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Intranasal mucociliary clearance of putative bioadhesive polymer gels

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Abstract

Rapid clearance of a drug away from the site of absorption is one factor that limits the bioavailability of compounds administered nasally. The effects of putative bioadhesive polymers including methylcellulose, sodium carboxymethylcellulose, hydroxypropyl-methylcellulose, chitosan glutamate, Carbopol 934P, polyethylene oxide 600K and Pluronic F127 on slowing nasal mucociliary clearance were investigated using a rat model. The clearance of these polymer gels from the nasal cavity was measured by following the removal of fluorescently labeled microspheres incorporated into the formulation. Due to the increased residence times of the gel formulations in the nasal cavity, the clearance rate of each polymer gel was slower than the clearance rate of a control microsphere suspension. The clearance rate constants were in the range of 7–57% of the control clearance constants. Methylcellulose gel (3%) resulted in the most prolonged nasal clearance whereas Carbopol 934P aqueous gel (0.2%) had the most rapid clearance. A Carbopol 934P gel with propylene glycol and glycerol formal as cosolvents was prepared to investigate the effect of an in situ gelling system on nasal clearance. The initial clearance of this cosolvent gel was not significantly different than the suspension, yet the total mass recovered was significantly lower than the control. The clearance of a 3% methylcellulose gel formulation from a damaged nasal mucosa was also investigated in order to obtain further information about the characteristics of nasal mucociliary clearance. Results showed that from 4 h through the 7th day following the initial damage, although the initial clearance rate constants were slightly higher, the time for 90% of the observed particle clearance was significantly extended and the total masses recovered were significantly lower than those obtained from a non-damaged mucosa.

Keywords: Bioadhesive polymer; Mucociliary clearance; Nasal delivery; Mucoadhesion; Methylcellulose; Sodium carboxymethylcellulose; Hydroxypropyl-methylcellulose; Chitosan glutamate; Carbopol 934P; Polyethylene oxide 600K; Pluronic F127

1. Introduction

Nasal drug delivery has been investigated as an alternative to the parenteral routes for administering drugs that show poor oral bioavailability. Systemic absorption from the nasal cavity has

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been reported for compounds ranging from traditional, well-absorbed compounds such as hydralazine and propranolol (Hussain et al., 1979; Kaneo, 1983) to macromolecular compounds, such as insulin, which have extremely poor oral bioavailabilities. Some efforts to increase the bioavailability of nasally administered drugs have attempted to increase the residence time in the nasal cavity since rapid clearance away from the site of absorption can significantly reduce bioavailability. Clearance times from the human nasal cavity have been reported to be in the range of 10–15 min (Proctor et al., 1973), and in a rat model, times for 90% clearance from the nasal cavity were reported to be between 14 and 35 min (Donovan and Zhou, 1995).

One method to lengthen nasal residence time has been to include a bioadhesive in the formulation. Detailed information regarding the interactions of bioadhesive polymers with the nasal mucosa is quite limited. Lin et al. (1993) studied the influence of bioadhesive polymers on mucociliary transport in an isolated frog palate model. All of the polymer gel formulations applied in their investigations showed a reduced transport rate compared to the normal rate. In vivo studies using formulations including bioadhesive microspheres for nasal administration in humans were reported by Illum et al. (1987) and using hydroxypropylmethylcellulose (HPMC) by Pennington et al. (1988). The intranasal clearances of three microsphere systems, albumin, starch and DEAE-dextran microspheres, were much slower than the intranasal clearances of control solutions or powders. Half times for clearance of the microsphere systems were 3 h or longer while the half times for clearance of control solutions or powders were approximately 15 min. For HPMC, the clearance half-times of the formulations increased with increasing concentration (0.6–1.25%). Addition of bioadhesive polymers in a powder nasal dosage form for insulin resulted in an increase in bioavailability as compared to an insulin solution (Nagai et al., 1984). In general, it is assumed that the polymers and microspheres interact with mucus and the tissue surface with a resulting increase in contact time.

Mucus is a macromolecular solution of glycoproteins which consist of approximately 20% protein and 80% carbohydrate. The cross-linked network of the glycoprotein molecules forms a viscoelastic gel. Alterations in the gel structure of mucus can result in alterations in clearance from the nasal cavity. The polymers selected for use in these studies all have the potential to interact with mucin, either through hydrogen bonding or ionic interactions.

Cilia on the surface of the nasal epithelium beat at a frequency of 10–20 times per second. Ciliary movement can be subdivided into two phases: one in which the cilium is extended and moves quickly called the 'effective stroke' and the other in which the cilium is bent and moves more slowly called the 'recovery stroke'. The tips of the cilia reach the mucus layer during the effective stroke resulting in movement of the mucus layer in the direction of the stroke. This results in the overall movement of mucus, entrapped particles and potentially toxic materials toward the nasopharynx.

The cilia on the surface of the nasal epithelium provide a shearing force at the polymer/mucosal tissue interface which may have a significant impact on the ability of these polymers to fully exert their bioadhesive effects. In comparison, bioadhesion at most other mucosal sites (ocular, buccal, intestinal, vaginal) is primarily affected by shearing forces at the external surface of the bioadhesive polymer/mucus/tissue layer, thus allowing strong bioadhesive interactions between the polymer and mucus or tissue surfaces.

Many commonly used bioadhesive polymers are anionic or nonionic compounds with active hydrophilic functional groups which can form hydrogen bonds along the polymer chain and with mucus (Pritchard, 1971). Using a different mechanism, chitosan, a high molecular weight cationic polysaccharide, has also been reported to be an effective mucoadhesive (Lehr et al., 1992). It possesses a high bioadhesive force in both tensile strength and rheological measurements (Hassan and Gallo, 1990; Lehr et al., 1992). Further studies using a nasal delivery system for peptide drugs containing chitosan has resulted in a remarkable increase in drug absorption across the nasal mucosa (Illum et al., 1994).

All of these studies provide evidence that the addition of bioadhesive polymers to drug formulations can increase bioavailabilities under many circumstances. Several of these putative bioadhesive polymers were used in these investigations to prolong the contact time of a formulation in the nasal cavity either through bioadhesive interactions, altered clearance due to increased viscosity, or a combination of these factors.

2. Materials and methods

2.1. Materials

Orange FluoSpheres®, sulfated, 4 μm latex particles (2% w/v suspension) were purchased from Molecular Probes Inc. (Eugene, OR). Ketamine hydrochloride (10%) was obtained from Aveco Co., Inc. (Fort Dodge, IA). Polyoxyethylene-9-lauryl ether (laureth-9), triethanolamine (TEA), propylene glycol and glycerol formal were obtained from Sigma Chemical Co. (St. Louis, MO). Sodium phosphate, (mono- and di-basic) was obtained from EM Industries (Gibbstown, NJ). The following bioadhesive or gelling agents were used as received: chitosan glutamate (SeaCure G 210) (Pronova Biopolymer, Inc., Drammen, Norway), hydroxypropylmethylcellulose (HPMC) (Scientific Polymer Products Inc., Ontario, NY), sodium carboxymethylcellulose 7MF (CMC) (Amend, Irvington, NJ), methylcellulose (MC) (City Chemical, New York, NY), Carbopol 934P (CBP) (B.F. Goodrich, Brecksville, OH), Polyox 600K (PEO) (Union Carbide, Danbury, CT), Pluronic F127 prill (poloxamer 407) (BASF Wyandotte Co., Parsippany, NJ)

2.2. Preparation of gel formulations

Pluronic F127 gel was prepared as described earlier (Miller and Donovan, 1982). An appropriate amount of Pluronic F127 was dissolved in a volumetric flask containing Sørensen's phosphate buffer (pH 6.5), and the flask was stored in the refrigerator overnight. Cold buffer was then added to volume and the gel was thoroughly mixed. The test gel formulation was prepared by mixing 1 part of polymer gel and 1 part of FluoSpheres® suspension with a resulting concentration of 25% Pluronic F127 and 1% FluoSpheres® (solid).

Carbopol 934P gel in propylene glycol and glycerol formal as cosolvents was prepared according to the method of Chu et al. (1991). A 1.5% Carbopol 934P solution in glycerol formal (GF) and a 1.5% Carbopol 934P gel in propylene glycol (PG) were both neutralized using a 1:1 equivalent ratio of Carbopol:triethanolamine (TEA). Two parts of Carbopol 934P gel in PG and one part of Carbopol 934P gel in GF were mixed. The final gel formulation was obtained by thoroughly mixing 0.95 g of this mixture with 50 μl FluoSpheres® suspension which contained 0.02 g solid. The final concentrations in the formulation were 1.5% Carbopol 934P, 65% PG, 32.5% GF and 1% FluoSpheres®.

Carbopol 934P gels were prepared in water and neutralized with TEA to a pH value of 6.5. The other polymer gels, PEO, CMC, HPMC, MC and chitosan glutamate, were prepared in phosphate buffer (pH 6.5). All gels were allowed to hydrate overnight, then were mixed with FluoSpheres® suspension in a w/w ratio of 1:1. The final polymer concentrations in the formulations are listed in Table 1.

2.3. Viscosity measurement

The complex viscosity of each polymer gel was measured using an RS-100 controlled stress rheometer (Haake, Paramus, NJ). The measurements were performed in the oscillatory mode using the two parallel plate configuration. In these measurements, the sample was subjected to a constant sinusoidal oscillatory shear stress of 5 Pa over a frequency range of 0.1–10 Hz at a temperature of 37°C and the response (strain) was monitored. A gap width of 0.3 mm was chosen such that the gel would be held between the two plates during the measurement. The stress applied in the oscillation measurement was previously found to be in the linear region of the stress versus strain response. At low frequencies, the complex viscosity (in mPas) is approximately equal to the dynamic viscosity (in cps).

Table 1
Initial clearance rates

Polymer	Rate constant k ± S.E. (min-1)			
	Control	Gel formulation (% decrease from control)	24 h	48 h
0.2% CBP	0.197 ± 0.017	0.113 ± 0.018* (43%)		
0.4% CBP	0.164 ± 0.007	0.0692 ± 0.0060* (58%)	0.169 ± 0.008	
5% PEO	0.116 ± 0.007	0.0534 ± 0.005* (63%)	0.0647 ± 0.011*	0.160 ± 0.024*
3% Chitosan G	0.150 ± 0.011	0.0427 ± 0.014* (58%)	0.0689 ± 0.030*	0.103 ± 0.005
1.5% CBP (PUIUF)	0.132 ± 0.013	0.121 ± 0.010 (8.3%)	0.060 ± 0.008*	
3% CMC	0.176 ± 0.013	0.0560 ± 0.0062* (68%)	0.213 ± 0.025*	
3% HPMC	0.161 ± 0.014	0.0448 ± 0.0074* (72%)	0.213 ± 0.025*	
3% MC	0.227 ± 0.016	0.0154 ± 0.0075* (93%)	0.102 ± 0.011*	
25% Pluronic	0.137 ± 0.019	0.0378 ± 0.0114* (72%)	0.101 ± 0.014*	

* statistically different from control ($P \leq 0.05$).

2.4. Animal studies

2.4.1. Clearance from normal nasal mucosa

A modification of the in vivo mucociliary clearance method previously reported was used to measure the clearance rates of the polymer gels from the nasal cavity (Donovan and Zhou, 1995). Male, Sprague Dawley rats, weighing 250–300 g were housed and experiments conducted in a temperature controlled environment. At least three animals were exposed to each gel formulation. The rats were slightly sedated by subcutaneous injections of 10% ketamine (25 μl every 30 min) throughout the experiment. Twenty five microliters of either FluoSpheres® suspension or a gel formulation were instilled into the left nostril with a positive displacement pipette. The FluoSpheres® particles exiting the nasal cavity were collected by swabbing the oral cavity of the rat with moistened, cotton-tipped applicators every minute for the following 30 min and then every 5 min for the next 90 min. Four milliliters of distilled water were used to extract the FluoSpheres® from the cotton applicators, and the resulting solutions were measured using fluorescence spectrophotometry (Shimadzu RF 540, Kyoto, Japan).

2.4.2. Clearance from damaged mucosa

One percent laureth-9 was used to induce damage to the nasal epithelium by instilling twenty-five microliters of the solution into one nostril of each rat (Zhou and Donovan, 1996). The clearance of 3% methylcellulose gel was studied using the previously described method at 4 h and 2, 3, 4, 5, 7, 10 and 14 days post-exposure to the surfactant.

2.4.3. Control studies

Since nasal clearance rates vary significantly with the temperature and humidity of the external environment, control clearance studies were performed in each animal for at least 2 days prior to any chemical solution or gel formulation instillation. This allowed each group of animals to serve as their own control, since temperature and humidity conditions did not vary significantly over the course of each 5-day experiment whereas there were some variations in conditions between the groups. The control experiments used the same experimental procedures as the gels studied except FluoSpheres® suspension was instilled into the nostrils in place of the gel formulation (Donovan and Zhou, 1995).

2.5. Data analysis and statistics

The clearance of the fluorescent particles from the nasal cavity was expressed by the percent of the total mass recovered at the end of a 120 min collection interval as a function of time (Donovan and Zhou, 1995). The initial clearance rates (k) were obtained from the monoexponential fit of the first 60 min of data. The time needed for 90% of the total mass recovered from the nasal cavity

to clear (t_{90}) was also calculated, and the area under the curve (AUC) was determined using the trapezoidal rule. The mean values for each of these parameters for each group of animals receiving a particular gel were compared to the observed mean control values found for that group using the Student's t-test ($p \leq 0.05$).

3. Results and discussion

The initial clearance profiles of the gel formulations and FluoSpheres® suspension are shown in Fig. 1. In most cases, the intranasal clearance of the gel formulations demonstrated monoexponential behavior and the calculated rate constants are summarized in Table 1. From these data, it appears that all the gel formulations effectively decrease the intranasal mucociliary clearance rate. The clearance of viscous solutions from the nasal cavity has been reported to follow a biphasic pattern with an initial rapid clearance (half time less than 30 min) followed by a very slow clearance (half time greater than 2 h) of the formulation from the nasal cavity (Hardy et al., 1985; Pennington et al., 1988). In the current studies, only the initial clearance rate constant was obtained. The total mass of FluoSpheres® recovered during the 2-h collection period was used to represent the overall slow clearance of the gel formulations from the nasal cavity. For the cellulose derivatives (HPMC, MC, CMC), a constant polymer concentration was used in order to compare their effects on residence time. Comparing the clearance rate constants, 3% methylcellulose gel resulted in a dramatic decrease to 7% of the initial rate constant, although the clearance pattern of 3% MC did not show monoexponential behavior. Therefore AUC or t_{90} values more accurately describe the changes in the clearance behavior (Table 2 and Table 3). MC gel showed the lowest AUC and the longest t_{90} values. Hussain et al. (1980) previously demonstrated the utility of this polymer with the report that the addition of 3% methylcellulose to a nasal solution of propranolol produced a sustained blood level over an extended time period.

While 3% chitosan only decreases the clearance rate to 63% of its initial value, the total mass of chitosan gel recovered was only 0.7% of the amount administered. These results indicate that very little of the chitosan gel cleared from the nasal cavity during the collection period. In this case, the total mass recovered within the experimental time period is a more accurate parameter than the initial rate constant to evaluate the effect on residence time.

Poly(acrylic acid) has been widely used as a bioadhesive agent in formulations to enhance bioavailability. It has been reported that the bioadhesiveness of poly(acrylic acid) gel is pH and ionic strength dependent (Mortazavi et al., 1992; Park and Robinson, 1985). The intranasal clearance profiles of 0.2 and 0.4% Carbopol 934P gel formulations in these in vivo studies are shown in Fig. 1e–f. While the clearance of 0.2% Carbopol 934P gel followed a monoexponential pattern, the clearance of 0.4% Carbopol 934P gel did not. Instead, a rapid clearance was observed at approximately 12 min following gel administration. The increased viscosity of the 0.4% Carbopol 934P gel may require a longer time period for interaction between polymer gel and mucus to take place than the 0.2% gel, thus resulting in bulk movement of the gel formulation out of the nasal cavity prior to maximal adhesion.

Comparing the clearance rate constant, AUC, t_{90} and total mass recovered for the Carbopol 934P gels with those of the cellulose derivatives (Tables 1–4), the Carbopol 934P gels (0.2% and 0.4%) show faster clearance rate constants, larger AUC's and total masses recovered along with lower t_{90} values. These all indicate that the residence time-enhancing actions of the low concentration Carbopol 934P gel formulations in the nasal cavity are less than those of the cellulose derivatives. Similar observations were reported by Lin et al. (1993) in their in vitro studies of mucociliary transport of polymer gel formulations on the frog palate.

The adhesive force of polyethylene oxide was reported to be greater than methylcellulose in tensile strength testing (Junginger, 1991). The residence time of 5% polyethylene oxide in these in vivo intranasal clearance studies was less than

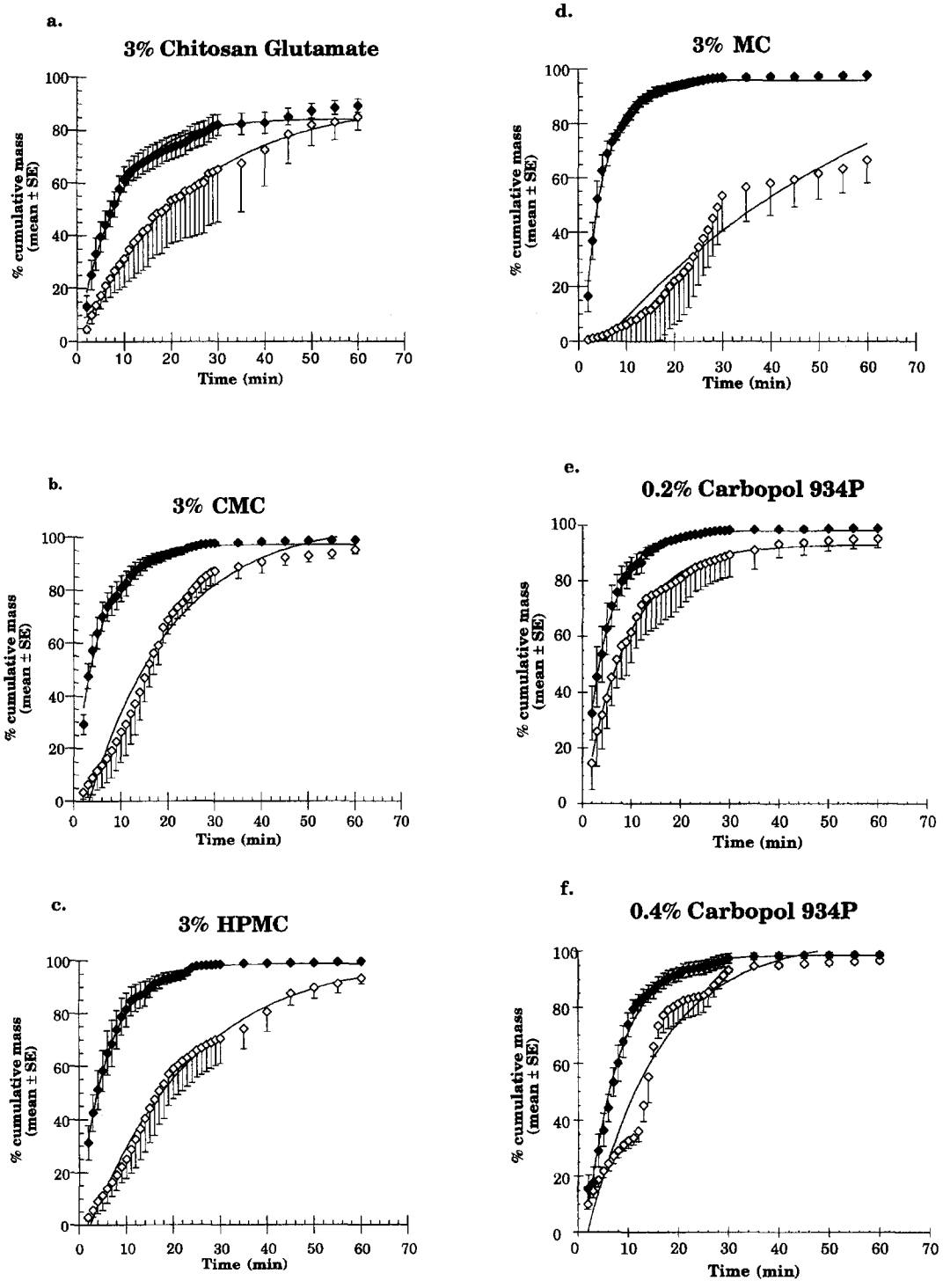
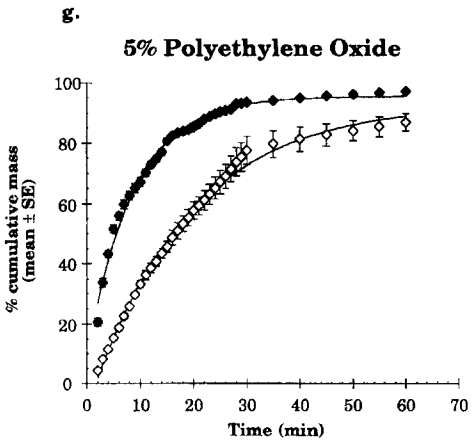
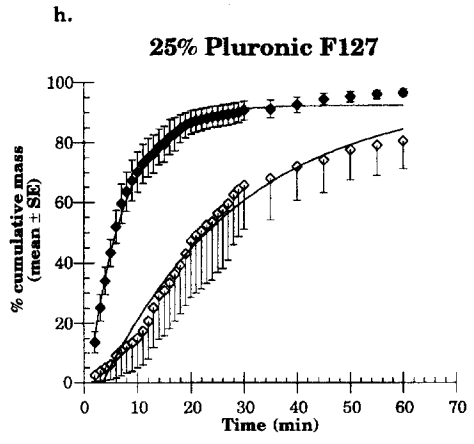
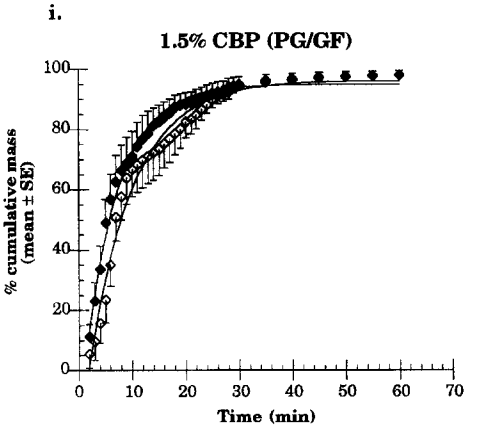


Fig. 1. Intranasal clearance profiles for polymer gels. Curves represent the monoexponential fit of the first 60 min of data. – ◆ Clearance of FluoSpheres® suspension during control studies. – ◇ –Clearance of polymer gel.







that of any of the cellulose derivatives and it was even slightly less than the 0.4% Carbopol 934P gel. It is expected that polymers with the greatest adhesive strength would result in the longest residence times. However, strongly adhesive, high-viscosity gel formulations are difficult to administer into the nasal cavity. In addition, if sufficient time to interact with mucus or the tissue surface is not allowed, these adhesive polymers may show poor in vivo retention.

In an attempt to develop liquid formulations that would gel following contact with the mucosal surface to eliminate these gel administration difficulties, 1.5% Carbopol 934P in a propylene glycol and glycerol formal solvent mixture (CBP-PG/GF) was prepared and used to study the bioavailability of intranasal propranolol in dogs (Chu et al., 1991). This formulation significantly increased the maximum plasma concentration and AUC of propranolol compared with a solution formulation prepared in normal saline. The proposed mechanism was that the non-aqueous Carbopol gel could take up water from the mucus layer in the nasal cavity resulting in a significant increase in the viscosity of the gel. Rheological studies of the nonaqueous Carbopol 934P gel with varying amounts of water added showed that a maximum viscosity was obtained with the addition of 30% water. A similar gel formulation mixed with Fluo-Spheres® was studied in these in vivo mucociliary clearance studies, and the clearance profile is shown in Fig. 1i. The rate constant, AUC and t90 values did not show significant differences following gel instillation compared with the control values (Tables 1–3). However, the total mass recovered values are significantly lower than the control (Table 4) which implies that the residence time was increased. These results suggest that this solvent system is useful in preparing a Carbopol 934P gel formulation with an extended residence time, yet the rapid initial clearance indicates that the formulation does not gel rapidly enough to eliminate the initial loss of some of the low viscosity solution.

Cellulose derivatives are capable of reverse thermal gelation where the viscosity of these gels increases rather than decreases with increasing temperature (Lieberman et al., 1988). Pluronic F127 is a polyoxyethylene-polyoxypropylene

Table 2
AUC values for FluoSpheres® recovery

Polymer	AUC ± S.D.(%min)			
	Control	Gel formulation	24 h	48 h
0.2%CBP	11 348 ± 181	10 642 ± 791*	10 223 ± 1136*	10 881 ± 316*
0.4%CBP	11 144 ± 251	10 435 ± 40*	11 292 ± 58	
5%PEO	10 932 ± 276	9873 ± 361*	10 769 ± 499	11 171 ± 373
3% Chitosan G	10 067 ± 775	9101 ± 1321	7442 ± 3038*	10 812 ± 22
1.5% CBP-PGIUF	10 895 ± 739	10 775 ± 287	10 190 ± 184	
3%CMC	11 325 ± 245	9994 ± 541*	11 455 ± 141	
3%HPMC	11 353 ± 273	9614 ± 750*	11 508 ± 171	
3%MC	11 256 ± 130	7939 ± 1604*	11 351 ± 166	
25%Pluronic	10 964 ± 578	8764 ± 1592*	10 830 ± 426	

* statistically different from control ($P \leq 0.05$).

Table 3
Time for 90% Fluosphere® clearance (t_{90})

Polymer	t_{90} (min) ± S.D.			
	Control	Gel formulation	24 h	48 h
0.2% CBP	11 ± 5	34 ± 27*	40 ± 31*	
0.4% CBP	19 ± 7	25 ± 6	15 ± 2	
5% PEO	25 ± 8	45 ± 18*	33 ± 18	17 ± 9
3% Chitosan G	53 ± 29	65 ± 30	83 ± 49	21 ± 5*
1.5% CBP-PU/UF	22 ± 12	23 ± 8	39 ± 12*	
3% CMC	15 ± 6	38 ± 15*	15 ± 9	
3% HPMC	14 ± 7	49 ± 12*	14 ± 3	
3% MC	15 ± 5	87 ± 14*	14 ± 6	
25% Pluronic	28 ± 18	69 ± 24*	31 ± 11	

* statistically different from control ($P \leq 0.05$).

Table 4
Total FluoSphere® mass recovered in 2 h

Polymer	Total mass ± S.D. (% of dose)			
	Control	Gel formulation (% of control)	24 h	48 h
0.2% CBP	10.8 ± 6.1	9.9 ± 5.4 (92%)	10 ± 5.6	
0.4% CBP	17.8 ± 8.9	6.5 ± 2.5* (37%)	8.1 ± 1.3	
5% PEO	13.1 ± 5.4	6.3 ± 0.8* (48%)	7.1 ± 2.4	6.8 ± 4.9
3% Chitosan G	18.0 ± 10.9	0.7 ± 0.3* (3.9%)	6.2 ± 6.6	26.0 ± 11.5
1.5% CBP-PG/GF	21.0 ± 5.1	9.1 ± 3.2* (43%)	15.5 ± 4.8	
3% CMC	18.8 ± 10.4	5.5 ± 1.6* (29%)	23.7 ± 16.5	
3% HPMC	27.9 ± 8.1	4.8 ± 2.0* (17%)	25.2 ± 1 0.6	
3% MC	27.0 ± 6.5	6.0 ± 2.8* (22%)	19.4 ± 8.7	
25% Pluronic	33.0 ± 11.9	12.6 ± 3.0* (38%)	22.4 ± 4.1	

* statistically different from control ($P \leq 0.05$.)

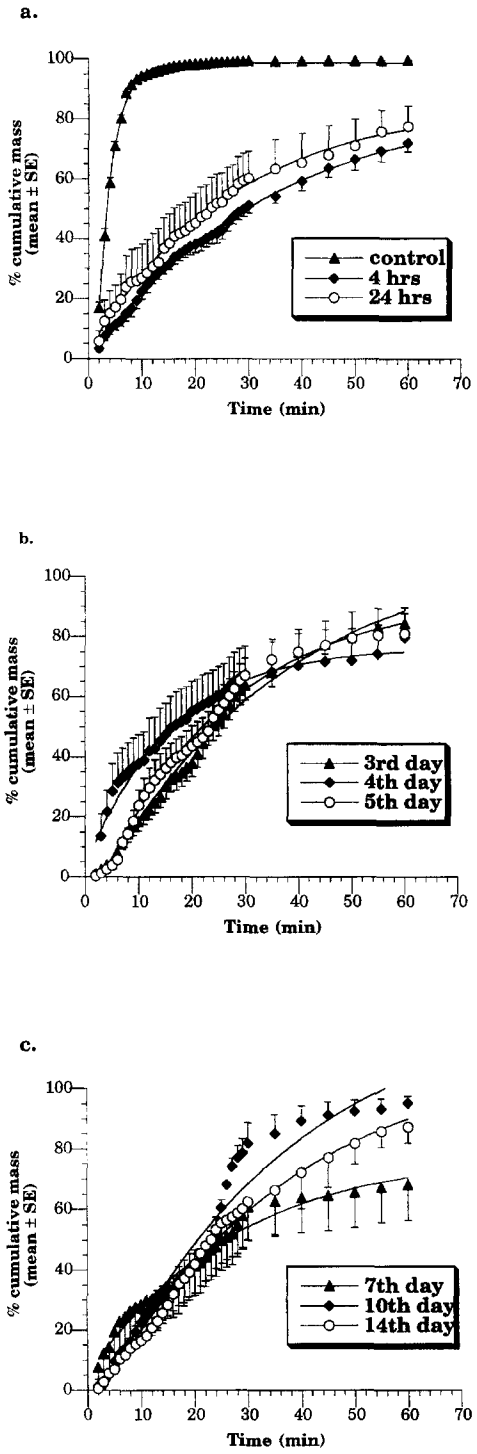


Fig. 2. Intranasal clearance profiles of MC gel following laureth-9 exposure. Curves represent the monoexponential fit of the first 60 min of data.

block copolymer that shows even more distinct reversible thermal gelation properties than do the cellulose derivatives. At 4°C, 25% Pluronic F127 in buffer is quite fluid, while at room temperature it forms a clear viscous gel. For these intranasal clearance studies, the gel was cooled to 4°C for instillation, the dosage form then gelled on contact with the warm mucosal tissue. The clearance results showed a significant decrease in the initial clearance rate constant and a lower AUC value for Pluronic F127 gel as compared to control. The overall effect was just slightly less than that of 3% methylcellulose. These results indicate that Pluronic F127 in the formulation can also significantly prolong the residence time in the nasal cavity.

While the viscosity of the gel may play a role in its clearance from the nasal cavity, the complex viscosities of the gels at low oscillatory frequency (0.1 Hz) alone were not found to correlate to the reduction in clearance rate or the percent mass recovered measured in vivo. The complex viscosities were in the order of 5% PEO (1.65 Pas) < 3% CMC (2.26 Pas) < 3% MC (10.3 Pas) < 3% HPMC (12.7 Pas) < 3% chitosan glutamate (48 Pas) < 0.2% CBP (210 Pas) < 0.4% CBP (630 Pas) < 25% Pluronic F127 (at 37°C, 8640 Pas).

In order to investigate the recovery of the normal mucociliary clearance pattern following gel administration, intranasal clearances were also measured at 24 and 48-h post gel exposure using the same FluoSpheres® suspension as was used for the control studies. The results are included in Tables 1–4. For most of the gels, although the clearance rate constants at 24 h are significantly different from the controls, the values were significantly higher than those observed in the presence of the gels. Exceptions to this occurred with 5% PEO and 3% chitosan. For these gels, the recovery of a normal clearance rate was not observed until 48 h post-exposure. The total mass recovered at 24 h was not significantly different when compared with their control values for any of the gels, however.

The methylcellulose gel formulation, which significantly decreased the clearance rate in the normal nasal cavity, was used for further investigation of the effects of damage to the nasal mucosa on intranasal clearance. Clearance studies using 3% MC gel were performed at various time points from

Table 5
Clearance properties for 3% MC following P-9-E (1%)

	Mean ± S.D.			
	Rate constant	AUC	t_{90}	Total mass
Control	0.3636 ± 0.086	11565 ± 84	7 ± 1	28.17 ± 7.98
4 h	0.0297 ± 0.0047	7928 ± 745	95 ± 9	0.92 ± 0.23
2nd day	0.0374 ± 0.0174	8628 ± 1887	76 ± 30	1.50 ± 0.66
3rd day	0.0278 ± 0.0049	8626 ± 582	79 ± 18	1.12 ± 0.62
4th day	0.0633 ± 0.0239	9093 ± 1946	56 ± 30	1.03 ± 0.68
5th day	0.0407 ± 0.0087	8854 ± 1501	61 ± 19	4.39 ± 3.50
7th day	0.0372 ± 0.0214	8138 ± 2573	82 ± 19	2.91 ± 1.48
10th day	0.0277 ± 0.0045	9629 ± 274	44 ± 20	8.88 ± 4.06
14th day	0.0277 ± 0.0078	9067 ± 1001	57 ± 16	7.42 ± 2.96

4 h to 14 days following exposure to a 1% laureth-9 solution. Laureth-9 has been reported to induce damage to the nasal mucosa (Chandler et al., 1991). Regrowth of the damaged mucosa has been recently reported to be completed in approximately 10 days, and cilia on the apical surface could only be observed after 10 days (Zhou and Donovan, 1996). Clearance profiles of 3% MC following laureth-9 exposure are shown in Fig. 2a–c and the clearance parameters are listed in Table 5. All of the clearance rate constants following gel instillation were significantly lower and the total masses recovered were also significantly lower than the control suspension. The other parameters listed in Table 5 (t_{90} and AUC) are also significantly different from the control. From 4 h through the 7th day following laureth-9 exposure, although the clearance rate constants were slightly higher than those from a non-damaged mucosa, the values of the total mass recovered were always lower than for the normal mucosa. It is likely that the clearance rate constants only reflect the early clearance phase. The results obtained at the 10th and 14th days were quite similar to the results found with MC gel in rats with a normal mucosa that were not exposed to laureth-9. These results show that the clearance of the MC gel formulation from the damaged mucosa was decreased for an extended period of time. The presence of cilia on the nasal epithelium resulted in a faster overall clearance after 10 days, thus further demonstrating the significance of viable cilia in effective mucociliary clearance.

4. Conclusions

All of the polymers used in these studies have been shown by previous investigators to exhibit bioadhesive characteristics either in in vitro studies such as tensile strength testing or in in vivo studies by increasing the bioavailability or prolonging the residence time of a drug formulation. In these studies, gel clearance from the nasal cavity was studied by measuring initial clearance rate constants, AUCs, t_{90}s, and the total mass recovered in 2 h of fluorescent microspheres incorporated into the gels. The results indicate that all of the formulations decreased intranasal mucociliary clearance, thus increasing the residence time of the formulations in the nasal cavity. The rank order of the percent decrease in clearance rate constants was found to be: 3% MC > 25% Pluronic F127 ~ 3% HPMC > 3% CMC 3% chitosan glutamate > 0.4% CBP > 5% PEO > 1.5% CBP-PG/GF > 0.2% CBP. Chitosan glutamate gel gave the lowest total mass recovered value indicating that the residence time of 3% chitosan glutamate would be greater than was represented by the clearance rate constant. The rank order of the percent mass recovered was: 0.2% CBP > 5% PEO > 1.5% CBP-PG/GF > 25% pluronic F127 ~ 0.4% CBP > 3% CMC > 3% MC > 3% HPMC > 3% chitosan glutamate. Thus, the clearance of a polymer gel formulation

from the nasal cavity is not well described by a single parameter. The initial clearance rate constant gives information about the initial rapid removal of a gel. Formulations that are very viscous or very fluid undergo rapid, bulk clearance during the first 60 min. Even with this rapid initial clearance, however, those formulations with strong bioadhesive capacities can significantly limit the total clearance from the nasal cavity. An optimal system for nasal drug delivery would therefore be fluid enough for easy administration yet would not undergo rapid initial clearance, and would have sufficient interaction with the mucosal surface to continue to limit clearance for extended time periods. Of the polymers studied, the cellulose derivatives appear to possess the best combination of these characteristics, with 3% methylcellulose exhibiting the best performance.

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