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Formulating Gels for Decreased Mucociliary Transport Using Rheologic Properties: Polyacrylic Acids

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ABSTRACT

The purpose of these studies was to identify the rheologic properties of polyacrylic acid gels necessary for optimal reductions in mucociliary clearance. The mucociliary transport of 2 bioadhesive polyacrylic acid polymers, polycarbophil and carbopol, was assessed in vitro by measuring their clearance rates across explants of ciliated bovine tracheal tissue. The viscoelastic properties of polymer gels were measured in the presence of mucus using controlled stress rheometry. Combinations of apparent viscosity (η) and complex modulus (G*) were found to be the most useful parameters in the identification of polyacrylic acid formulations capable of decreasing mucociliary transport rate (MTR). A narrow range of η and G* values suitable for reducing mucociliary clearance, while remaining sufficiently fluid for intranasal administration, were identified. The correlations between the rheologic parameters of the polycarbophil gels and their mucociliary transport rates were used to identify other polyacrylic acid gels that also had suitable mucociliary clearance properties, demonstrating that these parameters can be used to direct the optimization of formulations using simple in vitro rheologic testing.

KEYWORDS: Rheology, mucociliary clearance, polycarbophil, carbomer, bioadhesion.

INTRODUCTION

Inadequate gastrointestinal bioavailability of many important drug compounds has led to efforts to identify alternate routes for drug administration. The nasal cavity is easily accessible and highly vascularized, and compounds administered by this route are often rapidly absorbed, while avoiding hepatic first-pass metabolism. The majority of currently marketed nasal drug products are intended for local therapeutic action. However, several additional products show clinically effective bioavailability following nasal administration, demonstrating that the nasal mucosa can be a convenient and reliable route for systemic drug delivery.

One of the problems encountered with intranasal administration, however, is the rapid mucociliary clearance, which shortens intranasal residence time and can significantly limit the time available for drug absorption. Mucociliary clearance is the result of the coordinated motion of the cilia present on the mucosal surface resulting in the mucus layer being propelled unidirectionally toward the nasopharynx. In order for mucus to be efficiently transported, it must possess specific viscoelastic properties, and alterations in these properties can compromise the efficiency of mucociliary clearance.

Studies by Shih et al[1] and Majima et al[2] demonstrated that the maximum mucociliary transport rate of mucus across a frog palate was achieved when the values for G′ were between 1 and 2 Pa. Values greater than 2 Pa resulted in a decrease in mucociliary transport rate (MTR). Puchelle et al[3] reported that the optimum transport rate for xanthan gum across a mucus-depleted frog palate was observed at a viscosity value of 12 000 mPa s determined at a shear rate of 0.4 second^{-1}. Lin et al[4] showed a negative correlation between the G′ (40-1800 Pa) of linear polyanionic polymers (sodium carboxymethylcellulose, sodium alginate, Carbopol 934P) and their MTRs across frog palates. The studies of Lorenzi et al[5] and Macchione et al,[6] also using a frog-palate preparation, showed a negative correlation between both the tan δ and the overall mucus impedance (G*) with the measured in vitro mucus transport rates.

The bioadhesive properties of polyacrylic acids are well recognized,[7-11] and 2 of the most commonly used polyacrylic acids for improved bioadhesion are polycarbophil and carbomer. Polycarbophil (Noveon AA-1) is a homopolymer of acrylic acid cross-linked with divinylglycol. Carbomer (Carbopol 1342) is a copolymer of acrylic acid and a long-chain alkyl methacrylate cross-linked with allylethers of pentaerythritol. In most liquid systems, polycarbophil and carbomer require neutralization by a base to induce a significant increase in viscosity. Neutralization of the polymers causes increased ionization of the carboxyl functionalities and the resulting intramolecular repulsion results in the polymer chains taking on an extended conformation with increased polymer entanglements. This leads to the observed increase in gel viscosity. In contrast, increasing the ionic

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strength of the medium has been proposed to cause a shielding of the negative charges along the polymer chain, resulting in coiling of the macromolecule and a decrease in viscosity owing to the reduction in polymer-polymer interactions.

Polyacrylic acids interact with mucus and biological surfaces through hydrogen bonding of the ionized carbonyl functionalities.[12] Because of their bioadhesive properties, the polyacrylic acids have been investigated in nasal dosage forms for the enhancement of intranasal bioavailability. Morimoto et al reported an increase in the nasal absorption of insulin along with a corresponding decrease in plasma glucose levels using 0.1% and 1% wt/vol Carbopol 941 gel formulations.[13] They also observed a significant hypocalcemic effect when calcitonin was administered nasally in a 0.1% wt/vol Carbopol 941 gel. In a separate study, Dondeti et al[14] reported that nasal administration of insulin-loaded polyacrylic acid microparticles suspended in 1% wt/vol polyacrylic acid gel resulted in a significant and sustained hypoglycemic effect for up to 7 hours in normal rabbits.

Given the improved bioavailability reported for various compounds formulated for intranasal delivery with polyacrylic acids and the observed interactions of the polyacrylic acids with mucus, these studies were undertaken to quantify the viscoelastic properties of polyacrylic acid polymer gels, which can be used to delay mucociliary clearance in the nasal cavity. Initial studies were performed using polycarbophil to develop a correlation between its rheologic properties and mucociliary transport. The rheologic properties identified for polycarbophil were then used to formulate gels with the desired mucociliary transport rates using Carbopol 1342, a second polyacrylic acid polymer.

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). Novoen AA-1 (NOV, polycarbophil, molecular weight [MW] $>10^6$) and Carbopol 1342 (CAR, carbomer, MW $>10^6$) were gifts from Noveon Inc (Cleveland, OH).

Methods

Preparation of Polymer Gels

Polycarbophil or carbomer gels (Table 1) were prepared by slowly sifting the polymer into the vortex of stirred water in a beaker. Once the dry polymer was introduced, the required quantity of base (NaOH) needed to neutralize either 10% or 60% of the carboxyl functionalities was added with reduced agitation to avoid the entrapment of excess air. Mixing continued for 30 minutes. Each gel was allowed to hydrate overnight at room temperature. Gels were centrifuged at 3000 rpm for 3 minutes (Marathon 21K, Fisher Scientific, Hampton, NH) to remove entrapped air, and the gels were allowed to rest at room temperature for another 12 hours before any rheological measurements were made.

Table 1. Composition of Polyacrylic Acid Polymer Gels

Polymer Concentration (% wt/vol)	0.0625	0.125	0.25	0.5	0.75	1
Polycarbophil (10% neutralization)	—	•*	•	•	•	•
Polycarbophil (60% neutralization)	•*	•*	•	—	—	—

* indicates gel was too fluid to determine rheologic parameters using methods described; •, gel was prepared and studied in the described experiments; —, gel composition was not prepared.

Reconstitution and Purification of Mucus

A reconstituted porcine gastric mucus solution was prepared using a modification of the method reported by List et al.[15] Reconstituted mucus was selected for use to limit the variability in the rheologic and mucociliary transport measurements owing to variability in native mucus samples. Lyophilized porcine gastric mucin type II (40 mg/mL) was suspended in isotonic phosphate buffer (pH 6.6) and purified by dialysis to give a final mucus solution with a glycoprotein concentration of 3% to 3.5% wt/vol.[16] The final mucus solution was stored at −70°C prior to its use. A modified mucus solution prepared without buffer salts was made using the same methods, except distilled water was used as the dialysis exchange solvent. The pH of this mucus was adjusted to 6.6 with 1 N NaOH.

Determination of Rheologic Properties

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 temperature control system (Haake F3-CH refrigerated circulator) with V2.97 data acquisition software (Haake Mess-Technik GmbH, Karlsruhe, Germany). The measurement gap distance was fixed at 0.138 mm. All tests were conducted at 35ºC to simulate the physiologic temperature of the nasal cavity.[17] In order to minimize dehydration of the sample during testing, a solvent trap was used to cover the sample during analysis.

Stress amplitude sweep tests (0.1-40 Pa) at fixed frequencies of 1 and 3.16 Hz were conducted to measure the complex

modulus as a function of applied stress. These frequencies were selected because they are similar to the range of frequencies reported for ciliary beating in the respiratory tract.[18] A stress value (0.1 Pa), selected from the linear viscoelastic region, was used for the frequency sweep testing (0.05-5 Hz, within the linear viscoelastic region), and the rheological parameters determined from the oscillatory frequency sweep studies 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 seconds^{-1} for a period of 1 minute. The value reported for the apparent viscosity (η) was the average of the values obtained during the last 30 seconds of measurement.

Preparation of Polymer-mucus Mixed Gels

The rheological characterization of the polymers and polymer/mucus mixtures was performed to determine whether exposure to mucus caused a significant alteration in polymer rheology. To measure the viscoelastic parameters of the polymer gels following mixing with mucus, 5 parts of the polymer gel were gently mixed with 1 part (by weight) of mucus using a spatula.[16] Control polymer gels were mixed in an identical 5:1 ratio using water. The rheologic properties of these diluted gels were tested immediately. The concentration of the polymers reported for the polymer-mucus mixtures represents the initial concentration of the polymer before mixing with mucus.

Measurement of Mucociliary Transport Rate

A modified in vitro technique using bovine tracheal explants was used to study mucociliary transport.[19] Bovine tracheas were obtained from local abattoirs and maintained in Locke-Ringer's (LR) solution at room temperature during transport to the laboratory. Bovine tracheal segments (approximately 8 × 3 cm) were depleted of endogenous mucus by immersing them in 0.2 M DTT for 5 minutes. The explants were washed with Locke-Ringer's solution for ~10 minutes and placed in the refrigerator at 4°C for 30 minutes. Prior to use, each explant was placed in a chamber on a gauze pad saturated with LR and warmed to an epithelial temperature of 35°C (~5 minutes). Immediately prior to each transport experiment, the explant was quickly immersed in reconstituted porcine gastric mucus solution and reequilibrated in the chamber for an additional 5 minutes.

The MTR of the polymer gels across the bovine tracheal explants was measured by placing 10 μL of gel spiked with activated charcoal (~10 mg/mL) in the center of the explant.[4] The movement of the charcoal particles was followed using a Stereomaster stereomicroscope (Fisher Scientific, Hanover Park, IL) at original magnification of ×10 with a 1-cm calibrated eyepiece. Control studies were performed on the explant prior to each gel MTR measurement to verify that the explant still retained normal ciliary movement. A suspension of charcoal-spiked, reconstituted mucus was used as the control, and MTR measurements were made in an identical manner to the gel. After each measurement of the mucus control followed by sample gel, the surface of the explant was rinsed with LR to remove the sample and 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 prepared. To account for variability between tissues, the decrease in transport rate for each gel sample was reported as the percentage decrease in MTR for the sample compared with the MTR for the mucus control tested immediately preceding it (Equation 1).

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

Results and Discussion

Viscoelastic Properties of Polymer and Polymer-mucus Gels

Since polyacrylic acids have been reported to be sensitive to the ionic strength of the hydration medium,[20,21] a modified mucus solution was prepared that did not contain buffer salts. Comparisons between the rheologic parameters for polymer:mucus (buffer-free) and polymer:mucus (with buffer) gels were made to investigate changes in the viscoelastic behavior of the gel:mucus mixtures in the presence and absence of additional ions. Table 2 contains the viscoelastic parameters measured for the polymer:water and polymer:mucus mixtures containing buffer-free and buffered mucus. The polyacrylic acids showed substantial decreases in their viscous and elastic moduli following mixing with buffer-free reconstituted mucus, and drastic reductions in viscosity were seen when the polymer gels were mixed with mucus containing buffer salts. In fact, most of these samples became too fluid to allow for the measurement of their rheologic properties with the techniques used in these studies.

Previous investigators have reported on the strong dependence of the viscoelastic moduli of the polyacrylic acids on the pH and ionic strength of the hydration medium.[20-22] Rossi et al[22] prepared 0.96% wt/wt Carbopol 934P solutions in distilled water, pH 7.0 phosphate buffer, and pH 4.5 acetate buffer; both the viscosity (measured at 100 seconds^{-1}) and the viscoelastic moduli (G′ and G″ measured at 1 Hz) were found to be higher in distilled water than in pH 7.0 buffer, and both of these solvents gave values higher than

Table 2. Viscoelastic Parameters (at 3.16 Hz) of Anionic Polyacrylic Acids for Polymer and Polymer-mucus Mixtures Obtained From a Frequency Sweep*

Sample	η (mPa s)†	G′ (Pa)	G″ (Pa)	Tan δ	G* (Pa)
NOV 0.5% (10% n) / water	611 (552-651)	223 (214-237)	15 (14-16)	0.07 (0.06-0.07)	223 (215-237)
NOV 0.5% (10% n) / mucus + salt	NM	ND	ND	ND	ND
NOV 0.5% (10% n) / mucus − salt	348 (328-369)	87 (82-93)	8 (8-8)	0.09 (0.09-0.10)	88 (82-93)
NOV 0.75% (10% n) / water	1121 (1093-1162)	386 (371-401)	25 (24-26)	0.06 (0.06-0.07)	387 (372-402)
NOV 0.75% (10% n) / mucus + salt	NM	ND	ND	ND	ND
NOV 0.75% (10% n) / mucus − salt	903 (886-929)	226 (206-257)	17 (15-19)	0.07 (0.07-0.08)	226 (207-257)
NOV 1% (10% n) / water	1402 (1308-1541)	417 (393-431)	26 (23-28)	0.06 (0.05-0.07)	418 (394-432)
NOV 1% (10% n) / mucus + salt	187 (185-189)	34 (29-37)	5 (5-6)	0.16 (0.15-0.17)	34 (30-37)
NOV 1% (10% n) / mucus − salt	1297 (1181-1367)	287 (276-295)	22 (21-23)	0.08 (0.07-0.08)	288 (276-295)
NOV 0.25% (60% n) / water	690 (652-752)	222 (206-233)	25 (21-27)	0.11 (0.10-0.12)	223 (207-234)
NOV 0.25% (60% n) / mucus + salt	ND	ND	ND	ND	ND
NOV 0.25% (60% n) / mucus − salt	382 (327-432)	79 (63-107)	12 (10-14)	0.16 (0.13-0.18)	80 (64-108)
0.5% (10% n) C1342 / water	872 (826-908)	121 (108-133)	12 (11-14)	0.10 (0.10-0.11)	121 (109-134)
0.5% (10% n) C1342 / mucus + salt	94 (89-100)	14 (13-15)	1 (1-2)	0.10 (0.07-0.14)	14 (13-15)
0.5% (10% n) C1342 / mucus − salt	678 (598-674)	82 (79-85)	9 (9-9)	0.11 (0.11-0.11)	83 (80-86)
0.25% (60% n) C1342 / water	821 (804-852)	65 (62-67)	11 (10-11)	0.16 (0.16-0.17)	66 (63-68)
0.25% (60% n) C1342 / mucus + salt	138 (136-140)	15 (15-16)	3 (3-3)	0.19 (0.18-0.20)	16 (15-16)
0.25% (60% n) C1342 / mucus − salt	672 (625-703)	52 (50-54)	11 (10-12)	0.21 (0.21-0.21)	53 (51-55)

*NOV indicates Polycarbophil; (%n), % neutralization of the polymer; NM, not measured; ND, not detectable; and C1342, Carbopol 1342. All values are means of 3 replicate determinations; values in parentheses are range.
†Apparent viscosity obtained from constant rate flow curve at 100 seconds^{-1}.

those measured in pH 4.5 buffer. Carbomer, however, has been reported to be more resistant than polycarbophil to the presence of salts in the hydration medium owing to the presence of the hydrophobic alky methacrylate copolymer limiting the polymer from undergoing a conformational change to the less viscous coiled state.

Conflicting information regarding the changes in rheologic behavior of mixtures of polyacrylic acids with mucin exists in the literature. While Madsen et al reported an increase in the viscoelastic moduli of polyacrylic acids (0.33% to 1.67% wt/wt) after mixing with homogenized mucus, decreases in the viscoelastic moduli have also been reported when these polymers were mixed with either homogenized mucus and commercial mucins similar to those used in these studies.[22,23,24] For example, decreases in the elastic moduli (G′) with increasing pH (pH 5-8) were reported when 2% Noveon AA-1 (polycarbophil) was mixed with homogenized mucus.[24] The differences among the reported results have been attributed to the concentration and ion sensitivity of the polymer used, mucin type and concentration, methods used to mix the polymer gels with mucus, and instrumental factors.[25] Because the rheologic parameters for many of the gel-mucus (with buffer) mixtures in these studies were not able to be measured, and attempting to mimic physiologic conditions is complicated by (1) the changing composition of respiratory secretions in response to endogenous and exogenous stimuli, and (2) the variable mixing of the gels with the secretions, the parameters obtained from the gel:mucus (5:1) mixtures (without buffer) were used to develop the correlations with the measured mucociliary transport rates.

Rheologic Properties of Polycarbophil

Increasing polycarbophil concentrations (at 10% neutralization) resulted in significant increases in gel viscosity (η) (Table 2). Increasing the percentage neutralization of polycarbophil from 10% to 60% also caused a significant increase in the apparent viscosity as demonstrated by the similarity in the properties of a 0.5% polycarbophil gel (10% neutralization) and a lower concentration, 0.25% gel with 60% neutralization. The elastic (G′) and viscous (G″) moduli of the polycarbophil gels also increased with both increasing polymer concentration and neutralization in a manner similar to that previously reported by Madsen et al.[26] Polycarbophil, at the concentrations tested, formed predominantly elastic gels with tan δ (G″/G′) values all considerably less than 1. The complex modulus (G*, the vector sum of G′ and G″,

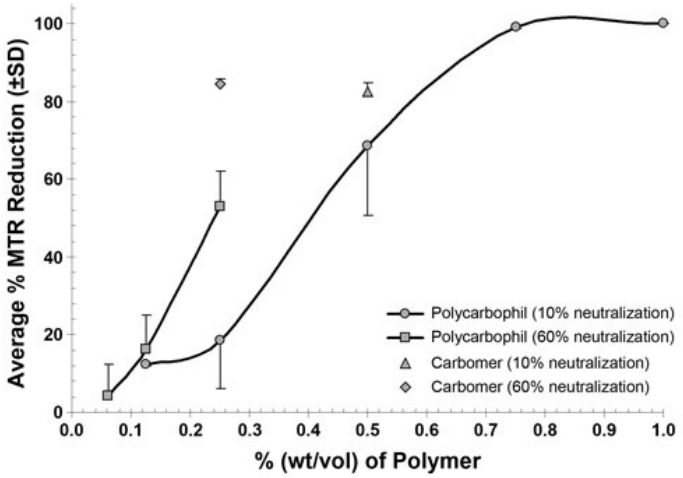

Figure 1. Reduction in mucociliary transport rate across bovine tracheal explants for various concentrations of polycarbophil and 2, rheologically matched concentrations of Carbopol 1342. Each point represents the mean of 3 replicates, and error bars represent the standard deviation (SD).

which indicates the rigidity and overall strength of the polymer gel), also increased with increasing polymer concentration and neutralization. These increases are strongly influenced by the significantly higher G′ as compared with G″ values and indicate an increase in interpolymer connectivity with increased resistance to deformation of the more highly concentrated, increasingly entangled, elastic polymer gels.

Transport of Polycarbophil

The mucociliary transport of the gels (10% neutralization) decreased in a nearly linear fashion with increasing polymer concentrations in the range of 0.25% to 0.75% polycarbophil (Figure 1, Table 3). Above 0.75%, mucociliary transport was nearly completely inhibited (>99%) during the 5-minute measurement interval, and it is likely that further increases in polycarbophil concentration would not significantly increase the nasal residence time, but instead, would merely result in a gel with increased stiffness and significant difficulty in administration.

Prediction of MTR From Rheologic Properties

The apparent viscosity (η) allows the estimation of the ease of administration, the flow characteristics of the formulation once inside the nasal cavity, and the ease with which energy can be transferred from the cilia into movement of the gel across the tissue surface. Polycarbophil gels with MTR reductions of greater than 80% all had apparent viscosities above 900 mPa s for polymer:mucus (buffer-free) mixtures (Table 2). Other, less concentrated, polycarbophil gels gave MTR reductions of ~60% and had values of η ~350 mPa s for polymer:mucus (buffer-free) mixtures. These observations indicate that the magnitude of η may be useful in the estimation of the MTR. Most of the polycarbophil gels mixed with buffer-free mucus that possessed G′ values greater than 200 Pa and G″ values greater than 15 Pa showed greater than 80% reductions in MTR. Thus, the elastic and viscous moduli (G′ and G″, respectively) also appear to be somewhat predictive of MTR. The tan δ was a very poor predictor of the MTR reduction for these gels, however. Because of the significantly greater G′ value as compared with G″, tan δ values for these gels were all much less than 1 and showed little discrimination between gels with significant or insignificant effects on MTR. G* values were much more predictive of an MTR decrease for the polyacrylic acids than tan δ, likely the result of the dominance of the G′ in the calculation of both of these parameters. Polycarbophil gels mixed with buffer-free mucus with G* values greater than 200 Pa resulted in reductions in MTR of more than 80%.

When the rheologic properties of the polycarbophil gels tested in these studies were evaluated, it was observed that gels with similar η and G* values also showed very similar MTR reductions. For example, 0.5% polycarbophil (10% neutralization) and 0.25% polycarbophil (60% neutralization) gels had very similar values for both of these parameters and both gels reduced the MTR by ~50% to 70%. A similar predictive relationship between η, G*, and tan δ values and the resulting MTR was observed with a series of polysaccharide polymers, yet the absolute values for each of the parameters that result in a given MTR reduction were quite different between these 2 classes of polymers.[27]

Table 3. Mucociliary Transport Rates of Control Mucus Solutions and Polyacrylic Acid Gels*

	MTR (cm/min)		
Polymer-Mucus Mixtures	Control Mucus	Polyacrylic Acid Gel	% Reduction
NOV 0.25% (60% n)	0.69 (0.50-0.75)	0.33 (0.17-0.40)	53 (47-66)
NOV 0.5% (10% n)	0.78 (0.75-0.86)	0.25 (0.16-0.50)	68 (42-79)
NOV 0.75% (10% n)	0.75 (0.75-0.75)	0.01 (0.00-0.01)	99 (99-100)
NOV 1% (10% n)	0.60 (0.60-0.60)	0 (0-0)	100 (100-100)
C1342 0.5% (10% n)	0.62 (0.60-0.67)	0.11 (0.10-0.12)	83 (81-85)
C1342 0.25% (60% n)	0.60 (0.60-0.60)	0.09 (0.09-0.10)	85 (83-86)

*MTR indicates mucociliary transport rate; NOV, Polycarbophil; and C1342, Carbopol 1342. The % MTR reduction was calculated using Equation 1. All values are means of 3 replicate determinations; values in parentheses are ranges.

Formulating Carbomer Gels With Desired Reductions in MTR

The rheology-MTR correlation for the polycarbophil:mucus (no buffer) mixed gels was used to guide the formulation of carbomer gels capable of achieving >70% reduction in MTR. Two samples of Carbopol 1342 containing different concentrations and neutralizations of polymer (0.5%, 10% neutralization; 0.25%, 60% neutralization) were selected from several preparations based on the similarity in their values of apparent viscosity (η) and G*. These values were also similar to those of the 0.5%, 10% neutralized and 0.25%, 60% neutralized polycarbophil gels previously tested (Table 2). As predicted, the carbomer gels gave MTR reductions of 82% to 84% when tested on the bovine tracheal explants. Compared with the polycarbophil gels, the carbomer gels were slightly more effective at reducing the MTR, likely because of their somewhat higher apparent viscosities (η).

CONCLUSION

The success of the selection of the carbomer gels from their rheologic properties demonstrates that the combination of η and G* values can be used to formulate polyacrylic acid gels with predictable effects on MTR. Similar methods could be extremely useful in the development and optimization of nasal dosage forms. Since the addition of active drug and excipients, such as buffers and preservatives, may significantly alter the formulation rheology compared with the original polyacrylic acid gel, the ability to increase or decrease the polymer concentration in order to reach the desired rheologic thresholds without the need for extensive MTR testing should significantly shorten the time and effort required for formulation optimization.

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