AAPS PharmSciTech 2007; 8 (2) Article 32 (http://www.aapspharmscitech.org).

Rheological Characterization of Neutral and Anionic Polysaccharides With Reduced Mucociliary Transport Rates

Submitted: January 24, 2006; Accepted: September 20, 2006; Published: April 20, 2007

Ankur J. Shah[1,2] and Maureen D. Donovan[1]

[1]Division of Pharmaceutics, University of Iowa, Iowa City, IA 52242
[2]Current address: Novartis Institutes of Biomedical Research, Chemical and Pharmaceutical Profiling, 4560 Horton St, Emeryville, CA 94608

ABSTRACT

The purpose of this research was to compare the viscoelastic properties of several neutral and anionic polysaccharide polymers with their mucociliary transport rates (MTR) across explants of ciliated bovine tracheal tissue to identify rheologic parameters capable of predicting the extent of reduction in mucociliary transport. The viscoelastic properties of the polymer gels and gels mixed with mucus were quantified using controlled stress rheometry. In general, the anionic polysaccharides were more efficient at decreasing the mucociliary transport rate than were the neutral polymers, and a concentration threshold, where no further decreases in mucociliary transport occurred with increasing polymer concentration, was observed for several of the neutral polysaccharides. No single rheologic parameter (η, G', G'', tan δ, G^*) was a good predictor of the extent of mucociliary transport reduction, but a combination of the apparent viscosity (η), tangent to the phase angle (tan δ), and complex modulus (G^*) was found to be useful in the identification of formulations capable of decreasing MTR. The relative values of each of the rheologic parameters were unique for each polymer, yet once the relationships between the rheologic parameters and mucociliary transport rate reduction were determined, formulations capable of resisting mucociliary clearance could be rapidly optimized.

KEYWORDS: Mucociliary clearance, rheology, carboxymethylcellulose, hydroxypropyl methylcellulose, methylcellulose, xanthan, alginate.

INTRODUCTION

Efficient mucociliary clearance depends on the mucus layer having particular rheological properties. The viscoelasticity of the mucus layer contributes to the effectiveness of mucociliary clearance, but the interaction between the mucus and the cilia also plays a critical role. Previous investigators have studied the relationship between a material's viscosity and/or elasticity and the resulting mucociliary transport rate.[1-3] Work performed by King et al showed that while there was no specific chemical requirement for transport, all of the systems found to be transported across a mucus-depleted frog palate possessed a slight degree of crosslinking, suggesting an important role for the elastic character of the system.[4] Shih et al showed, using reconstituted lyophilized canine tracheal mucus, that mucociliary clearance increased with increasing elasticity up to an elastic modulus value (G') of 1 Pa (determined at a frequency of 16 Hz) but then decreased again with further increases in elasticity above this value.[3] Majima et al[5] demonstrated that the maximum clearance rate of mucus on a mucus-depleted frog palate was achieved when the value of G' was 2 Pa at a frequency of 1 Hz at 25°C. Gelman and Meyer[6] were able to alter the elastic modulus of cervical mucus gels without significantly altering the viscous modulus by crosslinking the mucins with gluteraldehyde; the transport of these cervical mucus samples across the mucus-depleted frog palate correlated with the changes in elastic modulus and showed an optimum value for transport at 0.16 Pa. Chen and Dulfano[2] showed that the most rapid transport across a mucus-depleted frog palate was achieved by sputum with Newtonian viscosity values between 1000 and 3000 Poise determined at shear stresses less than 10 Pa, and Puchelle et al[7] reported the optimum transport rate for xanthan gum across a mucus-depleted frog palate was observed at a viscosity value of 120 Poise determined at a shear rate of 0.4 sec^{-1}. These results indicate that biorheological requirements do exist for optimal mucociliary clearance, yet the variety of methods and materials selected for testing makes it difficult to establish parameter values that can be used to a priori optimize mucociliary transport of drug formulations containing various polymers and added excipients. Other investigators have attempted to correlate rheologic properties of gels and gel-mucus combinations to the mucoadhesive character of the gel.[8-10] Unfortunately, the rheological measurements, while excellent for identifying gel-mucus interactions, were not able to accurately predict mucoadhesion as measured using tensile strength testing.[10]

Identifying the viscoelastic properties of formulations capable of transiently increasing the residence time in the

Corresponding Author: Maureen D. Donovan, Division of Pharmaceutics, University of Iowa, Iowa City, IA 52242. Tel: (319) 335-9697; Fax: (319) 335-9349; E-mail: maureen-donovan@uiowa.edu

respiratory tract while efficiently releasing the drug from the matrix may enable the formulation of bioadhesive systems with improved therapeutic efficacy and minimal toxicity. The objective of these studies was to systematically compare the rheological parameters of a series of chemically related, neutral, and anionic polysaccharide polymers to the reduction in their mucociliary transport across bovine tracheal explants. A knowledge of the parameter ranges that result in the greatest inhibition of mucociliary clearance should greatly improve the ability to rapidly optimize formulations with prolonged mucosal contact time on ciliated mucosal surfaces using in vitro methods.

Materials and Methods

Materials

Sodium chloride, potassium chloride, calcium chloride, sodium bicarbonate, dextrose, sodium hydroxide, potassium phosphate (dibasic), potassium phosphate (monobasic), porcine gastric mucin (Type II), sodium azide, activated charcoal, and dithiothreitol (DTT) were obtained from Sigma Chemical Co (St Louis, MO). Methocel A4C and A15C (methylcellulose [MC], molecular weight [MW] 41 000 and 63 000, respectively) and Methocel E4M (hydroxypropyl methylcellulose [HPMC], MW 86 000) were gifts from Dow Chemical Co (Midland, MI). Aqualon 7MF (sodium carboxymethylcellulose [CMC], MW 250 000) was a gift from Hercules, Inc (Wilmington, DE). Vanzan NF (xanthan gum [XAN], MW > 10^6) was obtained as a gift from R.T. Vanderbilt Co, Inc (Norwalk, CT). Dextran (DEX, MW ~500 000) was purchased from Sigma Chemical Co (St Louis, MO). Manugel GHB (sodium alginate [ALG], MW ~10^5) was a gift from International Specialty Products (Wayne, NJ).

Methods

Preparation of Polymer Gels

Both Methocel A4C and A15C (MC) gels were prepared in 1.25%, 2.5%, and 5% wt/vol concentrations, while Methocel E4M (HPMC) gels were prepared in 1.25% and 2.5% wt/vol concentrations. These concentrations were based on previous reports regarding the bioadhesion of these polymers and qualitative estimates of a useful range of viscosities appropriate for intranasal administration.[11-13] Approximately one third of the volume of water required for preparation of each gel was heated to ~90°C, and the polymer was added to the water with agitation by a lab stirrer. The remaining volume was added as cold water or ice. Agitation was continued for at least 30 minutes.

Other polymers investigated included 1%, 2.5%, and 3% wt/vol sodium CMC; 0.125%, 0.45%, and 0.5% wt/vol XAN; 3.5% and 6% wt/vol ALG; and 0.25% and 2.5% wt/vol DEX. These gel formulations were prepared by slowly sifting the polymer into the vortex of room temperature water stirring in a beaker. Mixing was continued for 30 minutes following addition of the polymer.

All of the polymer formulations were allowed to hydrate overnight at room temperature. They were centrifuged at 3000 rpm for 3 minutes (Marathon 21K, Fisher Scientific, Hampton, NH) to remove entrapped air, and the formulations were allowed to rest at room temperature for another 12 hours before any rheological measurements were conducted.

Reconstitution and Purification of Mucus

A reconstituted porcine gastric mucus solution was prepared using a modification of the method reported by List et al.[14] The use of lyophilized porcine gastric mucin was preferred in these studies because of the need for a matrix that contained limited nonglycoprotein contaminants that could contribute to the variability in the rheological properties of the final mucus gel. Lyophilized porcine gastric mucin Type II (40 mg/mL) was suspended in isotonic phosphate buffer (pH 6.6, the pH of mucus at the apical cell surface[15]) containing 0.02% wt/vol sodium azide and stirred overnight at 4°C. Sodium azide prevents the growth of mold and bacteria in the prepared mucus, and its presence has no significant effect on mucociliary clearance in the bovine explants. The resulting suspension was centrifuged at 16 000 rpm for 15 minutes using a refrigerated super-speed Sorvall RC26 Plus centrifuge (Kendro Laboratory Products, Newtown, CT). The supernatant was decanted and centrifuged once again under the same conditions. The final supernatant was placed into cellulose acetate dialysis tubing (MW cutoff 12 000-14 000; Spectrum Chemical Co, Houston, TX) and dialyzed for 24 hours against isotonic phosphate buffer (pH 6.6) at 4°C. The resulting mucus solution (3%-3.5% wt/vol) was stored at 4°C for 2 days before long-term storage at –70°C prior to use.

Preparation of Polymer-Mucus Mixed Gels

Since a polymer-containing formulation will interact with the mucus layer prior to any interaction with the mucosal surface, the rheological characterization of polymers and polymer/mucus systems was performed to gain a better understanding of the role that the viscoelastic properties of polymers and mucus play in mucoadhesion.[8] Polymer-mucus ratios that were reflective of in vivo conditions were prepared by gently mixing 5 parts (by weight) of the polymer gel with 1 part of mucus.[16] Control gels were prepared by diluting the polymer gel with water in a 5:1 ratio. The concentration of the polymers reported for the polymer-mucus

Table 1. Viscoelastic Parameters (at 3.16 Hz) of Neutral Polysaccharide Polymer and Polymer-Mucus Mixtures[†]

Sample	η[‡] (*mPa s*)	G′ (Pa)	G″ (Pa)	Tan δ	G* (Pa)
A4C 1.25%	36 (1.5)	8.9 (2.2)	2.6 (0.4)	0.30 (0.09)	9.3 (2.1)
A4C 1.25%/mucus	44[§] (1.9)	7.7 (1.7)	1.6 (0.6)	0.21 (0.03)	7.8 (1.8)
A4C 2.5%	278 (17)	19 (3.0)	9.5 (0.7)	0.51 (0.10)	21 (2.6)
A4C 2.5%/mucus	309 (11)	27[§] (3.0)	12 (2.2)	0.43 (0.03)	29[§] (3.6)
A4C 5%	2722 (335)	84 (15)	88 (9.2)	1.1 (0.09)	121 (17)
A4C 5%/mucus	2936 (143)	127[§] (14)	101 (10.7)	0.79[§] (0.04)	162[§] (17)
A15C 1.25%	58 (3.4)	7.2 (1.6)	2.3 (0.4)	0.33 (0.09)	7.6 (1.5)
A15C 1.25%/mucus	74[‡] (4.5)	9.5 (1.6)	2.8 (0.6)	0.30 (0.01)	10 (1.6)
A15C 2.5%	515 (7.0)	40 (7.4)	19 (2.5)	0.48 (0.10)	44 (7.0)
A15C 2.5%/mucus	535 (44)	22[§] (6.0)	17 (3.1)	0.80[§] (0.11)	28[§] (6.4)
A15C 5%	3157 (155)	100 (11)	105 (25)	1.0 (0.16)	145 (25)
A15C 5%/mucus	2817 (258)	99 (6.4)	93 (4.5)	0.94 (0.02)	136 (7.7)
HPMC 1.25%	133 (7.7)	3.4 (0.9)	3.8 (0.3)	1.2 (0.22)	5.1 (0.8)
HPMC 1.25%/mucus	128 (8.5)	4.9 (0.6)	4.4 (0.7)	0.91 (0.15)	6.6 (0.7)
HPMC 2.5%	928 (142)	23 (5.5)	28 (3.1)	1.3 (0.18)	36 (5.8)
HPMC 2.5%/mucus	718 (68)	30 (4.6)	33 (1.7)	1.1 (0.12)	45 (4.2)
DEX 0.5%	2.3 (0.1)	ND	ND	ND	ND
DEX 0.5%/mucus	2.5 (0.7)	ND	ND	ND	ND
DEX 2.5%	2.9[‡] (0.01)	ND	ND	ND	ND
DEX 2.5%/mucus	3.7 (0.1)	ND	ND	ND	ND

[†]All values are means of 3 replicate determinations; values in parentheses are SDs. A4C indicates methylcellulose [MW 41000]; A15C, methylcellulose [MW 63000]; HPMC, hydroxypropyl methylcellulose; DEX, dextran; ND, not detectable.
[‡]Values for polymer-mucus mixtures significantly different than polymer ($P<0.05$).
[§]Apparent viscosity obtained from constant rate flow curve at 100 s^{-1}.

mixtures (Table 1 and Table 2) indicates the concentration of the polymer before mixing with mucus.

Rheological Measurements

The rheological properties of the polymer formulations were determined with a Haake RS1 controlled stress rheometer using a cone and plate sensor system (C60/4, 60 mm diameter, 4° angle) connected to a Haake F3-CH temperature control system equipped with V2.97 data acquisition software (Haake Mess-Technik GmbH Co, Karlsruhe, Germany). The measurement gap distance was fixed at 0.138 mm. All tests were run at 35ºC to simulate the temperature of the nasal mucosal surface.[17] To minimize dehydration of the sample during rheologic testing, a solvent trap was used to cover the sample during analysis.

Stress amplitude sweep tests (0.1-40 Pa) at a fixed frequency of 3.16 Hz were conducted to determine the complex modulus as a function of applied stress. This frequency was selected to mimic the reported in vivo ciliary beat frequency.[18] A stress value (0.1 Pa), selected from the linear viscoelastic region, was used for frequency sweep testing where the oscillatory frequency was increased from 0.05 to 5 Hz. The rheological parameters measured during the oscillatory testing included the elastic modulus (G′), viscous modulus (G″), complex modulus (G*), and tan δ (G″/G′). The apparent viscosity of the sample was measured after applying a constant shear rate of 100 sec^{-1} for a period of 1 minute. The value reported for the apparent viscosity was the average of the values obtained during the final 30-second interval of the measurement period.

Measurement of Mucociliary Transport Rate

A modified in vitro technique using bovine tracheal tissues was used to measure the reduction in mucociliary clearance induced by the gels.[2] Tracheal tissues were obtained from local abattoirs and maintained in Locke-Ringer's solution (LR) at room temperature during transport to the laboratory.

Table 2. Viscoelastic Parameters (at 3.16 Hz) of Anionic Polysaccharide Polymers and Polymer-Mucus Mixtures[†]

Sample	η[‡] (mPa s)	G′ (Pa)	G″ (Pa)	Tan δ	G* (Pa)
CMC 1%	23 (0.5)	ND	ND	ND	ND
CMC 1%/mucus	20 (0.03)	ND	ND	ND	ND
CMC 2.5%	278 (22)	3.6 (0.7)	6.9 (0.2)	1.9 (0.4)	7.8 (0.4)
CMC 2.5%/mucus	299 (14)	4.2 (0.1)	7.4 (0.7)	1.8 (0.2)	8.5 (0.6)
CMC 3%	493 (9.3)	7.3 (0.5)	12 (0.6)	1.6 (0.05)	14 (0.7)
CMC 3%/mucus	556 (54)	8.2 (0.5)	13 (0.8)	1.6 (0.02)	15 (0.9)
XAN 0.125%	15 (0.2)	ND	ND	ND	ND
XAN 0.125%/mucus	9.7 (0.2)	ND	ND	ND	ND
XAN 0.45%	67 (1.2)	5.4 (0.1)	2.3 (0.2)	0.43 (0.02)	5.9 (0.2)
XAN 0.45%/mucus	58 (1.8)	6.1[§] (0.3)	2.4 (0.2)	0.40 (0.02)	6.6[§] (0.3)
XAN 0.5%	72 (4.5)	6.3 (0.4)	2.7 (0.2)	0.43 (0.02)	6.9 (0.5)
XAN 0.5%/mucus	73 (2.0)	7.2 (0.4)	2.7 (0.1)	0.38 (0.02)	7.7 (0.4)
ALG 3.5%	513 (17)	1.5 (0.5)	12 (1.2)	8.6 (3.4)	12 (1.1)
ALG 3.5%/mucus	439 (42)	2.4 (0.8)	13 (3.3)	5.4 (1.0)	13 (3.4)
ALG 4%	678 (18)	2.3 (0.1)	16 (0.6)	7.2 (0.5)	16 (0.6)
ALG 4%/mucus	714 (1.2)	3.3 (0.5)	19 (2.1)	5.8 (0.3)	20 (2.2)

[†]All values are means of 3 replicate determinations; values in parentheses are SDs. CMC indicates sodium carboxymethylcellulose; XAN, xanthan gum; ALG, sodium alginate; ND, not detectable.
[‡]Apparent viscosity obtained from constant rate flow curve at 100 s^{-1}.
[§]Values for polymer-mucus mixtures significantly different from those for polymer gel ($P < .05$).

Tracheal segments (~8 × 3 cm) were prepared, and the explants were depleted of endogenous mucus by immersion in 0.2 M DTT for 5 minutes prior to the use of the explants for mucociliary transport rate (MTR) measurement.[4] The explants were washed with LR for ~10 minutes and stored at 4°C for 30 minutes. Prior to measurement, each explant was placed within a closed chamber on a gauze pad saturated with LR and warmed to an epithelial temperature of 35°C (~5 minutes). Immediately prior to the conduct of a transport experiment, the explant was quickly immersed in reconstituted porcine gastric mucus solution and then placed back into the chamber for a final 5-minute equilibration period.

The rate of each gel's movement across the tracheal explant was measured by following the movement of ~10 μL of gel placed in the center of the explant. The gels were spiked with charcoal particles (~10 mg/mL) to assist with visualization. Prior to each gel transport measurement, the tracheal explant was calibrated with a control, charcoal-containing mucus suspension placed on the explant in the same manner as the gels.[12] The movement of the charcoal particles entrapped within the gel or mucus was followed using a Stereomaster stereomicroscope (Fisher Scientific, Hanover Park, IL) at a 10× magnification with a 1-cm calibrated eyepiece. The cilia swiftly and cleanly carry the control mucus along the explant at a rate of ~0.6 to 1 cm/min. The transport rate for each polymer gel was reported as the percentage decrease in MTR in the presence of gel compared with the mucus suspension control (Equation 1):

$$\%\ \text{MTR decrease} = \left(\frac{\text{control MTR} - \text{gel MTR}}{\text{control MTR}}\right) \times 100 \qquad (1)$$

After each control and sample pair, the surface of the explant was rinsed with LR to remove any sample or mucus from the previous measurement, and the surface was replenished with reconstituted mucus. Whenever the control clearance rate of the charcoal suspension was observed to be less than 25% of the initial control clearance rate, the explant was discarded and a new explant was conditioned for use. All mucociliary clearance values reported are the mean of 3 replicate determinations (Table 3).

Results and Discussion

Rheological Measurement of Polymer-Mucus Mixtures

The viscoelastic moduli of the polymer-mucus mixtures exhibited qualitatively similar rheological profiles to those of the pure polymer gels. Methocel A4C 2.5%, A4C 5%,

Table 3. Reduction in Mucociliary Transit Rate Across Bovine Tracheal Explants in the Presence of Polysaccharide Gels*

	Mucociliary Transit Rate (cm/min)		
Sample	Control	Sample	% Reduction
A4C 1.25%	0.75 (0.00)	0.41 (0.02)	35 (12)
A4C 2.5%	0.67 (0.09)	0.19 (0.03)	72 (3.0)
A4C 5%	0.72 (0.05)	0.06 (0.05)	91 (7.9)
A15C 0.625%	0.71 (0.10)	0.61 (0.10)	15 (4.1)
A15C 1.25%	0.67 (0.10)	0.18 (0.06)	74 (7.1)
A15C 2.5%	0.61 (0.10)	0.10 (0.06)	87 (6.8)
A15C 5%	0.75 (0.00)	0.10 (0.05)	87 (6.3)
HPMC 1.25%	0.52 (0.03)	0.15 (0.01)	70 (2.8)
HPMC 2.5%	0.60 (0.00)	0.07 (0.01)	88 (0.98)
DEX 0.5%	0.87 (0.13)	0.76 (0.10)	13 (1.6)
CMC 1%	0.90 (0.08)	0.44 (0.05)	51 (1.9)
CMC 2.5%	0.56 (0.03)	0.07 (0.01)	88 (1.2)
CMC 3%	0.56 (0.03)	0.02 (0.00)	96 (0.69)
ALG 3.50%	0.69 (0.05)	0.02 (0.01)	97 (0.50)
ALG 4%	0.67 (0.00)	0.02 (0.00)	97 (0.49)
XAN 0.125%	0.69 (0.0*)	0.29 (0.14)	60 (16)
XAN 0.45%	0.62 (0.04)	0.03 (0.00)	95 (0.40)
XAN 0.5%	0.67 (0.00)	0.02 (0.01)	97 (1.8)

*All values are means of 3 replicate determinations; values in parentheses are SDs. A4C indicates methylcellulose [MW 41 000]; A15C, methylcellulose [MW 63 000]; HPMC, hydroxypropyl methylcellulose; DEX, dextran; CMC, sodium carboxymethylcellulose; ALG, sodium alginate; XAN, xanthan gum.

A15C 2.5%, and XAN 0.5% were the only polymer gels with somewhat increased elastic, viscous, and complex moduli for the polymer-mucus mixtures as compared with the pure polymer gels. DEX (0.5%-2.5% wt/vol), 0.125% XAN, 1% CMC, and reconstituted mucus were too fluid to be measured using the same methodology described for the other gels. As a result of the minimal change in the rheologic parameters at the polymer concentrations likely to be used in topical formulations, the rheologic parameters of the polymers themselves, rather than of the polymer-mucus mixtures, were compared with their mucociliary transport rates.

Comparison Between MTR and Apparent Viscosity (η)

As the concentration of each polymer was increased, increases in apparent viscosity and corresponding decreases in MTR were observed (Table 1 and Table 2; Figure 1 and Figure 2). The curves included in the figures have no theoretically derived relationship to the data, but it can be clearly seen for MC that a limiting polymer concentration was reached beyond which no further decreases in MTR were measured. This maximal clearance reduction occurred at concentrations of 5% Methocel A4C and 2.5% Methocel A15C. HPMC also showed a decrease in MTR with increasing polymer concentration (1.25%-2.5% wt/vol), but sufficiently high concentrations were not prepared to investigate the occurrence

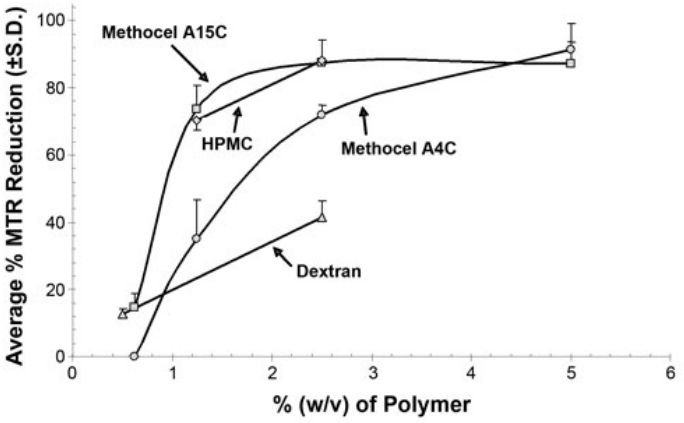

Figure 1. Effect of polymer concentration on reduction in mucociliary transport rate for neutral polysaccharide gels. Each point represents the mean of 3 replicates. Methocel indicates methylcellulose; HPMC, hydroxypropylmethylcellulose.

of a clearance threshold for this polymer. Complete inhibition of transport (for a 5-minute interval) occurred for concentrations of XAN > 0.45%, CMC > 2.5%, and ALG > 3% (Figure 2). Increasing the concentration of DEX, in comparison, did not significantly affect the viscosity of the solution, and mucociliary transport was not appreciably reduced (<50% MTR decrease) over the concentration range investigated (0.5-2.5%) (Table 1, Figure 1).

For the neutral polysaccharides, gels with apparent viscosities above 500 mPa s showed reductions in MTR of >80% (Table 1 , Table 3). CMC and ALG polymer gels with viscosities above 250 mPa s also showed reductions in MTR of this magnitude. It is interesting to note, however, that XAN produced reductions in MTR of >90% with apparent viscosities as low as 60 mPa s.

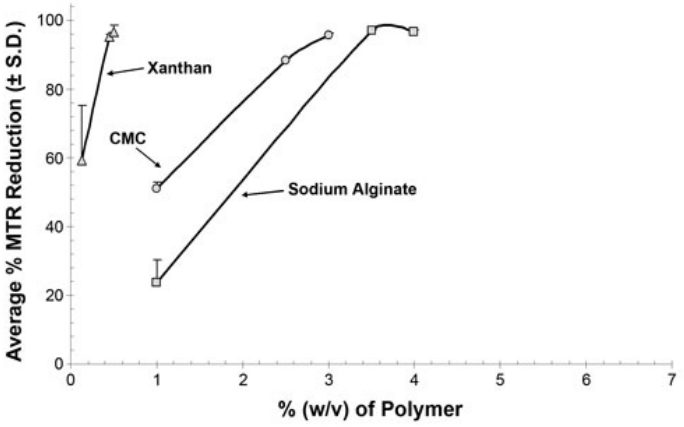

Figure 2. Effect of polymer concentration on reduction in mucociliary transport rate for anionic polysaccharide gels. Each point represents the mean of 3 replicates. CMC indicates carboxmethylcellulose.

Comparison Between MTR and Elastic and Viscous Moduli

All of the polymer gels investigated showed increasing elastic (G′) and viscous (G″) moduli with increasing polymer concentration (Table 1 ,Table 2). MC (A4C and A15C) gels were predominantly elastic, with G′ greater than G″ at lower concentrations (<2.5%). As the polymer concentration increased, the difference between the elastic and viscous moduli decreased until they were approximately equal at a concentration of ~5% wt/vol. In comparison, HPMC gels had similar values of G′ and G″ at all concentrations tested. CMC and ALG gels were predominantly viscous (G″ > G′) at all concentrations, while XAN was predominantly elastic (G′ > G″).

Since G′ is closely linked to the connectivity of the polymer network, it is not unexpected that the elastic modulus of the polysaccharide polymers increased with increasing concentration. Highly elastic gels are more difficult to clear efficiently, however, because the cilia have difficulty penetrating into the gel because of its increased "solid-like" (elastic) behavior. Instead, they slip underneath the mucus/polymer layer during the effective stroke, resulting in little net movement of the mucus-polymer blanket. The viscous modulus (G″), which is a measure of the resistance to deformation, would also be expected to increase with increasing polymer concentration because of the greater resistance to deformation of the more highly concentrated polymer network. Increasing values of G″ result in increased energy dissipation during the mechanical coupling between the mucus and the cilia, which interferes with the efficiency of mucus transport and results in a decrease in the MTR.

Neutral polysaccharides with G′ or G″ values greater than 20 Pa reduced the MTR by more than 80%, while most of the anionic polysaccharides with similar magnitudes of MTR reduction had G′ values between 1 and 25 Pa and G″ values above 2 Pa (Table 1 , Table 3). Decreases in MTR with increasing G′ have also been reported for HPMC, CMC, and ALG (G′ = 10 to 1000 Pa) by Lin et al[11] and for polyethylene oxide, HPMC, and Carbopol 934P (G′ = 5 to 1000 Pa) by Yu et al.[19] using a frog palate model. Yet the absolute value of G′ was not observed to be predictive of the extent of decrease in MTR. Polymers with similar G″ values (A4C 1.25% and A15C 1.25%) also had different effects on MTR, demonstrating that G″ values alone are insufficient to predict the effect of polymer gels on MTR.

Comparison Between MTR and Tan δ (G″/G′)

Tan δ (G″/G′) describes the relative viscous to elastic behavior of the sample. Gels with tan δ > 1 (G″ > G′) are more viscous, while gels with tan δ < 1 (G′ > G″) are more elastic. MC and HPMC showed increasing tan δ values with increasing polymer concentrations, which indicated the polymer gels were becoming increasingly viscous. HPMC, CMC, and ALG had tan δ values > 1 at all concentrations tested. XAN was observed to be predominantly elastic, with tan δ values < 1. Increases in the tan δ values for these polysaccharides were predictive of reduced mucociliary transport rates (Table 1 and Table 3). For example, most of the anionic polymers that reduced the MTR by more than 85% had tan δ values greater than 0.5 (Table 2 and Table 3). A similar predictive capability for tan δ values has also been reported by previous investigators.[20-22] Since tan δ is a ratio, however, it is quite insensitive to the actual magnitude of the individual G′ or G″ values. As a result, relatively large changes in the values of the individual parameters may not be apparent when the tan δ values are compared, especially when both parameters increase or decrease proportionally. As a result, tan δ is not sufficiently sensitive to accurately predict, as a single parameter, the effect of a polymer gel formulation on MTR.

Comparison Between MTR and Complex Modulus (G)*

The complex modulus (G*) is the vector sum of G′ and G″ and describes the rigidity and overall strength of the polymer gel. Increasing the polymer concentration of the neutral polysaccharide gels resulted in increasing G* values (10-250 Pa). These stiffer gels had slower mucociliary transport rates because of the inability of the cilia to penetrate effectively into the gel, decreasing the efficiency of energy transfer to the mucus/polymer layer. Most of the anionic polysaccharides, in comparison, showed G* values that were not significantly different from each other (7-20 Pa) and were lower than those of the neutral polysaccharides. The MTR reductions for the anionic polysaccharides varied over a smaller percentage range (51%-97%) than the neutral polysaccharides (15%-91%), indicating that the magnitude of the G* value may be useful in the a priori prediction of the ability of a formulation to reduce MTR.

Using Rheological Properties to Predict MTR

Previous investigators have suggested optimizing formulations based solely on complex modulus (G*) or tan δ (G″/G′) values. The studies of Lorenzi et al[20] and Macchione et al,[21] using a frog palate preparation, showed a negative correlation between both the tan δ and the complex modulus (G*) of mucus and the in vitro mucus transport rate. King postulated that increasing tan δ values (increased viscous modulus compared with elastic modulus) allowed for increased dissipation of ciliary energy, resulting in a decrease in the overall transport velocity.[22] The current studies, using chemically similar polymers over a range of concentrations, have

demonstrated that tan δ is too insensitive to be used alone as a predictor of mucociliary clearance rate. G*, in comparison, can be used alone as a general estimator of the extent of reduction in MTR, but attention to several parameters—apparent viscosity (η), complex modulus (G*), and tan δ—improves the ability to identify formulations capable of decreasing MTR.

To further demonstrate this, the MTRs for 2 different compositions of MC, 5% A4C and 5% A15C, were selected based on their similar values of apparent viscosity (η), tan δ, and G*. When tested on the bovine explants, they were observed to yield reductions in MTR that were not significantly different from each other (Student *t* test, $P < .05$) (Table 1 and Table 3). In comparison, 1.25% A4C and 1.25% A15C, which have similar tan δ and G* values but differ significantly in their apparent viscosities, did not yield similar MTR reductions.

Role of Polymer Structure

The anionic polysaccharides (CMC, XAN, ALG) were observed to be more efficient at reducing MTR than the neutral polysaccharides, suggesting that there may be a difference in the manner in which anionic polysaccharides interact with mucus or cilia compared with neutral celluloses. Previous investigators have claimed that the repulsion between the negatively charged groups on the CMC backbone keeps the polymer in an expanded conformation, allowing it to have a greater number of physical interactions with the mucus glycoproteins.[13] Similarly, the unique effects of low concentrations of XAN on MTR are believed to be the result of its branched structure and anionic nature. The branched structure results in a lower viscosity relative to the polymer's actual MW and enables gels containing lower polymer concentrations to spread easily over the mucus layer, resulting in an increased surface area available for entanglement. The repulsion between the anionic charges on the polymer allows it to be in a more favorable conformation for interaction with mucus glycoproteins, even though they are both negatively charged. DEX, in comparison, is also a branched polysaccharide, yet it contains no ionizable functionalities and exists in a helical molecular conformation. It has minimal interactions with mucus because of its limited ability to form hydrogen bonds with the mucin glycoprotein network,[23] which demonstrates the importance of hydrogen bonding or ionic interactions between mucoadhesive polymers and the mucin glycoproteins.

CONCLUSIONS

These studies demonstrate that the rheologic parameters, tan δ, G*, and η, can be used to identify gel formulations capable of reducing mucociliary transport. Each polymer has a unique range of parameter values that result in optimal MTR reduction, and once defined, these parameters can be used to optimize formulations containing viscoelastic polymers, drugs, and excipients via rheological profiling for maximal retention on ciliated mucosal surfaces.

REFERENCES

1. Dulfano M, Adler K. Physical properties and mucociliary transport, VII: rheologic properties and mucociliary transport. *Am Rev Respir Dis.* 1975;112:341–347.

2. Chen TM, Dulfano M. Mucus viscoelasticity and mucociliary transport rate. *J Lab Clin Med.* 1978;91:423–431.

3. Shih C, Litt M, Khan M, Wolf P. Effect of nondialysable solids concentration and viscoelasticity on ciliary transport of tracheal mucus. *Am Rev Respir Dis.* 1977;115:989–995.

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

5. Majima Y, Sakakura Y, Matsubara T. Rheological properties of middle ear effusions from children with otitis media with effusion. *Ann Otol Rhinol Laryngol.* 1986;124:1–4.

6. Gelman R, Meyer F. Mucociliary transference rate and mucus viscoelasticity: dependence on dynamic storage and loss modulus. *Am Rev Respir Dis.* 1979;120:553–557.

7. Puchelle E, Zahm JM, Quemada D. Rheological properties controlling mucociliary frequency and respiratory mucus transport. *Biorheology.* 1987;24:557–563.

8. Hassan EE, Gallo JM. A simple rheological method for the in vitro assessment of mucin-polymer bioadhesive bond strength. *Pharm Res.* 1990;7:491–495.

9. Edsman K, Hagerstrom H. Pharmaceutical applications of mucoadhesion for the non-oral routes. *J Pharm Pharmacol.* 2005; 57:3–22.

10. Hagerstrom H, Edsman K. Limitations of the rheological mucoadhesion method: the effect of choice of conditions and the rheologic synergism parameter. *Eur J Pharm Sci.* 2003;18: 349–357.

11. Lin SY, Amidon GL, Weiner ND, Goldberg AH. Viscoelasticity of cellulose polymers and mucociliary transport on frog palates. *Int J Pharm.* 1993;95:57–65.

12. Lin SY, Amidon GL, Weiner ND, Goldberg AH. Viscoelasticity of anionic polymers and their mucociliary transport in the frog palate. *Pharm Res.* 1993;10:411–417.

13. Madsen F, Eberth K, Smart JD. A rheological examination of the mucoadhesive/mucus interaction: the effect of mucoadhesive type and concentration. *J Control Release.* 1998;50:167–178.

14. List SJ, Findlay BP, Forstner GG, Forstner JF. Enhancement of the viscosity of mucin by serum albumin. *Biochem J.* 1978;175:565–571.

15. Khanvilkar K, Donovan MD, Flanagan DR. Drug transfer through mucus. *Adv Drug Deliv Rev.* 2001;48:173–193.

16. Shah AJ. *Viscoelastic Gels Resistant to Mucociliary Clearance: Rheological and Chemical Optimization for Prolonged Mucosal Contact [thesis].* Iowa City, IA: University of Iowa; 2005.

17. Keck T, Leiacker R, Riechelmann H, Rettinger G. Temperature profile in the nasal cavity. *Laryngoscope.* 2000;110:651–654.

18. Widdicombe JG, Wells UM. Airway Secretions. In: Proctor DF, Anderson IP, eds. *The Nose: Upper Airway Physiology and the*

Atmospheric Environment. New York, NY: Elsevier Biomedical Press; 1982:215–244.

19. Yu DM, Amidon GL, Weiner ND, Fleisher D, Goldberg AH. The role of rheological properties in mucociliary transport by frog palate ciliated model. *Pharm Res.* 1994;11:1785–1791.

20. Lorenzi G, Bohm G, Guimaraes E, Vaz M, King M, Saldiva P. Correlation between rheological properties and in vitro ciliary transport of rat nasal mucus. *Biorheology.* 1992;29:433–440.

21. Macchione M, King M, Lorenzi G, et al. Rheological determinants of mucociliary transport in the nose of the rat. *Respir Physiol.* 1995;99:165–172.

22. King M. Relationship between mucus viscoelasticity and ciliary transport in guaran gel/frog palate model system. *Biorheology.* 1980;17:249–254.

23. Duchene D, Touchard F, Peppas NA. Pharmaceutical and medical aspects of bioadhesive systems for drug administration. *Drug Dev Ind Pharm.* 1988;14:283–318.