despite prolonged exposure to aqueous solutions, and the matrix effects of the biological samples used.

Equipment

Plasma centrifugation was performed on a Microfuge 12 microcentrifuge from Beckman Instruments (Fullerton, CA, USA). All analyses were performed on an HPLC Waters (Stockport, UK) system consisting of two pumps (Waters Model 501); sample introduction was *via* a Rheodyne Model 7125 injection valve, fitted with a 25 μl loop for direct injection. For the purpose of extraction by column switching,[18,19] the injection valve was fitted with a 1 ml loop and a second pump (pump A), and the concentration column was connected to the analytical assembly *via* a Rheodyne Model 7000 six-port switching valve. Oxidative amperometric measurements were performed using a Model 400 EC potentiostat (EG&G) connected to the flow cell by crocodile clips. The resultant signals were recorded on a Philips Model PM 8261 *x-t* recorder (Eindhoven, The Netherlands) at a chart speed of 33 mm h^{-1}. The peak currents (measured as peak heights on the recorder) as a function of concentration were then measured for quantitative analysis.

Fabrication of micro-electrode flow cell

The method used in construction of the microflow cell was as fully described previously.[17,20] Placing the auxiliary electrode in this position reduces the uncompensated resistance to a negligible value even with the low ionic strength of the mobile phase in use. With such a low uncompensated resistance the interfacial electrode potential is not significantly influenced by sample concentration, and a wide linear range was obtained.

Standard solutions, linearity and recovery studies

Plasma standards were prepared from blank human plasma spiked with L-dopa, C-dopa, and their metabolites (3-OMD, and 3-OMC; 3-orthomethylcarbidopa), in the range 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, and 4.0 μg ml^{-1}, which is enough to cover the range of expected patient values (usually up to 3.5 μg ml^{-1}. Aliquots of the drug in plasma (1 ml) were introduced *via* the injector port. Each calibration point was run in triplicate over five consecutive days, and the observed RSD for each standard curve was calculated from the least-squares regression equations. The overall performance of the system was verified periodically by injection of standard samples. The extraction efficiency and recovery of the assay was assessed at concentrations of 0.5, 1.0 and 1.5 μg ml^{-1}. The recovery of both drugs from plasma was assessed by comparing the peak current of the extracted plasma samples with the peak current of authentic (unextracted, aqueous) standards, which were directly injected (*i.e.*, without column switching) onto the analytical column at the same concentration levels.

Clinical experiments

This study was carried out in two separate weeks on five healthy non-patient fasted male/female volunteers in the age range 20–37 years and weighing between 60 and 78 kg. A written informed consent was obtained from each subject. The

volunteers were required to pass a routine medical examination which included ECG and routine standard biochemical and haematological screens; the criteria for exclusion included the presence of a post history of active disease, major illness, the use of any medication 24 h prior to the study, the chronic use of any medication, and any history of drug abuse or hypersensitivity to either of the drugs. Any major illness or surgery occuring during the study would terminate the subjects' involvement.

The time-course of both drugs and their main metabolite concentrations was monitored in the volunteers after receiving repeated 8 h administration of Sinemet conventional tablets, controlled-release formulation of Sinemet CR3 and CR4 '50–200mg' (F. Hoffmann-La Roche, Basel, Switzerland).

Stability studies

In these studies human plasma samples transferred to a polyethylene tube containing 10 mg of sodium metabisulfite were spiked with 0.8 μg ml^{-1} L-dopa. The plasma was centrifuged for 60 s at 1500 rpm (1000*g*) to remove protein residues. The sample was then divided into sub-samples and stored at +4, −30 and −70 °C; concentration values from these sub-samples were determined in triplicate over a one month period on days 2, 5, 10, 15, 20, 25 and 30. The stability of the electrode and the analytes were assessed using these values.

Selectivity studies

These studies were carried out in order to examine the presence of any interferences from other impurities that might exist in the pharmaceutical preparations. A standard solution of each of the three compendial substances was repetitively injected while the detector operating voltage was increased from roughly 0.0 to 1.0 V in 25 mV increments and the change in peak current *versus* applied oxidation potential was observed. The applied potentials (*E*) were then plotted against the peak currents obtained.

Comparison studies

The proposed method was compared with detection involving the use of a macroglassy carbon electrode. The macroelectrode was polished successively with small particle-size silicon carbide, alumina, diamond paste and then subjected to ultrasonic cleaning.[21] Replicates of the same set of samples which contained a range but unknown concentrations of the drugs were analyzed using the glassy carbon electrode and the system was developed. The mean values found using the carbon fibre flow cell were plotted against the mean values obtained using the conventional glassy carbon electrode. The results were analysed statistically using the linear regression analysis of the one set of assay results *versus* the other.[22,23]

Results and discussion

Optimisation of analyte separation in human plasma

The microelectrodes used were fabricated from carbon fibres, the diameter of which is 14 μm. There are two prominent features of the electrochemistry attained at these electrodes: first, their small size results in predominantly diffusion-controlled currents that are time-independent within a very short time of the initial application of potential; secondly, surface conditions can contribute significantly to the electrochemical reactions at the electrode. Each of the analytes contains a phenol or catechol functionality, which can be easily detected by direct anodic oxidation at relatively low potential.[14] The detector operated in amperometric mode with a fixed potential sufficiently positive enough to force the ortho-hydroxy groups to undergo loss of two electrons and two protons, yielding orthoquinone. A single anodic peak obtained in the voltammetric range studied verified that both compounds underwent a single oxidation process within this range. A working potential of +0.8 V afforded a significant reduction in background current, which resulted in better signal-to-noise ratios and this potential was selected for the reminder of the LC-EC studies.

Reverse-phase chromatography appears to be the most useful in the analysis of mixtures of such substances which possess both acidic and basic organic functional groups.[22] Deproteinized plasma samples were injected directly into the HPLC system; every four samples were followed by a standard. The first step in the set-up of the switching/separation system involved the selection of a suitable pre- (or concentration) column, which would retain the analytes while other endogenous components were eluted to waste. Among the different columns used for this purpose, an Octyl-bonded reversed phase column (12.5 cm × 4.0 mm id; 5 μm particle size, Lichrosorb RP-8 Hibar) showed the most favourable retention characteristics for both drugs and their metabolites. The next stage was to find two compatible eluents of different elutropic strengths.[23] One should have a poor elution capability to concentrate the analytes on the pre-column and the second a strongly eluting solvent to elute the analytes off the pre-column and onto the analytical column. The washing eluent should also be miscible with the mobile phase, as even slight incompatibility could result in a plug of solvent travelling down the analytical column partially carrying sample components which may cause band broadening. The use of a 0.05 M solution of perchloric acid allowed the purification of plasma samples by washing out neutral and acidic compounds while the analytes (containing amino groups) were retained by the pre-column. A wash time (defined as the length of time between injection and switching of the valve) of 1.5 min was found to be satisfactory to provide a good clean-up of the plasma components without causing the analytes to elute. Fig. 2 shows the chromatogram of a spiked plasma sample containing 0.3 μg ml^{-1} of L-dopa/3-OMD and 0.35 μg ml^{-1} C-dopa/3-OMC. The figure clearly indicates that under the experimental conditions, both drugs were well resolved from their main metabolites and the endogenous plasma peaks. The importance of separating and detecting these metabolites lies in the side effects caused by their presence during long term therapies. The mobile phase was optimised by careful examination of all major variables, including pH and ionic strength, to determine their influence on enhancing peak separation without prolonging the analysis time for each sample. In this case, an acidic pH (3.04) proved optimal; modest pH adjustments can produce significant changes in the retention time characteristics of these substances. Side-effects (dyskinesia, on-off phenomena and psychoses) is attributed to the high levels of 3-OMD in plasma and/or the plasma 3-OMD/L-dopa ratio.[24] Furthermore hypokinesia (a situation associated with low plasma levels of levodopa) can occur due to a competition for transport across the blood–brain barrier between L-dopa and 3-OMD;[25,26] the determination of *o*-methylated products might be of clinical significance in psychiatric disorders.[27] Other metabolic pathways are unimportant.[28] Linear calibration graphs of peak heights of the standards *versus* the concentration of the standards were constructed by analysing spiked blank plasma over five days in the concentration range 0.02–4.0 μg ml^{-1}; within this range, the resulting current was directly proportional to the concentration of the oxidisable species. The curves showed a good linearity between the detector response and the analyte concentrations. For each of the five regression lines the correlation coefficients (r^2) were all greater than 0.993. The RSD of the slopes (n = 5) of the regression lines calculated

from the least-squares regression equations, ranged from 3.0–8.74%, with intercepts all close to zero.

The limit of detection (LOD) of the method was found to be 5 ng ml^{-1} for L-dopa and 8 ng ml $^{-1}$ for C-dopa. The limit of quantitation (LOQ) was *ca.* 8 ng ml^{-1} for L-dopa and 10 ng ml^{-1} for C-dopa. This limit of quantitation is well below the values monitored in our patients and adequate for clinical analysis and pharmacotherapeutic studies. The LOQ was also comparable to those values obtained by previous workers.[29] The precision of the method based on peak height measurements was evaluated by replicate injection and exhibited a RSD of less than 6.0%. A further precision study was performed by blind analysis of three patient samples, which were analysed in duplicate on six separate occasions. Table 1 summarises these results. The mean absolute recovery achieved with the described extraction procedure was always greater than 91.0% for L-dopa and 89% for C-dopa. Within-day variabilities were established for three concentrations (0.25, 0.5 and 1.5 μg ml^{-1}) for L-dopa and C-dopa by adding the drugs to blank plasma over five consecutive days. Peak current values were interpolated on the individual regression lines to yield three new values of amount found at each concentration level for every day. Between-day variability was then calculated by obtaining the mean RSD (%); the overall assay variations at 0.25, 0.5 and 1.5 μg nl^{-1} were less than 7% for both drugs.

Acidic solutions of C-dopa can decompose in air to give 3,4-dihydroxyphenylacetone;[30] this decomposition process was avoided by the addition of the antioxidant sodium metabisulfite to the samples.

The evaluated effect of storage temperature on the stability of L-dopa is given in Fig. 3. When incubation was carried out at +4.0 °C, the graphs show that the plasma concentration found for both analytes decreased, while the concentration increased at a lower temerpature, suggesting that the storage of the samples containing these compounds at low temperature can increase the stability for at least one month. This low temperature was used subsequently to achieve maximum stability for these compounds. Acidic solutions of C-dopa can decompose in air to give 3,4-dihydroxyphenylacetone; this decomposition process was avoided by the addition of the antioxidant sodium metabisulfite to the samples.

Various possible potential foreign compounds were studied; these compounds were selected according to (a) compounds which can present as an impurity in combination with the drugs in different pharmaceutical formulations and can affect the analytical process and (b) other drugs that might be administered with these drugs. The United States Pharmacopoeia (USP)[30] specifies methyl dopa, 3-methoxytyrosine, and 3-*o*-methylcarbidopa as impurities in the analysis of L-dopa–C-dopa combination formulations. Interference from some of these substances in the determination of the drugs was tested in two different studies using the proposed technique. Hydrodynamic voltammograms can provide a qualitative identification capability by calculation of the half-wave potential ($E_{1/2}$).[14] The correlation between chemical structure and use of oxidation is clearly observed (Table 2). The trihydroxy compound has the lowest $E_{1/2}$, the dihydroxy species has an intermediate $E_{1/2}$ and the monohydroxy substance has the largest value. This information together with the identity of impurity peaks provided enough qualitative information to identify such impurities which can exist. The separation, peak height, retention times and the recovery of the analytes were not affected by the presence of these substances. Errors less than 2% were calculated by comparing the results with the correspond-

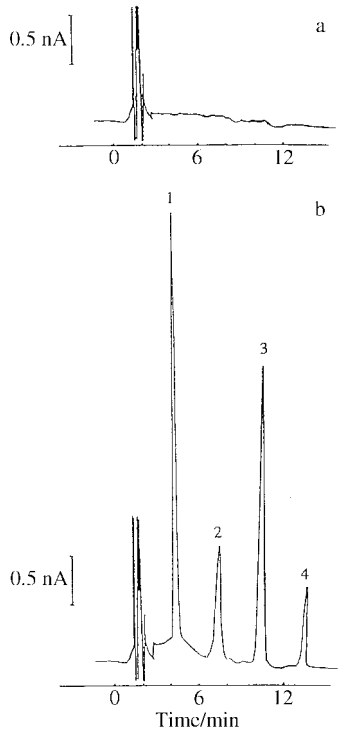

Fig. 2 A chromatogram obtained by direct injection of (a) drug-free plasma extract and (b) plasma sample containing L-dopa, 3-OMD, C-dopa and 3-OMC. Peaks: 1, L-dopa; 2, 3-OMD; 3, C-dopa; 4, 3-OMC. The conditions were: the mobile phase was a mixture of 16.5 g of NaH_2PO_4, 1.0 ml of 0.1 M EDTA, 20 ml of methanol and 102 ml of 0.5 mM sodium heptanesulfonate, pH 3.4 and a flow rate of 1.0 ml min^{-1} at ambient temperature.

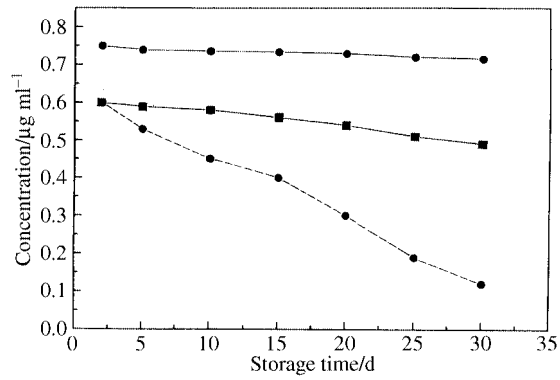

Fig. 3 Stability of L-dopa (0.8 μg ml^{-1}) in plasma samples stored at +4 (-- ● --), −30 (■) and −70 °C (— ● —) and analysed at different times.

Table 1 Precision of analysis for three patient samples

Patient	Target value/μg ml^{-1}	Mean value/μg ml^{-1}	*n*	RSD(%)
C1	0.23	0.21	6	4.30
C2	0.60	0.54	6	5.75
C3	1.00	0.93	6	4.34

Table 2 Hydrodynamic voltammetric half-wave potentials ($E_{1/2}$) of both drugs and some impurities using the different hydrodynamic voltammograms

Compound	Concentration/ μg ml^{-1}	Retention time[a]/min	$E_{1/2}$/V (*vs.* Ag_3PO_4)
Levodopa	0.4	4.2	0.40
Carbidopa	0.4	10.0	0.41
3-Methoxytyrosine	10.0	8.0	0.61
Methyl dopa	1.0	6.2	0.40
3-(3,4,6-Trihydroxy-phenyl)alanine	2.0	8.0	0.61

[a] 25 cm Reversed-phase column (Ultrasphere) at a flow rate of 1.0 ml min^{-1}.

ing results obtained with a solution containing only both drugs. Other drugs such as a tricyclic antidepressant (Desipramine) that might be co-administered with both drugs was not observed under the experimental conditions. The selectivity and the resolution of the column was enhanced by the use of a pairing-ion species (HAS), and by the admixture of an organic modifier such as methanol in the mobile phase.

Clinical results

The time courses of L-dopa, C-dopa and their metabolite concentrations for monitoring the plasma levels after the administration of Sinemet CR4 formulation is given in Fig. 4. In all the plasma level profiles a post-dosing peak plasma level followed by a mono-exponential decline of drug concentration with time was observed. The profiles show a rapid, but short-lasting, increase of the parent drugs. In contrast the methylated derivatives, unlike their parent compounds, increased less rapidly. The high levels of 3-OMD can be caused by its interfering with the penetration of L-dopa into the brain, reducing its utilisation at the synaptic level. As a result of its long half-life, plasma levels of the 3-*o*-methylated metabolite exceeded the parent drug plasma levels by as much as a factor of four for Sinemet CR3 and five for Sinemet CR4 and Sinemet. Factors of five to 10 have been reported with long-term therapy.[18] Fig. 5 provides a comparison of the plasma levels of L-dopa in healthy, fasting volunteers following the administration of the various oral formulations. The calculated peak concentration (C_{max}), the time taken to obtain this level (T_{max}), elimination half-life ($T_{1/2}$), average plasma concentrations and the areas under the plasma concentration curves (AUC) values for the different formulations of Sinemet are included in Tables 3 and 4. C_{max} and T_{max} and the average plasma concentrations were obtained from the plasma concentration–time curve, the half-time ($T_{1/2}$) values were determined from the elimination rate constant (K_{el})[19,21] by the equation $T_{1/2} = 0.693\,K_{el}$. K_{el} was calculated by least squares regression analysis of the data points in the elimination phase of the semilogarithmic plot of Sinemet concentrations in plasma *versus* time curve after oral dose.[31,32] The phamacokinetic data obtained in this study are in fairly good agreement with those previously published (healthy

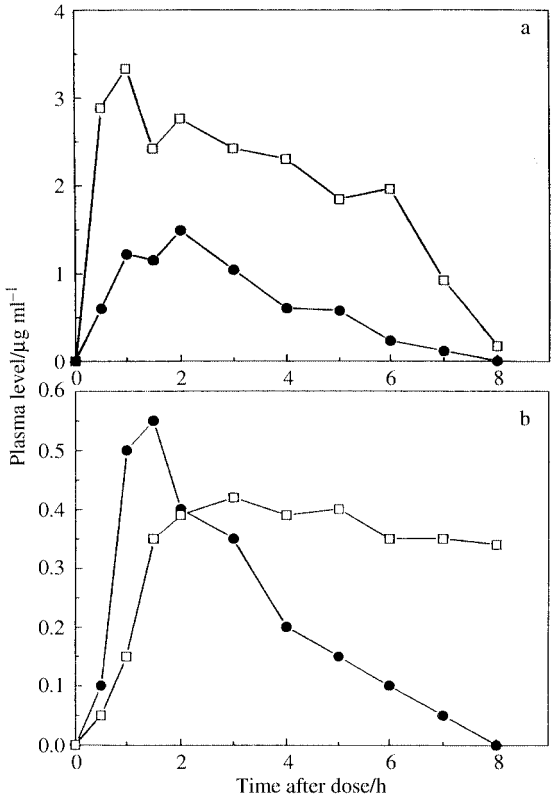

Fig. 4 Time course of the plasma concentrations of: (a) free L-dopa (●) and 3-OMD (□); and (b) C-dopa (●) and 3-OMC (□), in a patient after administration of Sinemet CR4.

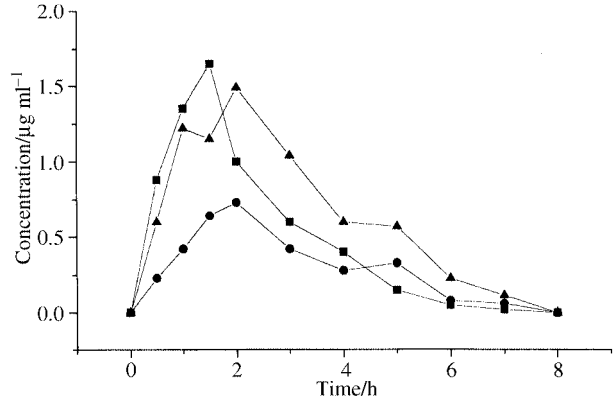

Fig. 5 A comparison of plasma levels of L-dopa after administration of Sinemet (■), Sinemet CR3 (●), and Sinemet CR4 (▲).

Table 3 Pharmacokinetic variables for levodopa from a conventional tablet and two different controlled release formulations

	Dosage formulation		
Pharmacokinetic variables	Conventional tablet	Sinemet CR3	Sinemet CR4
C_{max}/μg ml^{-1}	1.65	0.75	1.50
C_{min}/μg ml^{-1}	0.01	0.03	0.05
C_{av}/μg ml^{-1}	0.67	0.35	0.78
F[a]	2.45	2.06	1.86
T_{max}/h	2.00	2.00	
$T_{1/2}$/h	0.90	1.40	1.50
AUC[b]/μg ml^{-1}			
Plasma level	3.91	2.36	5.16
Ratio[c]/μg ml^{-1}	4.16	4.00	4.15
K_{el}[d]	0.93	2.02	2.16

[a] FI (fluctuation index) = $(C_{max} - C_{min})/C_{av}$. [b] AUC = Area under the curve per mg dose (0–8 h). [c] Plasma level ratio = mean ratio of 3-OMD level to L-dopa level. [d] K_{el} = Elimination rate constant determined by least squares regression analysis of the data points in the elimination phase of the plasma *versus* time curve.

Table 4 Pharmacokinetic variables for C-dopa from a conventional tablet and two different controlled release formulations of Sinemet administered orally

	Dosage formulation		
Pharmacokinetic variables	Conventional tablet	Sinemet CR3	Sinemet CR4
C_{max}/μg ml^{-1}	0.15	0.19	0.55
C_{min}/μg ml^{-1}	0.01	0.06	0.12
C_{av}/μg ml^{-1}	0.08	0.12	2.40
FI[a]	1.75	1.08	0.18
T_{max}/h	1.50	2.00	1.50
$T_{1/2}$/h	1.00	1.50	1.40
AUC[b]/μg ml^{-1} h			
Plasma level	0.78	1.73	
Ratio[c]/μg ml^{-1}	2.29	1.84	2.21
K_{el}[d]	1.44	2.16	2.02

[a] FI (fluctuation index) = $(C_{max} - C_{min}/C_{av})$. [b] AUC = area under the curve per mg dose (0–8 h). [c] Plasma level ratio = mean ratio of 3-OMD level to L-dopa level. [d] K_{el} = Elimination rate constant determined by least squares regression analysis of the data points in the elimination phase of the plasma *versus* time curve.

Table 5 Incremental urinary excretion of C-dopa and L-dopa in one volunteer following adminstration of Sinemet and Sinemet CR formulations

Formulation	Analyte	Excretion/mg 0–2 h	2–4 h	4–6 h	6–8 h
Sinemet[a]	L-dopa	4.50 (1.8%)[b]	4.00 (1.6%)	0.50 (0.2%)	0.25 (0.1%)
	C-dopa	0.53 (2.1%)	0.63 (2.5%)	0.15 (0.6%)	0.03 (0.1%)
Sinemet CR3[c]	L-dopa	3.40 (1.7%)	3.00 (1.5%)	0.60 (0.3%)	0.24 (0.12%)
	C-dopa	1.00 (2.0%)	1.10 (2.2%)	0.35 (0.7%)	0.10 (0.20%)
Sinemet CR4	L-dopa	3.60 (1.8%)	3.00 (1.5%)	0.60 (0.35%)	0.32 (0.16%)
	C-dopa	1.05 (2.1%)	1.15 (2.3%)	0.20 (0.40%)	0.15 (0.30%)

[a] Sinemet dose contains 250 mg L-dopa and 25 mg C-dopa. [b] Values in parenthesis indicate recoveries of % dose. [c] Sinemet CR dose contains 200 mg L-dopa and 50 mg C-dopa.

volunteers) for Sinemet CR and conventional levodopa formulations.[33,34]

Sinemet CR releases both carbidopa and levodopa by first-order release rate. In a zero-order release system, the delivery ratio remains constant until the device is almost exhausted of its active agent. In a first-order release system, the delivery rate is proportional to the amount of drug remaining within the system and this declines with time. The two controlled release dosage forms have a different controlled release mechanism. Sinemet CR4 is designed to have both of its active compounds released by surface dissolution and erosion, while CR3 is designed to release its content by a diffusion-controlled release system. The erosion-controlled release systems have a faster release times than diffusion-controlled release systems. When CR4 was given, all volunteers measured showed that the L-dopa and C-dopa delivered enters the bloodstream and reaches a peak significantly faster than CR3 (2 h *vs.* 3 h), as illustrated in Fig. 5. This would suggest that a CR4 Sinemet formulation would begin to exert its therapeutic effects more quickly than CR3. The reason for the rapid-release profile can be attributed to the release mechanism. When a Sinemet CR4 tablet enters the *in vivo* dissolution medium, drug components initially pass into solution from the surface. The solid matrix also begins to erode into aggregates or granules, and these in turn deaggregate into fine particles that also release their drug content by dissolution. Within a short time, erosion, deaggregation, and dissolution are occurring simultaneously to release both carbidopa and levodopa.

C-dopa levels released by Sinemet CR4 were always higher; this is in agreement with previous studies.[35] These higher levels can diminish L-dopa conversion to dopamine in the peripheral tissues thus permitting low doses to be used effectively for the treatment of parkinsonism and reducing the relative side-effects. Another advantage of Sinemet CR4 is that the delivery device will essentially disappear after delivering its drug content which helps to minimise the potential for any gastro-intestinal irritation, while CR3, diffusion-controlled, will pass through the body as empty shells after delivering its drug contents. The conventional Sinemet tablet had a slightly higher C_{max} than the CR4 formulation. The minor differences in plasma concentration–time profiles for L-dopa between Sinemet and Sinemet CR4 shown in this study (Fig. 5) are of no clinical importance in the treatment of Parkinson's patients. Meanwhile, the plasma concentrations were more stable during Sinemet CR4 (Fig. 5) than during Sinemet alone. Such controlled variations in L-dopa levels are preferred to reduce the fluctuations in motor performance, which can cause the so-called 'on–off' phenomenon and hence providing a better control of parkinsonian symptoms. This side effect can be a major problem after long-term treatment.

Urine is a complex medium that contains many electroactive and fluorescent biological materials that can interfere with the performance of the analysis. The occurrence of matrix effects (defined as the relative sensitivity in the presence of a matrix to that in the absence of a matrix) during urine analysis was significant. It was necessary to use an electrochemical pre-treatment to counteract these effects and to obtain a good resolution and recovery. Table 5 shows the incremental urinary excretion of L-dopa and C-dopa in urine using the enrichment and separation procedure. The data indicate a very low accumulation of both L-dopa and C-dopa following the administration of the drugs which was consistent with their relatively short half-lives (less than 2 h). In contrast, the half-life for 3-OMD is in the order of 13 h. As a result, there can be an extensive accumulation of 3-OMD and its level will be significantly higher than that of L-dopa upon repeated administration. The majority of L-dopa and C-dopa was recovered during the first 4 h.

Comparison studies

A comparison of this method and detection using a glassy carbon electrode in the plasma concentration range 0.2–1 μg ml^{-1} indicated a good correlation. A plot (Fig. 6) of the mean values found using the microelectrode against the mean values obtained using a conventional glassy carbon macro-electrode indicated a good correlation. When the results were

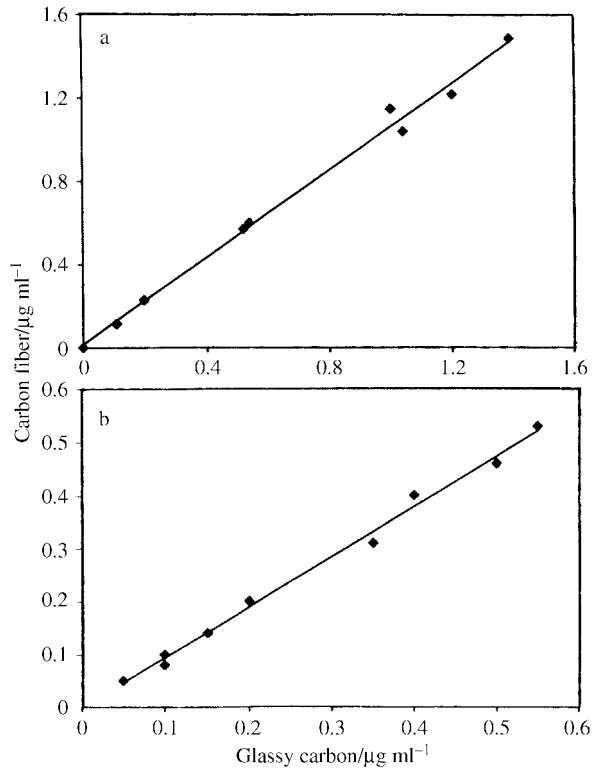

Fig. 6 Correlation curves between the concentrations of L-dopa (a) and C-dopa (b) in blood samples measured by a conventional glassy carbon electrode and those measured by the micro-electrode.

compared by calculating the respective limits of detection, there was a good agreement between the two methods. Considering the laborious preparation time required for polishing the glassy carbon electrode to renew the electrode surface and regain its sensitivity, the detection with the microelectrode flow cell is labour saving and less time consuming. The low cost of replacing the flow cell and the lower dependence on flow rate fluctuations using these electrodes are another advantage over the use of the glassy carbon electrode.[36,37] As to amperometric detectors utilising the carbon-paste electrode, they can require frequent standardisation and repacking and their performance can deteriorate in the presence of admixed organic solvents.

Conclusion

The HPLC-EC method has been used for monitoring and evaluation of the plasma and urine profiles and pharmacokinetics and bioavailability of L-dopa, C-dopa and their metabolites in healthy volunteers after ingestion of various oral formulations. The formulation of choice for use in treating parkinsonism would be Sinemet CR4 due to its superior pharmacokinetics and bioavailability parameters; CR4 has also shown a rapid release profile and less fluctuation index than the other formulations studied.

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