ELSEVIER

International Journal of Pharmaceutics 180 (1999) 59–67

international journal of pharmaceutics

The transport of polymeric microspheres across the ciliated epithelia of the bullfrog

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Received 4 September 1998; received in revised form 23 November 1998; accepted 2 December 1998

Abstract

The influence of some hydrophilic polymers on the clearance of particles across the ciliated epithelium of the bullfrog palate has been examined. The polymers studied were Carbopol 907 cross-linked with maltose to provide microspheres of varying cross-link density, Carbopol 934P, hydroxypropylmethylcellulose, chitosan and poly(vinyl alcohol). Transport rates were determined relative to glass spheres. The polymers in dilute solution (0.1 and 0.5% w/v) resulted in a reduction in the transport rate of the glass spheres. For non-cross-linked microspheres, Carbopol 934P exhibited a lower transport rate than the more slowly hydrating chitosan. The cross-linked poly(acrylic acid) microspheres showed clearance rates which were dependent on the cross-link density. Incorporation of some preservatives (EDTA, methylhydroxybenzoate, chlorbutol and chlorocresol), known to reversibly retard clearance, into the cross-linked poly(acrylic acid) microspheres produced effects dependent on cross-link density: lightly cross-linked microspheres were cleared more slowly than the preservative-free microspheres whilst for more heavily cross-linked particles the converse was observed.

Keywords: Bullfrog; Ciliated epithelium; Polymeric microspheres

1. Introduction

Rapid clearance from the site of absorption is a limiting factor to nasal drug delivery. Macromolecules such as proteins and polypeptides are poorly absorbed from the nasal cavity and therefore have low nasal bioavailabilities. Prolonging the residence time of such macromolecular medications in the nasal cavity can increase their absorption and hence improve their bioavailabilities.

The residence time of the drug in the nasal cavity is determined by the nasal condition (determines mucus secretion), delivery device (deposition profile) and formulation (deposition profile and possibly nasal condition). Mucociliary clearance ensures that the nasal cavity is continually

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0378-5173/99/$ - see front matter
PII: S0378-5173(98)00412-8

lined by a fresh, clean, moist surface. Clearance is achieved by the numerous cilia of the pseudostratified epithelium, whose beating causes the overlying mucous layer and upper surface of the periciliary fluid to move in a co-ordinated direction. Mucociliary clearance removes particles in a backward and/or forward direction depending upon the area of impaction. Particles deposited anteriorly are propelled forward to be wiped away. Posterior deposition leads to backward propulsion followed by ingestion. The co-ordination of cilia motility is controlled by neural innervation, chemical pacemaking, hormonal stimulation and by the effects of ions such as calcium and potassium.

The nature of the mucus has a profound effect on mucociliary clearance, though the cilia have been shown to be capable of propelling fluids of widely differing rheological properties. Polymer solutions and gels [gelatin, agar, poly(vinyl alcohol), methocel], with a wide range of viscosities and elasticities, in addition to mucus from the rat, hen, rabbit, cat trachea and cow cervix, were found to be transported along the frog/toad palate (Sadé et al., 1970; King et al., 1974; Lin et al., 1993). This was attributed to the three-dimensional structure and the slight degree of cross-linking within the gels. The characterization of mucus has shown that it has four properties that make mucoadhesion possible: namely (1) the network of linear, flexible chains; (2) the negative charges; (3) the cross-linked network; and (4) the high degree of hydration. Changes in the above properties could result in the retardation of mucociliary clearance and hence increase the contact time of the drug with the nasal mucosa.

Duchateau et al. (1985) have shown a correlation between ciliary beat frequency (CBF) and the log of the mucus transport time showing that CBF is the main factor in mucociliary clearance. Therefore, any cessation or decrease of ciliary activity will result in impaired or no mucociliary artclearance. Mucociliary clearance along the frog palate ceases when the mucus has been removed, and Lucas (1933) suggested that the addition of particles onto the palate stimulated cilia activity. More recent investigations by Spungin and Silberberg (1984) have revealed that the addition of

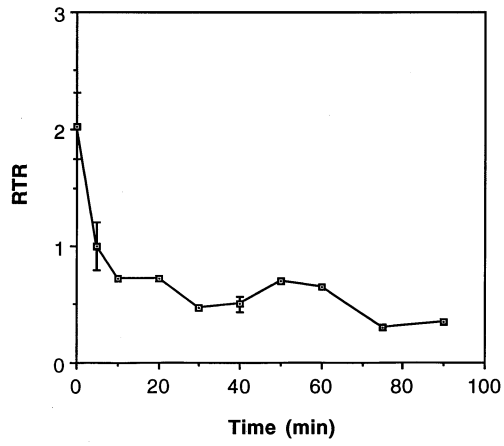

Fig. 1. Effect of 0.5% (w/v) Carbopol 934P solution on mucociliary transport of glass spheres ($n = 4$, mean $\pm$ S.D.).

particles stimulates the secretion of mucus, which in turn stimulates cilia activity.

The removal of surface liquid or particles from the nasal cavity is governed by many factors including the rate of mucus secretion, the viscoelastic properties of the mucus, the ciliary beat pattern and the ciliary beat frequency (Seybold et al., 1990). There are, therefore, several procedures by which the retardation of mucociliary clearance can be achieved, such as modifying the CBF by chemical agents (preservatives and absorption enhancers) and altering mucus secretion. Alternative approaches involve expanding the glycoproteinic

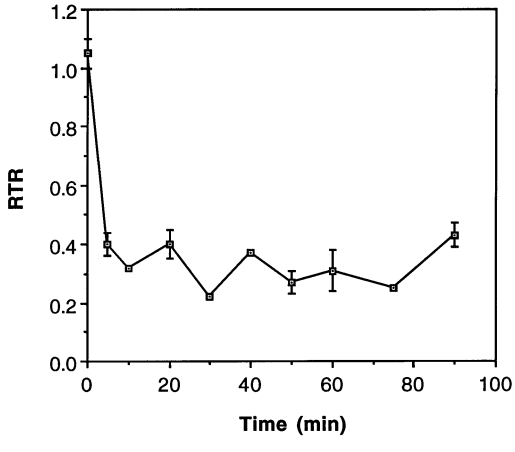

Fig. 2. Effect of 0.5% (w/v) HPMC solution on mucociliary transport of glass spheres ($n = 4$, mean $\pm$ S.D.).

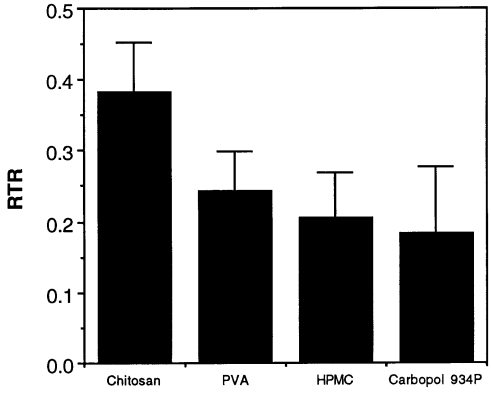

Fig. 3. Relative transport rates of polymer microspheres ($n = 4$, mean ± S.D.).

network and modifying the polymeric formulation. The latter can result in changes to the elastic modulus and/or viscosity of the mucus achieved by control of charge on the polymer, its degree of cross-linking and the extent to which water is extracted from the mucus to hydrate the polymer.

Various in vitro or ex vitro and in vivo methods have been developed to study mucociliary function. Merkus et al. (1992) Merkus et al. (1993) and others (Svartengren et al., 1989) investigated the influence of drugs and additives on mucociliary clearance by determining the CBF. Alternative methods measure the mucociliary clearance directly by using either visible or invisible tracers such as dyes, teflon or aluminium disks (Van Ree and Van Dishoeck, 1962; Ewert, 1965).

Table 1
Effect of particle size on the RTR of Carbopol 934P and chitosan microspheres ($n = 4$, mean ± S.D.)

Microsphere	Microsphere size (μm)		
	<50	50–106	>106
Carbopol 934P	0.24 ± 0.06	0.10 ± 0.02	0.04 ± 0.01
Chitosan	0.63 ± 0.02	0.33 ± 0.03	0.13 ± 0.02

The frog palate is similar to human nasal epithelium, being ciliated with numerous mucus producing and secreting glands (Puchelle et al., 1980). The frequency of the cilia beats range from 9 to 15 beats/s for bullfrog, which is close to the cilia beat frequency in the human nasal cavity (8–13 beats/s). However, the bullfrog has a faster mucociliary transport rate (8–12 mm/min) compared to the human nasal clearance rate (normally 1–10 mm/min) (Phillips et al., 1990). The frog palate can clear solid substances with a high elastic modulus such as charcoal powder, glass beads and aluminium disks at the same transport rate as mucus. Pritchard et al. (1996) have used the frog palate model to investigate the bioadhesive properties of hyaluronan derivatives.

In the current study of mucociliary transport, polymeric solutions and microspheres have been investigated utilizing the bullfrog (*Rana castesbiana*) palate. The influence of some preservatives incorporated within the matrix of microspheres on transport rates has also been evaluated.

2. Materials and methods

2.1. Materials

Carbopol 907 (450 kDa) and 934P (3×10^6 Da) were a gift from B.F. Goodrich. Hydroxypropylmethylcellulose (HPMC; 22 kDa), chitosan (1.25×10^6 Da), poly(vinyl alcohol) (PVA; 30 kDa), palmitic acid (grade II), lactose, olive oil (purified), mineral oil Arlacel A and fluorescein isothiocyanate (FITC)–dextran (4000 Da) were purchased from Sigma UK. All other materials were Analar grade (BDH Chemicals Ltd., UK). Deionized water was used throughout.

2.2. Preparation of polymer solutions

Polymer solutions of 0.1 and 0.5% (w/v) in Frog Ringer buffer were prepared to give solutions of Carbopol 934P 0.1% (pH 7.03) and 0.5% (pH 7.13), chitosan 0.1% (pH 6.62) and 0.5% (pH 4.83), PVA 0.1% (pH 8.2) and 0.5%

Table 2
The relative transport rates ($n = 15$, mean ± S.E.M.) of cross-linked mucoadhesive microspheres across the bullfrog palate

Microsphere maltose:PAA	Curing time (h)			
	1.5	2.0	3.0	4.0
1:10	0.81 ± 0.05	0.00 ± 0.00	0.07 ± 0.02	0.01 ± 0.01
1:5	0.29 ± 0.03	0.14 ± 0.05	0.15 ± 0.03	0.10 ± 0.04
1:2	0.36 ± 0.07	0.08 ± 0.02	0.15 ± 0.01	0.34 ± 0.04

(pH 7.9), and HPMC 0.1% (pH 7.7) and 0.5% (pH 7.8). The pH of the solutions was adjusted to pH 7.8 using HCl and NaOH.

2.3. Preparation and characterization of non-cross-linked microspheres

Microspheres were prepared by the water-in-oil emulsification solvent evaporation technique employing a method previously described (Abd El-Hameed and Kellaway, 1997). The model drug FITC–dextran (if required) was dissolved in an aqueous polymer solution in drug:polymer ratios ranging from 1:99 to 15:85 (w/w) to provide a total concentration (polymer + drug) of 1% w/v. The solution (30 ml) was poured into 200 g of mineral oil containing 0.5% w/w Arlacel A (mannide monooleate) as an 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 achieved from a Janke & Kunkel Ika-Werk RW 20 stirring motor. The flask and its contents were heated by an electrothermal isomantle at 80°C for 4.5 h to evaporate the water. The oil was decanted and the 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.

The microspheres were characterized as previously described (Abd El-Hameed and Kellaway, 1997). Particle size measurements were made by Malvern 2600 Laser Diffraction Spectroscopy, and morphological examination by optical microscopy.

2.4. Preparation of mucoadhesive cross-linked microspheres

The aqueous phase was prepared by the dissolution of Carbopol 907 (0.25 g) and maltose (0.025, 0.050 and 0.125 g) in 50 ml of deionized water by mechanical shaking overnight thereby ensuring thorough mixing. The maltose to poly(-acrylic acid) (PAA) concentration ratios were therefore 1:10, 1:5 and 1:2. The organic phase consisted of olive oil (150 ml) and palmitic acid (3 g), present as a droplet stablizer. Since palmitic acid is an endogenous lipid, any residual amounts present in the microspheres should be pharmaceutically acceptable. The organic phase was heated to 110–115°C by an electrothermal isomantle whilst stirring at 1400 rpm (Janke & Kunkel Ika-werk RW20 DZM) with an homogenizer stirring rod in a 250-ml wide-necked round-bottomed flask.

Once the organic phase had attained a stable temperature, the aqueous phase was added dropwise to the flask. Temperature regulation was facilitated by the use of adhesive temperature-sensitive labels attached to the surface of the reaction vessel. Stirring and heating were maintained until the oil appeared clear, indicating the evaporation of all the water derived from the polymer solution and therefore onset of the polycondensation reaction, and for a further period (1.5, 2, 3 and 4 h) to ensure that the particles were cured. The microspheres were harvested from the oil–fatty acid mixture by repeatedly washing with acetone and collected by centrifugation (4000 rpm for 10 min). The supernatant was discarded after each washing procedure. The microparticles were dried at 30°C under vacuum and stored in a vacuum desiccator.

2.5. Loading preservatives into the mucoadhesive cross-linked microspheres

Solutions of the preservatives in deionized water were prepared with the following concentrations, which had been suggested by previous work. Chlorbutol 0.5% w/v (Greenwood et al., 1946; Bond et al., 1984; Duchateau et al., 1986), methylhydroxybenzoate (MHB) 0.05% w/v (Gallay, 1960; Bond et al., 1984; Duchateau et al., 1986; Hastie et al., 1987; Batts et al., 1989 Batts et al., 1990; Jian and Po, 1993), EDTA 0.1% w/v (Marriott, 1990) and chlorocresol (CC) 0.05% w/v (Duchateau et al., 1986). Cilia activity was halted for all of the above preservatives expect EDTA, where cilia activity was retarded.

The preservatives were incorporated into the synthesized microspheres by swelling the particles in a solution of the compound followed by dehydration under vacuum.

2.6. Determination of mucociliary clearance by the frog palate method

Bullfrog palate specimens were obtained by decapitation and pithing of the frog (*Rana catesbiana*) without sedation. The upper jaw was dissected free and pinned to a cork bed on the mounting stage in a controlled perspex chamber at 30°C and $>96\%$ relative humidity (RH). The temperature and humidity of the chamber were maintained by the circulation of water through the chamber by a Grant Operation Pump, type FH15A and periodically monitored. A Swift Optical Microscope ($\times 30$ magnification) equipped with a calibrated eyepiece micrometer was positioned above the palate to observe the movement of particles along the mucosal tissue through the viewing orifice in the roof of the chamber.

There are conflicting reports in the literature of the viability of the palate to transport particles, ranging from 1 h (Dulfano and Adler, 1975) to 36 h (Sadé et al., 1970). Hilding (1957) showed that ciliary activity could be maintained for many hours even on an isolated palate, and the cessation of particulate movement was due to mucus depletion and not reduced ciliary activity. The palate surface was washed with Frog Ringer solution (pH 7.8) and wiped down after each comparative experiment with clinical tissue to remove mucus build-up. The problem of decreased mucus secretion and thus decreased mucociliary clearance with time was overcome in this study by using glass spheres (44–110 μm in a diameter) as a standard, and expressing all rates of transport along the palate as relative transport rate (RTR) with respect to the standard. Reporting RTRs also eliminated differences in transport values from palate to palate (Shih et al., 1977). Experiments with a given palate were completed in 4–5 h to avoid errors associated with mucus secretion decline.

The influence of the polymers on mucociliary clearance was investigated in a series of six experiments:

1. The influence of the four polymer solutions (0.1 and 0.5% w/v versus Ringer solution) on mucociliary clearance using glass spheres as marker ($n=4$, ten time points conducted over 90 min).

Table 3
The relative transport rates ($n=15$, mean ± S.E.M.) of mucoadhesive microspheres of maltose:PAA 1:10 loaded with preservatives across the bullfrog palate

Preservative	Curing time (h)			
	1.5	2.0	3.0	4.0
EDTA	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.01	0.04 ± 0.01
CC	0.10 ± 0.03	0.03 ± 0.01	0.16 ± 0.00	0.00 ± 0.00
MHB	0.03 ± 0.01	0.16 ± 0.03	0.06 ± 0.01	0.05 ± 0.03
CB	0.05 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
None	0.81 ± 0.05	0.00 ± 0.00	0.07 ± 0.02	0.01 ± 0.00

Table 4
The relative transport rates ($n = 15$, mean ± S.E.M.) of mucoadhesive microspheres of maltose:PAA 1:5 loaded with preservatives across the bullfrog palate

Preservative	Curing time (h)			
	1.5	2.0	3.0	4.0
EDTA	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.02
CC	0.16 ± 0.04	0.05 ± 0.01	0.08 ± 0.04	0.00 ± 0.00
MHB	0.02 ± 0.02	0.11 ± 0.04	0.14 ± 0.03	0.04 ± 0.01
CB	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.14	0.14 ± 0.05
None	0.29 ± 0.03	0.14 ± 0.05	0.15 ± 0.03	0.10 ± 0.04

2. The effect of mucociliary transport rate of the four polymer non-cross-linked microspheres versus glass spheres as control.
3. The influence of particle diameter of Carbopol 934P and chitosan microspheres on the rate of clearance. The microsphere batches (10–200 μm) were sieved to provide three fractions (< 50, 50–106 and > 106 μm).
4. The effect of FITC–dextran content (0–12% w/v) of Carbopol 934P microspheres on mucociliary transport rates.
5. The influence of cross-linked density in PAA microspheres on mucociliary clearance. This was achieved by utilizing different maltose:PAA ratios (1:10, 1:5 and 1:2) together with different curing times (1.5, 2, 3 and 4 h).
6. The effect of preservatives in cross-linked PAA microspheres on the RTR.

2.7. Influence of polymer solutions on ciliary transport rate

The effects of polymers on the mucociliary clearance were determined by placing a small aliquot (0.2 ml) of the polymer solution on top of the mucus layer of the palate. The transport rate for four glass particles (ten time points each) was then measured by recording the time taken to travel a given distance along the midline of the palate (2.73 mm) from the anterior part of the hard palate, just behind the vomerine teeth, towards the posterior part near the oesophagus. The results were normalized by the baseline transport rate (glass particles across a Ringer-washed palate) and expressed in terms of the RTR.

2.8. The transport of microspheres across the palate

The microspheres were placed on the upper central region of the palate out of the view of the microscope. Once the mucociliary clearance system was activated (particle deposition is known to stimulate mucus secretion and thus indirectly stimulate clearance; Spungin and Silberberg, 1984), the microspheres were transported along the palate, and the time required to transverse three 0.9-mm sections (as measured by the eyepiece micrometer) was recorded. For non-cross-linked microspheres, four microspheres were studied and ten timings recorded for each. For cross-linked microspheres 15 microspheres of each formulation were employed. The transport rates were expressed as RTR by dividing the transport rate of the standard (glass particles, $n = 15$) by the transport rate of the sample, thereby ensuring reproducibility.

3. Results and discussion

An initial increase in RTR was observed with palates exposed to 0.2 ml of Carbopol 934P (0.1 and 0.5% w/v), chitosan (0.5% w/v) and PVA (0.5% w/v); however, this transient increase was followed by a subsequent decrease and plateauing of the RTR (Fig. 1). In contrast, a rapid decrease in RTR with subsequent plateauing was observed with all other solutions (Fig. 2).

All non-cross-linked microspheres were cleared more slowly than the control, with RTR values

< 1 (ANOVA, $P < 0.05$) (Fig. 3). Carbopol 934P microspheres cleared more slowly than chitosan microspheres, while there was no significant difference between microspheres of Carbopol 934P and microspheres of HPMC or PVA. Also no significant difference was observed between HPMC, PVA and chitosan microspheres (ANOVA, $P > 0.05$).

The RTRs of Carbopol 934P and chitosan microspheres were inversely proportional to particle size (Table 1). The large microspheres have the potential to absorb more water from the underlying mucus to become hydrated, consequently the mucus rheology would be expected to change to a greater degree. This may well lead to the ciliary beat frequency decreasing further and, as a result, reduced clearance rates would follow. Although abnormal viscosity is often cited as the cause of faulty clearance systems (Chodosh and Segal, 1964; Denton et al., 1968), it is likely that the more critical parameter is the elastic modulus. Giordano et al. (1978) observed that a ten-fold increase in the elastic modulus halved the mucociliary clearance rate.

The ability of the frog palate to transport gels such as guaran, gelatin, agarose and acrylamide was suggested by King et al. (1974) to be attributable to the three-dimensional structure and slight degree of cross-linking within the gels. However, Duchateau et al. (1985) have shown a correlation between ciliary beat frequency (CBF) and the log of the mucus transport time, suggesting that CBF is the main factor in mucociliary clearance.

No effect was seen in the rate of mucociliary clearance over the palate when the FITC–dextran content of the microspheres ranged from 0 to 12% w/w (ANOVA, $P > 0.05$). Previously, the same concentration range was shown to have no influence on in vitro mucoadhesion (Abd El-Hameed and Kellaway, 1997). The FITC–dextran was dispersed homogeneously throughout the Carbopol 934P matrix; therefore the hydrating surface of the microsphere largely comprises Carbopol 934P. The properties of the microspheres are largely due to the mucoadhesive Carbopol 934P and the effect of FITC–dextran is negligible.

For all the cross-linked PAA microspheres cured for 2 and 3 h, mucociliary clearance was reduced considerably, having RTRs of < 0.2. For microspheres cured for 1.5 h, a reduced effect on RTR was observed with 0.81, 0.29 and 0.36 for maltose:PAA ratios of 1:10, 1:5 and 1:2, respectively (Table 2). These RTR values are higher than that recorded for Carbopol 934P (0.18; Fig. 3) although Carbopol 934P was comparable with 1:5 maltose:PAA cured for 2 and 3 h. An increase in RTR (i.e. mucociliary clearance is less retarded) was observed as the concentration of the maltose cross-linker in the microsphere was increased. This effect is more pronounced for particles cured for 4 h. It should also be noted that the RTR is ≥ 0.3 for microspheres cured for 1.5 h and the maltose:PAA 1:2 preparation cured for 4 h, indicating that a low or high degree of cross-linking does not retard mucociliary clearance as effectively. These results suggest that a critical range of cross-linking is required to provide maximum mucoadhesion. The limited bioadhesive properties of

Table 5
The relative transport rates ($n = 15$, mean $\pm$ S.E.M.) of mucoadhesive microspheres of maltose:PAA 1:2 loaded with preservatives across the bullfrog palate

Preservative	Curing time (h)			
	1.5	2.0	3.0	4.0
EDTA	0.07 ± 0.03	0.19 ± 0.05	0.02 ± 0.01	0.66 ± 0.02
CC	0.00 ± 0.00	0.05 ± 0.02	0.25 ± 0.02	0.47 ± 0.04
MHB	0.12 ± 0.05	0.00 ± 0.00	0.16 ± 0.02	0.42 ± 0.08
CB	0.04 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.47 ± 0.08
None	0.36 ± 0.07	0.08 ± 0.02	0.15 ± 0.01	0.34 ± 0.04

heavily cross-linked networks is attributed to the interpenetration of only the smaller chains and chain ends into the mucus layer (Peppas and Buri, 1985).

From Table 2, information can be obtained on the cross-linker density providing greatest mucoadhesion for each maltose:PAA ratio. For the 1:10 maltose:PAA, a significant increase in RTR with increasing curing time cannot be seen since the cross-linker density achieved had not reached the critical level after 4 h of curing. The 1:5 maltose:PAA is also still approaching the critical level of cross-linking, but the minimum RTR for the 1:2 maltose:PAA was achieved after 2 h of curing, indicating that this preparation has attained the optimum cross-linking density for the retardation of mucociliary clearance.

All of the microspheres, with the exception of the maltose:PAA 1:2 preparation cured for 3 or 4 h, loaded with EDTA (0.1% w/v), MHB (0.5% w/v) and CC (0.05%w/v) gave RTRs < 0.2 (Tables 3–5). The high cross-linking density of the maltose:PAA 1:2 preparation cured for 4 h may contribute to the higher RTR values since the restricted chain movement does not allow for sufficient diffusion of the preservative into the microsphere during the loading process and out of the microsphere whilst hydration and swelling are taking place.

All the preservatives lowered the RTR value obtained for microspheres cured for 1.5 h, whereas the particles cured for 2 and 3 h show no such pattern (however, their very low and close RTR values make trend detection difficult). In contrast, microspheres cured for 4 h and containing preservatives gave faster clearance rates compared to the original (i.e. preservative-free) microspheres. The transport of particles ceased irreversibly for microspheres with maltose:PAA 1:5 and loaded with CB or EDTA (except for the preparations cured for 4 h).

4. Conclusions

The bullfrog palate model has enabled the following conclusions to be drawn regarding particle clearance across ciliated epithelia. Clearance was retarded by the hydrophilic polymers studied whether in solution or microsphere form. For the latter, an optimum cross-linking density range exists. The inclusion of preservatives known to reversibly retard clearance produced variable effects on microsphere clearance. For lightly cross-linked microspheres the tendency was to reduce the RTR, whereas for more heavily cross-linked microspheres the converse was observed. Utilization of microspheres formed from mucoadhesive polymers may well provide a formulation approach to increase drug residence time in the nasal cavity.

References

Abd El-Hameed, M.D., Kellaway, I.W., 1997. Preparation and in vitro characterisation of mucoadhesive polymeric microspheres as intra-nasal delivery systems. Eur. J. Pharm. Biopharm. 44, 53–60.

Batts, A.H., Marriott, C., Martin, G.P., Bond, S.W., 1989. The effect of some preservatives on mucociliary clearance. J. Pharm. Pharmacol. 41, 156–159.

Batts, A.H., Marriott, C., Martin, G.P., Wood, C.F., Bond, S.W., 1990. The effect of some preservatives used in nasal preparations on the mucus and ciliary components of mucociliary clearance. J. Pharm. Pharmacol. 42, 145–151.

Bond, S.W., Hardy, J.G., Wilson, C.G., 1984. Deposition and clearance of nasal sprays. In: Aiache, J.M., Hirtz, J. (Eds.), Proceedings of the Second European Congress of BioPharmaceutics and Pharmacokinetics. Salamanca, Spain, pp. 93–98.

Chodosh, S., Segal, M.S., 1964. Medical intelligency. Current concepts. Chronic bronchitis. New Engl. J. Med. 270, 894–897.

Denton, R., Forsman, W., Hwang, S.H., Litt, M., Miller, C.E., 1968. Viscoelasticity of mucus. Its role in ciliary transport of pulmonary secretions. Am. Rev. Respir. Dis. 98, 380–391.

Duchateau, G.S.M.J.E., Graamans, K, Zuidema, J., Merkus, F.W.H.M., 1985. Correlation between nasal ciliary beat frequency and mucus transport rate in volunteers. Laryngoscope 95, 854–859.

Duchateau, G.S.M.J.E., Zuidema, J., Hermens, W.A.J.J., Merkus, F.W.H.M., 1986. In vitro ciliotoxicity of propranolol isomers. Int. J. Pharm. 31, 275–276.

Dulfano, M.J., Adler, K.B., 1975. Physical properties of sputum. VII. Rheologic properties and mucociliary transport. Am. Rev. Respir. Dis. 112, 341–347.

Ewert, G., 1965. On the mucus flow rate in human nose. Acta Oto-Laryngol. 200, 1.

Gallay, C., 1960. Essays physiologiques des gouttes nasales. Pharm. Acta Helv. 35, 358–374.

Giordano, A.H., Holsclaw, D., Litt, M., 1978. Mucus rheology and mucus clearance: normal physiological state. Am. Rev. Respir. Dis. 118, 245–250.

Greenwood, G., Pittenger, R.E., Constant, G.A., Ivy, A.C., 1946. Effect of zephiron chloride, tyrothricin, penicillin and streptomycin on ciliary action. Arch. Otolaryngol. 43, 623–628.

Hastie, A.T., Lögering, D.A., Gleich, G.J., Küppers, X.., 1987. The effect of purified eosinophil major basic protein on mammalian ciliary activity. Am. Rev. Respir. Dis. 135, 848–853.

Hilding, A.C., 1957. Ciliary streaming in the bronchial tree and the time element in carcinogenesis. New Engl. Med. J. 256, 634–640.

Jian, L., Po, A.L.W., 1993. Kinetic evaluation of the ciliotoxicity of methyl- and propyl-*p*-hydroxybenzoates using factorial experiments. J. Pharm. Pharmacol. 45, 98–101.

King, M., Gilboa, A., Meyer, F.A., Silberberg, A., 1974. On the transport of mucus and its rheological simulants in ciliated systems. Am. Rev. Respir. Dis. 110, 740–745.

Lin, S.Y., Amidon, G.L., Weiner, N., Goldberg, A.H., 1993. Viscoelasticity of anionic polymers and their mucociliary transport on the frog palate. Pharm. Res. 10, 411–417.

Lucas, A.M., 1933. Comparison of ciliary activity under in vitro and in vivo conditions. Proc. Soc. Exp. Biol. Med. 30, 501–506.

Marriott, C., 1990. Mucus and mucociliary clearance in the respiratory tract. Adv. Drug Delivery Rev. 5, 19–35.

Merkus, F.W.H.M., Hermens, W.A.J.J., Schipper, N.G.M., Rameijn, S.G., Verhoef, J.C., 1992. Present and future trends in pharmaceutical dosage forms for nasal application. In: Junginger, H.E. (Ed.), Drug Targeting and Delivery: Concepts in Dosage Form Design. Ellis Horwood, Chichester, pp. 233–242.

Merkus, F.W.H.M., Schipper, N.G.M., Hermens, W.A.J.J., Rameijn, S.G., Verhoef, J.C., 1993. Absorption enhancers in nasal drug delivery: efficacy and safety. J. Control. Release 24, 201–208.

Peppas, N.A., Buri, P.A., 1985. Surface, interfacial and molecular aspects of polymer bioadhesion. J. Control. Release 2, 257–275.

Phillips, P.P., McCaffrey, T.V., Kern, E.B., 1990. Measurement of human nasal ciliary motility using computerised microphotometry. Otolaryngol. Head Neck Surg. 103, 420–426.

Pritchard, K., Lansley, A.B., Martin, G.P., Helliwell, M., Marriott, C., Bendetti, L.M., 1996. Evaluation of the bioadhesive properties of hyaluronan derivatives: detachment weight and mucociliary transport rate studies. Int. J. Pharm. 129, 137–145.

Puchelle, E., Zahm, J.M., Girard, F., Betrand, A., Polu, J.M., Aug, F., Sadoul, P., 1980. Mucociliary transport in vivo and in vitro. Relations to sputum properties in chronic bronchitis. Eur. J. Respir. Dis. 61, 254–264.

Sadé, J., Eliezer, N., Silberberg, A., Nevo, A.C., 1970. The role of mucus in transport by cilia. Am. Rev. Respir. Dis. 102, 48–52.

Seybold, Z.V., Mariassy, A.T., Stroh, D., Kim, C.S., Gazeroglu, H., Wanner, A., 1990. Mucociliary interaction in vitro: effects of physiological and inflammatory stimuli. J. Appl. Physiol. 68, 1421–1426.

Shih, C.K., Litt, M.A., Khan, M.A., Wolf, D.P., 1977. Effect of non dialyzable solids concentration and viscoelasticity on clarity transport of tracheal mucus. Am. Rev. Resp. Dis. 115, 989–995.

Spungin, B., Silberberg, A., 1984. Stimulation of mucus secretion, ciliary activity and transport in frog palate epithelium. Am. J. Physiol. 247, 299–308.

Svartengren, K., Wiman, L.G., Thyberg, P., Rigler, R., 1989. Laser light scattering spectroscopy: a new method to measure tracheo-bronchial mucociliary activity. Thorax 44, 539–547.

Van Ree, J.H.L., Van Dishoeck, H.A.E., 1962. Some investigations on nasal ciliary activity. Pract. Oto-Rhino-Laryngol. 24, 383–390.