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Microencapsulation of apomorphine HCl with gelatin

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Abstract

Microspheres of apomorphine HCl were prepared, with gelatin as polymer coating material, using the emulsification solvent extraction method. The microencapsulation efficiency, particle size and release profile were compared to those of riboflavin sodium phosphate (RSPh). An efficiency of approximately 95% was obtained with RSPh while the quantity of acetone and ether used as preparative solvents resulted in low microencapsulation efficiency for apomorphine HCl. A reduction of the volumes of these solvents improved the efficiency by a factor of more than two but the mean particle size range became slightly higher due to agglomeration as compared to the mean particle size when more solvent was used. A faster release rate was found for RSPh than for apomorphine HCl, according their water solubilities. Crosslinking of gelatin-RSPh microspheres with formaldehyde and glutharaldehyde (10 and 20% v/v for 1, 6, and 24 h) did not extend the duration of release of RSPh and reduced the quantity of drug microencapsulated. © 1997 Elsevier Science B.V.

Keywords: Gelatin microsphere; Microencapsulation; Apomorphine HCl; Riboflavin sodium phosphate; Nasal drug delivery; Controlled release

1. Introduction

Apomorphine HCl is a dopamine agonist which is effective in the management of Parkinsonism, especially the 'on-off' motor response fluctuations (Corsini et al., 1979; Obeso et al., 1987; Stibe et al., 1987, 1988; Poewe et al., 1988; Pollak et al., 1989; Gancher et al., 1991; Sam et al., 1994). It has advantages over the other dopamine agonists, bromocriptine and pergolide, because of its fast onset of action, potent decrease in 'off' periods and lower psychologic complications (Corboy et al., 1995). The absorption of apomorphine HCl

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from various routes is variable and the subcutaneous route has almost exclusively been used until recently when the intranasal administration (Kapoor et al., 1991) was proposed. Parenteral routes of drug administration when used in the treatment of chronic diseases are both expensive and inconvenient to the patient. This makes a nonparenteral route such as the nose very attractive. Preliminary reports of the efficacy of intranasal apomorphine HCl in Parkinsonism was quite promising in terms of the degree of absorption and onset of action (Kapoor et al., 1991; van Laar et al., 1992a,b). The response was equivalent to the pharmcodynamic profile of apomorphine HCl following subcutaneous administration (Corboy et al., 1995). Similar results were obtained in the studies carried out in our laboratory (Sam et al., 1995). The duration of action was rather short as expected from the elimination half-life of 30 min. Considering the chronic nature of the disease and the debility of the patients, this is unacceptable. Prolonged administration using infusion pumps has been tried, but tolerance at injection site was poor. A mucoadhesive dosage form which can extend drug residence time and prolong drug absorption could be a valuable alternative to improve the duration of drug effect. This has prompted further research in our laboratory for a nasal mucoadhesive formulation of apomorphine HCl. Such a dosage form should resist the mucociliary action of the nose, thereby increasing drug residence time on the nasal mucosa and as such its duration of action. Riboflavin sodium phosphate (RSPh) was chosen as a model water-soluble drug for the microencapsulation study. Indeed, it is much more stable than apomorphine HCl and as such easier to handle. This paper examines the microencapsulation of RSPh, using the mucoadhesive polymer gelatin (Longer and Robinson, 1986) and by the emulsification solvent extraction method, and its release from crosslinked and noncrosslinked microspheres. Two crosslinking agents, glutharaldehyde and formaldehyde, were investigated to elucidate their effects on the entrapment and release of RSPh since they are reported to extend the duration of release (Luzzi and Gerraughty, 1967; Leucuta, 1989; Raymond et al., 1990; Vandelli et al., 1991a; Vyas et al., 1991). Subsequently, using the optimized method, microspheres of apomorphine HCl were prepared and its properties compared to those of RSPh.

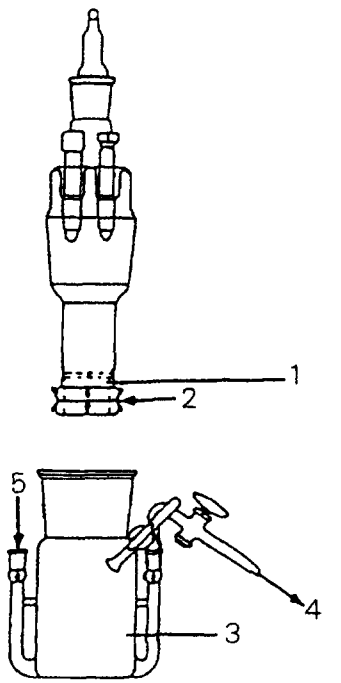

Fig. 1. Schematic diagram of Desaga Resomat® 147030. 1, Donor compartment; 2, membrane filter; 3, receptor compartment; 4, to flow-through cell; and 5, from flow-through cell.

2. Materials and methods

2.1. Materials

Gelatins of Bloom 240 was kindly donated by Sanofi Bio-Industry (B-Brussels) and used as

Table 1
Particle size (μm) distribution of gelatin-apomorphine HCl microspheres

Batch	1	2	3	*4	*5	*6
Mean	36.14	32.06	34.79	45.70	42.24	40.21
Median	27.22	29.63	33.42	46.23	42.04	25.23
10.0%>	75.08	57.71	55.00	75.21	67.31	94.44
10.0%<	9.20	9.38	12.05	13.09	13.96	9.13

* See Section 3.2.

such. Liquid paraffin, apomorphine HCl, riboflavin sodium phosphate (RSPh), were European pharmacopoeial grade; glutharaldehyde and formaldehyde, reagent grade.

2.2. Methods

2.2.1. Preparation of gelatin microspheres

The microencapsulation method is a modification of the emulsification solvent extraction method (Tanaka et al., 1963) as we reported (Ugwoke and Kinget, 1996).

2.3. Analysis of microencapsulation efficiency

For noncrosslinked microspheres, an aliquot from each batch (30.0 mg) was sonicated for 30 min in 0.1 N HCl (100 ml) to break up the drug-containing microspheres. The gelatin-RSPh microspheres were sonicated in 0.1 M phosphate buffer of pH 7.0. After sonication, the mixtures were filtered through a sintered glass filter and the absorbance measured spectrophotometrically (HP 84752 A Hewlett Packard, Santa Clara, CA) at 272 nm for apomorphine HCl and at 266 nm for RSPh. The amount of drug encapsulated was calculated from standard calibration curves (correlation coefficient 0.9998 for apomorphine HCl and 0.9991 for RSPh).

In case of crosslinked microspheres, the microcapsules (100 mg) had to be first crushed in a porcelain mortar, since crosslinked gelatin microspheres do not break up with sonication. A 30.0 mg aliquot of the powdered mass was then sonicated in 100 ml of 0.1 M phosphate buffer for 30 min and the liquid filtered through a sintered glass filter.

2.4. Chemical crosslinking

Chemical crosslinking of the gelatin-RSPh microspheres was performed with formaldehyde and glutharaldehyde solutions in acetone. The concentrations of the crosslinking agents used were 10 and 20% (v/v). Crosslinking agent (20 ml) was added to gelatin-RSPh (500 mg) and allowed to stand at room temperature for 1, 6, or 24 h, filtered and rinsed with 20 ml of acetone.

2.5. Particle size analysis

It was necessary to reduce the agglomeration in the presence of water by crosslinking the microspheres with formaldehyde before measuring the particle size as we previously described (Ugwoke and Kinget, 1996). An aliquot of the microspheres (500 mg) was treated with 20 ml of a solution of formaldehyde in acetone (10% v/v) for 6 h, filtered and rinsed with 20 ml of acetone. Particle size analysis was performed with the Coulter Multisizer II (Coulter Electronics, GB-Beds) in sodium chloride solution (0.9% w/v).

2.6. Measurement of the release rate of apomorphine HCl from gelatin microspheres

Desaga Resomat® 147030 (Desaga, D-Heidelberg), shown in Fig. 1 was the apparatus chosen for the study of drug release. A situation similar to the in vivo deposition of the microspheres in the nose can be simulated with this apparatus. A membrane filter which separates the donor from the receptor compartment provides a barrier to the release medium just like the nasal epithelium but only to the extent that the microspheres absorbs just enough water for hydration. The pore size of 0.45 μm is such that it does not act as a limiting barrier to drug release. The only limiting factor to drug release thus is from the dosage form.

Gelatin microspheres (apomorphine HCl microspheres, 100 mg and RSPh microspheres, 70 mg) were weighed on a membrane filter (0.45 μm) and secured firmly between the ground glass surfaces of the Desaga Resomat® with a rubber band. The release medium, (0.1 N HCl for apomorphine HCl microspheres and 0.1 M phosphate buffer pH 7.0 for RSPh microspheres) was introduced into the receptor compartment in a water-bath maintained at 37°C and stirred continuously. The donor compartment was then lowered into the receptor compartment, connected to a flow-through cell and continuous circulation of the medium was maintained with a pump (Gilson Minipuls 3, Gilson, F-Villiers Le Bel). The absorbance of the drug released was measured at 272 nm for apomorphine HCl and 266 nm for

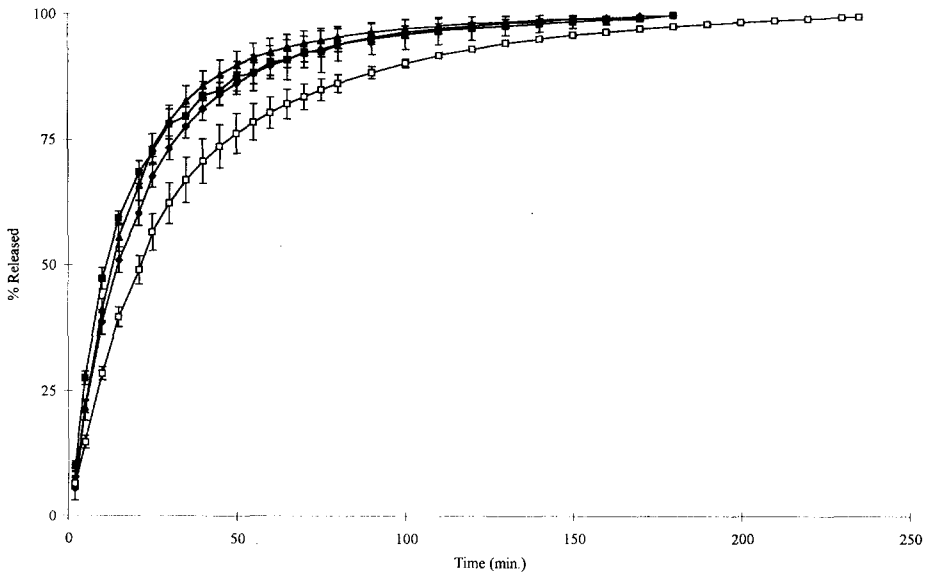

Fig. 2. Release profiles of riboflavin sodium phosphate from noncrosslinked microspheres for batches 1 (◆), 2 (■), 3 (▲) and 4 (□).

RSPh with a spectrophotometer (HP 84752 A Hewlett Packard, Santa Clara, CA) from the flow-through cell at specific time intervals.

2.7. Treatment of in vitro release data

Statistical analysis of the $t_{50\%}$ and $t_{85\%}$ was performed with a computer software, Instat (Graphpad Software, San Diego, CA). Repeated measures ANOVA was used for statistical treatment of the data at the 5% level.

To deduce the mechanism of drug release from the microspheres, the release data were fitted to the general exponential equation which is often used to describe the drug release behaviour from polymeric materials:

$$M_t/M_\infty = kt^n$$

where M_t/M_∞ is the fraction of drug released (≤ 0.6) 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). The release data were fitted with the equation using the software XY-math (Shareware Version 2.4, C. Taylor, Sacramento, CA).

3. Results and discussion

3.1. Microencapsulation efficiency

Microencapsulation efficiency as used here is defined as the ratio of the actual quantity of drug encapsulated within the microspheres to the theoretical quantity added during the emulsification phase, expressed as a percentage. Microencapsulation efficiency of RSPh was very high (92.7, 91.8 and 97.4% from three batches of microspheres) and reproducible. Following crosslinking for 6 h, only 70% of the original RSPh content was left within the microspheres. Although RSPh is not soluble in acetone, the reason for such amount of drug-loss is not far-fetched. This is because the crosslinking agent, formaldehyde is supplied as an aqueous solution (37.0% w/v). Since RSPh is freely soluble in water (112 mg/ml), the small quantity of water present in the crosslinking solution was capable to extract a substantial amount of RSPh from the microspheres during crosslink-

Table 2
$t_{50\%}$ and $t_{85\%}$ (min) of riboflavin sodium phosphate

Microsphere batch	Crosslinking time (h)	$t_{50\%}$	$t_{85\%}$
[a]Noncrosslinked microspheres	0	(1) 14, (2) 11, (3) 13, (4) 20	(1) 46, (2) 44, (3) 37, (4) 75
Drug powder sample	0	3	9
Formaldehyde solution (% v/v)			
10	1	11	51
10	6	8	31
20	1	8	35
20	6	7	26
20	24	8	43
Glutharaldehyde solution (% v/v)			
10	1	34	100
10	6	12	40
20	1	15	85
20	6	18	50
20	24	15	40

[a] Batches 1–3 were used in crosslinking with formaldehyde, and batch 4 with glutharaldehyde.

ing. Contrary to the results with RSPh, very low microencapsulation efficiencies were recorded for the batches of microspheres containing apomorphine HCl prepared with the normal quantity of solvents (11.9, 14.8 and 24.0%). Physical observation of the microspheres showed no sign of extensive oxidation (green coloration) that could have resulted in the low degree of drug within the microspheres. A possible cause of the low microencapsulation efficiencies obtained was from the solubility of apomorphine HCl in the solvents used, especially acetone which was used in the largest amount. A reduction in the quantity of acetone used confirmed this by the higher microencapsulation efficiency values of the batches prepared using only 50 ml of acetone (56.5, 59.6 and 54.9%). Such situation of reduced microencapsulation efficiency due to the solvent effect has earlier been reported for alginate microspheres using isopropanol as the dehydrating solvent (Wan et al., 1992). In order to surmount this problem, alternative solvents such as methanol, ethanol or isopropanol could be used but apomorphine HCl remains soluble too in these solvents.

3.2. Particle size of the microspheres

Table 1 shows the particle size distribution of gelatin-apomorphine HCl microspheres. 10% > and 10% < denote the upper and lower limits of particle size (μm) of microspheres, i.e. 10% of the total number of microspheres are greater or less than the particle size stated. The mean particle size for the batches of apomorphine HCl microspheres prepared with a smaller quantity of solvents (*4, *5, *6) were higher than the others. This increase in mean particle size can be ascribed to agglomeration of the small sized microspheres as observed under the microscope. Using a smaller quantity of dehydrating solvent increases the microencapsulation efficiency, but also leads to agglomeration of the microspheres.

3.3. Release profiles of RSPh

The release profiles of RSPh from noncrosslinked microspheres are shown in Fig. 2. The times taken for the release of 50 and 85% of the microencapsulated drug are denoted by $t_{50\%}$ and $t_{85\%}$ respectively and are used to describe the drug release. The $t_{50\%}$ and $t_{85\%}$ for the release of RSPh from noncrosslinked gelatin microspheres are listed in Table 2. These rates of release are faster than that of apomorphine HCl but somewhat slow when compared to the drug powder. A prolonged extension in duration of release could not be said to have been achieved. Figs. 3 and 4 show the release profiles of RSPh from microspheres

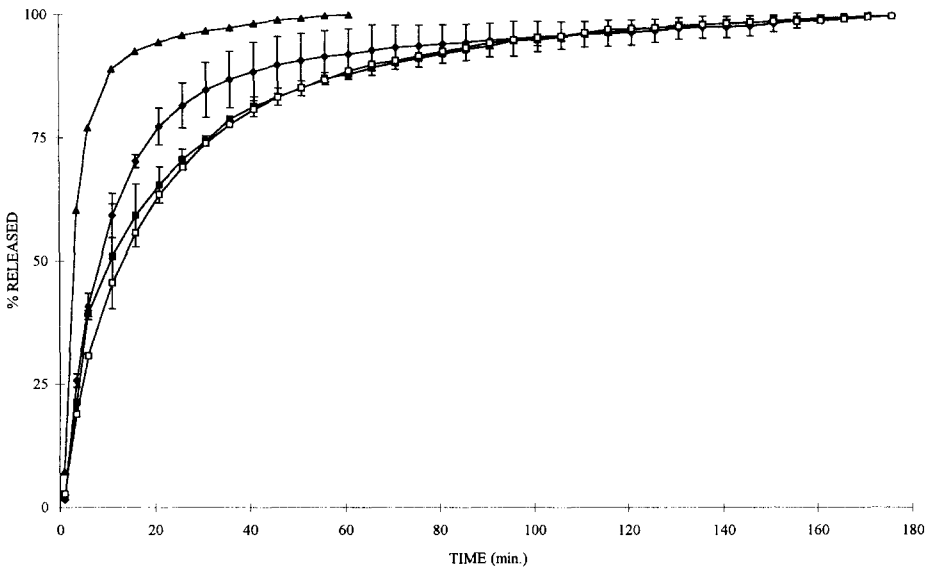

Fig. 3. Release profiles of riboflavin sodium phosphate from microspheres crosslinked with 10% formaldehyde solution for 1, (■), and 6 h (◆), noncrosslinked microspheres (□) and drug powder sample (▲).

crosslinked with formaldehyde and the drug powder sample. The release of the drug powder sample was also determined in order to serve as a control in understanding the contributory effects of the experimental set-up and the microspheres in extending the duration of drug release. Figs. 5 and 6 show the release profiles of RSPh from microspheres crosslinked with glutharaldehyde.

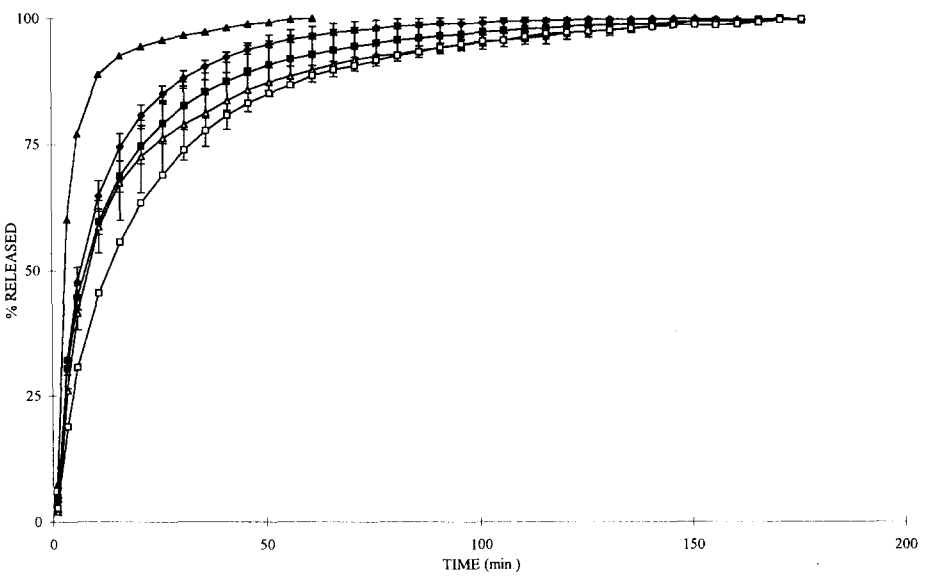

Fig. 4. Release profiles of riboflavin sodium phosphate from microspheres crosslinked with 20% formaldehyde solution for 1, (■), 6, (◆) and 24 h (△), noncrosslinked microspheres (□) and drug powder sample (▲).

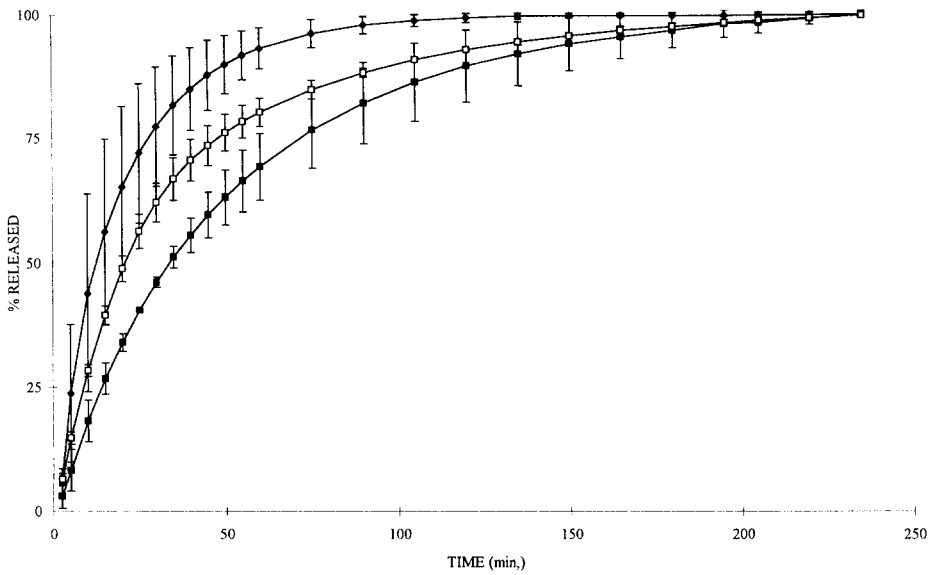

Fig. 5. Release profiles of riboflavin sodium phosphate from microspheres crosslinked with 10% glutharaldehyde solution for 1, (■), and 6 h (◆) and noncrosslinked microspheres (□).

The $t_{50\%}$ and $t_{85\%}$ (min) of RSPh from gelatin microspheres crosslinked with formaldehyde and glutharaldehyde solutions in acetone are shown in Table 2. An increase in the concentrations and crosslinking periods with both crosslinking agents did not extend the duration of RSPh release since the $t_{50\%}$ and $t_{85\%}$ were not significantly different to the corresponding values for noncrosslinked mi-

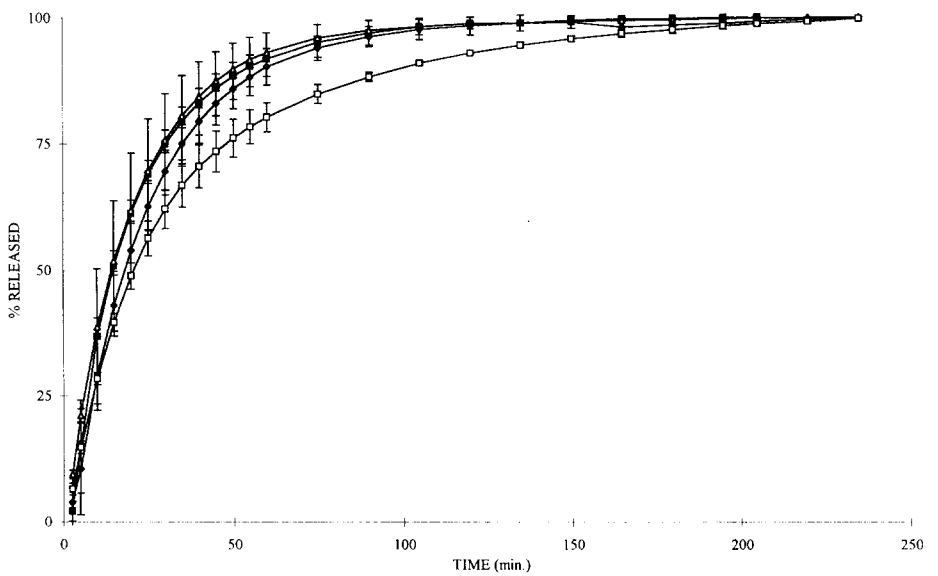

Fig. 6. Release profiles of riboflavin sodium phosphate from microspheres crosslinked with 20% glutharaldehyde solution 1, (■), 6, (◆) and 24 h (△) and noncrosslinked microspheres (□).

Table 3
Values of the exponent, n and correlation coefficients of the exponential equation for the release of riboflavin sodium phosphate from gelatin microspheres

Microspheres batch	Crosslinking time (h)	n value	Correlation coefficient
[a]Noncrosslinked microspheres	0	(1) 0.866 (2) 0.877 (3) 0.988 (4) 0.840	(1) 0.993 (2) 0.990 (3) 0.994 (4) 0.997
Formaldehyde solution (% v/v)			
10	1	0.654	0.963
10	6	0.728	0.996
20	1	0.587	0.994
20	6	0.607	0.994
20	24	0.709	0.993
Glutharaldehyde solution (% v/v)			
10	1	0.783	0.997
10	6	0.794	0.996
20	1	0.748	0.998
20	6	0.838	0.997
20	24	0.781	0.997

[a] Batches 1–3 were used in crosslinking with formaldehyde, and batch 4 with glutharaldehyde.

crospheres (for microspheres crosslinked with formaldehyde, $P = 0.1504$, and glutharaldehyde, $P = 0.1000$).

When compared to the release profiles of RSPh from noncrosslinked microspheres, the denser network of gelatin microsphere matrix formed following crosslinking with up to 20% v/v glutharaldehyde or formaldehyde solution in acetone does not retard the release of RSPh enough to show an extension of the duration of release of RSPh even after crosslinking for 24 h. These results are contrary to some published results (Tanaka et al., 1963; Mortazavi and Smart, 1993; Nastruzzi et al., 1994) where the duration of release of drugs from gelatin microspheres were extended by crosslinking the microspheres with formaldehyde or glutharaldehyde. Our results imply that properties other than that of the polymer alone play a role when duration of drug release is increased by crosslinking. The physicochemical properties of the drug may also be important as the properties of crosslinked gelatin. A strong influence of the solubility of the drug microencapsulated on the duration of release is suggested by the difference in release rates between apomorphine HCl and RSPh.

The values of the diffusional exponent n, according to the general exponential equation, for RSPh release from noncrosslinked microspheres (Table 3) suggests a stronger influence of polymer relaxation over the diffusive process (Vandelli et al., 1991b). This is contrary to the situation following crosslinking where the n values show that the release mechanism is by the anomalous (non-Fickian) transport (Ritger and Peppas, 1987). The release mechanism but not the overall rate of RSPh release is thus influenced by crosslinking gelatin microspheres with formaldehyde and glutharaldehyde.

3.4. Release profile of apomorphine HCl

The in vitro release profile of apomorphine HCl from noncrosslinked gelatin microspheres is shown in Fig. 7. The release of apomorphine HCl is characterized by an initial burst-effect of about 125 min during which about 80% of the entrapped apomorphine HCl was released. This was followed by a period of a much slower rate of release that decreased progressively until complete release of the entrapped drug. Such a burst effect was reported previously with gelatin microspheres

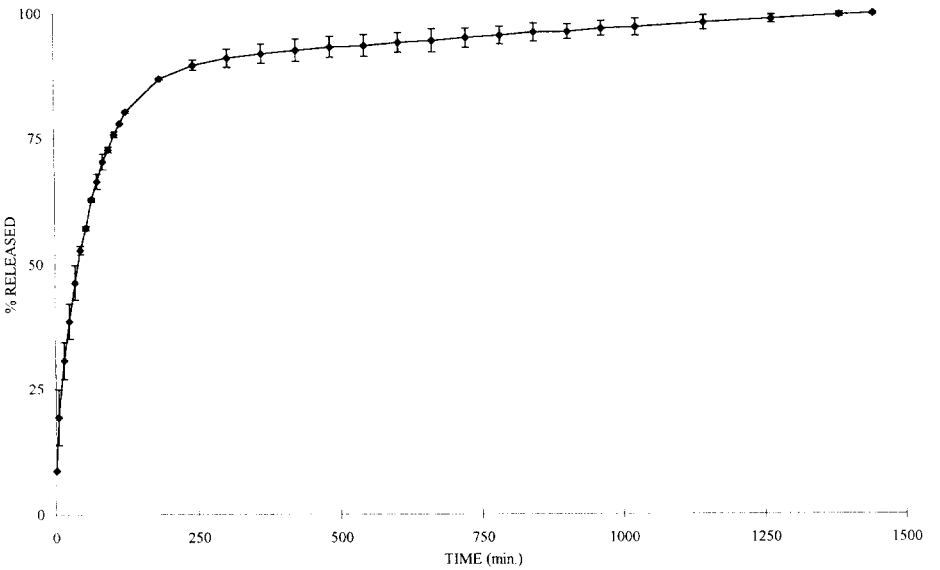

Fig. 7. Release profiles of apomorphine HCl from noncrosslinked microspheres.

(Leucuta, 1989). The $t_{50\%}$ and $t_{85\%}$ (min) for the release of apomorphine HCl were 38 and 165 respectively. This shows an extension of the duration of release and if such release profile is reproduced in vivo, drug effect will be obtained almost immediately to start therapeutic action and the slow release period serving to maintain its plasma concentration. In such a way, it is possible to record a complete drug release from the dosage form before it is cleared from the nose by the nasal mucociliary clearance after an extended time period. Apomorphine HCl is reported to be absorbed rapidly from the nose with an absorption half-life of 8.62 ± 2.6 min (Sam et al., 1995). Bearing in mind the in vitro and in vivo variability, replication of such a rate of release in vivo would result in the release of apomorphine HCl from the dosage forms being the rate limiting step to plasma drug concentration and not its rate of absorption across the epithelium. In addition, a prolonged duration of release and as such pharmacological action may be achieved.

From the general exponential release equation, the diffusional exponent (n) for the release of apomorphine HCl is 0.469 (correlation coefficient = 0.9996) showing that the release follows the anomalous (non-Fickian) transport mechanism for swellable spherical matrices. The release mechanism is thus different to that of RSPh probably due to their different solubility characteristics.

4. Conclusion

We have shown that the emulsification solvent extraction method is suitable for the microencapsulation of an easily oxidized drug such as apomorphine HCl. The solubility of the microencapsulated drug: (a) in the solvents used seems to influence the efficiency of the method; and (b) in water, the extension of duration of release. For apomorphine HCl this period was longer than for RSPh even after crosslinking.

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