Design and Evaluation of Levodopa Methyl Ester Intranasal Delivery Systems

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Abstract. *Objectives:* This study aimed to examine the feasibility of nasal powder formulations for the delivery of levodopa (L-dopa) into the brain using highly water-soluble levodopa methyl ester hydrochloride (LDME).

Methods: For designing nasal LDME powders, pH-rate stabilities of LDME in buffer solutions and their enzymatic degradations in rabbit nasal mucosal and serosal extracts were investigated. *In vitro* permeation studies were carried out with four LDME nasal powders.

Results: LDME was degraded fast in weakly acidic and neutral solutions, but relatively stable in acidic solutions. In nasal extracts, LDME (50 and 200 μg/mL) was rapidly hydrolyzed, forming L-dopa, and there were no significant differences in first-order degradation rates between mucosal and serosal extracts. From the *in vitro* permeation studies, LDME powder formulations resulted in faster appearance rates (1.07 ± 0.39 mg/cm^2/hr) of L-dopa than solution formulations (0.35 ± 0.08 mg/cm^2/hr).

Conclusions: These results suggested that LDME nasal powder formulations could be useful delivery systems of L-dopa.

Keywords: Levodopa methyl ester, levodopa, nasal delivery, stability, permeation

INTRODUCTION

Parkinson's Disease (PD) is a condition precipitated by decreased dopamine (DA) in the central nervous system—therefore, treatment is geared towards ensuring an adequate supply of DA to the striatum to rectify the imbalance. However, DA cannot be administered directly because it cannot penetrate the blood brain barrier. Levodopa (L-dopa), the metabolic precursor of DA, has been regarded as the standard for treating parkinson's disease [1]. L-dopa exerts its action by replacing the DA that would be normally released by the substantia nigra. It can cross the blood brain barrier via a saturable transporter and is converted to DA by L-aromatic amino acid decarboxylase (also known as dopa decarboxylase) in the brain [2, 3].

Currently, the oral route is the most popular clinically available route for L-dopa administration in PD patients. However, oral administration of L-dopa causes variable and unreliable clinical responses. Since L-dopa is immediately absorbed in the proximal duodenum by an active transport system, its plasma concentrations rapidly fluctuate. The rates and extents of L-dopa absorption may be affected by diet and other gastric factors. Saturable large neutral amino acids such as leucine, valine, phenylalanine, tyrosine, and methionine contained in food may compete with L-dopa for intestinal absorption [4, 5]. Thus, a high-protein diet reduce the absorption of L-dopa from the intestines. Constipation and slowing the gastric emptying influence the intestinal absorption in a negative way.

In addition, dysphagia is a well-recognized manifestation of PD; the prevalence of dysphagia is uncertain

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but may be as high as 52% in this disease. Patients experiencing this manifestation cannot be expected to comply with oral administration or to obtain optimal bioavailability of L-dopa [6, 7].

A nasal delivery system has been considered as an alternative dosage form to oral delivery. The nasal cavity is easily accessible, extensively vascularized, and highly permeable. The large surface area of the nasal mucosa affords rapid absorption, fast onset of therapeutic effect, and higher bioavailability. Compounds administered via this route are absorbed directly into the systemic circulation, avoiding the hepatic first-pass effect [8–10]. In our previous study [11], nasal delivery systems were formulated using L-dopa and found to be a favorable administration route. However, due to its low solubility, it was not possible to load a sufficient amount of L-dopa.

Levodopa methylester hydrochloride (LDME) is a highly soluble prodrug produced by the esterification of L-dopa. The molecular weight of LDME is 314 mg, which is equivalent to 250 mg of L-dopa. LDME is known to be about 250 times more soluble than L-dopa [12]. Therefore, it was expected that this compound could successfully overcome the solubility problem of L-dopa. Kao et al. (2000) reported the nasal administration of the water soluble prodrugs of L-dopa, but they mainly used pure butyl ester of L-dopa without further formulation for nasal delivery [13].

The objective of this study was to evaluate the feasibility of nasal delivery of LDME by investigating its physicochemical and enzymatic stability, formulating nasal delivery systems, and characterizing the permeation of L-dopa through rabbit nasal mucosae.

MATERIALS AND METHODS

Animals

Male New Zealand White rabbits weighing 2.5–3.5 kg were obtained from Samtako Bio Co., Ltd. (Osan, Korea).

Materials

Levodopa (L-dopa), carbidopa (C-dopa), l-3,4-dihydroxyphenylalanine methyl ester hydrochloride (levodopa methyl ester, LDME) and α-methyldopa were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Silicated microcrystalline cellulose (SMCC, Mendell, Patterson, NY, USA), 2-hydroxypropyl-β-cyclodextrin (2-HPβCD, Cargill Inc., Minneapolis, MN, USA), hydroxypropyl cel-

lulose (HPC, Aqualon, Wilmington, DE, USA), Carbopol 974 P (Noveon Inc., Cleveland, OH, USA), carboxymethyl cellulose-Na (CMC-Na, Junsei Chem. Co. Ltd., Kyoto, Japan), Xylazine (Bayer Korea, Ansan, Korea), tiletamine-xolazepam (Virbac Korea Medicine, Seoul, Korea), ether (Daejung Chemicals and Metals, Siheung, Korea), heptanesulphonate, disodium ethylenediamine tetraacetic acid (EDTANa$_2$), citric acid, sodium citrate, perchloric acid, orthophosphoric acid, maleic acid, and triethanolamine (Duksan Pure Chemical, Ansan, Korea) were also obtained. Methanol was used for HPLC analysis. Other reagents were of analytical grade.

Chromatographic conditions

Samples were analyzed by high-performance liquid chromatography (HPLC). The HPLC system consisted of a pump (PU-2080, Jasco, Tokyo, Japan) with an electrochemical detector (CouloChem III, ESA, Muskegon, MI, USA). The first electrode in the analytical cell was set at -400 mV (Range 100 μA) and the second one at 450 mV (Range 100 nA) with a flow-rate of 1 mL/min. A Gemini 5μ C18 column (4.6 × 150 mm, 5 μm bead size, Phenomenex, Torrance, CA, USA) was used. The mobile phase was composed of 16.5 g of potassium phosphate, 1.0 mL of 0.1 M EDTANa$_2$, 1.2 mL of 0.5 mM heptanesulphonate, and 19.5 mL of methanol with pH adjusted to 3.4 with phosphoric acid. The mobile phase was filtered through a 0.2-μm cellulose membrane (Whatman, Maidstone, England).

Solution stability studies

LDME solutions (100 μg/mL) were prepared in various buffer solutions at pH 1.72, 3.20, 4.10, 4.96, 5.81 and 6.72. Each LDME solution was stored in water bath set at 37°C. Samples (100 μL each) were periodically taken from each solution and mixed with IS (20 μg/mL in pH 1.2 buffer) 100 μL and 0.1 N phosphoric acid 800 μL. LDME and L-dopa concentrations in the samples were determined by assaying 50 μL of the resultant mixture by the HPLC method summarized above.

Dissection of mucosa specimens

Male New Zealand White rabbits were sacrificed by injecting air into the marginal ear vein. Nasal mucosa was collected by making an incision from the tip of the nose all the way up with a surgical scissor and

cutting the cartilage along the length of the lateral wall of the nose on each side of the nasal septum from the bottom with a heavy-duty scissor. This was followed by cutting the nasal septum across the top and the bottom with the surgical scissor and pulling off the nasal bone anteriorly to fully expose the nasal cavity. The nasal cartilage plate, which is covered with nasal membrane, was carefully removed by making three cuts on the top, bottom, and along side. Nasal membranes were separated from the underlying cartilage by carefully pushing off the cartilage, with a gloved thumb fingertip, from the lower end.

Preparation of mucosal and serosal extracts

Mucosal and serosal extraction was carried out in Valia-Chien permeation cells. Freshly excised nasal mucosae were mounted on the cell opening between the two half-cells, with the mucosal epithelium facing the donor half-cell. Both the donor and receptor half-cells were filled with 3.5 mL of isotonic phosphate buffer at pH 7.4, and mucosal and serosal extracts were collected separately by exposing the mucosal and serosal surfaces of each mucosa, respectively, to the isotonic phosphate buffer at 37°C for 8 hr. The extraction procedure was repeated three times. The mucosal and serosal extracts, three each, were combined individually and stored in a refrigerator.

LDME degradation study in mucosal and serosal extracts

The degradation study was conducted by adding 100 μL of two LDME solutions (50 and 200 μg/mL) into 900 μL of each extract and incubating them in a shaker bath at 37°C for up to 180 min. As a control, 200 μg/mL LDME solution in 1 : 1 mixture of pH 6.8 and pH 7.4 isotonic phosphate buffer was used. At predetermined time intervals, 100 μL was withdrawn from the incubation solution and mixed with 100 μL IS solution (20 μg/mL in pH 1.2 buffer). Fifty microliters of the mixed solution was analyzed by HPLC.

Formulation of nasal delivery systems

Five nasal compositions were formulated as shown in Table 1. For powder formulations, LDME was mixed with additives and stored in darkness until use. A solution formulation E was used as a control.

Table 1
LDME formulations for nasal delivery

Ingredients	Formulations				
	A	B	C	D	E
LDME (mg)	600	600	600	600	30
N-acetyl-L-cysteine (mg)	50	50	50	50	2.5
2-HPβCD (mg)	230	230	230	250	15
SMCC (mg)	100	100	100	100	–
Carbopol 974 P (mg)	20	–	–	–	–
HPC (mg)	–	20	–	–	–
CMC-Na (mg)	–	–	20	–	–
Water	–	–	–	–	Qs
Total (mg)	1000	1000	1000	1000	1 mL

LDME: levodopa methyl ester, 2-HPβCD: 2-hydroxypropyl-β-cyclodextrin, SMCC: silicated microcrystalline cellulose, HPC: hydroxypropyl cellulose, CMC-Na: carboxymethyl cellulose-Na

Permeation studies

Franz Diffusion cells were used for LDME permeation study through rabbit nasal mucosae. Formulated powder (Formulation A~D) and solution (Formulation E) for nasal delivery from Table 1 were loaded to the donor compartment at a dose of 3 mg of LDME; the serosal side was in contact with the receptor compartment. Receptor compartment cells were filled with pH 7.4 phosphate buffer, and the media were stirred by a Teflon-coated magnetic bar to keep them well mixed. The permeation media were maintained at 37 °C. The surface area of the receiver cell opening was 1.766 cm^2, and the cell volume was 5 mL. At predetermined time intervals, 100 μL was withdrawn from the receiver cells and diluted with 900 μL of 0.1 N phosphate solution. The diluted solution 100 μL was mixed with 200 μL IS solution. Fifty microliters of the mixed solution was analyzed by HPLC.

RESULTS AND DISCUSSION

The physicochemical stability of LDME was evaluated according to solution pH. As pH increased, LDME was rapidly hydrolyzed, only 58% of LDME remaining 5 hr after storage at pH 6.7 as shown in Table 2. The degradation profile of LDME followed first-order kinetics. The elimination rate constants were 0.001, 0.002, 0.002, 0.006, 0.029, and 0.106 at pH 1.72, 3.20, 4.10, 4.96, 5.81, and 6.72, respectively. As shown in Fig. 1, the effect of pH on the solution stability of LDME in the pH range above 4.1 was much greater than that in the range below pH 4.1. The log k-pH profile showed a linear slope value in pH ranges above 4.1 ($r^2 = 0.9955$). The total percentages of remaining LDME and forming L-dopa were far less than 100% at

Table 2
Remaining (%) levodopa methyl ester and formed (%) levodopa in various buffered aqueous solutions

Time (hr)	pH 1.72		pH 3.20		pH 4.10		pH 4.96		pH 5.81		pH 6.72	
	LDME	L-dopa	LDME	L-dopa	LDME	L-dopa	LDME	L-dopa	LDME	L-dopa	LDME	L-dopa
0	100	0	100	0	100	0	100	0	100	0	100	0
1	99.4 ± 1.2	0.8 ± 0.02	102.7 ± 3.1	0.6 ± 0.03	98.9 ± 1.9	0.5 ± 0.01	98.1 ± 1.0	1.0 ± 0.08	97.6 ± 1.2	0.9 ± 0.03	92.4 ± 1.9	6.9 ± 0.09
3	101.8 ± 2.3	0.8 ± 0.03	101.8 ± 2.1	0.8 ± 0.01	97.2 ± 1.5	1.0 ± 0.07	100.4 ± 2.9	1.2 ± 0.04	98.9 ± 2.9	1.4 ± 0.08	73.2 ± 2.9	15.5 ± 0.08
5	98.0 ± 3.2	0.8 ± 0.1	102.3 ± 1.5	0.9 ± 0.1	103.1 ± 2.7	1.0 ± 0.02	99.4 ± 1.8	1.5 ± 0.04	92.9 ± 1.4	2.1 ± 0.09	58.4 ± 1.9	23.1 ± 1.0
21	97.9 ± 1.3	1.5 ± 0.2	97.7 ± 1.0	1.9 ± 0.07	96.1 ± 2.5	3.2 ± 0.08	87.7 ± 0.9	8.9 ± 0.08	55.1 ± 1.1	24.2 ± 0.9	11.0 ± 0.8	43.1 ± 1.1

Data are expressed as the mean ± S.D. ($n = 3$). LDME: levodopa methyl ester. L-dopa: levodopa.
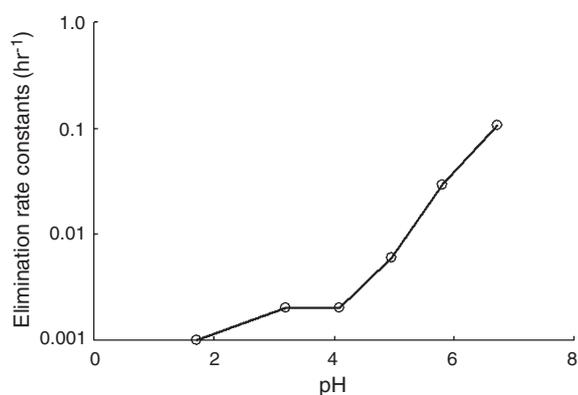
Fig. 1. Elimination rate constants of LDME in buffer solutions with various pH. LDME: levodopa methyl ester.

pH 6.72. This was thought to be due to the involvement of other degradative reactions in LDME metabolism, in addition to hydrolysis.

To determine the amount of LDME enzymatically degraded before and after permeation, mucosal and serosal extracts were employed. Degradation profiles were investigated by monitoring the disappearance of LDME and the appearance of its main metabolite, L-dopa, using two concentrations (50 and 200 µg/mL) of LDME. The disappearance of LDME in the mucosal and serosal extracts followed first-order kinetics as shown in Fig. 2 (A). LDME was very rapidly degraded in nasal mucosa extracts; more than 90% of LDME was degraded at 30 min while more than 90% of LDME remained in the isotonic buffer (control) which was used for extracting enzymes from nasal mucosae. There were no significant differences in degradation

rates between the extracts from the mucosal and serosal surfaces. Elimination rate constants were calculated to be 0.08 and 0.07 min^{-1} in the mucosal and serosal extracts, respectively. In addition, degradation rates were not significantly different between low and high concentrations of LDME, of which the half-life was 8.9 and 8.8 min, respectively. This was a much more rapid degradation, compared to levodopa butyl ester (degradation half-life of 144 min) in a study by Kao et al. (2000) [13], although they used nasal perfusates.

As shown in Fig. 2 (B), more than 80% L-dopa was formed at 30 min regardless of which extract and drug concentration were used. On the contrary, LDME was less degraded in isotonic buffer—therefore, L-dopa was not formed much. Based on the results, it was speculated that LDME could be a favorable prodrug of L-dopa, due to its rapid degradation and appearance of L-dopa in nasal mucosa extracts.

Since LDME as a solution formulation was very unstable, a powder formulation was obtained to ensure stability. The addition of Carbopol, HPC, and CMC-Na at a concentration of 2% in Formulation A, B, and C (Table 1), respectively, allowed adhesiveness of the powder formulations [14, 15]. SMCC was added to the adhesives to enhance absorption—according to a study, the addition of MCC to HPC enhanced nasal absorption of drugs such as leuprolide, calcitonin, and FITC-dextran. In the study, MCC was considered to function as an absorption enhancer by causing a locally high concentration of drugs near the nasal mucosa surface, while HPC was thought to increase retention of drugs on the nasal mucosa, due to its gel-forming property [14].
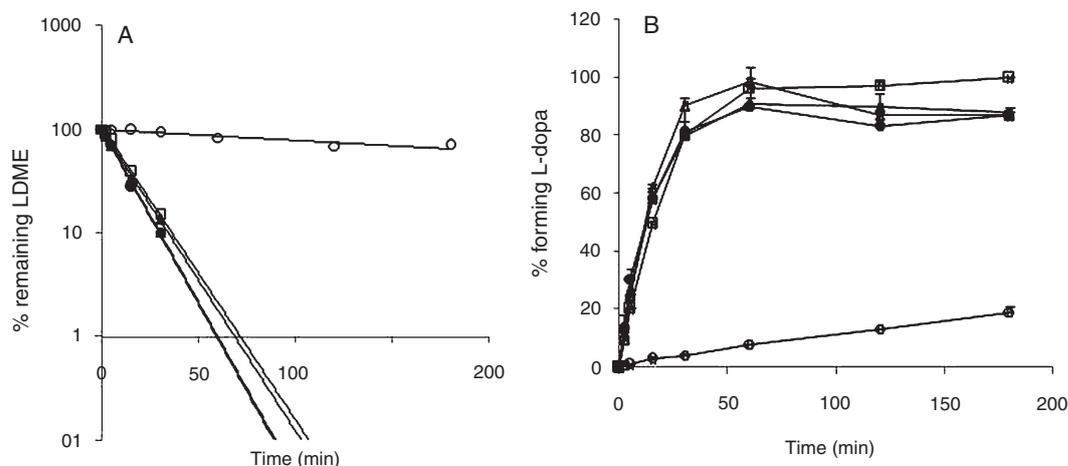
Fig. 2. Profiles of LDME degradation (A) and L-dopa formation (B) in nasal mucosa extracts (mean ± S.D., $n = 3$). LDME: levodopa methyl ester, L-dopa: levodopa. ○: control, ●: low concentration in mucosal extract, △: high concentration in mucosal extract, ▲: low concentration in serosal extract, □: high concentration in serosal extract.
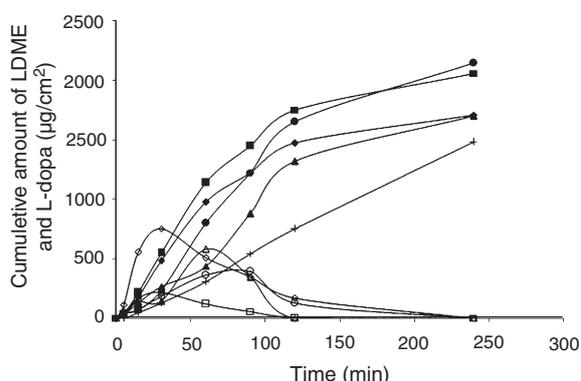
Fig. 3. Appearance profiles of LDME and L-dopa in the receptor cells through nasal mucosa following various formulations (mean ± S.D., $n = 3$). LDME: levodopa methyl ester, L-dopa: levodopa, FN: formulation. ○: LDME from FN-A, ●: L-dopa from FN-A, △: LDME from FN-B, ▲: L-dopa from FN-B, □: LDME from FN-C, ■: L-dopa from FN-C, ◇: LDME from FN-D, ◆: L-dopa from FN-D, +: L-dopa from FN-E.

Table 3
Appearance rates of levodopa in the receptor cell

FN	Flux (μg/cm^2/hr)	Lag time (hr)
A	1.07 ± 0.39	0.26 ± 0.10
B	0.68 ± 0.15	0.09 ± 0.06
C	0.90 ± 0.46	0.09 ± 0.07
D	1.05 ± 0.32	0.08 ± 0.05
E	0.35 ± 0.08	0.14 ± 0.04

Data are expressed as the mean ± S.D. ($n = 3$). FN: formulation.

N-acetyl-L-cysteine, a mucolytic agent, was employed to reduce nasal fluid viscosity and improve accessibility of the drug to the epithelial membrane [16]. 2-HPβCD (23%) was used for improving nasal drug absorption either by increasing aqueous drug solubility or by enhancing nasal drug permeability [17]. In addition, it is known to reduce nasal toxicity [18]. Due to its lack of mucosal adhesive agents, Formulation D was used as a control for Formulation A, B, and C. As a control for powder formulations, solution formulation E was constructed.

The permeation study was conducted using powder formulations. As shown in Fig. 3, LDME concentrations increased in the receptor cell for the first several min and then declined. Table 3 shows the appearance rates of L-dopa in the receptor compartment. LDME from solution formulation was not detected in the receptor cell. This was possibly because LDME was rapidly hydrolyzed to L-dopa in donor cells before permeation.

The appearance rates of L-dopa from powder formulations were higher than those from solution per-

meation. Among adhesive agents, Carbopol revealed the highest permeation enhancing effects, while HPC showed the lowest. Lag times were very short, ranging between $5 \sim 15$ min. The appearance rate of L-dopa was not affected by the presence of adhesive agents (Formulation A, C and D).

Further *in vivo* pharmacokinetic study is required to confirm these *in vitro* results because the *in vitro* study is not able to provide the information on the distribution and elimination of a drug. In addition, considering that nasal irritation was reported to be a limiting factor of apomorphine nasal delivery systems [19], investigation of the nasal irritation with the formulated nasal systems is needed. Despite the limitations, the results of this study can be valuable as a preliminary data for formulating the LDME nasal delivery systems.

CONCLUSIONS

These results suggested that LDME nasal powder formulations would be useful delivery systems of L-dopa, owing to its high appearance rates and stability, compared to solution formulations.

DECLARATION

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest to disclose.

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REFERENCES

[1] Cotzias CG, Van Woert MH, & Schiffer LM (1967) Aromatic amino acids and modification of parkinsonism. *New Engl J Med*, **276**, 374-379.

[2] Okereke CS (2002) Role of integrative pharmacokinetic and pharmacokydnamic optimization strategy in the management of Parkinson's disease patients experiencing motor fluctuations with levodopa. *J Pharm Pharma Sci*, **5**, 146-161.

[3] Chen JJ, Nelson MV, & Swope DM (2008) Parkinson's disease. In *Pharmacotherapy: A Pathophysiologic Approach*, Dipiro JT, Talbert RL, Yee GC, Matzke GR, Wells BG, Posey ML, eds. McGraw Hill, New York, pp. 977-988.

[4] Simon N, Gantcheva R, Bruguerolle B, & Viallet F (2004) The effects of a normal protein diet on Levodopa plasma kinetics

in advanced Parkinson's disease. *Parkinsonism Relat Disord*, **10**, 137-142.

[5] Müller T, Erdmann C, Bremen D, Schmidt WE, Muhlack S, Woitalla D, & Goetze O (2006) Impact of gastric emptying on levodopa pharmacokinetics in Parkinson disease patients. *Clin Neuropharmacol*, **29**, 61-67.

[6] Johnston BT, Li Q, Castell JA, & Catell DO (1995) Swallowing and esophageal function in Parkinson's disease. *Am J Gastroenterol*, **90**, 1741-1746.

[7] Nyholm D (2006) Pharmacokinetic optimization in the treatment of Parkinson's disease: an update. *Clin Pharmacokinet*, **45**, 109-136.

[8] Illum L (2000) Transport of drugs from the nasal cavity to the central nervous system. *Eur J Pharm Sci*, **11**, 1-18.

[9] Dahlin M, Jansson B, & Björk E (2001) Levels of dopamine in blood and brain following nasal administration to rats. *Eur J Pharm Sci*, **14**, 75-80.

[10] Costantino HR, Illum L, Brandt G, Johnson PH, & Quay SC (2007) Intranasal delivery: physicochemical and therapeutic aspects. *Int J Pharm*, **337**, 1-24.

[11] Kim TK, Kang W, Chun IK, Oh SY, Lee YH, & Gwak HS (2009) Pharmacokinetic evaluation and modeling of formulated levodopa nasal delivery systems. *Eur J Pharm Sci*, **38**, 525-532.

[12] Zangaglia R, Stocchi F, Sciarretta M, Antonini A, Mancini F, Guidi M, Martignoni E, & Pacchetti C (2010) Clinical experiences with levodopa methylester (Melevodopa) in patients with Parkinson disease experiencing motor fluctuations: an open-label observational study. *Clin Neuropharmacol*, **33**, 61-66.

[13] Kao HD, Traboulsi A, Itoh S, Dittert L, & Hussain A (2000) Enhancement of the systemic and CNS specific delivery of L-dopa by the nasal administration of its water soluble prodrugs. *Pharm Res*, **17**, 978-984.

[14] Suzuki Y & Makino Y (1999) Mucosal drug delivery using cellulose derivatives as a functional polymer. *J Contr Rel*, **62**, 101-107.

[15] Bonacucina G, Cespi M, Misici-Falzi M, & Palmieri GF (2005) Rheological, adhesive and release characterization of semisolid carbopol/tetraglycol systems. *Int J Pharm*, **307**, 129-140.

[16] Matsuyama T, Morita T, Horikiri Y, Yamahara T, & Yoshino H (2006) Improved nasal absorption of salmon calcitonin by powdery formulation with N-acetyl-L-cysteine as a mucolytic agent. *J Contr Rel*, **115**, 183-188.

[17] Challa R, Ahuja A, Ali J, & Khar RK (2005) Cyclodextrins in drug delivery: an updated review. *AAPS PharmSciTech*, **14**, E329-E357.

[18] Zhang Y, Jiang XG, & Yao J (2001) Nasal absorption enhancement of insulin by sodium deoxycholate in combination with cyclodextrins. *Acta Pharmacol Sin*, **22**, 1051-1056.

[19] Dewey RB Jr, Maraganore DM, Ahlskog JE, & Matsumoto JY (1998) A double-blind, placebo controlled study of intranasal apomorphine spray as a rescue agent for off-states in Parkinson's disease. *Mov Disord*, **13**, 782-787.