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Nasal mucoadhesive delivery systems of the anti-parkinsonian drug, apomorphine: influence of drug-loading on in vitro and in vivo release in rabbits

Michael Ikechukwu Ugwoke, Exaud Sam, Guy Van Den Mooter, Norbert Verbeke *, Renaat Kinget

Laboratorium voor Farmacotechnologie en Biofarmacie, Campus Gasthuisberg O&N, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

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Abstract

Lyophilized polyacrylic acid powder formulations loaded with apomorphine HCl were prepared and the influence of drug loading on in vitro release and in vivo absorption studied after intranasal administration in rabbits. These formulations prepared with Carbopol 971P, Carbopol 974P and polycarbophil sustained apomorphine release both in vitro and in vivo. The in vitro release rate and mechanism were both influenced by the drug loading. There was no large influence of drug loading on the time to achieve the peak (T_{max}) for a particular polymer, but T_{max} differed between different polymers. For a particular drug loading, the T_{max} from Carbopol 971P was the slowest compared with that for Carbopol 974P and polycarbophil; however, only the T_{max} from Carbopol 971P loaded with 15% w/w of apomorphine was significantly longer than polycarbophil of similar drug loading ($P = 0.0386$). The trend further observed was that increasing drug loading led to increased peak plasma concentration and area under the curve (AUC). In the second part of this study, a mixture containing an immediate release component and sustained release formulation was administered in an attempt to increase the initial plasma level, as this could be therapeutically beneficial. Only one peak plasma concentration was observed and the initial plasma concentrations were no higher than those obtained with solely sustained release formulation. The T_{max}, the peak plasma drug concentration (C_{max}) and AUC from the lactose-containing formulation were lower than the formulation without lactose but the differences were only marginally statistically significant for C_{max} ($P = 0.0911$) and AUC ($P = 0.0668$), but not T_{max} ($P = 0.2788$).

Keywords: Apomorphine; Parkinson's disease; Nasal drug delivery; Controlled release; Carbopol; Polycarbophil.

* Corresponding author. Tel.: + 32-16-34-58-24; fax: + 32-16-34-59-96.
E-mail address: Norbert.Verbeke@farm.kuleuven.ac.be (N. Verbeke)

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1. Introduction

Polycarbophil (Noveon AA1), Carbopol 971P and Carbopol 974P are polyacrylates differing in cross-linking conditions. Carbopol 974P, a more cross-linked, higher viscosity analogue of Carbopol 971P, is non-irritant to the skin (man), eye (rabbit), and is non-toxic orally (rats and dogs), while all three polymers are considered as GRAS (Generally Recognized As Safe) for oral use by the FDA (BF Goodrich, 1997). Due to its ability to absorb and hold water, polycarbophil has both antidiarrhea and laxative properties (See et al., 1987). Water absorption, swelling, drug release and mucoadhesion of these polyacrylic acids are influenced by both pH and ionic strength of the medium (Ch'ng et al., 1985; Morimoto and Morisaka, 1987; French et al., 1995; Neau et al., 1996). Their mucoadhesive properties have also been well documented (Mortazavi, 1995; Nagai, 1985; Park and Robinson, 1985; Peppas et al., 1985; Ponchel et al., 1985, 1987; Tobyn et al., 1996; Edsman et al., 1996). Their use for nasal drug delivery is thus expected to be beneficial as drug residence time within the nasal cavity should be prolonged due to mucoadhesion and decreased mucociliary clearance rate. The mucociliary clearance half-time values of albumin, starch and DEAE–Sephadex microspheres, all of which are mucoadhesive, were 3 h or more in man compared with 15 min for solutions and non-mucoadhesive powders (Illum et al., 1987). In addition to its mucoadhesive properties in drug delivery, polycarbophil has been found to sustain the release of atropine sulphate both in vitro and in vivo after oral administration (See et al., 1987).

It has previously been reported that apomorphine in aqueous solution is well absorbed from the nasal route in Parkinsonian patients at rates comparable with subcutaneous injection (van Laar et al., 1992; Sam et al., 1995). However, the low intranasal bioavailability (Kapoor et al., 1990; Sam et al., 1995) may have been due to drainage of the solution from the nose, as this was not corroborated by the study of van Laar et al. (1992). The time to onset of therapeutic action was fast (within a mean of 8.9 min) but the duration of drug effect was very short (mean of 44 min) (Corboy et al., 1995). Further difficulties associated with the aqueous solution is that apomorphine oxidizes easily in solution turning green (Merck Index, 1983). A mucoadhesive and sustained release powder dosage form will no doubt improve on some of the disadvantages associated with the solution given as subcutaneous injection or intranasal solution.

Sustained release apomorphine HCl powder dosage forms for nasal administration were prepared with these excipients and the influence of drug loading on their release both in vitro and in vivo investigated. In the second part of this publication, experiments are reported in which an immediate release component consisting of a lyophilized mixture of lactose and apomorphine was mixed with Carbopol 971P also loaded with the drug. This was with the aim of incorporating an immediate release portion to the sustained release preparation in order to increase the initial plasma levels of the drug such that, while having a prolongation of duration of drug action, the fast onset of action noted for apomorphine (Corboy et al., 1995; Chaudhuri and Clough, 1998) will still be retained.

2. Materials and methods

2.1. Materials

Carbopol 971P, Carbopol 974P and polycarbophil (Noveon AA1) were gifts from BF Goodrich (Cleveland, OH, USA); Apomorphine HCl (Alpha Pharma, Zwevegem, Belgium) and anhydrous lactose were of European Pharmacopoeial quality, diethylether and acetonitrile were of high-performance liquid chromatography (HPLC) grade and other chemicals used of reagent grade.

2.2. Dosage form preparation

Apomorphine HCl (300–600 mg) was dissolved with enough Milli-Q water in lyophilizing vials with bath sonication (Branson 2200; Branson Ultrasonics, Danbury, CT, USA) until complete dissolution. To each of the vials, Carbopol 971P,

Carbopol 974P, polycarbophil or lactose was added. The amount of these excipients varied according to the final drug loading needed to give a total formulation weight of 2 g for 15 and 30% w/w drug loading, and 1 g for 60% w/w drug loading. After stirring properly to disperse Carbopol 971P, Carbopol 974P, polycarbophil or dissolve lactose, they were frozen overnight (− 35°C) and lyophilized (Alpha, Christ, Osterode, Germany) at − 10°C for 24 h followed by, 0°C for 24 h. They were sieved (180 μm mesh) and stored at room temperature until use. The percentage drug loading of the formulations was determined by HPLC with electrochemical detection (Sam et al., 1994).

2.3. In vitro release studies

The in vitro release study was carried out with a modified rotating basket placed in USP XXII dissolution vessel of SR8-Plus dissolution test station (Hanson Research, Chatsworth, CA, USA). This modification consisted of fixing an inverted aluminum cup to a steel bar. The internal dimensions of the cup were 3.6 cm (diameter) and 1.5 cm depth (Ugwoke et al., 1998). The powders (20 mg) were weighed on a membrane filter (0.45 μm) and secured firmly between an aluminum ring filter holder and the cup (both surfaces smeared with silicon grease) with clamps. Two grooves on the side of each filter-holder facing the filter were air vents allowing complete contact between release medium and filter. We previously reported on the advantages of a similar in vitro release apparatus over the paddle method (Filipovic-Grcic and Jalsenjak, 1992; Okada et al., 1992; Gunder et al., 1995) for studying drug release from formulations for nasal administration (Ugwoke et al., 1997) Five hundred milliliters of the release medium, phosphate buffer of pH 6.0 containing 0.01% 2-mercapto-ethanol as anti-oxidant (Sam et al., 1994), maintained at 37°C, was used to study the influence of drug loading on in vitro release rate. The donor compartments were connected to a flow-through cell and the medium circulated with a peristaltic pump (Gilson Minipuls 3; Gilson, Villiers Le Bel, France). The modified baskets were rotated at 50 rpm and released drug analyzed spectrophotometerically at 272 nm (DU® 640 spectrophotometer; Beckman Instruments, Fullerton, CA, USA).

2.4. Drug administration, sample collection and analysis

Male New Zealand white rabbits were used, six animals for the first study and five for the second. The rabbits were purchased from and fed throughout the period of the experiment by the Animalium department of K.U. Leuven (Leuven, Belgium). They weighed 3.49 ± 0.30 kg (SD) at the beginning and 3.53 ± 0.21 kg at the end of the study period. They were housed individually in stainless steel cages, fed a commercial laboratory diet and had free access to water.

In the first study, the percentage drug loading of apomorphine HCl studied were about 15 and 30 in all cases, and, additionally, 60% w/w for Carbopol 971P and polycarbophil. In the second study, experiments were carried out with a powder mixture containing an immediate release portion. This formulation was prepared by mixing lyophilized lactose (32.3% w/w) and Carbopol 971P formulation (17.95% w/w) both loaded with apomorphine HCl at a ratio of 1:7.2. The drug content contributed by the lactose mixture accounted for 20% of the total dose administered. The quantified amount of apomorphine in the mixture of Carbopol 971P and lactose was 18.73% w/w. The dose of apomorphine for both studies was 0.61 mg/kg body weight, except for the control study with lyophilized mixture of lactose and apomorphine where the dose was 0.305 mg/kg body weight.

The formulations were administered by insufflation with a home-made device made of an air-filled 10 ml syringe compressed to 2.5 ml, and released with an electrically-actuated valve that expulsed the powder through a plastic tip inserted into the nostril. The animals were conscious throughout the duration of the experiments and were held in rabbit restrainers during blood collection. Blood samples were taken from the marginal ear vein at 0, 5, 10, 15, 20, 30, 45, 60, 90, 120, 180, 240, 300, 360 and 480 min, except for the lactose-containing immediate release formula-

tions where additional samples were taken at 2, 7 and 12 min. Sample handling and HPLC analysis were carried out as reported previously (Sam et al., 1994, 1995).

2.5. Data treatment and statistics

To deduce the mechanism of drug release from the formulations, the release data were fitted to two different equations. The general exponential equation (Eq. (1)) is often used to study the drug release behaviour from polymeric drug delivery systems:

$$M_t/M_\infty = kt^n \quad (1)$$

where M_t/M_∞ is the fraction of drug released (≤ 0.8) at time t, k denotes a constant including the properties of the polymer and the drug, and n is a diffusional exponent characteristic of the release mechanism. For example, with swellable spherical matrices, $n = 0.43$ for Fickian diffusion, $0.43 < n < 0.85$ for anomalous (non-Fickian) transport, and $n = 0.85$ for Case II transport (Ritger and Peppas 1987). To further clarify the contributions to drug release by Fickian diffusion and polymer relaxation, the data were fitted to Eq. (2).

$$M_t/M_\infty = k_1 t^{1/2} + k_2 t \quad (2)$$

where M_t/M_∞ is the fractional amount of drug released (≤ 0.8), while k_1 and k_2 are the drug diffusional and polymer relaxational constants (Gander et al., 1988). The release data were fitted with the equations using the software XYMATH (Shareware Version 2.4; C. Taylor, Sacramento, CA, USA).

The plasma concentration–time data were analyzed using TOPFIT Pharmacokinetics and Pharmacodynamics data analysis system software, version 2.0 (Gustav Fischer Verlag, D-7000 Stuttgart, Germany). The absorption rate constants (k_a) were calculated using the Wagner–Nelson method. The peak plasma drug concentration (C_{max}) and time to achieve the peak (T_{max}) values shown are the empirical data. The reported C_{max} and T_{max} are the mean values from all the rabbits and not the values obtainable from the average curves shown in the figures. The area under the curve (AUC) was calculated using the linear trapezoidal method without extrapolation to infinity.

Results are reported as mean $\pm$ SD ($n = 3$) for in vitro experiments and mean $\pm$ SE of mean ($n = 5$ or 6) for in vivo experiments. Statistical tests of significance were performed using INSTAT® (Graphpad Software, San Diego, CA, USA); differences were considered to be statistically significant when $P < 0.05$ using a two-tailed unpaired t-test.

3. Results and discussion

Table 1 shows the actual drug loading of the different formulations. In contrast to the formulations containing about 15 and 30% w/w of apomorphine HCl, those loaded with 60% w/w developed a green coloration with time due to atmospheric oxidation of the drug upon storage. These observations suggest that these polymers have protective action against the atmospheric oxidation of the drug and that this protective action fails in the presence of a high amount of apomorphine HCl.

3.1. In vitro release of apomorphine HCl

The drug loading influenced the in vitro release of apomorphine. The release rate increased with increasing drug loading. This was the case with Carbopol 971P and polycarbophil (Fig. 1a,c) but

Table 1
Drug loading of the formulations

Formulation (theoretical drug loading in %w/w)	Actual mean drug concentration (% w/w of apomorphine HCl) ($n = 2$)
Carbopol 971P (15)	17.95
Carbopol 971P (30)	32.3
Carbopol 971P (60)	64.6
Carbopol 974P (15)	14.3
Carbopol 974P (30)	32.1
Polycarbophil (15)	15.6
Polycarbophil (30)	33.0
Polycarbophil (60)	65.2
Lactose (30)	32.3

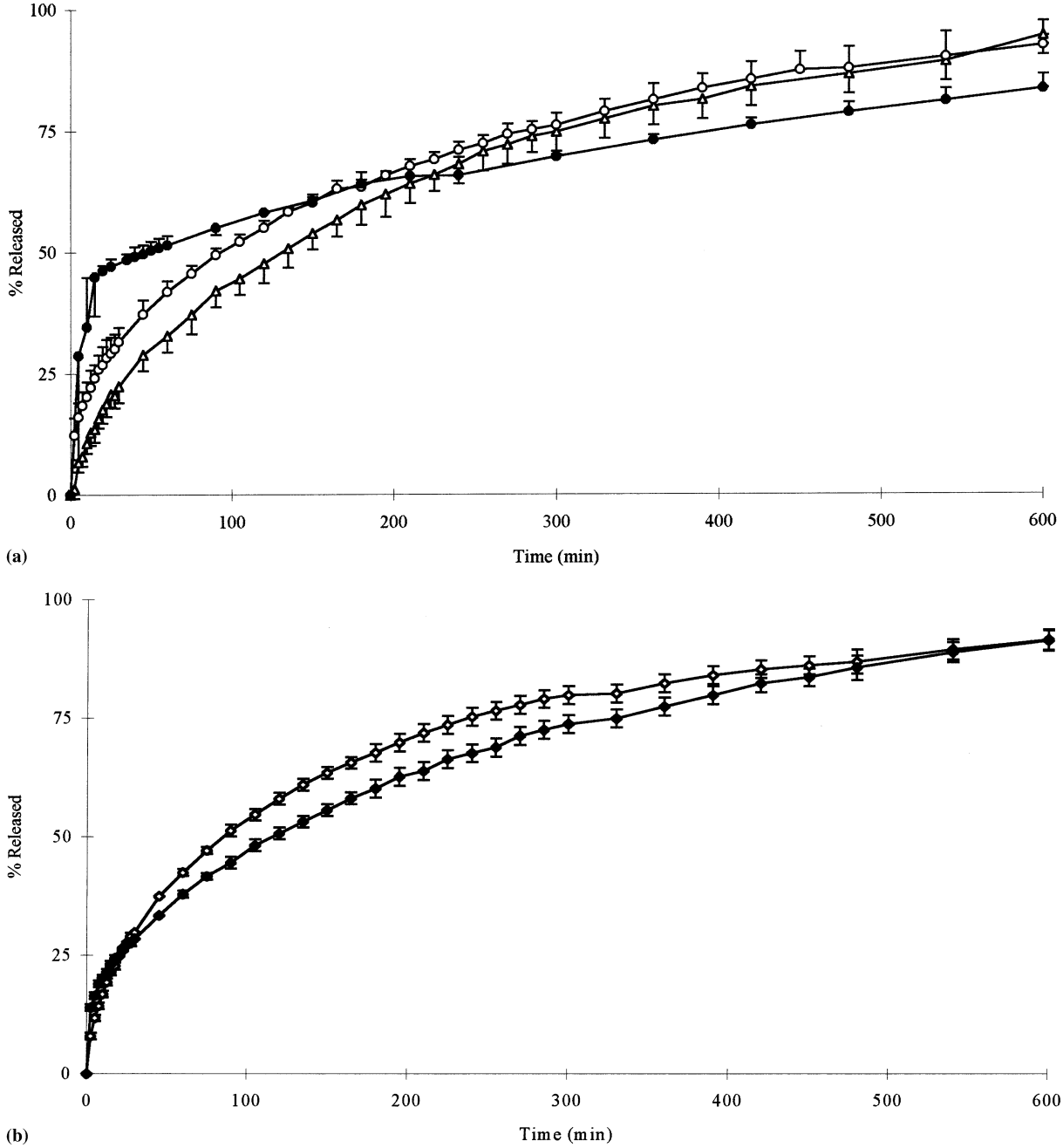
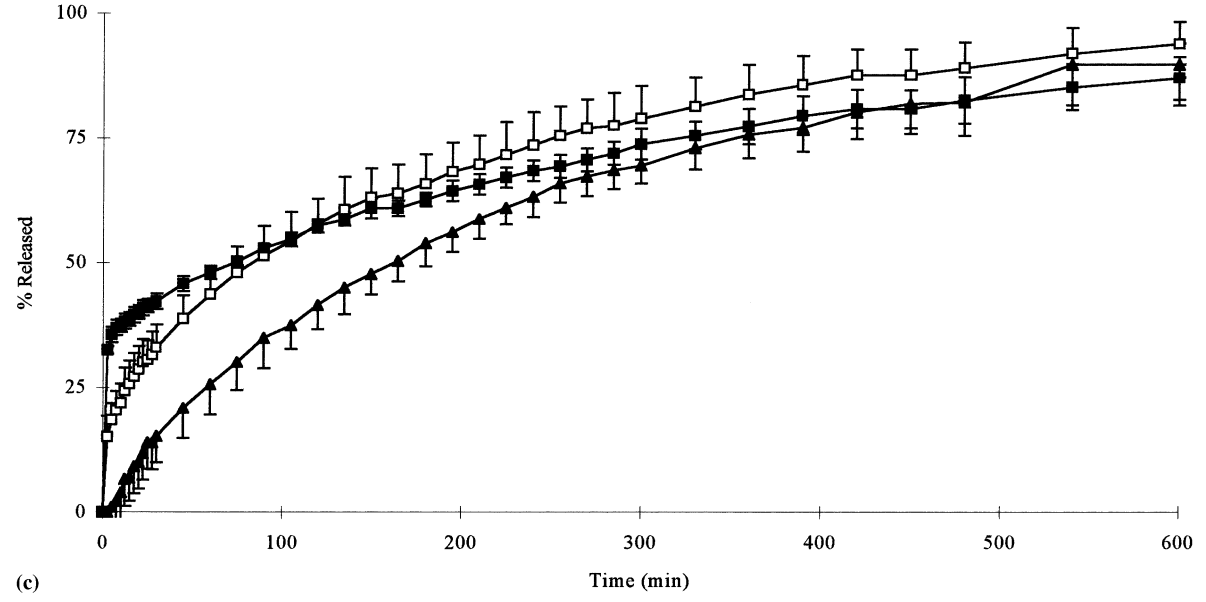


Fig. 1. In vitro release profiles showing the influence of drug loading on apomorphine release from Carbopol 971P (a), Carbopol 974P (b), and polycarbophil (c), and a comparison of release from the different polymers with similar (30% w/w) drug loading and lactose (d):
–◆– Carbopol 971P (15%) –◇– Carbopol 971P (30%) –■– Carbopol 971P (60%) –●– Carbopol 974P (15%) –□– Carbopol 974P (30%) –○– Polycarbophil (15%) –▲– Polycarbophil (30%) –△– Polycarbophil (60%) –X– Lactose mixture



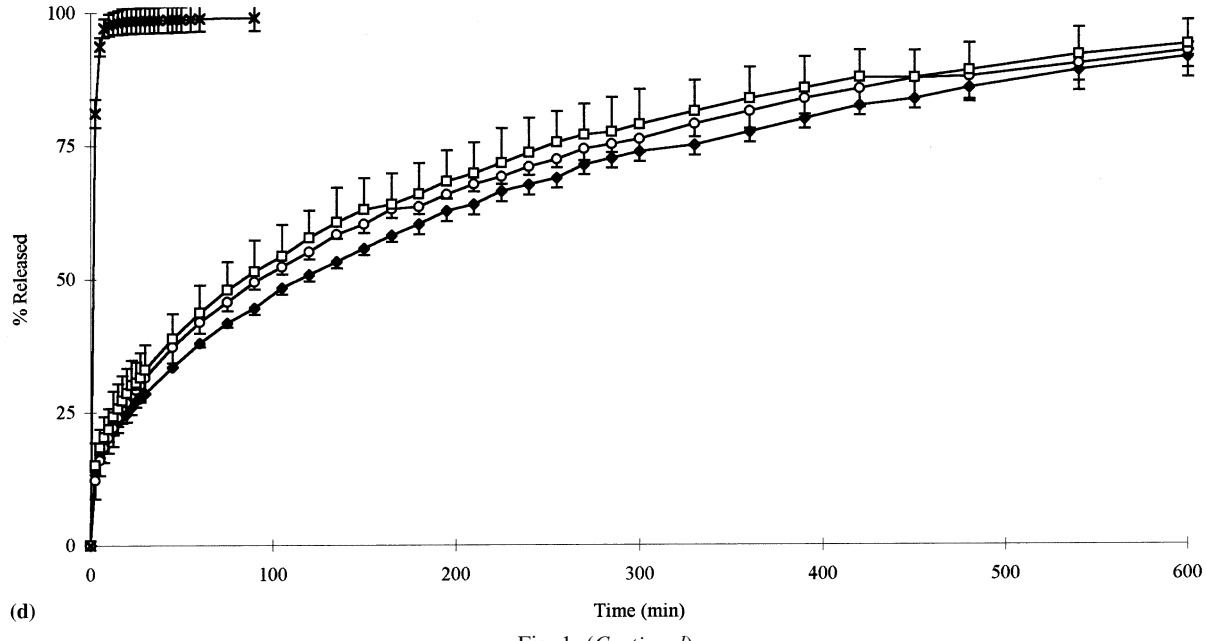


Fig. 1. (*Continued*)

not for Carbopol 974P (Fig. 1b). This influence of drug loading on in vitro release rate is in agreement with previous studies using Carbopol 934P, Carbopol 974P and polycarbophil (Vidgren et al., 1992; Hosny, 1993; Neau et al., 1996). No large differences in release rates were observed between different polymers of similar drug loading (Fig. 1d). Furthermore, sustained release of apomorphine was obtained compared with the lactose powder where more than 90% of the drug was released within 5 min. This rapid release also shows that the membrane filter separating the

drug from the release medium does not impede drug release appreciably.

Changing the drug loading also influenced the mechanism of release of apomorphine. According to Eq. (1), for a particular polymer, the highest value for the release exponent, n, was always obtained at 15% w/w drug loading (Table 2), showing a strong contribution by polymer relaxation to the overall drug release. There was a progressive decrease in the value of n with increasing drug loading, showing a change in the release mechanism from a state of strong influence by polymer relaxation at 15% w/w loading to that of diffusion controlled release (Vandelli et al., 1991) at higher drug loading. The relative contribution to drug release by polymer relaxation and Fickian diffusion is very evident from the values of k_1 and k_2 of Eq. (2) (Table 3). Except for 15% w/w drug-loaded polycarbophil, the influence of diffusion was always stronger than that of polymer relaxation. In agreement with Eq. (1), the trend is still maintained whereby diffusional contribution was strongest with the highest drug loading. Unfortunately, the influence of both drug loading and polymer type on the release mechanism could not be crosschecked with previous studies using similar dosage forms since no mechanistic treatment of the in vitro release data was carried out (Vidgren et al., 1992; Neau et al., 1996).

3.2. Pharmacokinetics analysis

As demonstrated in Fig. 2a–c, the plasma concentration–time profiles of apomorphine showed a lot of inter-animal variation. The time-courses of the drug in plasma following absorption from the various formulations were mixed with no large differences between different drug loading. However, it could be observed that, compared with the lactose control (Fig. 3), sustained release of apomorphine was achieved, and that absorption from the formulations loaded with about 60% w/w of apomorphine HCl was faster. This could probably be due to the smaller quantity administered at this drug loading, while unlike the in vitro release, those loaded with about 30 and 15% w/w of apomorphine were comparable except Carbopol 974P. Contrary to the in vitro release, the in vivo release from Carbopol 974P of 15% w/w drug loading was inexplicably slower than from 30% w/w drug loading. Comparing absorption from similar drug loading between different polymers, the absorption region of the plasma curves were similar at 15 and 60% w/w drug loading for all the polymers, whereas at 30% w/w loading, this region for Carbopol 974P was steeper (Fig. 2d). Nagai et al. (1984) previously reported nasal delivery of insulin using freeze-dried, neutralized Carbopol 934. While in that study insulin absorption was enhanced by neutralized Carbopol 934, apomorphine is well absorbed from the nose (van Laar et al., (1992). What our study shows, compared with previous studies on nasal delivery of apomorphine, is a sustained release.

3.3. T_{max} and absorption rate constant (k_a)

The empirical value $T_{\max}$ together with absorption rate constant (k_a), which offer independent criteria to evaluate the influence of drug loading on the in vivo drug release from drug formulations, were used for the comparison. The mean pharmacokinetics parameters are shown in Table 4. There was no large influence of drug loading on

Table 2
Release exponent, n, and constant, k, obtained from Eq. (1) showing the effect of drug loading on the in vitro release of apomorphine

	Carbopol 971P		Carbopol 974P		Polycarbophil	
	n	k	n	k	n	k
60%	0.1946	23.4016			0.1912	23.44
30%	0.3896	8.4202	0.3749	8.4672	0.3691	9.6618
15%	0.7562	1.1834	0.4752	5.7504	0.9422	0.377

Table 3
Drug diffusion (k_1) and polymer relaxation (k_2) constants according to Eq. (2) from the polymers with different drug loading.

	Carbopol 971P		Carbopol 974P		Polycarbophil	
	k_1	k_2	k_1	k_2	k_1	k_2
60%	9.2966	−0.2831			9.8106	−0.3418
30%	6.7339	−0.1418	6.2836	−0.1266	7.2976	−0.1714
15%	1.1694	0.2386	5.637	−0.0461	0.1016	0.2687

the in vivo release rates when the times to peak plasma concentrations (T_{max}) were compared (Table 4). With Carbopol 971P, T_{max} decreased above 30% w/w drug loading but was similar for drug loading 15 and 30% w/w. The difference between the T_{max} of Carbopol 971P loaded with 60% w/w of apomorphine and that with 15 and 30% w/w of the drug were not significant ($P =$ 0.2703 and 0.4617, respectively). For polycarbophil and Carbopol 974P, the T_{max} was similar for all drug loading.

For a particular drug loading, the T_{max} differs between different polymers with the T_{max} from Carbopol 971P being the slowest compared with Carbopol 974P or polycarbophil. At 15% w/w drug loading, Carbopol 971P had the longest T_{max} while Carbopol 974P and polycarbophil were similar. However, T_{max} from 15% w/w loaded Carbopol 971P was significantly different only to polycarbophil ($P = 0.0386$). At 30% w/w drug loading, Carbopol 971P still has the longest T_{max} but the difference was not statistically significant compared with polycarbophil ($P = 0.4267$) or to Carbopol 974P ($P = 0.2227$), which had the shortest T_{max}. At 60% w/w drug loading, T_{max} for both Carbopol 971P and polycarbophil were comparable.

Similar to T_{max}, the absorption rate constant, k_a and the $T_{1/2abs}$ values (Table 4) showed no large differences between the formulations loaded with about 15 and 30% w/w of apomorphine HCl. In four out of the 48 cases, very low k_a values (and, as such, large $T_{1/2abs}$ values) were obtained, resulting in a large standard error of the mean. Increasing the drug loading showed an increase in the absorption rate constants (and thus decreased $T_{1/}$ 2abs) for polycarbophil, while for Carbopol 971P, this occurred only above 30% w/w drug loading. Contrary to the T_{max}, at 15% w/w drug loading, Carbopol 971P has the shortest $T_{1/2abs}$ compared with Carbopol 974P and polycarbophil, which were similar. Applying a statistical test of significance on $T_{1/2,abs}$ as performed for T_{max} revealed no significant differences except those between polycarbophil with 60 and 15% w/w ($P = 0.0598$) and 30% w/w ($P = 0.0633$) drug loading, both of which were but marginally significant. It is noteworthy that these parameters for assessing the in vivo release and absorption rates sometimes gave different overall results. For instance, very long $T_{1/2abs}$ values were obtained in some cases (data from individual rabbits not included), but this did not correspond to prolonged T_{max} values. Additionally, the trend of the means of these parameters gave different pictures.

Even though the reported viscosity values (0.5% dispersion) of Carbopol 974P (29 400–39 400 mPa s) and polycarbophil ($\approx$ 20 000 mPa s) are higher than that of Carbopol 971P (4000–11 000 mPa s) (BF Goodrich, 1997), the release rates of apomorphine HCl from them were no slower than that of Carbopol 971P. Differences between the hydrated macromolecular structure of the polymers may have contributed to this observation. The postulated 'fish-net' gel structure for lightly cross-linked polymers like Carbopol 971P may have contributed more resistance to drug release than the heterogeneous 'fuzzball' gel structure for more highly cross-linked polymers such as Carbopol 974P and polycarbophil (Dittigen et al., 1997). Passive diffusion out of an inert polymer matrix may also not be the only or dominant factor contributing to the release of the drug from these polyacrylic acids. Interaction between the poly-

Table 4
Mean pharmacokinetics parameters for each formulation and/or drug loading (± SEM) in rabbits following intranasal administration of 0.61 mg/kg body weight of apomorphine

	Carbopol 971P			Carbopol 974P		Polycarbophil		
	15%	30%	60%	15%	30%	15%	30%	60%
AUC (ng/ml min)	27752.3 (± 1716.94)	36862.5 (± 2412.516)	34869.2 (± 2096.256)	26993.0 (± 1534.53)	35881.8 (± 1589.38)	28831.4 (± 1356.746)	32894.96 (± 2435.51)	34415.9 (± 3463.16)
C_{max} (ng/ml)	309.6 (± 30.64)	369.4 (± 70.22)	382.5 (± 19.31)	285 (± 54.95)	463.5 (± 59.85)	292.6 (± 41.65)	342.6 (± 49.0)	476.1 (± 87.53)
T_{max} (min)	52.7 (± 3.39)	52.9 (± 8.45)	45.2 (± 5.46)	42.7 (± 4.61)	40.1 (± 5.05)	42.7 (± 2.48)	45.2 (± 3.87)	42.8 (± 6.02)
k_a (min^{-1})	0.0177 (± 0.0015)	0.0144 (± 0.0023)	0.0245 (± 0.0053)	0.0158 (± 0.0031)	0.0170 (± 0.0026)	0.0140 (± 0.002)	0.0158 (± 0.0022)	0.0242 (± 0.002)
$T_{1/2abs}$ (min)	40.44 (± 3.09)	58.95 (± 14.23)	34.15 (± 5.58)	61.08 (± 20.28)	45.43 (± 6.30)	59.64 (± 15.27)	51.60 (± 11.58)	27.04 (± 1.70)

mers and apomorphine HCl may have played a role, as reported previously between Carbopol 934 and some cationic drugs (Elgindy, 1976).

3.4. Area under the curve

The AUC from 15% w/w loaded polymers were consistently lower than those with higher drug loading, whereas those loaded with 30 and 60% w/w apomorphine HCl were similar (Table 4). These differences were statistically significant between the following drug loadings of Carbopol 971P, 15 and 30% w/w ($P = 0.0117$), and between 15 and 60% w/w ($P = 0.0253$). There was also a very significant difference between Carbopol 974P of 15 and 30% w/w ($P = 0.0024$), but none be-

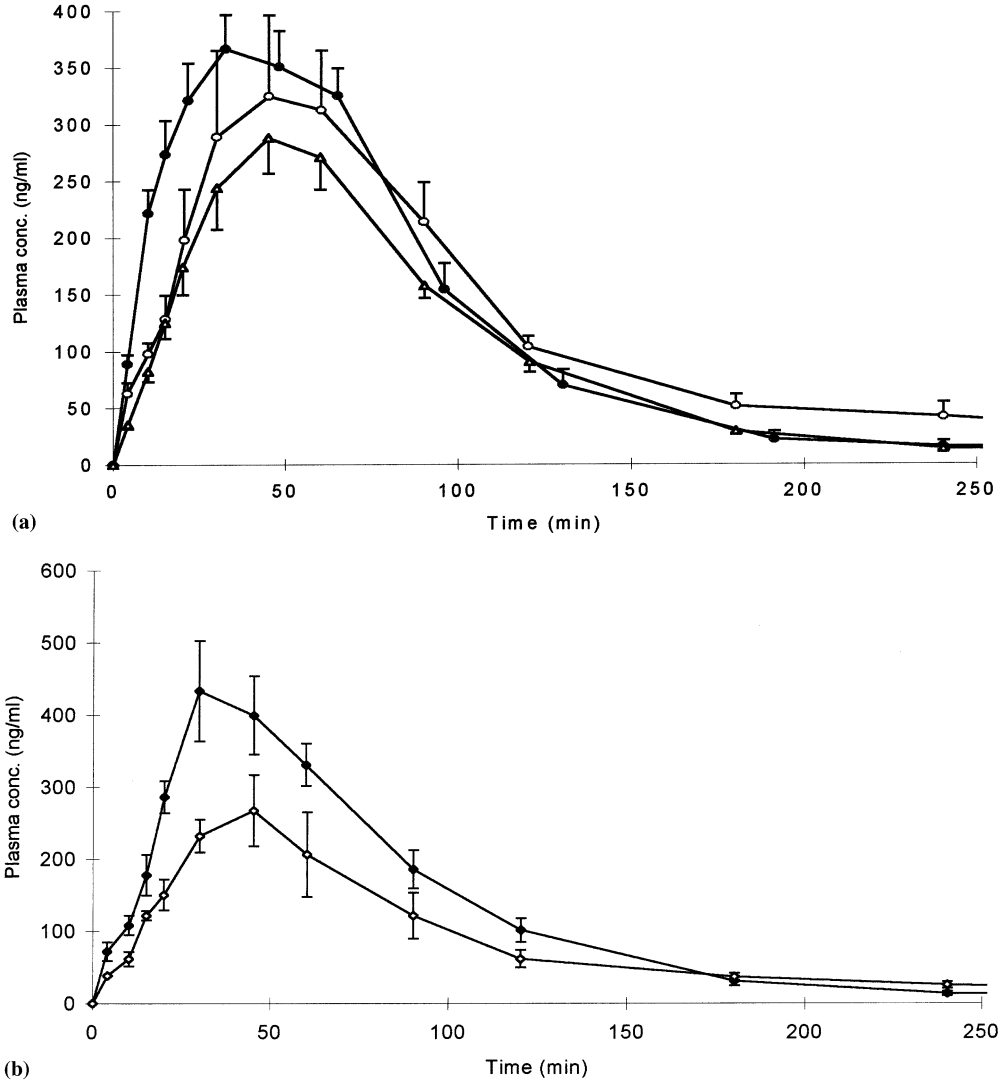
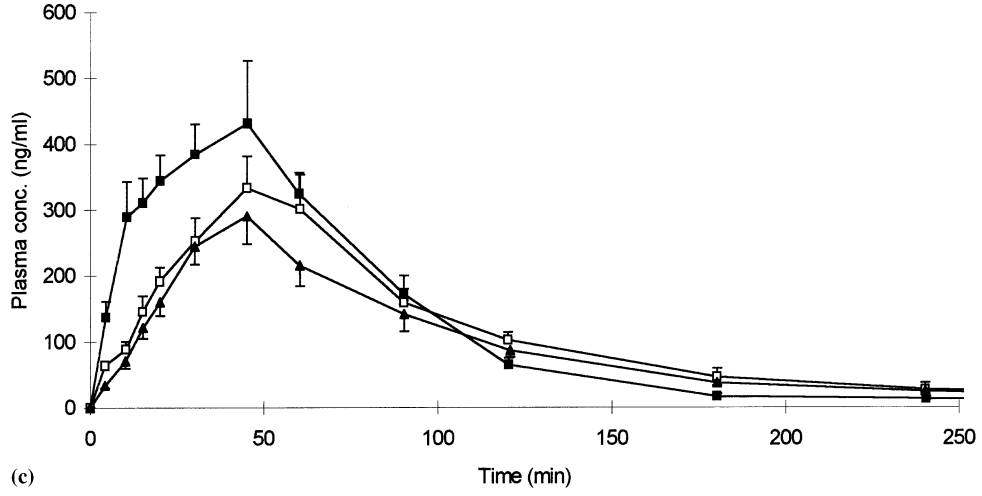


Fig. 2. Plasma concentration–time plots showing the influence of drug loading on in vivo release following the intranasal administration (0.61mg/kg of apomorphine) to rabbits of lyophilized powder formulations of Carbopol 971P (a), Carbopol 974P (b), and polycarbophil (c), and a comparison of release from the different polymers with similar drug loading (d): –◆– Carbopol 971P (15%) –◇– Carbopol 971P (30%) –■– Carbopol 971P (60%) –●– Carbopol 974P (15%) –□– Carbopol 974P (30%) –○– Polycarbophil (15%) –▲– Polycarbophil (30%) –△– Polycarbophil (60%)



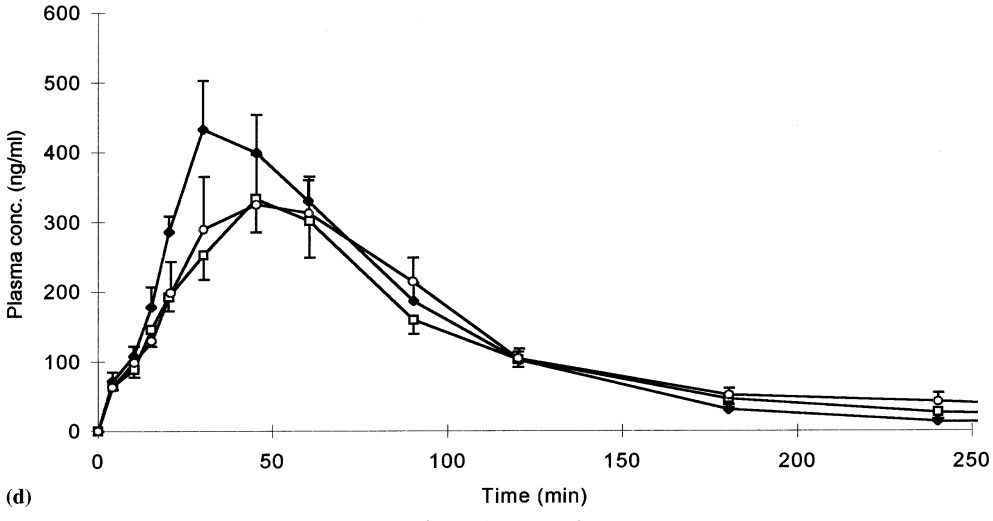


Fig. 2. (*Continued*)

tween following drug loadings of polycarbophil 15 and 30% w/w ($P = 0.1756$), and 15 and 60% w/w ($P = 0.1641$). It has been reported that deposition site and pattern influence intranasal absorption (Chien, 1992; Provasi et al., 1994). Lower drug loading entails a larger quantity of the powder formulation to be administered for an equivalent dose; therefore, the deposition site and pattern may have played a role in this case. The smaller quantity administered may have been deposited at that part of the nasal mucosa where absorption is optimum. A larger quantity administered would inevitably be deposited over a wider surface area within the nasal cavity. This will lead to a faster rate of clearance of the fraction deposited at the posterior part of the nasal cavity, as suggested by Provasi et al. (1994). This may have, together with drug release, especially for the drug loading of 60% w/w, contributed to the absorption being faster, leading to higher C_{max} and AUC. Similar influence of quantity of a formulation administered intranasally was also reported by Harris et al. (1989), where it was found that absorption of desmopressin was higher with 100 μl than with 200 μl of solution. Also, the nasal clearance time of 200 μl of the solution was faster.

Given the slow release and absorption of apomorphine from the formulations with 15 and 30% w/w drug-loadings, the AUC accomplished with lower peak plasma drug concentration (that can, in clinical situations, reduce side-effects), these drug loadings can be selected as compromise formulae. They also appear to be more physically stable.

3.5. Peak plasma concentration

A trend similar to the AUC was noted for the peak plasma concentrations. It was observed that, for a particular polymer, increasing the drug loading led to increased peak plasma concentration (Table 4). This difference was only marginally significant between Carbopol 971P with 15 and 60% w/w ($P = 0.0718$), Carbopol 974P with 15 and 30% w/w ($P = 0.0527$), and polycarbophil with 15 and 60% w/) ($P = 0.0876$) drug loadings. This observation was the same with all polymers used and may be connected with the lower AUC obtained from the formulations with lower drug loading. No particular polymer consistently had a higher or lower C_{max} across all the drug loading.

3.6. Immediate release-containing formulation

A formulation consisting of a lyophilized immediate release lactose–apomorphine HCl mixture mixed with lyophilized Carbopol 971P powder also loaded with apomorphine was compared with a formulation consisting of only lyophilized Carbopol 971P also loaded with apomorphine. The pharmacokinetics parameters compared were release profile, T_{max}, C_{max} and AUC (Fig. 3 and Table 5). Only in one case was there two plasma concentration peaks. Additionally, the initial plasma concentrations (0–15 min) from the formulation containing an immediate release portion were not higher than those given by only the slow release formulation. The mean T_{max}, C_{max} and AUC values from the lactose-containing formulation were all lower than those without lactose, but the difference between the T_{max} was not significant ($P = 0.2788$), while the C_{max} and AUC were only marginally significant (0.0911 and 0.0668, respectively). Therefore, the lactose mixture was dispersed within the Carbopol matrix and drug release from the latter overshadowed that from the former. This may have prevented the occur-

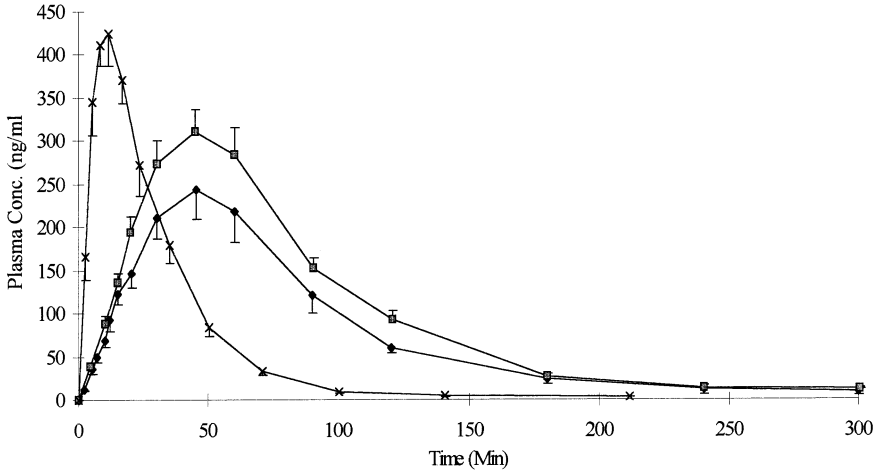


Fig. 3. Plasma concentration–time curves following administration of a formulation containing an immediate release portion, Carbopol 971P/lactose, compared with Carbopol 971P (0.61 mg/kg of apomorphine) and lactose mixture (0.305 mg/kg of apomorphine) all containing apomorphine:
–◆– Carbopol 971P + Lactose –□– Carbopol 971P –X– Lactose mixture.

Table 5
Pharmacokinetics parameters of apomorphine from Carbopol 971P formulation of apomorphine compared with Carbopol 971 P NF containing an immediate release lactose portion[a]

	Carbopol 971P	Carbopol 971P+lactose mixture	Lactose mixture
AUC (ng/ml min)	27120 ($\pm$1720.87)	21960 ($\pm$1720.64)	14189 ($\pm$1061.26)
C_{max} (ng/ml)	330.61 ($\pm$27.29)	245.67 ($\pm$34.81)	450.72 ($\pm$30.94)
T_{max} (min)	52.21 ($\pm$3.72)	45.21 ($\pm$4.74)	11.4 ($\pm$1.28)

[a] The dose of apomorphine was 0.61 mg/kg body weight for Carbopol 971P and Carbopol 971P+lactose mixture, and 0.305 mg/kg body weight for lactose mixture alone.

rence of two peaks and also leading to the observed lack of statistical significant differences between the two formulations.

Apomorphine has been reported to be rapidly absorbed from the nose with the therapeutic effect being felt rapidly, within a mean of 8.9 min (Corboy et al., 1995). It was therefore tempting to combine such fast therapeutic action using an immediate release lactose powder with the sustained release formulations. Unfortunately, this objective could not be achieved with only one formulation.

4. Conclusion

These results show that increasing the drug loading influences the in vitro release rate and release mechanism but does not increase to a large extent the rate of release and absorption in vivo in rabbits. The trend observed was that increasing the drug loading led to increased C_{max} and AUC, as well as a faster rate of absorption and decreased time to achieve the peak plasma drug concentration. Mixing of an immediate release powder entraps the drug within the Carbopol matrix with no resultant initial increase in plasma drug concentration and this may be an explanation for the absence of an immediate release peak.

References

BF Goodrich, 1997. Product information provided by BF Goodrich. Cleveland, OH, USA.

Ch'ng, H.S., Park, H., Kelly, P., Robinson, J.R., 1985. Bioadhesive polymers as platforms for oral controlled drug delivery II: synthesis and evaluation of some swelling, water-insoluble bioadhesive polymers. J. Pharm. Sci. 74, 399–405.

Chaudhuri, K.R., Clough, C., 1998. Subcutaneous apomorphine in Parkinson's disease: Effective yet underused. Br. Med. J. 316, 641.

Chien, Y.W., 1992. Novel Drug Delivery Systems. Marcel Dekker, New York.

Corboy, D.L., Wagner, M.L., Sage, J.I., 1995. Apomorphine for motor fluctuation and freezing in Parkinson's disease. Ann. Pharmacother. 29, 282–288.

Dittigen, M., Durrani, M., Lehmann, K., 1997. Acrylic polymers, a review of pharmaceutical applications. Stp Pharma 7, 403–437.

Edsman, K., Carlfors, J., Harju, K., 1996. Rheological evaluation and ocular contact time of some carbomer gels for ophthalmic use. Int. J. Pharm. 137, 233–241.

Elgindy, N.A., 1976. Molecular entrapment of cationic drugs by Carbopol 934. Can. J. Pharm. Sci. 11, 32–34.

Filipovic-Grcic, J., Jalsenjak, I., 1992. Microspheres of human serum albumin with barbiturates: Effect of drug partition coefficient on preparation and release. J. Pharm. Pharmacol. 45, 394–399.

French, D.L., Himmelstein, K.J., Manger, J.W., 1995. Physicochemical aspects of controlled release of substituted benzoic and naphtoic acids from carbopol gels. J. Control. Release 37, 281–289.

Gander, B., Gurny, R., Doelker, E., Peppas, N.A., 1988. Crosslinked poly(alkylene oxides) for the preparation of controlled release micromatrices. J. Control. Release 5, 271–283.

Gunder, W., Lippold, B.H., Lippold, B.C., 1995. Release of drugs from ethyl cellulose microcapsules (diffusion pellets) with pore formers and pore fusion. Eur. J. Pharm. Sci. 3, 203–214.

Harris, A.S., Ohlin, M., Svensson, E., Lethagen, S., Nilsson, I.M., 1989. Effect of viscosity on the pharmacokinetics and biological response to intranasal desmopressin. J. Pharm. Sci. 78, 470–471.

Hosny, E.A., 1993. Polycarbophil as a controlled release matrix. Int. J. Pharm. 98, 235–238.

Illum, L., Jorgensen, H., Bisgaard, H., Krogsgaard, O., Rossing, N., 1987. Bioadhesive microspheres as a potential nasal drug delivery system. Int. J. Pharm. 39, 189–199.

Kapoor, R., Turjanski, N., Frankel, J.P., Kleedorfer, B., Lees, A., Stern, G., 1990. Intranasal apomorphine: a new treatment in Parkinson's disease. J. Neurol. Neurosurg. Psychiatry 53, 1015.

Merck Index, 1983. Tenth edition. Merck & Co., NJ, USA.

Morimoto, K., Morisaka, K., 1987. In vitro release and rectal absorption of barbital and aminopyrine from aqueous polyacrylic acid gel. Drug Dev. Ind. Pharm. 13, 1293–1305.

Mortazavi, S.A., 1995. An in vitro assessment of mucus/mucoadhesive interactions. Int. J. Pharm. 124, 173–182.

Nagai, T., Nishimoto, Y., Nambu, N., Suzuki, Y., Sekine, K., 1984. Powder dosage form of insulin for nasal administration. J. Control. Release 1, 15–22.

Nagai, T., 1985. Adhesive topical drug delivery systems. J. Control. Release 2, 121–134.

Neau, S.H., Chou, M.Y., Durrani, M.J., 1996. Fabrication and characterization of extruded and spheronized beads containing carbopol 974P, NF resin. Int. J. Pharm. 131, 47–55.

Okada, J., Masuyama, Y., Kondo, T., 1992. An analysis of first-order release kinetics from albumin microspheres. J. Microencapsulation 9, 9–18.

Park, H., Robinson, J.R., 1985. Physico-chemical properties of water insoluble polymers important to mucin/epithelial adhesion. J. Control. Release 2, 47–57.

Peppas, N.A., Ponchel, G., Duchene, D., 1985. Bioadhesive analysis of controlled-release systems. II. Time dependent bioadhesive stress in poly(acrylic acid)-containing systems. J. Control. Release 5, 143–149.

Ponchel, G., Touchard, F., Duchene, D., Peppas, N.A., 1985. Bioadhesive analysis of controlled-release systems. I. Fracture and interpenetration analysis in poly(acrylic acid)-containing systems. J. Control. Release 5, 121–141.

Ponchel, G., Touchard, F., Wouessidjewe, D., Duchene, D., Peppas, N.A., 1987. Bioadhesive analysis of controlled-release systems. III. Bioadhesive and release behaviour of metronidazole-containing poly(acrylic acid)-hydroxypropyl methylcellulose systems. Int. J. Pharm. 38, 65–70.

Provasi, D., De Ascentiis, A., Minutello, A., Colombo, P., Catellani, D.L., 1994. Nasal powders of progesterone: manufacturing and bioavailability. Eur. J. Pharm. Biopharm. 40, 223–227.

Ritger, P.L., Peppas, N.A., 1987. A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. J. Control. Release 5, 23–36.

Sam, E., Augustijns, P., Verbeke, N., 1994. Stability of apomorphine in plasma and its determination by high-performance liquid chromatography with electrochemical detection. J. Chromatogr., B 658, 311–317.

Sam, E., Jeanjean, A.P., Maloteaux, J.M., Verbeke, N., 1995. Apomorphine pharmacokinetics after intranasal and subcutaneous application. Eur. J. Drug Metab. Pharmacokinet. 20, 27–33.

See, N.A., Russell, J., Connors, K.A., Bass, P., 1987. Adsorption of inorganic ions to polycarbophil as a means of sustained release dosage formulation. Pharm. Res. 4, 244–247.

Tobyn, M.J., Johnson, J.R., Dettmar, P.W., 1996. Factors affecting in vitro gastric mucoadhesion II. Physical properities of polymers. Eur. J. Pharm. Biopharm. 42, 56–61.

Ugwoke, M.I., Verbeke, N., Kinget, R., 1997. Microencapsulation of apomorphine HCl with gelatin. Int. J. Pharm. 148, 23–32.

Ugwoke, M.I., Sam, E., Van Den Mooter, G., Verbeke, N., Kinget, R., 1998. Eur. J. Pharm. Sci. (submitted).

van Laar, T., Jansen, E.N.H., Essink, A.W.G., Neef, C., 1992. Intranasal apomorphine in parkinsonian on–off fluctuations. Arch. Neurol. 49, 482–484.

Vandelli, M.A., Forni, F., Coppi, G., Cameroni, R., 1991. The effect of the crosslinking time period upon the drug release and dynamic swelling of gelatin microspheres. Pharmazie 46, 866–869.

Vidgren, P., Vidgren, M., Arppe, J., Hakuli, T., Laine, E., Paronen, P., 1992. In vitro evaluation of spray-dried mucoadhesive microspheres for nasal administration. Drug Dev. Ind. Pharm. 18, 581–597.