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Preparation and in vitro characterisation of mucoadhesive polymeric microspheres as intra-nasal delivery systems[1]

M.D. Abd El-Hameed, I.W. Kellaway *

Welsh School of Pharmacy, Cardiff, UK

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Abstract

Microspheres of hydrophilic polymers were prepared by the w/o emulsification solvent evaporation technique with a potential application as drug carriers for nasal administration by insufflation. The microspheres had a median particle size of 38 ± 1.7 μm (Carbopol 934P), 38.6 ± 1.8 μm (Chitosan), 33.6 ± 7.2 μm (HPMC), and 16.5 ± 4.3 μm (PVA) prepared using a stirring speed of 2000 rpm for 4 or 4.5 h at 80°C. Chitosan and PVA microspheres were spherical and smooth-surfaced while Carbopol 934P and HPMC were of irregular shape with a rough surface morphology. For Carbopol 934P, the microsphere size was inversely proportional to stirring speed (1200–2000 rpm), and to the percentage of the emulsifier (Arlacel A, 0.2–1% w/w) and proportional to the percentage of core materials (0.2–0.5% w/w). FITC-dextran (Mw 4300 Da) was incorporated into the microspheres with an efficiency of 81 ± 3.6% (Carbopol 934P), 55 ± 20% (PVA), 44 ± 6.7% (HPMC), and 36 ± 2.7% (Chitosan). The change in initial concentration of FITC, dextran (0–15% w/w) had no effect on the particle size of Carbopol 934P microspheres. The rank order of mucoadhesion for the polymeric microspheres was Carbopol 934P > Chitosan = PVA = HPMC, although Chitosan was > HPMC. The change in content of FITC-dextran showed no significant effect on the mucoadhesive strength of Carbopol 934P microspheres within the concentration range of 0–12% w/w. The FITC-dextran was released from the microspheres initially at a constant rate. However the release rate subsequently decreased over the 24 h test period. No differences were observed for release from Carbopol 934P, PVA and HPMC: all exhibited faster release than that achieved from the Chitosan microspheres which exhibited a size-dependent release effect. © 1997 Elsevier Science B.V.

Keywords: Mucoadhesive polymers; Microsphere preparation: Nasal delivery

1. Introduction

For peptides and proteins, the nasal cavity offers an attractive alternative route to parenteral administration. It is easily accessible and more suitable for self medication. The nasal mucosa exhibits greater permeability than other mucosal surfaces including the various regions of the gastrointestinal tract, buccal and vaginal cavities [1]. The nasal mucosa is well supplied with a rich vasculature which directly flows into the systemic circulation thus avoiding hepatic metabolism. Currently, medications are given intransally for treatment of local inflammation, rhinitis and nasal constriction. Also a few small peptides like oxytocin, vasopressin, desmopressin and calcitonin are delivered nasally for systemic medication [2]. However nasal absorption decreases sharply for hydrophilic molecules with a molecular weight greater than 1000 Da [3].

Several attempts have been made and ongoing to improve the absorption of peptides and proteins from the nasal cavity, by employing different strategies. These include: I, synthesis of stabilised and more lipophilic analogues such as metkephamide [4] and desmopressin [2]; II, co-administration of aminopepti-

* Corresponding author. Welsh School of Pharmacy, UWC, Cardiff CF1 3 XF, UK. Tel./fax: +44 1222 874159; e-mail: kellaway@cardiff.ac.uk

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dase inhibitors (e.g. boroleucine) to enhance the absorption of leucine enkephalin [5]; III, using absorption enhancers, e.g. surfactants, bile salts, chelators and fatty acids [6,7]; and IV, the application of bioadhesive polymers to increase drug absorption by increasing the residence time in the nasal cavity. Bioadhesion has been investigated by adding the polymer to the drug in solution [8] or by mixing powders of the drug and the polymer [9]. Nagai et al [9] reported an increased intranasal absorption of insulin when mixed with microcrystalline cellulose powder and an even greater enhancement was achieved when freeze dried insulin was mixed with Carbopol 934P powder. Also incorporating drugs into degradable starch [1,10,11], dextran [12], albumin [13], and polyvinyl alcohol [14] microspheres increased their bioavailability after intranasal delivery.

The mucoadhesive polymers provide a relatively short-term adhesion between the drug delivery system and mucus and/or the epithelial cell surface. They have the advantage of not being absorbed and therefore would not be expected to display systemic toxicity. Mucoadhesive polymers when used as drug carriers for nasal delivery, may achieve (I) an increased residence time within the nasal cavity, (II) an intensified contact between the nasal mucosa and the drug, (III) increased drug concentration at the site of deposition and (IV) facilitated drug permeation through the mucosa by opening the tight junctions between the epithelial cells. There is therefore the possibility of increased drug bioavailability. Utilising mucoadhesive polymers in the form of microspheres provides protection to the incorporated drugs from enzymes and due to their sustained drug release, may also result in desirable blood concentration profiles. A temporary widening of the tight junctions between the epithelial cells induced by degradable starch microspheres in a cell culture environment has also been reported [12].

The site of deposition of particles within the nasal cavity is important; size control of intranasally administered microspheres will guarantee their deposition in the respiratory region, where maximum absorption will occur. The deposition is governed by the individuals nasal resistance to airflow [15] and by particle size diameter. Particles less than 1 μm will escape to the lungs, whereas particles larger than 10 μm will deposit in the nasal mucus membrane, with larger ones depositing more anteriorly [13].

The aims of the work described in this paper are to investigate:

(1) the optimal conditions to produce microspheres from anionic, cationic and non-ionic hydrophilic polymers with a suitable size for nasal delivery and to study the factors affecting their preparation process.

(2) The in-vitro mucoadhesive properties of the prepared microspheres versus non-mucoadhesive lactose and to study the effects of the different variables on their mucoadhesive properties.

(3) The entrapment of a model drug (FITC-dextran, 4300 Da) within the microspheres and to study the in-vitro release rates.

FITC-dextran is a hydrophilic marker and is available in several molecular weight fractions. It has been routinely employed as a model compound to study the delivery of hydrophilic macromolecules [7,16,17].

2. Materials and methods

2.1. Materials

Carbopol 934P 3×10^6 Da was supplied by BF Goodrich, Cleveland, USA. Hydroxypropylmethylcellulose 22 kDa, Chitosan 1.25×10^6 Da, Poly (vinyl alcohol) 30 kDa, Fluorescein isothiocyanate, dextran 4300 Da (FITC-dextran), Lactose, Mineral oil, Arlacel A (Mannide Monooleate) were purchased from Sigma. All the chemicals were of analytical grade and used as received.

2.2. Preparation of the microspheres

Microspheres were prepared by the water-in-oil (w/o) emulsification solvent evaporation technique employing a method modified from that described previously [18].

The model drug (FITC-dextran) was dissolved in each polymeric aqueous solution in a drug/polymer ratio of 5/95 w/w to provide a total concentration (polymer + drug) of 1% w/v. The solution was poured into 200 g of mineral oil containing 0.5% (w/w) Arlacel A (Mannide Monooleate) as emulsifying agent. The aqueous phase was emulsified into the oily phase by stirring the system in a wide-necked, round-bottomed flask.

Constant agitation at 2000 rpm was carried out using an homogeniser stirring rod and a Janke and Kunkel Ika-Werk RW 20 stirring motor. The flask and its contents were heated by an electrothermal isomantle at 80°C. Stirring and heating were maintained for 4.5 h (except PVA—4 h) until the aqueous phase was completely removed by evaporation.

The light mineral oil was decanted and the collected microspheres were washed three times with 100 ml aliquots of n-hexane, filtered through Whatman filter paper (grade 1), dried in an oven at 50°C for 2 h and stored in a desiccator at room temperature.

2.3. Microsphere evaluation

To evaluate the preparation process, the percentage-yield (w/w) was calculated as the weight of the dried microspheres recovered from each batch divided by the

Table 1
Particle size, yield and loading efficiency of microspheres

Polymer	Median particle size diameter (μm)	Percentage yield (w/w)	Loading efficiency (percentage)
Carbopol 934P	38.0 ± 1.7	62.6 ± 13.8	81.2 ± 3.6
PVA	16.5 ± 4.3	79.6 ± 2.2	55.2 ± 20
HPMC	33.6 ± 7.2	76.4 ± 5.5	43.8 ± 6.7
Chitosan	38 ± 1.8	49.2 ± 8.9	36.4 ± 2.7

sum of the initial dry weight of the starting materials × 100.

2.3.1. Particle size measurement

The prepared microspheres were sized by using a Malvern 2600 Laser Diffraction Spectrometer. The size of the microspheres was determined in 1-hexane as a non-dissolving dispersion medium and the particles were suspended mechanically by magnetic stirring during the measurement.

2.3.2. Morphological examination

Optical microscopy (Swift, Stereo eighty, Instruments International, USA) of the microspheres was undertaken in 1-hexane.

For scanning electron microscopy (SEM), the microspheres were thinly sprinkled onto a metal stub which was precoated with double- sided adhesive tape. The samples were then coated with a thin layer of gold using a sputtering coater (Emscope AE 1231, Kent, UK) and examined in a Philips EM 400T electron microscope fitted with a scanning electron detector STEM 400T.

2.3.3. FITC-dextran content

The FITC-dextran content of microspheres was determined by an extraction method. Microspheres of each polymer type (50 mg) was added to 100 ml of 0.02 M phosphate buffer (pH 7) and stirred over night to extract the entrapped FITC-dextran. Samples were withdrawn, filtered through a 0.2 μm syringe filter (Nalgene Syringe Filters, New York, USA) and assayed fluorometrically at 495 nm (excitation) and 520 nm (emission). The concentration was calculated from a calibration curve for FITC-dextran in phosphate buffer. FITC-dextran entrapped in Carbopol 934P microspheres was detected by using high-performance, size-exclusion chromatography (HPSEC) [19], as Carbopol 934P absorbed at the selected wavelength. By using HPSEC, a separation was achieved on a 4.6 mm × 25 cm column with 5 μm size-exclusion packing material (Hydropore-5-SEC, Rainin Instrument, Woburn, MA). A small cartridge (Mini-Cartridge, 4.6 mm × 1.5 cm, Rainin) of the same packing material was used as a pre-column. The mobile phase, 0.05 M phosphate buffer (pH 7) was delivered at a flow rate of 0.5 ml/min. The column was connected to a chromatography system consisting of a solvent delivery pump (LDC/Milton Roy, Consta Metric 3000), a variable-wavelength fluorometric detector (Perkin-Elmer LS-5 Luminescence Spectro-photometer), and an integrator (Spectra-Physics SP 4270). The concentration of FITC-dextran was calculated by using peak area and absorbance based on calibration curves constructed on the day of the experiment.

The amount of FITC-dextran loaded in the microspheres relative to the initial amount of FITC-dextran was calculated according to the following equation and expressed as the loading efficiency.

$$\text{Loading efficiency} = \frac{\text{Weight of FITC} - \text{dextran in microspheres}}{\text{Total initial weight of FITC} - \text{dextran}} \times 100$$

2.4. Effect of process variables on microsphere properties

Carbopol 934P microspheres were prepared

1. at different stirring rates (1200, 1400, 1600, 1800 and 2000 rpm).
2. with various initial drug/polymer ratios 1/99, 3/97, 5/95, 10/90 and 15/85 w/w with a fixed total weight of 0.3 g.
3. with different core percentages (core to oil phase) of 0.2, 0.3, 0.4 and 0.5%w/w and
4. with an emulsifying agent at concentrations of 0.2. 0.4, 0.6, 0.8 and 1% w/w contained within the mineral oil. The amount of solvent was maintained constant in all experiments. The effect of rate of agitation, drug/polymer ratio, core percentage, and percentage emulsifying agent on particle size and particle size distribution of Carbopol 934P microspheres was therefore studied.

In order to prepare Chitosan microspheres of differing sizes, the rotational speed of the stirrer was varied (1200, 1400, 1600 rpm) and either 0.2 or 0.3% of Arlacel A was employed. The microspheres were fractionated by sieving in order to investigate the effects of particle size on mucoadhesion and drug release.

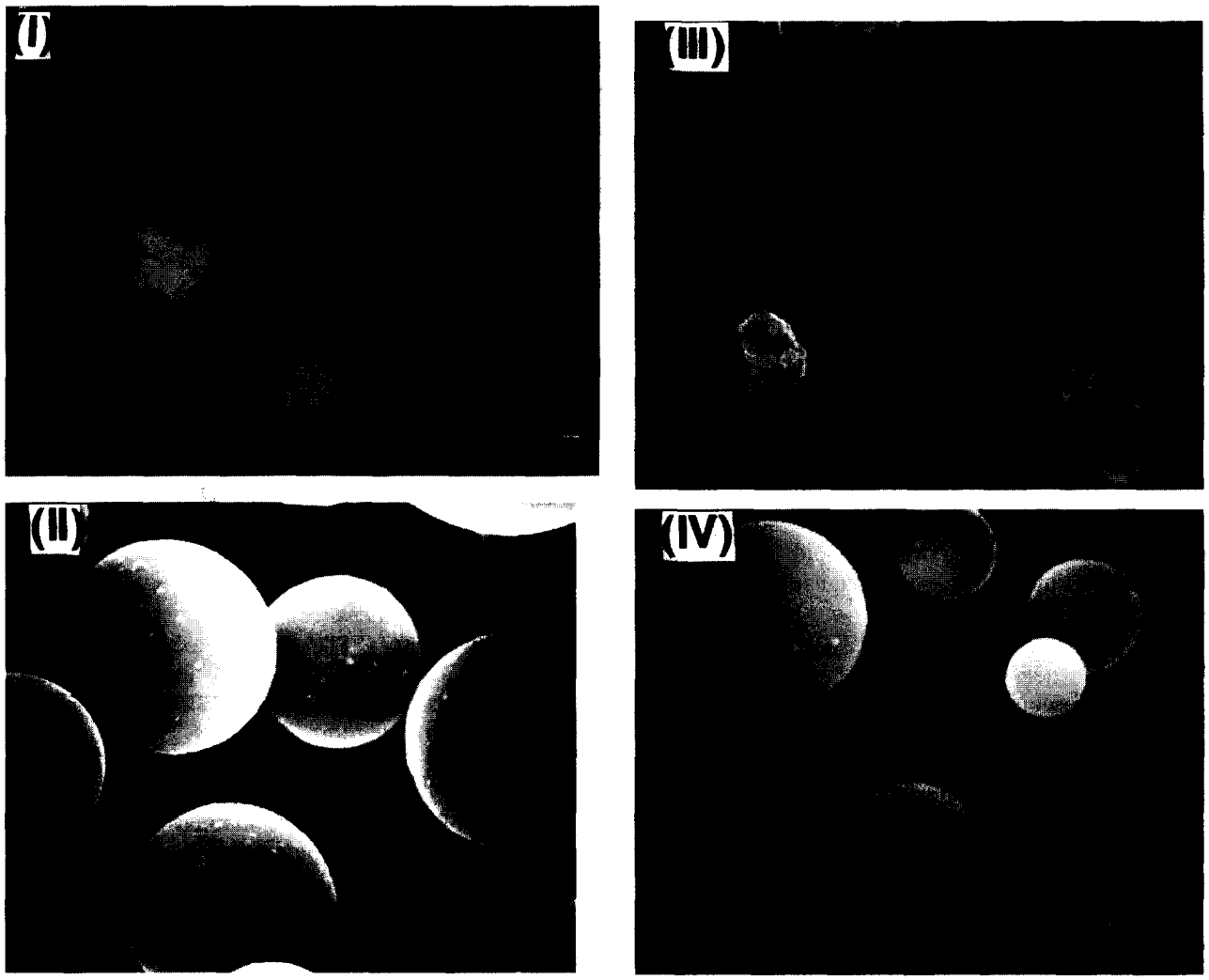

Fig. 1. Scanning electron micrographs of microspheres of: (I) Carbopol 934P; (II) Chitosan; (III) HPMC; (IV) PVA. Scale bar = 10 μm.

2.5. In-vitro evaluation of mucoadhesive properties of microspheres

The in-vitro mucoadhesion evaluation was performed on the microspheres by employing a tensile method described previously [20] but with a slight modification to enable dry microspheres to be examined.

Excess of microspheres were thinly sprinkled onto a circle of 9 mm diameter cellulose nitrate filtration membrane (0.2 μm) which was pre-coated with a thin film of Carbopol gel in order to retain the microspheres. This gel film had no effect on subsequent tensile test measurements. The membrane was dried in an oven for a few minutes before a stream of air was blown over the membrane to remove microspheres in excess of a monolayer. The residual weight of microspheres was 100 ± 5 mg. The membrane was held firmly by vacuum to the upper probe and the detachment force required to separate the membrane from the mucous layer, comprising 0.3 g of a mucin gel (20%) after 20 s contact, was measured. The results ($n = 4$) were expressed as the force required to separate the microspheres from the mucous layer and not as a force per unit area as the area of the contact face varies according to the size of the microspheres and cannot readily be measured. Carbopol 934P and Chitosan microspheres with different FITC dextran contents ranging from 0–12% and with different size ranges (< 50, 50–105, 106–178 and 178–200 μm) separated by sieving were investigated.

2.6. In-vitro release kinetics from polymeric microspheres

The drug release experiments employed Franz diffusion cells, since this model would allow the microspheres to hydrate slowly in a humid environment, conditions designed to be similar to those encountered in the nasal cavity.

Microspheres, 20 mg, were applied evenly across a pre-hydrated dialysis membrane (Scientific Industries

International, UK, Mw cut of 12–14 000, average pore radius 2.4 nm) mounted in a Franz diffusion cell. The lower chamber was filled with a known amount of buffer solution (0.2 M phosphate buffer pH 6) as a receptor medium, which was stirred with a magnetic stirring bar. The assembled cells ($n = 4$) were placed in a water bath (37 ± 0.2°C) in a 100% humidity. At set time intervals, 0.3 ml was removed via the side arm and assayed for FITC-dextran. The removed samples were replaced with an equal quantity of pre-warmed fresh buffer. Each of the four polymeric microspheres was studied for their release properties, and factors affecting release from Carbopol 934P and Chitosan were investigated.

3. Results and discussion

3.1. Microsphere characterisation

Discrete microspheres were produced with a relatively high percentage-yield (Table 1) which ranged from 49.2 to 79.6% w/w. A 100% yield could not be achieved principally due to adhesion of microspheres to the stirring rod of the homogeniser.

The microspheres median size ranged from 16.5 to 38.6 μm (Table 1) and are therefore suitable for nasal administration by insufflation.

Table 2
Effects of variation of stirring rate, % emulsifying agent, % core quantity and % FITC-dextran content on particle size of Carbopol 934P microspheres

Variable	Particle size diameter (μm)
(I) Effect of stirring rate (rpm)	
1200	173.3 ± 2.33
1400	132.3 ± 1.19
1600	108.5 ± 1.40
1800	41.2 ± 0.22
2000	30.8 ± 0.84
(II) Effect of percentage emulsifying agent (w/w)	
0.2	131.6 ± 4.03
0.4	86.1 ± 2.61
0.6	73.6 ± 2.93
0.8	53.3 ± 1.83
1.0	41.5 ± 1.00
(III) Effect of percentage core (polymer + FITC-dextran to oil phase w/w)	
0.2	50.5 ± 0.37
0.3	84.5 ± 0.66
0.4	95.2 ± 4.39
0.5	119.1 ± 0.47
(IV) Effect of percentage FITC-dextran content	
1.1	88.0 ± 7.43
2.2	84.5 ± 0.66
4.1	83.7 ± 1.87
8.2	83.8 ± 0.97

Results of mean ± S.D. of four batches.

FITC-dextran was entrapped within Carbopol 934P and PVA microspheres with a higher efficiency than HPMC and Chitosan. The entrapment of FITC-dextran in polymeric microspheres is a function of aqueous solubility and concentration of both the polymer and dextran. Steady evaporation of water content from droplets of dextran/polymer solution during the preparation is followed by precipitation of the polymer and dextran in different proportions relative to their individual solubility. Most of the less soluble Chitosan is precipitated from the solution earlier than dextran. Therefore less dextran is entrapped in the Chitosan matrix producing a lower loading efficiency.

Hassan et al. [21] reported a similar percentage-yield of Chitosan microspheres prepared with the solvent evaporation technique which ranged from 39.1 to 80.6%, while the percentage of entrapped drug ranged from 0.9 to 4.77% which is significantly lower than our finding. This may relate to different drugs and the use of a cross-linked Chitosan.

Electron microscopy showed that Chitosan and PVA produced smooth spherical microspheres, while Carbopol 934P and HPMC microspheres were of irregular shape with a rough surface morphology (Fig. 1). PVA microspheres previously prepared by using spray-drying and a spray-desolvation method had the same morphological characteristics as those currently prepared by the solvent evaporation technique [14].

3.2. Effect of process variables on microsphere properties

The effect of the various process parameters on microsphere properties has been previously investigated [22–24]. Factors affecting particle size, shape, size distribution and yield of microspheres can be the degree of agitation, shape of the reactor and blade, rate of heating, polymer/drug ratio and the concentration of additives [24]. In this paper we focused on some of these variables. The effect of stirring rate during the preparation of Carbopol 934P microspheres on the particle size and particle size distribution is shown in Table 2. The median particle size was inversely proportional to stirring speed in the range of 1200–2000 rpm. A similar finding was reported previously for the preparation of polyacrylate (Eudragit retard) microspheres [25].

The particle size was also inversely proportional to the percentage of emulsifying agent in the range of 0.2–1% w/w, and proportional to the percentage of core material (polymer + drug to oil phase) in the range of 0.2–0.5% w/w. Amperiadou and Georgarakis [26] also reported a decrease in the particle size of microparticles prepared by the solvent evaporation method with increasing the percentage of the emulsifying agent. The change in drug/polymer ratio in the range of 1/99–8/92 within a fixed total core percentage showed no effect on the size of the microspheres produced.

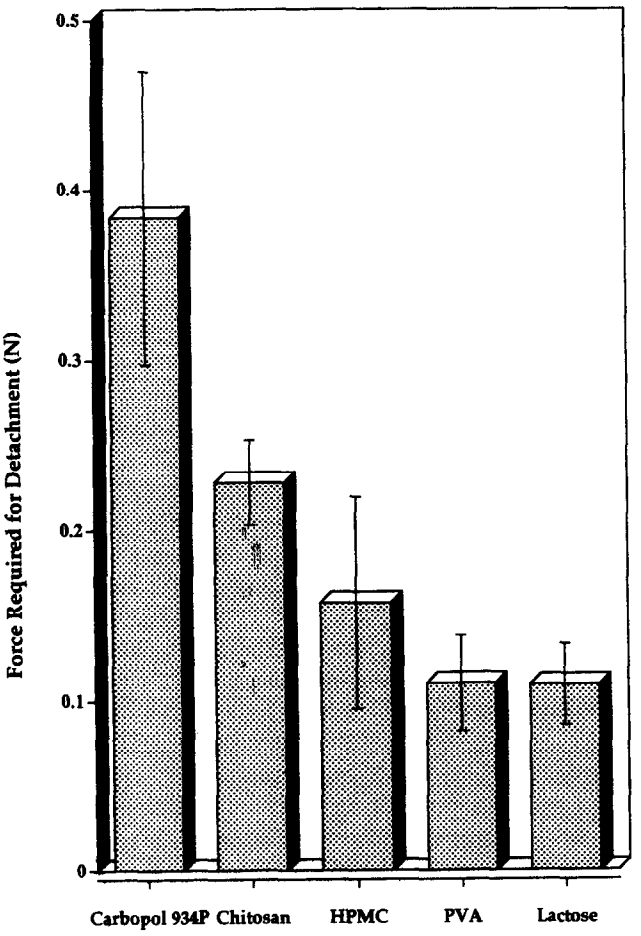

Fig. 2. Mucoadhesive strength of microspheres ($n = 4$; $\pm$ S.D.).

3.3. In-vitro evaluation of mucoadhesive properties of microspheres

The rank order of mucoadhesion for the polymeric microspheres was Carbopol 934P > Chitosan = HPMC = PVA = lactose, although Chitosan was significantly more mucoadhesive than PVA and lactose ($P > 0.05$; ANOVA, $n = 4$; $\pm$ S.D.) (Fig. 2). The poor mucoadhesion of HPMC and PVA may be due to these relatively low molecular weight (22 and 30 kDa), non-ionic polymers possessing low hydrogen bonding capability with mucus glycoproteins. The mucoadhesive properties of C934P microspheres showed no dependence on FITC-dextran concentration over the range of 0–12% w/w ($P > 0.05$; ANOVA).

Mucoadhesion was not dependent on microsphere size for Carbopol 934P in the range 50–200 μm ($P > 0.05$; ANOVA). However for Chitosan microspheres within the same size range, mucoadhesion was inversely proportional to the particle size (Table 3).

These results may be explained on the bases of hydration rates. The rapidly hydrating Carbopol 934P showed no size dependence whilst for the less soluble Chitosan, the smaller microspheres hydrated more rapidly to provide a more highly mucoadhesive system.

3.4. In-vitro release kinetics of FITC-dextran microspheres

FITC-dextran was released from the microspheres resting on a moist membrane and transported across the membrane to appear in the receptor phase at rates which were indistinguishable for Carbopol 934P, PVA, HPMC and lactose microspheres. All these microspheres hydrate relatively rapidly. Solute release initially occurred at a constant rate (approximately 0.06% per min) and subsequently decreased toward the end of the 24 h test period. At this time approximately 40% of the FITC-dextran had been released. However for Chitosan, hydration was slower and consequently about 12% was transported over the same time. Only Chitosan exhibited microsphere size-dependent release rates (Fig. 3), which probably relates to slower hydration of the polymer. Faster and less reproducible release was observed for microspheres from a sieve fraction > 50 μm diameter compared with a sieve fraction of < 50 μm. Benita and co-workers [23] previously reported an increase in the release rate of nifedipine from polyacrylate microspheres with a decrease in particle size from 600 to 300 μm. Decrease in particle size increases the surface area and hence release rates. Morath and Edman [27] showed an increase in insulin absorption when a reduction in particle size of Chitosan microspheres was achieved.

Table 3
The effects of particle size on mucoadhesive properties of Carbopol 934P and Chitosan microspheres

Effect of variables (particle size and drug content)	Force (N) required for detachment of microspheres	
	Carbopol 934P	Chitosan
(I) Effect of particle size (μm)		
< 50	0.31 ± 0.10	0.38 ± 0.07
50–106	0.49 ± 0.08	0.27 ± 0.07
106–178	0.35 ± 0.16	0.22 ± 0.03
178–200	0.49 ± 0.15	0.21 ± 0.04
(II) Effect of % FITC-dextran content		
0	0.40 ± 0.08	—
4	0.35 ± 0.11	—
8	0.31 ± 0.10	—
12	0.31 ± 0.11	

Results ± S.D. ($n = 4$).

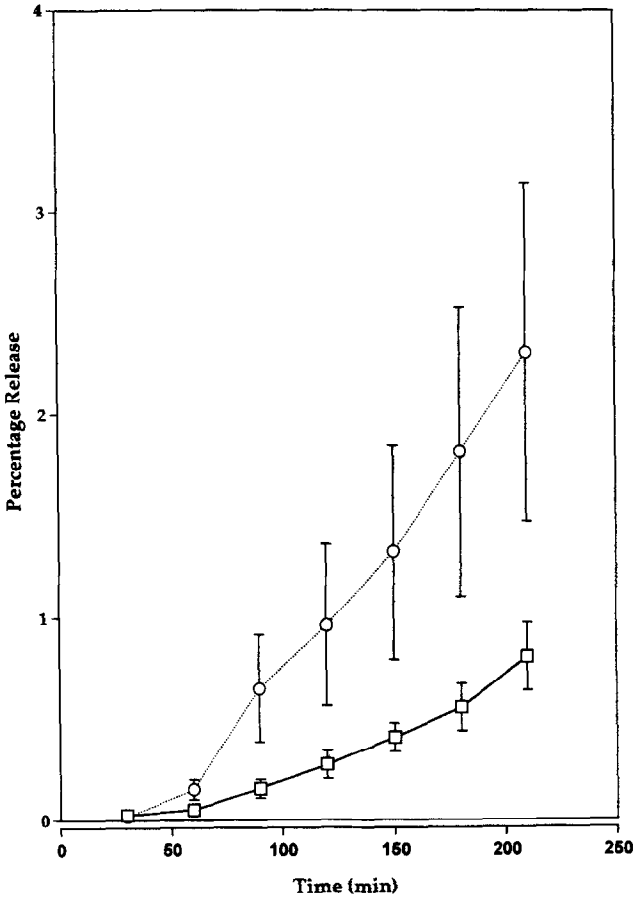

Fig. 3. Release of FITC-dextran from Chitosan microspheres of different particle size ($n = 4$; ± S.D.). ···○··· > 50 μm, – □ – < 50 μm.

4. Conclusions

The solvent evaporation technique for the entrapment of FITC-dextran in Carbopol 934P, Chitosan, HPMC, and PVA produced a high yield of discrete microspheres with minimal agglomeration, reproducible drug loading efficiency and release profiles from batch to batch. The release rate and mucoadhesion of Carbopol 934P and Chitosan microspheres could be modified by varying the process parameters. Therefore we conclude that:

1. The water-in-oil emulsion solvent evaporation process produced microspheres of a suitable size for nasal administration.
2. The in-vitro mucoadhesive study demonstrated that Carbopol 934P adhered to mucus to a greater extent than the Chitosan, HPMC and PVA.
3. Carbopol 934P, HPMC and PVA microspheres released FITC-dextran at a higher rate than Chitosan which may be due to their more rapid hydration rates as observed by microscopy.

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