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Bioadhesive properties and rheology of polyether-modified poly(acrylic acid) hydrogels

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Abstract

Transient rheological properties and mucoadhesion of hydrogels composed of poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) (PEO–PPO–PEO, or Pluronic) block copolymers and poly(acrylic acid) were explored. Nine Pluronic copolymers ranging in nominal molecular weight and PPO/PEO content were grafted to PAA through C–C bonds, with or without the use of divinyl cross-linker, ethylene glycol dimethacrylate (EGDMA). The hydrogel elasticity increased with the PPO content in the copolymers, as well as in the presence of EGDMA. Tensile tests were conducted to measure the fracture strength and the work of adhesion between the hydrogels and rat intestinal tissue. The fracture strength was proportional to the gel pseudoequilibrium modulus and depended on the nominal length of the PPO segments in the parent Pluronic copolymer. The work of mucoadhesion and gel cohesion declined with the loss angle measured in oscillatory shear experiments. The length of the PEO segments in Pluronic affected the work of adhesion. Applications of the Pluronic-PAA gels as vehicles in oral drug delivery are discussed. The longest Pluronic copolymers bonded to PAA resulted in copolymeric gels with strongest mucoadhesive properties.

Keywords: Poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-(polyethylene oxide)-*g*-poly(acrylic acid) copolymers (CAS #186810-81-1); Hydrogels; Mucoadhesion; Cohesion; Rheology; Rat intestine

1. Introduction

The term mucoadhesion is implied when there is an adhesion between a polymer and a mucous tissue (Chickering and Mathiowitz, 1999). Mechanistically, mucoadhesion is a complex process that typically involves bringing the polymer and mucus into intimate contact, followed by interpenetration and mechanical interlocking (Chickering and Mathiowitz, 1999; Junginger, 1991). The work of adhesion assessed in the tensile tests is dependent on the viscoelastic properties of the hydrogels and is a direct measure of the hydrogel bioadhesive characteristics (Huang et al., 2000). Hydrogels based on poly(acrylic acid) (PAA) are among the most accepted mucoadhesives widely used in topical and oral drug delivery (Singla et al., 2000; Ahuja et al., 1997). There has been much work dedicated to studies of the possible correlation between the rheological properties and mucoadhesiveness of the PAA-based polymers (Ahuja et al., 1997; Barry and Meyer, 1974; Bernkop-Schnürch, 2000; Hägerström and Edsman, 2001, 2003; Hassan

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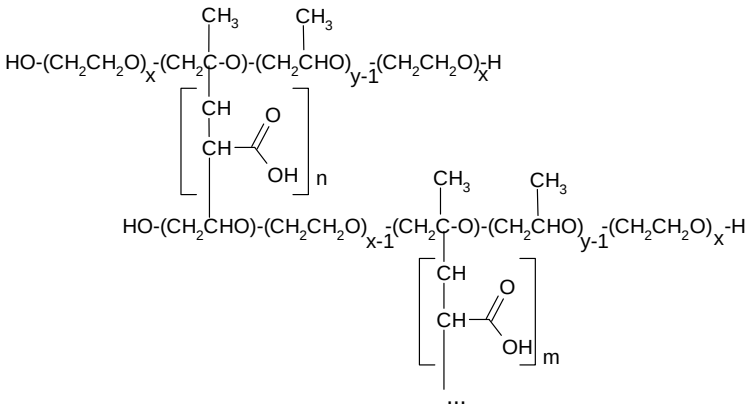
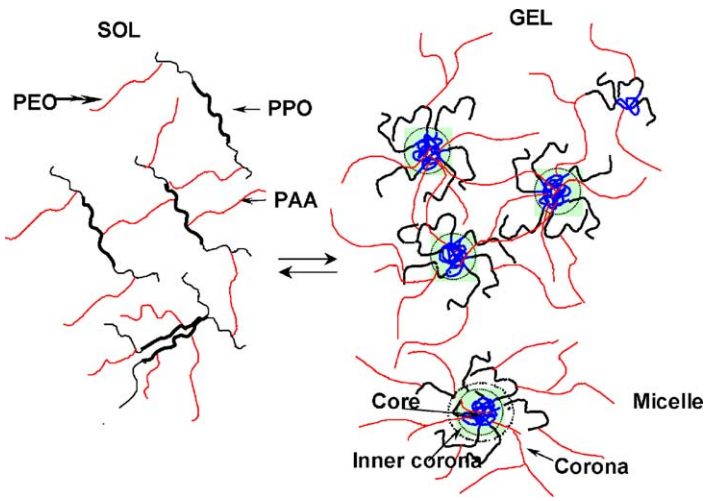
and Gallo, 1990; Gu et al., 1988; Madsen et al., 1998; Mortazavi and Smart, 1994; Riley et al., 2001; Rossi et al., 1999; Singla et al., 2000; Tamburic and Craig, 1995, 1997). These polymers, typically known under trade names Carbopol, Carbomer, Polycarbophil, and Noveon, comprise PAA cross-linked by various vinyl cross-linkers to varying degrees. Despite some positive synergism found in many studies between rheological parameters and mucoadhesion of the PAA polymers (Caramella et al., 1999) the relationships between those parameters are not without controversy (Hägerström and Edsman, 2003), primarily because the mucoadhesion testing itself is not well established for the gel materials. The "rheological synergism" widely implied in many works for the mucoadhesive polymers assumes that the rheological response of a gel–mucin mixture should be larger than the sum of the contributions from the gel and the mucin alone (Bromberg et al., 1997; Hassan and Gallo, 1990). However, despite some successes in ranking PAA-based polymers in order of their mucoadhesive properties (Tamburic and Craig, 1995, 1997), there has been some debate regarding correlation of the properties of elastic, cross-linked microgels with their mucoadhesion, as estimated via rheological synergism. Elastic microgels that tend to be very adhesive can rank poorly in rheological tests when mixed with mucin (Hägerström and Edsman, 2003). Furthermore, since the rheology of the mucin–polymer blends does depend on conformation of either component, which is, in turn, governed by the polymer structure and origin of the mucin and the ionic strength and the nature of ions present, the results of such rheological evaluations may vary considerably in some uncontrollable fashion (Caramella et al., 1999).

The present work concerns the copolymers of triblock PEO–PPO–PEO polyethers and PAA (Pluronic-PAA) that recently emerged as a novel class of PAA-based materials with mucoadhesive properties (Bromberg et al., 1997, 2002, 2003; Bromberg, 1998a,b,c,d, 1999, 2001a,b,c,d, 2002a,b; Bromberg and Ron, 1998). These copolymers have a unique graft-comb structure (Scheme 1) whereby the polyether chains (primarily PPO segments with tertiary carbons) are bonded to PAA via C–C bonding (Bromberg, 1998a).

Because of the prominent ability of the PPO segments to aggregate in response to temperature increases, the Pluronic-PAA polymers form physical gels in semidilute aqueous solutions at the temperature of the human body (Bromberg, 1998a,b,c,d, 2001a). Physical associations lead to the appearance of the micelle-like aggregates that act as cross-links (Scheme 2). The Pluronic-PAA solutions thus exhibit reversible sol–gel transitions and exist in the form of viscoelastic gels at body temperatures. Pharmacological formulations based on Pluronic-PAA and various generic drugs have been tested in topical and oesophageal applications (Bromberg and Ron, 1998; Bromberg, 2002a,b). When such formulation is injected or sprayed as a liquid onto the mucosal surface it quickly gels. The gelation lowers the rate



Scheme 1. Structure of the poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-(polyethylene oxide)-*g*-poly(acrylic acid) Pluronic-PAA copolymers (CAS #186810-81-1).

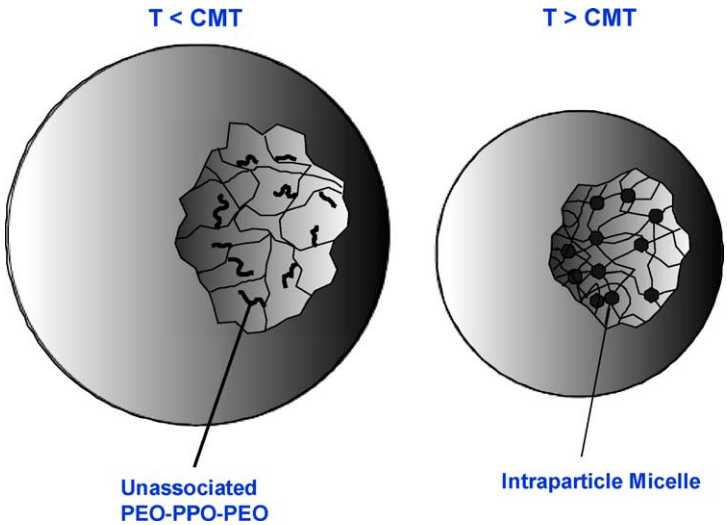


Scheme 2. Schematic of the sol–gel transitions in the Pluronic-PAA aqueous solutions.

of diffusion and erosion of the polymer and associated drug, thereby enhancing the drug retention and bioavailability.

The Pluronic-PAA copolymers can be additionally cross-linked by divinyl cross-linkers, resulting in permanent microgel particles (Bromberg et al., 2002) (Scheme 3).

The highly swollen microgel particles form viscoelastic, crowded suspensions in water, much like Carbopol systems. These microgels can be loaded with anti-cancer agents (Bromberg et al., 2002) and undergo large volume transitions in response to changing pH, which is useful in gastrointestinal applications, since the pH in the stomach is in the 1–2 range ensuring that the microgels remain collapsed and thus preventing an undesirable drug release in the stomach. The pH in the intestine is 6.2–7.4, leading to a highly swollen state of the microgels and to a facile drug release from these particles. The rheology of the Pluronic-PAA solutions strongly depends on the abil-



Scheme 3. Structure of the cross-linked Pluronic-PAA microgel particles.

ity of the Pluronic segments to aggregate, and the presence of mucin leads to a "negative synergism", i.e. weakening of the Pluronic-PAA gels because of hydrophobic polymer–mucin interactions (Bromberg, 1999). Therefore, in the present work we set out to test how rheological properties of the gels evaluated without the mucin present correlate with the gel mucoadhesion measured in independent tensile experiments. Excised rat intestine was chosen as a substrate for the mucoadhesion tests, because it has been shown to be a reliable model (Miyazaki et al., 2003; Tirosh and Rubinstein, 1998; Kakoulides et al., 1998) and also because of the intended oral application of our hydrogels. Since the content of acrylic acid, the bioadhesive component in the gel, strongly affects the overall gel adhesion (Park and Robinson, 1987) for the comparative study we used the same relative content of acrylic acid in all polymers. Also, we alleviated the variances in adhesion due to effects of different kinetics of gel swelling (Smart, 1999; Peppas and Mikos, 1990) by allowing our hydrogels to equilibrate with water. This enabled us to reveal how structural parameters of the polyethers bonded with PAA affect the observed mucoadhesion.

2. Experimental

2.1. Materials

Nonionic copolymers (trade name Pluronic) were obtained from BASF Corp. (Parsippany, NJ) and used without further treatment. Properties of the Pluronic copolymers under study are collected in Table 1. Acrylic acid (99%, vinyl monomer), ethylene glycol dimethacrylate (98%, divinyl cross-linker), dodecane (99+%, solvent), and 4,4′-azobis(4-cyanovaleric acid) (75+%, azo initiator) were purchased from Aldrich Chemical Co. and used as received. Lauroyl peroxide (97%, redox initiator) was obtained from Fluka Chemie AG (Switzerland). Poly(vinylpyrrolidinone-*co*-1-hexadecene) (Ganex V-216) (dispersion stabilizer) was obtained from International Specialty Products (Wayne, NJ). PAA-based materials Carbopol C907 and C941P NF were obtained from Noveon, Inc. (Cleveland, OH). All other chemicals, gases and organic solvents of the highest purity available were obtained from commercial sources.

2.2. Polymer synthesis and characterization

Synthesis was carried out on a laboratory scale in an adiabatic mode. Cross-linked and uncross-linked poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-(polyethylene oxide)-*g*-poly(acrylic acid) copolymers (CAS #186810-81-1) were synthesized using analogous procedures, with slight variations, as follows. Acrylic acid (40 mL) was partially neutralized by addition of 5 M NaOH aqueous solution (0.5 mL). Pluronic (24 g) was dissolved in the resulting solution under nitrogen. For the synthesis of the cross-linked gels, ethylene glycol dimethacrylate (EGDMA) (1.1 mL) was added resulting in a molar ratio of

Table 1
Properties of the Pluronic copolymers used in this study (Bromberg, 1998a)

Polymer[a]	Composition	Average MW	M_{PPO}	PEO (%)	CP (°C)[b]	HLB[c]
L61	$EO_3PO_{30}EO_3$	1950	2495	10	20	1–7
L92	$EO_8PO_{50}EO_8$	3650	2900	20	26	1–7
F38	$EO_{42}PO_{17}EO_{42}$	4700	990	80	>100	>24
P103	$EO_{17}PO_{60}EO_{17}$	4950	3465	30	86	12–18
P105	$EO_{37}PO_{56}EO_{37}$	6500	3250	50	91	12–18
F68	$EO_{76}PO_{29}EO_{76}$	8400	1680	80	>100	>24
F88	$EO_{103}PO_{39}EO_{103}$	11400	2280	80	>100	>24
F127	$EO_{100}PO_{65}EO_{100}$	12600	3780	70	>100	18–23
F108	$EO_{132}PO_{50}EO_{132}$	14600	2920	80	>100	>24

[a] L, F and P mean liquid, flakes, and paste, respectively.
[b] Cloud point in aqueous 1 wt.% solution, °C.
[c] Hydrophilic–lipophilic balance.

EGDMA to acrylic acid, XL, of 1 mol%. Lauroyl peroxide (100 mg) and 4,4′-azobis(4-cyanovaleric acid) (100 mg) were dissolved in 2 mL of acrylic acid and added to the solution of Pluronic in acrylic acid. The resulting solution was deaerated by nitrogen bubbling for 0.5 h and added to a 3-necked 0.5 mL flask containing 1 wt.% solution of Ganex V-216 in dodecane (200 mL). The flask was vigorously stirred by a mechanical stirrer and deaerated by constant nitrogen purge from the bottom. Then the flask was heated to 70 °C using an oil bath and kept at that temperature under stirring and nitrogen purge. After about 1 h, formation of white particles was observed on the flask walls. The reaction was continued at 70 °C for another 1 or 7 h for the synthesis of uncross-linked and cross-linked copolymers species, respectively. Then the reactor was disassembled, and the contents of the reactor were filtered using Whatman filter paper (retention size 10 μm). The copolymer particles were extensively washed by hexane and dried under vacuum. The average particle size was measured in hexane by dynamic light scattering and was typically in the range 10–20 μm. Herein, uncross-linked and cross-linked copolymers are termed Pluronic-PAA and Pluronic-PAA-EGDMA, respectively, with Pluronic specified by the manufacturer's abbreviation as given in Table 1. The particles were further purified and the copolymers characterized as described in detail previously (Bromberg, 1998b, 2001a; Bromberg et al., 2002). The yield of the copolymers in all cases exceeded 90% per acrylic acid component. The weight ratio of Pluronic to poly(acrylic acid) in the copolymers was measured by ^{1}H NMR (Bromberg, 1998d) to be 45:55. The weight-average molecular mass of the uncross-linked copolymers was measured by aqueous size-exclusion chromatography (Bromberg, 1998c) and in all cases exceeded 200 kDa and polydispersity index varied from 2.1 to 5.4. The particles resulting from the synthesis and purification were dispersed in deionized water at a known concentration and were allowed to equilibrate at 4–8 °C for 2 days. To ensure absence of unattached Pluronic admixtures, a gel fraction was exhaustively dialyzed (membrane molecular weight cut-off 30 kDa) against excess deionized water (pH adjusted to 7.0) at 4–8 °C for a week, followed by lyophilization, until constant weight, of the samples snap-frozen in liquid nitrogen, using a Labconco Lyph-Lock 4.5 Freeze-Dry system. The lyophilized samples were reconstituted with deionized water and allowed to equilibrate at 4–8 °C for at least 48 h, followed by pH adjustment to 7.4 prior to further experiments. The heat capacity measurements using modulated differential scanning calorimetry were conducted as described in detail elsewhere (Bromberg et al., 2004). Scanning electron microscopy of the lyophilized samples was performed as described previously (Bromberg et al., 2002).

2.3. Rheology study

Rheological measurements within angular frequency (ω) range of 0.628 mrad/s to 62.8 rad/s (minimum strain 6×10^{-5}) were performed using a controlled stress Rheolyst Series AR1000 Rheometer (TA Instruments, Inc., New Castle, DE) with a cone and plate geometry system (cone: diameter, 4 cm; angle, 2°, truncation, 57 μm) equipped with a solvent trap. Temperature control (internal resolution 0.016 °C) was provided by two Peltier plates. Oscillatory shear experiments were performed in both frequency and temperature ramp modes. Minimum strain and stress were 0.0143% and 6 mPa, respectively. Care was taken to ensure that the measurements of the viscoelastic moduli were in the viscoelastic regime. Rheological measurements in creep retardation and recovery modes were performed using a C-VOR digital rheometer (Bohlin Instruments, Inc., East Brunswick, NJ) equipped with coaxial cylinder double gap geometry and a solvent trap. The samples were pre-equilibrated inside the geometry at a given temperature for 24 h prior to the measurements.

2.4. Bioadhesion study

2.4.1. Animal studies

Experimental protocols involving animals were reviewed and approved by the animal experimentation committee at Supratek Pharma, Inc. Adult, pathogen-free male Sprague–Dawley rats (Harlan, 280–320 g) were acclimated to the environmentally controlled quarters (25 ± 1 °C and 12:12 h light–dark cycle) for 5–6 days prior to the experiments. The animals had free access to the laboratory rodent diet and water, ad libitum, until 12 h prior to being used in experiments, at which time food only was withdrawn. At the start of experiments, the rats ($n = 4$) were

sacrificed by cervical dislocation after isofluorane anesthesia. The rat gastrointestinal tract (GIT) was rapidly removed from the animal. Stomach, proximal small intestine, distal small intestine, and colon were quickly isolated and flushed with ice-cold physiological (pH 7.0) phosphate buffer to remove the lumenal contents. After washing, the jejunum mucosa excised from the small intestine and all other tissues were immediately snap-frozen in liquid nitrogen and stored at −70 °C. The frozen tissue, utilized within 24 h of dissection, was found to possess identical adhesive parameters to those observed with material used immediately after isolation from the animal. Analogous successful experiments on bioadhesion using rat intestine have been reported elsewhere (Kakoulides et al., 1998).

2.4.2. Texture analysis

Tensile strength measurements were performed in order to access mucoadhesion and cohesion parameters. TA.XT2i Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) equipped with a 5 kg load cell was used in tensile strength measurements. The setup had a force measurement accuracy of 1 mN and a distance resolution of 1 μm. Two types of measurements were applied as follows.

2.4.3. Mucoadhesion study

In the first series of experiments, bioadhesion between the gels and mucosa was studied (Madsen et al., 1998). An equilibrium swollen gel sample was placed in a thermostatted cell with a 4.0 cm-circular opening and the cell was kept in a refrigerator. In order to achieve approximately isoviscous gel preparations, the Pluronic-PAA solutions were tested at 5 wt.% polymer concentrations (C_{pol}), while the cross-linked Pluronic-PAA-EGDMA and Carbopol suspensions were tested at 1 wt.% concentrations. The cell was briefly sonicated to ensure that air bubbles were not entrapped in the gel and was consequently mounted on a stationary surface of the instrument and the gel was brought to 37 °C. A piece of the mucosa was thawed to room temperature and attached to the upper movable stainless steel cylindrical probe using Vetbond™ Tissue Adhesive (3 M Company, St. Paul, MN). Care was taken to ensure that a flat surface of the ex vivo mucosa (total 1.54 cm^2 area) was exposed parallel to the stationary surface and the surface of the gel. The mucosa was lowered toward the gel surface at a speed of 1 cm/min. Upon contacting the gel surface, as detected by a triggering force of 2 mN, the mucosa penetrated into the gel with a speed of 0.1 mm/s to 1.0 mm depth. After 2.0 min contact time, the mucosa was withdrawn, again at the speed of 0.1 mm/s. Preliminary experiments where the withdrawal speed and contact time varied in 0.1–5 mm/s and 2–10 min ranges, respectively, indicated high precision and reproducibility of these measurements, as compared to higher withdrawal speeds and longer contact times. No inhomogeneities in the gel preparations were observed in these experiments.

2.4.4. Cohesion study

In the cohesion study, the self-adhesion of the gels and of the mucus itself was examined. Cohesiveness of the mucus was measured by attaching mucosa to both the stationary surface and the probe. The penetration depth was only 0.5 mm, which ensured complete contact between the two mucous surfaces. For mucus cohesiveness, 25 measurements were performed, and the mucosa used was obtained from at least four different rats. Literature data indicate that the above conditions are optimal in the hydrogel studies (Madsen et al., 1998; Caramella et al., 1999). In the study of mucosa self-adhesion, the mucus was attached to both the probe surface and a stationary polycarbonate surface, and the force between the mucous surfaces was kept at 10 mN during the 2 min contact period, and then the probe with the mucosa was withdrawn with a speed of 0.1 mm/s. In the, the stainless steel probe without mucosa attached was used against the gels. The gels were kept in a thermostatted cell and the measurements were conducted as described above. In all experiments, except in mucosa self-adhesion, each measurement was performed at least four or five times. The tissue was also examined in order to identify the position at which the fracture of the adhesive joint had occurred.

2.4.5. Statistical analysis

Analysis of variance (two-way ANOVA for bioadhesion obtained with different rats) and evaluation of the regressions (one-way ANOVA to correlate gel properties and bioadhesion data) were performed using a Systat® Version 10.2 software (Systat Software, Inc., Richmond, CA). The comparison between work

of adhesion data was conducted using an unpaired, two-tailed *t*-test assuming unequal variances. Only statistically significant data (an acceptable probability of $P \leq 0.05$) resulting from *t*-tests (Dunn and Clark, 1974) are reported.

3. Results and discussion

Rheological properties of the Pluronic-PAA copolymer solutions and suspensions exhibit an exclusive temperature-sensitivity lacking in hydrogels of PAA typically used in bioadhesive formulations (Park and Irvine, 1997). In the present work, the most detailed rheological study was performed on L92-PAA-EGDMA and F127-PAA-EGDMA gels, the structures of which have been most studied (Bromberg et al., 2002). Fig. 1 illustrates the temperature-induced viscoelasticity of a 1 wt.% aqueous suspension of F127-PAA-EGDMA and a 1 wt.% aqueous solution of uncross-linked F127-PAA copolymer.

The temperature ramp of the oscillatory shear experiment is overlapped with the DSC thermogram obtained on the same sample. Both systems demonstrated endothermic peaks on heating with positive entropy and enthalpy changes, characteristic of aggregation transitions (Bromberg et al., 2004). The maxima of the peaks were close to the onset of the increase of both storage (G') and loss (G'') moduli. The elasticity of the aqueous systems (as judged by G') reached a plateau upon completion of the aggregation indicated by the end of the endothermic peaks. The formation of micelle-like aggregates in the Pluronic-PAA aqueous systems at elevated temperatures (i.e., critical aggregation temperatures, or CAT) has been demonstrated previously (Bromberg, 2002a,b). The transition from a viscous-dominated response at temperatures below CAT to an elastic-dominated response above CAT is indicative of the formation of the networks of aggregates. Analogous viscoelastic networks have been observed in aqueous solutions of hydrophobically modified polyelectrolytes and other associative polymers (Bromberg, 2001b; Rubinstein and Dobrynin, 1999) as well as in latex dispersions (Pham et al., 1999; Quatrat and Snuparek, 1990; English et al., 2002; Wang et al., 2000; Berli and Quemada, 2000). The solution of the uncross-linked F127-PAA undergoes a typical sol–gel transition from a region with $G' < G''$ below CAT to a $G' > G''$ region above CAT. The transition point can be characterized by a specific transition temperature $T^* = 33.7\,^\circ$C measured at $G' = G''$. This T^* is significantly higher than the temperature of the onset of the G' increase (23.4 °C) and roughly corresponds to the completion of the endothermic peak (32.5 °C). Notably, the suspension of the cross-linked F127-PAA-EGDMA shows gel-like properties ($G' > G''$) throughout the temperature range, but the moduli increase 1.5–1.7-fold above the CAT (the maximum and completion of the endothermic peak are at 20.8 and 30.8 °C, respectively). Such behavior is typical of viscoelastic microgel particles forming networks due to interparticle association and bridging by the dan-

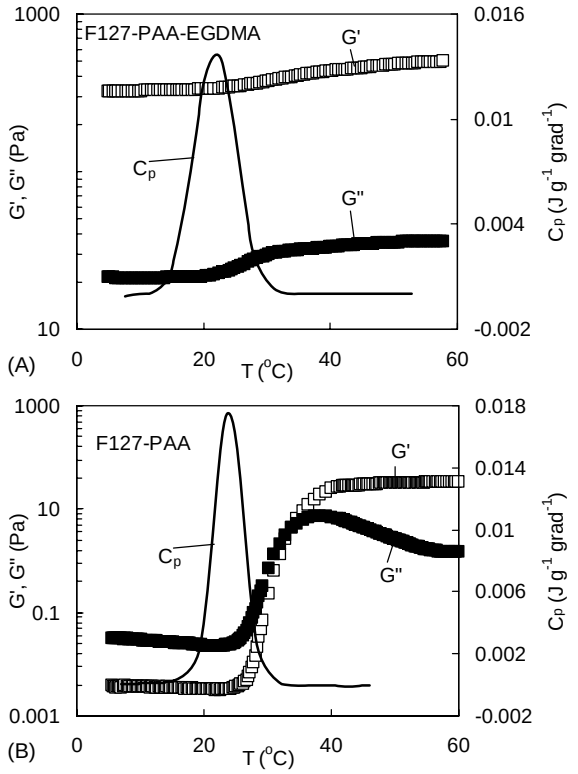


Fig. 1. Temperature dependencies of the storage (G', open points) and loss (G'', filled points) moduli and heat capacity (C_p, solid line) of 1 wt.% aqueous solution of 1 wt.% aqueous suspension of F127-PAA-EGDMA (A) and aqueous solution of F127-PAA copolymer (B). The viscoelastic moduli were measured in the oscillatory shear experiment (oscillatory stress, 0.6 Pa, frequency, 1 Hz). The DSC thermogram was obtained on the same samples. Underlying heating rate is 1 grad/min, amplitude, ±0.16 K; and frequency, 0.1 rad/s. The pH is 7.4 throughout.

gling chains of the uncross-linked polymer (English et al., 2002).

Further insight into the formation of the microgel networks at physiological pH and temperature was obtained from the frequency spectra of the dynamic moduli using L92-PAA-EGDMA microgel dispersions as an example (Fig. 2). As expected, at low frequencies dilute microgel dispersions ($C_{pol} \leq 0.5$ wt.%) exhibited liquid-like behavior (Fig. 2).

The characteristic relaxation times ($\tau = 2\pi/\omega$ at $G' = G''$) ranging from 1 to 5 s indicated the onset of the frequency range over which the microgel dispersions of intermediate concentrations ($C_{pol} < 1.5$ wt.%) behaved as interconnected networks. These estimated values of τ are typical for highly swollen latexes from hydrophobically modified polyelectrolytes with added surfactants (English et al., 2002) and are about 10–100-fold higher than in dispersions of hard-core latexes with adsorbed associative copolymers (Pham et al., 1999). Yet, the relaxation times found for the lightly cross-linked microgels were 10–100-fold smaller than in aqueous solutions of uncross-linked Pluronic-PAA of comparable concentrations. These trends are apparently a reflection of the lifetime of a strand of the reversible network, which decreases with decreasing length (Rubinstein and Semenov, 1998). At concentrations of 2 wt.% and above no crossover of $G'(\omega)$ and $G''(\omega)$ was observed at available frequencies. In concentrated dispersions, the elastic microgel particles may become compressed, so that both the storage and loss moduli become almost frequency-independent. Such weak frequency dependence of $G'(\omega)$ and $G''(\omega)$ in solutions of associative polymers containing latex particles is attributed to microstructures of close-packed microgel particles (English et al., 1999, 2002; Ketz et al., 1988). Compressibility of such microstructure determines the elasticity of the system.

The connectivity of the network can be expressed in terms of a pseudoequilibrium modulus (G_e), which, together with the characteristic relaxation time of the network (τ_{gel}), can be ascertained from the frequency-dependent moduli (Regalado et al., 1999; Wientjes et al., 2000; Ferry, 1980). That is, G_e can be found from the value of G' corresponding to $\omega = 2\pi/\tau_{gel}$ or from the integer of the loss modulus, G'', found in the area corresponding to the maximum of G'' occurring at the onset of the elastic plateau modulus (Ferry, 1980). Neither the plateau $G'(\omega)$ nor the maximum $G''(\omega)$ could be clearly defined in our oscillatory shear experiments (Fig. 2). Therefore, we used the dynamic creep method to determine the

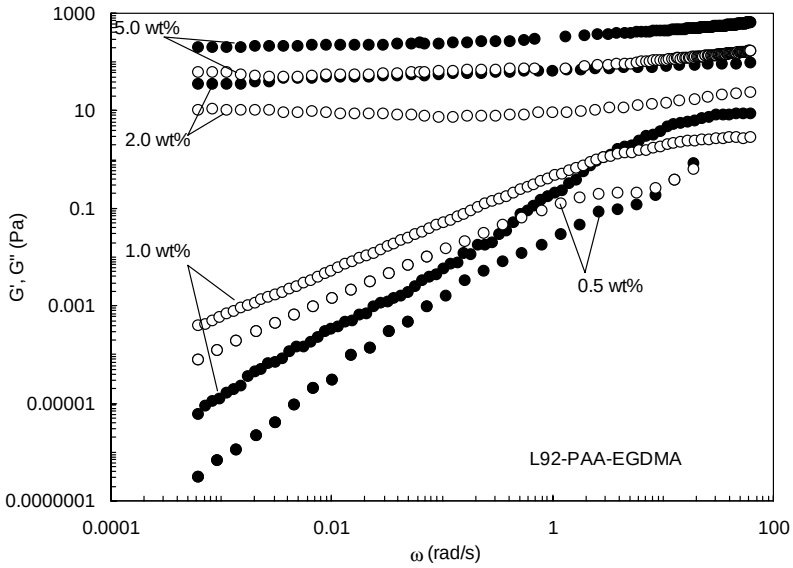


Fig. 2. Frequency dependencies of the viscoelastic moduli of aqueous suspensions of L92-PAA-EGDMA. The pH and temperature are 7.4 and 37 °C throughout, polymer concentration is in wt.%. Open and filled symbols designate G'' and G', respectively.

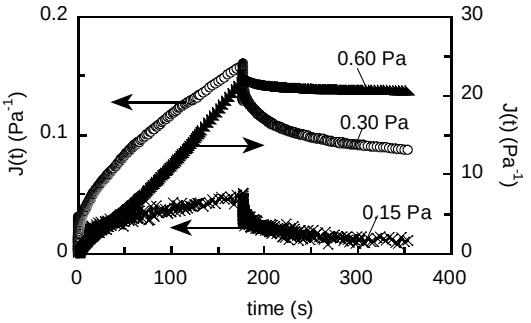


Fig. 3. Creep retardation and recovery curves obtained for 2 wt.% L92-PAA-EGDMA aqueous dispersions at varying initial stresses. Equilibrium moduli were determined from the steady state compliance values measured in shear recovery experiments. The plateau compliance was estimated using the instrument's built-in software. The pH and temperature are 7.4 and 37 °C throughout.

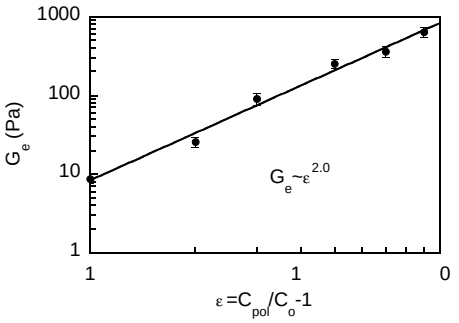


Fig. 4. Scaling of the pseudoequilibrium modulus ($G_e = 1/J_e$) of the L92-PAA-EGDMA aqueous dispersions with the distance from the gelation threshold ($\varepsilon = C_{pol}/(C_0 - 1)$). The threshold concentration C_0 was arbitrarily set at 0.5 wt.%. The experiments were conducted in triplicate.

steady-state compliance of the gel networks in the recovery mode (Fig. 3). Relatively concentrated ($C_{pol} \geq 2$ wt.%) L92-PAA-EGDMA dispersions exhibited elastic behavior, manifested by a large recoverable compliance $J(t) = \gamma(t)/\sigma$, where γ and σ are strain and stress, respectively. At low stresses ($\sigma \leq 0.3$ Pa), the dispersion deformed elastically, but when the applied stress increased above 0.35–0.45 Pa, the material began to flow as a viscous liquid (Fig. 3). This is an indication that σ in the 0.35–0.45 Pa range is close to the yield stress, σ_y, for this dispersion. Analogous transient creep responses have been reported for concentrated ($C_{pol} \geq 3$ wt.%) aqueous dispersions of lightly cross-linked poly(acrylic acid) microgels (Carbopol 941) (Ketz et al., 1988).

Despite a considerable deformation at larger stresses, our microgel dispersions were able to recover up to 90% of their compliance, i.e., they exhibited a "structural memory", which is indicative of significant number of reversible interparticle and intraparticle associations that restore the structure.

Equilibrium compliance reached in creep recovery experiments at minimal applied stresses is reciprocal to the pseudoequilibrium modulus of the (unperturbed) network: $J_e = 1/G_e$ (de Rooij et al., 1994). As the recovery results were somewhat noisy, an average of 10 independent measurements was used to obtain each J_e value.

The network moduli scaled with the relative polymer concentration as demonstrated in Fig. 4. The obtained scaling relationship $G_e \sim \varepsilon^{2.0}$ is much flatter than those with the power-law exponents of 4.0–5.0 often reported for flocculated gels of latex dispersions (de Rooij et al., 1994; Larson, 1999; Rueb and Zukoski, 1997). The exponent of 2 is much closer to, although smaller than, the scaling exponent predicted for associative polymer solutions in the concentration range above the gelation threshold, but below the percolation concentration: $G_e \sim \varepsilon^3\mu$, where $\mu \cong 0.85$ is the percolation critical exponent (Rubinstein and Semenov, 1998). Scaling exponents of about 2.6 in this concentration regime have been reported for the physical gels from F127-PAA copolymers devoid of covalent cross-links and for pectin hydrogels (Bromberg, 1998d).

Above the percolation threshold, where polymer chains overlap strongly, the concentration dependence of the modulus levels off: $G_e \sim \varepsilon$ (Rubinstein and Semenov, 1998; Pashkovski et al., 2003). On the other hand, entangled polymer solutions in a good solvent demonstrate scaling close to that observed in the present work: $G_0 \sim \varepsilon^{9/4}$ (Rubinstein, 1997). It would appear, therefore, that the scaling behavior of the lightly cross-linked microgels in our case can be explained by a very high swelling degree, where the microgels "unravel" into a loose core of the cross-linked chains that are bound to long, extended chains that entangle at a certain C_{pol}. It should be noted that such a structure is in sharp variance with the dispersions of F127-PAA-EGDMA microgels, where distinct spherical particles predominate even

after equilibrium swelling (Bromberg et al., 2002). The abundance of elastic microparticles results in the F127-PAA-EGDMA dispersions being more elastic overall than the corresponding L92-PAA-EGDMA dispersions. We have reported that the differences in the structure of the Pluronic-PAA polymers and gels are related to the differences in the Pluronic structure such as the length and overall content of the PPO segments, primarily responsible for the efficiency of the bonding between the polyether and PAA chains in the process of synthesis (Bromberg, 1998a; Bromberg et al., 2002). In the present work, we have taken interest in relating rheological and bioadhesive properties of hydrogels based on a variety of the polyether–PAA copolymers to the structure of the parent Pluronic (Table 1).

For the comparative study, overall polymer concentrations of 1 and 5 wt.% were chosen for the Pluronic-PAA-EGDMA dispersions and Pluronic-PAA solutions, respectively. These concentrations ensured that dramatic differences were observed in the hydrogel rheology, depending on the Pluronic used in the copolymer. All measurements were performed after equilibration of the hydrogels at 37 °C and pH 7.4. The viscoelastic parameters employed were the gel modulus G_e as well as the loss angle (δ°; $\tan\delta = G''/G'$) measured in the oscillatory stress experiments at arbitrarily fixed $\sigma = 0.6$ Pa and $\omega = 6.28$ rad/s. The choice of the deformation stress (0.6 Pa) ensured tests within the viscoelastic regime. The tensile tests were conducted concurrently with the rheology study using the same gel samples. Using a texture analyzer, the probe surface with (*mucoadhesion* test) or without (*cohesion* tests) rat intestinal tissue attached was withdrawn from the gels, and the maximum peak force (F_p) and the tensile work (equal to the area under the force–distance curve, W) were measured using the software provided with the instrument (Fig. 5).

The force–distance curves in Fig. 5 show that when the uncross-linked system was replaced with the cross-linked one the maximum stress of detachment, equal to F_p, increased dramatically, while the deformation to peak, x_1, decreased. Yet, the tensile work equal to the integrated area under the F–x curve, changed insignificantly. This result is qualitatively understood in light of a linear elastic fracture mechanics analysis based on the treatment of Johnson, Kendall, and Roberts (JKR) (Johnson et al., 1971). Modifications to the JKR theory are often required when thermodynamic work of adhesion, W, between gels and flat surfaces produces a substantial contact area (Mowery et al., 1997). In the situation like ours, when

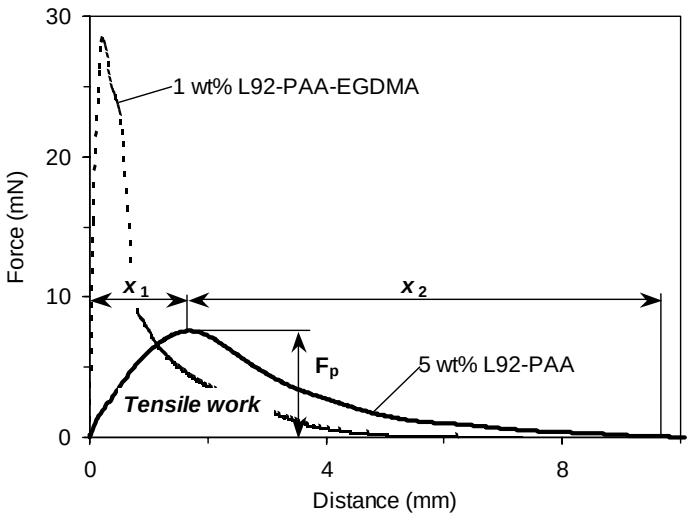


Fig. 5. Representative force–distance diagrams obtained at 37 °C and pH 7.4 in tensile tests on 5 wt.% uncross-linked solution of L92-PAA (solid line) and 1 wt.% dispersion of the cross-linked L92-PAA-EGDMA (dashed line). Indexes F_p, x_1, and x_2 stand for the fracture strength, deformation to peak, and deformation to failure, respectively. Rat jejunum mucosa was attached to the probe. For other experimental detail, see Section 2.

a flat cylindrical probe with a radius a is withdrawn from a compliant gel layer of a thickness h, the energy release rate, Ω, given by (Ahn and Shull, 1996) $\Omega = -(F^2/2)(\mathrm{d}J/\mathrm{d}A)$, corresponds to the direct measure of energy dissipation such as the integrated area under the F–x curve, W (Mowery et al., 1997). Here, $J = x/F$ is the compliance, and for our axisymmetric system, $A = \pi a^2$. The relationship between W, F, and the elastic Young's modulus of the system, $E = (\Delta\sigma/\Delta\gamma)|_{\Delta\gamma\to 0}$, can then be approximated by

$$W = \frac{3\Delta F^2}{32\pi E a^2}, \qquad \frac{W}{E} = \frac{2\Delta x^2}{3\pi a}\left(1 - \frac{a}{h}\right)^2$$

where $\Delta F = F' - F$, $\Delta x = x' - x$; F' and x' are the force and distance, respectively, in the absence of adhesive interactions. The above expressions enable estimation of the W/E values of thermoreversible gels from the tensile tests (Mowery et al., 1997) and show that at a given W a more elastic sample (higher E) must possess a shorter deformation to failure (smaller Δx). Consequently, elastic gel preparations with high W have been shown to extend during the withdrawal phase to form an elongated string that broke at long Δx (Hägerström and Edsman, 2001).

In the present work, we observed statistically significant correlation between the pseudoequilibrium modulus (G_e) and fracture strength (F_p) of the same gels, measured in either mucoadhesion or cohesion experiments (Fig. 6).

For Pluronic-PAA solutions there appeared to be no difference between the results of the mucoadhesion and cohesion tests, while for the Pluronic-PAA-EGDMA the fracture strength for cohesion experiments was slightly larger than that for the mucoadhesion tests, indicating that viscoelastic gel microparticles adhere slightly better to each other than to the mucosal tissues. It is interesting to note, however, that several Pluronic-PAA-EGDMA microgel dispersions appeared to be more mucoadhesive than the lightly cross-linked PAA such as Carbopol C934P.

The observed correlation points to the interrelated nature of the G_e and F_p parameters, both stemming from the gel elasticity. Furthermore, it appeared that the F_p parameter correlated positively with the compositional parameter of the gels such as the length of the PPO segment (N_{PO}) in the parent Pluronic bonded

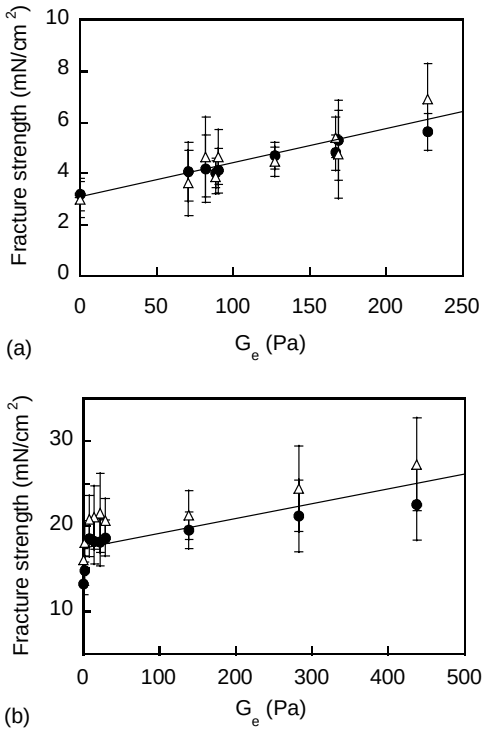


Fig. 6. Dependencies of the fracture strength measured in tensile tests on the pseudoequilibrium modulus (G_e) obtained with 5 wt.% Pluronic-PAA solutions (a) and 1 wt.% Pluronic-PAA-EGDMA dispersions (b). Filled circles and open triangles show results of the mucoadhesion and cohesion tests, respectively. Correlation between the fracture strength and the G_e values was tested by ANOVA algorithm considering fracture strength as a dependent variable and G_e as an independent variable. Cases of the Pluronic-PAA solutions and the Pluronic-PAA-EGDMA suspensions as well as cohesion and mucoadhesion were analyzed separately. The correlations were found to be statistically significant ($P < 0.005$) in all cases. Solid line is shown to guide the eye only.

with PAA (Fig. 7). This finding is, again, a reflection of the gel elasticity, which has been shown to increase with the N_{PO} (Bromberg, 1998a, 2001a). The fundamental reason for this dependency lies in the increasing density of the cross-links, either physical or covalent, in the gels, with the increasing length of the PPO segments. Firstly, the lifetime of associations between the longer and thus more hydrophobic PPO segments increases, and thus the stability of the associations between the polymer chains and the network elasticity increase (Bromberg, 1998c,d). Secondly, because the probability of the hydrogen abstraction from the PPO chains compared to the PEO

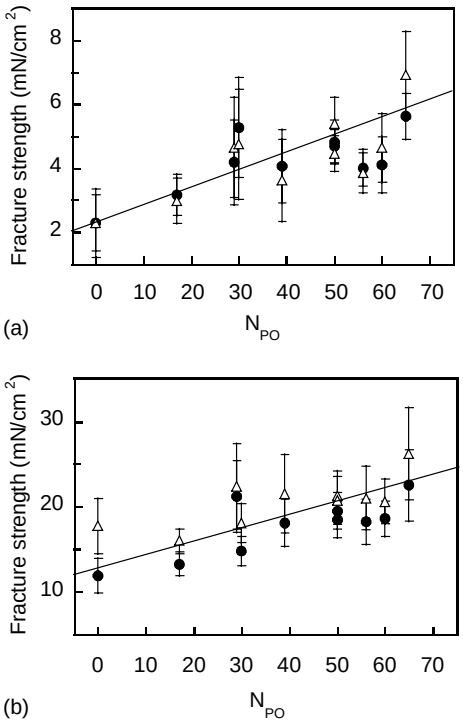


Fig. 7. Dependencies of the fracture strength of 5 wt.% Pluronic-PAA solutions (a) and 1 wt.% Pluronic-PAA-EGDMA dispersions (b) on the nominal length of the PPO segments in the parent Pluronic (expressed via number of the PO units, N_{PO}). Filled circles and open triangles show results of the mucoadhesion and cohesion tests, respectively. Correlation between the fracture strength and the G_e values was tested by ANOVA algorithm considering fracture strength as a dependent variable and G_e as an independent variable. Cases of the Pluronic-PAA solutions and the Pluronic-PAA-EGDMA suspensions were analyzed separately. The correlations were found to be statistically significant ($P < 0.005$) in all cases. The data for $N_{PO} = 0$ were obtained with 5 wt.% Carbopol C907 (A) and 1 wt.% Carbopol C941 (B). Correlation between the fracture strength and the length of the PPO segments was found to be statistically significant ($P < 0.005$) in all cases. Solid line is shown to guide the eye only.

is much higher (Bromberg, 1998b), increasing N_{PO} leads to a higher probability of recombination of the PPO-radicals and thus to the appearance of covalent cross-links (Bromberg, 2001a).

The observed correlation probably reflects on the stronger interpenetration between more viscoelastic gels and mucin and/or could be a measure of the gel elasticity, should the fracture occur within the gel's bulk. After the tensile test was completed, the probe surface was always examined in order to identify the position at which the fracture of the adhesive joint had occurred. In the cohesion tests, a small amount of gel was always observed on the probe surface, indicating fracture within the gel. In the mucoadhesion experiments, visual inspection was inconclusive, and therefore, a comparison of the cohesiveness of the individual components (i.e., mucin or gel alone) should in some instances aid interpretation as to the site of the joint failure. With mucous tissue alone, the work of adhesion was measured to be 24 ± 1.5 μJ ($n = 25$), which was significantly higher ($P < 0.005$) than with 5 wt.% solutions of F38-PAA (6.9 ± 1.4 μJ), but did not differ significantly ($P \geq 0.1$) from the results of mucoadhesion tests with 5 wt.% solutions of other Pluronic-PAA polymers or Carbopol C907 (18 ± 1.2 μJ). The work of cohesion with the uncross-linked hydrogels alone was significantly lower than the self-adhesion of mucin in the cases of 5 wt.% F38-PAA and Carbopol C907, but did not differ significantly from all other uncross-linked hydrogel samples, except for the 5 wt.% of F127-PAA, which was significantly higher (36 ± 2 μJ; $n = 5$; $P < 0.005$). Hence, a strengthening of the mucus layer due to interpenetration can be reliably assumed only with the F127-PAA hydrogels, which also are the most viscoelastic among the uncross-linked hydrogels studied. Among the cross-linked samples, both mucoadhesion and cohesion W values were significantly ($P < 0.005$) higher than in mucin self-adhesion tests with 1 wt.% F127-PAA-EGDMA, F108-PAA-EGDMA, and F88-PAA-EGDMA. Therefore, these microgels did strengthen the mucin interface. It is interesting to note that the Pluronic polymers used in these gels are the longest of the polyethers studied herein (Table 1). Mucoadhesion results with all other Pluronic-PAA-EGDMA gels as well as 1 wt.% Carbopol C934P did not differ significantly from the mucin self-adhesion. Overall, these results allow us to conclude that the mucoadhesion of the Pluronic-modified PAA gels is comparable to, and in many instances exceeds that, of the Carbopol cross-linked and linear polymers, which are the industry standard for mucoadhesive materials. The mucoadhesive bond strength is proportional to the elasticity of our hydrogels, whether or not the exact location or the joint failure is perceptible.

Further insight into the nature of the mucoadhesive properties was obtained from the loss angle

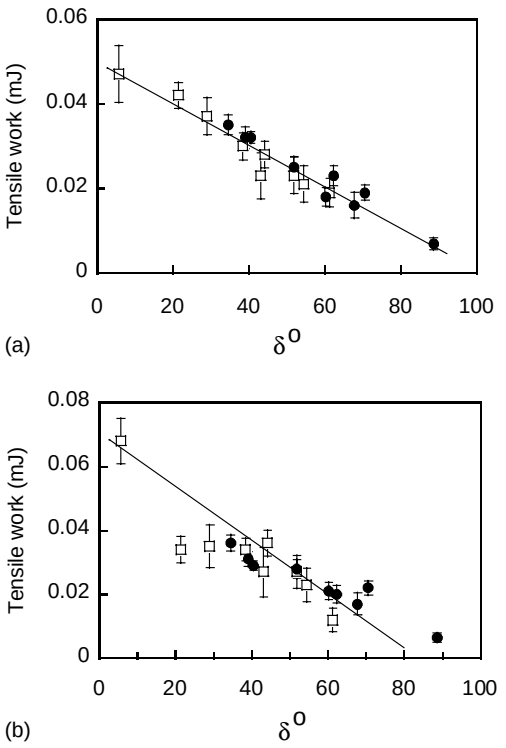


Fig. 8. Relationship between the tensile work in mucoadhesion (a) and cohesion (b) tests and the loss angle (δ) measured in oscillatory shear experiments at frequency of 1 Hz. Filled circles and open squares show results for 5 wt.% Pluronic-PAA solutions and 1 wt.% Pluronic-PAA-EGDMA dispersions, respectively. See text for other experimental detail. Linear regressions ($P < 0.005$ in all cases) were obtained with the square of the Pearson's correlation coefficient (R^2) equal to 0.956 (Pluronic-PAA-EGDMA) and 0.954 (Pluronic-PAA) in mucoadhesion tests, and 0.802 (Pluronic-PAA-EGDMA) and 0.925 (Pluronic-PAA) in cohesion tests. Solid line is shown to guide the eye only.

($\delta^\circ = \arctan G''/G'$) measurements. Fig. 8 depicts the tensile work–loss angle plots obtained in the oscillatory shear, mucoadhesion and cohesion experiments. As is seen, the W value declined linearly with δ° in all cases.

This interesting finding can be explained by the fact that the work of adhesion is a function of the free energies, per contact surface, of the gel elements and/or mucin–gel interface during the deformation leading to the joint fracture (Chickering and Mathiowitz, 1999). In turn, the loss angle reflects on the components of the viscoelastic energy of a gel. That is, the viscous stress is the rate of energy dissipation per unit volume, per unit shear rate; the elastic stress is the maximum energy stored during the cycle per unit volume, per unit strain. Therefore, $\tan\delta$ is descriptive of the reciprocal energy flow per cycle within a unit volume of the material. In other words, what we experimentally observed is that the energy stored in an elastic material such as a Hookean spring ($\delta \rightarrow 0^\circ$) necessitates a higher work for the fracture to occur than in a Newtonian dampening, viscous liquid ($\delta \rightarrow 90^\circ$) (Fig. 8). It should be noted that a linear correlation between the loss angle and the work of mucoadhesion of various hydrogels has been reported previously, and is believed to be a link between the intrinsic properties of the gel and the mucus–gel interface (Caramella et al., 1999; Tamburic and Craig, 1995, 1997).

In a further effort to shed light on the relationship between the intrinsic properties of our hydrogels and their mucoadhesion, we were able to correlate the tensile work obtained in mucoadhesion tests with a structural parameter, the nominal length of the PEO segments in the Pluronic that is bonded to PAA in our gel (Fig. 9). Two distinct trends can be seen in Fig. 9. As the N_{EO} increases up to 100, the PPO content in the corresponding Pluronic-PAA copolymers decreases from over 30 to about 6 wt.% and the gel modulus (G_e) decreases concomitantly (Fig. 10). We have discussed above the relationship between the PPO content (which contributes to the density of cross-linking)

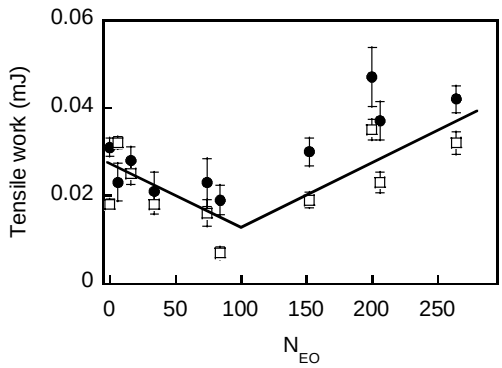


Fig. 9. Relationship between the tensile work measured in mucoadhesion tests and nominal length of the PEO segment in parent Pluronic (N_{EO}). Filled circles and open triangles show results with 1 wt.% Pluronic-PAA-EGDMA dispersions and 5 wt.% Pluronic-PAA solutions, respectively. The data for $N_{EO} = 0$ were obtained with 5 wt.% Carbopol C907 (A) and 1 wt.% Carbopol C941 (B). Solid lines are shown to guide the eye only.

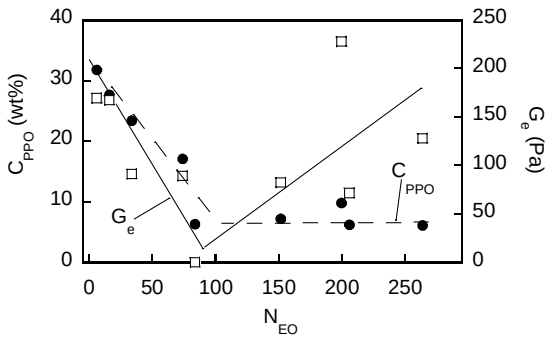


Fig. 10. Effect of nominal length of the PEO segment in parent Pluronic (N_{EO}) on the content of PPO in the resulting Pluronic-PAA copolymers (C_{PPO}, filled circles) and the storage modulus (G', open squares) of 5 wt.% Pluronic-PAA solutions measured at 37 °C, pH 7.4, frequency 1 Hz, and oscillatory shear 0.6 Pa. The C_{PPO} was measured by ^{1}H NMR (Bromberg, 1998c) and is expressed in wt.% per dry weight of the copolymer. Solid and dashed lines are shown to guide the eye only.

and G_e. For $N_{EO} > 100$, the overall PPO content in the gels plateaus at about 6–7 wt.%, but the gel elasticity expressed through G_e increases (Fig. 10), contributing to the rise in the work of mucoadhesion (Fig. 9). This result correlates well with the observation discussed above that the longest Pluronic copolymers such as F127, F108, and F88 bonded within the gel structure strengthen the gel–mucous tissue interface.

Weak adhesion via hydrogen bonding has been reported between mucins and PEO or PEG chains tethered to gel substrates (Efremova et al., 2002; Huang et al., 2000; Shojaei and Li, 1997). In the case of copolymeric PEG-*co*-PAA hydrogels, the PEG molecular weight can contribute to the interpenetration and thus enhanced anchoring of the polymer chains on the mucosa (Huang et al., 2000). The grafting of PEG to PAA affects the dispersive and polar components of the surface free energy of the PEG-*co*-PAA hydrogel surfaces such that the free energy characteristics of the gel surface and mucus layers become similar (Shojaei and Li, 1997). This lowers the interface free energy and facilitates spreading and the intimate contact between the mucus and gel surfaces, promoting interpenetration and ultimately the higher mucoadhesive bond strength. Analogous trends can be suggested in the case of the Pluronic-PAA hydrogels. The presence of the PPO chains in the Pluronic-PAA adds another dimension to the gel–glycoprotein interactions due to hydrophobic forces (Bromberg, 2002a,b). It has been correctly pointed out (Huang et al., 2000; Hwang et al., 1998; Efremova et al., 2002) that successively mucoadhesive gel formulations should specifically bind to the mucus layer but not to the free glycoproteins in the lumen, because the glycoprotein–polymer complex is unable to bind to the epithelial mucous layers. Significantly, the weakening of the Pluronic-PAA gel elasticity due to the interactions between PPO and mucin (Bromberg, 1999) should be minimized with the minimal PPO content in the gel (Fig. 10) and thus contribute to the higher mucoadhesion when N_{EO} is high (compare Figs. 9 and 10). It may well appear that at $N_{EO} > 100$ our hydrogels offer an optimum composition as far as mucoadhesive properties. The enhanced nasal, vaginal, and oesophageal retention of these gels in vivo (Bromberg and Ron, 1998; Bromberg, 2001c, 2002a,b) seems to suggest just that.

4. Conclusions

In the present work, we observed previously unexplored relationships between the composition and rheological properties of the Pluronic-PAA copolymers and gels and their mucoadhesion. The fracture strength and the work of adhesion between the gels and the rat intestine correlated with the viscoelastic characteristics of gels such as the pseudoequilibrium modulus obtained in creep recovery tests and the loss angle measured at fixed oscillatory stress and frequency. The gels' fracture strength is linearly proportional to their viscoelasticity stemming from the chain entanglement and physical cross-links due to aggregation of the hydrophobic PPO segments, as well as covalent cross-links. The viscoelastic energy stored in a gel as characterized by the loss angle correlates with the work of mucoadhesion, an observation that has potential for use in the designing gels with the best mucoadhesive properties. Combined with the pH- and temperature-sensitivity (Bromberg et al., 2002), ability to solubilize and stabilize hydrophobic steroids, anti-cancer drugs, and proteins (Bromberg and Ron, 1998; Bromberg, 2001b,d) the mucoadhesive properties described herein make the Pluronic-PAA gels a feasible vehicle for oral and topical drug delivery.

References

Ahn, D., Shull, K.R., 1996. JKR studies of acrylic elastomer adhesion to glassy polymer substrates. Macromolecules 29, 4381–4390.

Ahuja, A., Khar, R.K., Ali, J., 1997. Mucoadhesive drug delivery systems. Drug Dev. Ind. Pharm. 23, 489–515.

Barry, B.W., Meyer, M.C., 1974. Viscoelastic and continuous shear properties of Carbopol gels. J. Pharm. Pharmacol. 26, 129P–130P.

Berli, C.L.A., Quemada, D., 2000. Rheological modeling of microgel suspensions involving solid–liquid transition. Langmuir 16, 7968–7974.

Bernkop-Schnürch, A., 2000. Mucoadhesive polymers. In: Dumitriu, S. (Ed.), Polymeric Biomaterials. Marcel Dekker, New York, pp. 147–165.

Bromberg, L., 1998a. A novel family of thermogelling materials via C–C bonding between poly(acrylic acid) and poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide). J. Phys. Chem. B 102, 1956–1963.

Bromberg, L., 1998b. Polyether-modified poly(acrylic acid): synthesis and properties. Ind. Eng. Chem. Res. 37, 4267–4274.

Bromberg, L., 1998c. Properties of aqueous solutions and gels of poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide)-*g*-poly(acrylic acid). J. Phys. Chem. B 102, 10736–10744.

Bromberg, L., 1998d. Scaling of rheological properties of hydrogels from associating polymers. Macromolecules 31, 6148–6156.

Bromberg, L.E., 1999. Interactions between hydrophobically modified polyelectrolytes and mucin. Polym. Prepr. 40, 616–617.

Bromberg, L., 2001a. Synthesis and self-assembly of poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide)-*g*-poly-(acrylic acid) gels. Ind. Eng. Chem. Res. 40, 2437–2444.

Bromberg, L., 2001b. Hydrophobically modified polyelectrolytes and polyelectrolyte block-copolymers. In: Nalwa, H.S. (Ed.), Handbook of Surfaces and Interfaces of Materials, vol. 4: Solid Thin Films and Layers. Academic Press, pp. 369–404 (Chapter 7).

Bromberg, L., 2001c. Enhanced nasal retention of hydrophobically modified polyelectrolytes. J. Pharm. Pharmacol. 53, 109–114.

Bromberg, L., 2001d. Interactions among proteins and hydrophobically modified polyelectrolytes. J. Pharm. Pharmacol. 53, 541–547.

Bromberg, L., 2002a. Hydrophobically modified polyelectrolytes and polyelectrolyte block-copolymers for biomedical applications. In: Tripathy, S.K., Kumar, J., Nalwa, H.S. (Eds.), Handbook of Polyelectrolytes and their Applications. American Scientific Publishers, pp. 23–46 (Chapter 51).

Bromberg, L., 2002b. Poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide)-*g*-poly(acrylic acid) copolymers as in situ gelling vehicle for nasal delivery. In: Rathbone, M.J., Hadgraft, J., Roberts, M. (Eds.), Modified-Release Drug Delivery Technology. Marcel Dekker, pp. 749–758 (Chapter 62).

Bromberg, L.E., Ron, E.S., 1998. Protein and peptide release from temperature-responsive gels and thermogelling polymer matrices. Adv. Drug Delivery Revs. 31, 197–221.

Bromberg, L.E., Orkisz, M.J., Ron, E.S., 1997. Bioadhesive properties of polyoxyethylene-*b*-polyoxypropylene-*b*-polyoxyethylene-*g*-poly(acrylic acid) polymers (Smart Hydrogel^{TM}). Polym. Prepr. 38, 626–627.

Bromberg, L., Temchenko, M., Hatton, T.A., 2002. Dually responsive microgels from polyether-modified poly(acrylic acid): swelling and drug loading. Langmuir 18, 4944–4952.

Bromberg, L., Temchenko, M., Hatton, T.A., 2003. Smart microgel studies. Polyelectrolyte and drug-absorbing properties of microgels from polyether-modified poly(acrylic acid). Langmuir 18, 4944–4952.

Bromberg, L., Temchenko, M., Moesser, G.D., Hatton, T.A., 2004. Thermodynamics of temperature-sensitive polyether-modified poly(acrylic acid) microgels. Langmuir 20, 5683–5692.

Caramella, C.M., Rossi, S., Bonferoni, M.C., 1999. A rheological approach to explain the mucoadhesive behavior of polymer hydrogels. In: Mathiowitz, E., Chickering, D.E., Lehr, C.-M. (Eds.), Bioadhesive Drug Delivery Systems. Fundamentals, Novel Approaches, and Development. Marcel Dekker, New York, pp. 26–65.

Chickering, D.E., Mathiowitz, E., 1999. Definitions, mechanisms, and theories of bioadhesion. In: Mathiowitz, E., Chickering, D.E., Lehr, C.-M. (Eds.), Bioadhesive Drug Delivery Systems. Fundamentals, Novel Approaches, and Development. Marcel Dekker, New York, pp. 1–10.

de Rooij, R., van den Ende, D., Duits, M.H.G., Mellema, J., 1994. Elasticity of weakly aggregating polystyrene latex dispersions. J. Phys. Rev. E 49, 3038–3049.

Dunn, O.J., Clark, V.A., 1974. Applied Statistics: Analysis of Variance and Regression. Wiley, New York.

Efremova, N.V., Huang, Y., Peppas, N.A., Leckband, D.E., 2002. Direct measurement of interactions between tethered poly(ethylene glycol) chains and adsorbed mucin layers. Langmuir 18, 836–845.

English, R.J., Raghavan, S.R., Khan, S.A., Jenkins, R.D., 1999. Associative polymers bearing *n*-alkyl hydrophobes: rheological evidence for microgel-like behavior. J. Rheol. 43, 1175–1194.

English, R.J., Laurer, J.H., Spontak, R.J., Khan, S.A., 2002. Hydrophobically modified associative polymer solutions: rheology and microstructure in the presence of nonionic surfactants. Ind. Eng. Chem. Res. 41, 6425–6435.

Ferry, J.D., Viscoelastic Properties of Polymers, 3rd ed. Wiley, New York, 1980.

Gu, J.M., Robinson, J.R., Leung, S.H.S., 1988. Binding of acrylic polymers to mucin/epithelial surfaces: structure–property relationships. Crit. Rev. Ther. Drug Carrier Syst. 5, 21–67.

Hägerström, H., Edsman, K., 2001. Interpretation of mucoadhesive properties of polymer gel preparations using a tensile strength method. J. Pharm. Pharmacol. 53, 1589–1599.

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

Hassan, E.E., Gallo, J.M., 1990. A simple rheological method for the in vitro assessment of mucin–polymer bioadhesive bond strength. Pharm. Res. 7, 491–495.

Huang, Y., Leobandung, W., Foss, A., Peppas, N.A., 2000. Molecular aspects of muco- and bioadhesion: tethered structures and site-specific surfaces. J. Contr. Rel. 65, 63–71.

Hwang, S.-J., Park, H., Park, K., 1998. Gastric retentive drug-delivery systems. Crit. Rev. Ther. Drug Carrier Syst. 15, 243–284.

Johnson, K.L., Kendall, K., Roberts, A.D., 1971. Surface energy and the contact of elastic solids. Proc. R. Soc. London Ser. A 324, 301–313.

Junginger, H.E., 1991. Mucoadhesive hydrogels. Pharm. Ind. 53, 1056–1065.

Kakoulides, E.P., Smart, J.D., Tsibouklis, J., 1998. Azocrosslinked poly(acrylic acid) for colonic delivery and adhesion specificity: in vitro degradation and preliminary ex vivo bioadhesion studies. J. Contr. Rel. 54, 95–109.

Ketz, R.J., Prud'homme, R.K., Graessley, W.W., 1988. Rheology of concentrated microgel solutions. Rheol. Acta 27, 531–539.

Larson, R.G., 1999. The Structure and Rheology of Complex Fluids. Oxford University Press, Oxford, 1999, p. 343.

Madsen, F., Eberth, K., Smart, J.D., 1998. A rheological examination of the mucoadhesive/mucus interaction: the effect of mucoadhesive type and concentration. J. Contr. Rel. 50, 167–178.

Miyazaki, Y., Ogihara, K., Yakou, S., Nagai, T., Takayama, K., 2003. In vitro and in vivo evaluation of mucoadhesive microspheres consisting of dextran derivatives and cellulose acetate butyrate. Int. J. Pharm. 258, 21–29.

Mortazavi, S.A., Smart, J.D., 1994. Factors influencing gel-strengthening at the mucoadhesive–mucus interface. J. Pharm. Pharmacol. 46, 86–90.

Mowery, C.L., Crosby, A.J., Ahn, D., Shull, K.R., 1997. Adhesion of thermally reversible gels to solid surfaces. Langmuir 13, 6101–6107.

Park, N.A., Irvine, T.F., 1997. Anomalous viscosity–temperature behavior of aqueous Carbopol solutions. J. Rheol. 41, 167–173.

Park, H., Robinson, J.R., 1987. Mechanisms of mucoadhesion of poly(acrylic acid) hydrogels. Pharm. Res. 4, 457–464.

Pashkovski, E.E., Masters, J.G., Mehretab, A., 2003. Viscoelastic scaling of colloidal gels in polymer solutions. Langmuir 19, 3589–3595.

Peppas, N.A., Mikos, A.G., 1990. Kinetics of mucus–polymer interactions. In: Gurny, R., Junginger, H.E. (Eds.), Bioadhesion—Possibilities and Future Trends. Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, pp. 65–85.

Pham, Q.T., Russel, W.B., Thibeault, J.C., Lau, W., 1999. Polymeric and colloidal modes of relaxation in latex dispersions containing associative triblock copolymers. J. Rheol. 43, 1599–1615.

Quatrat, O., Snuparek, J., 1990. Structure and flow properties of lattices containing carboxylic groups. Prog. Org. Coating 18, 207.

Regalado, E.J., Selb, J., Candau, F., 1999. Viscoelastic behavior of semidilute solutions of multisticker polymer chains. Macromolecules 32, 8580–8588.

Riley, R.G., Smart, J.D., Tsibouklis, J., Dettmar, P.W., Hampson, F., Davis, J.A., Kelly, G., Wilber, W.R., 2001. An investigation of mucus/polymer rheological synergism using synthesised and characterised poly(acrylic acid)s. Int. J. Pharm. 217, 87–100.

Rossi, S., Bonferoni, M.C., Ferrari, F., Caramella, C., 1999. Drug release and washability of mucoadhesive gels based on sodium carboxymethylcellulose and polyacrylic acid. Pharm. Dev. Technol. 4, 55–63.

Rubinstein, M., 1997. Slow modes in dynamic light scattering from semi-dilute solutions of polymeric and polymer-like systems. In: McLeish, T. (Ed.), Theoretical Challenges in the Dynamics of Complex Fluids. NATO ASI Series, Dodrecht (Chapter 3).

Rubinstein, M., Dobrynin, A.V., 1999. Associations leading to formation of reversible networks and gels. Curr. Opin. Coll. Interf. Sci. 4, 83–87.

Rubinstein, M., Semenov, A.N., 1998. Thermoreversible gelation in solutions of associating polymers. 2. Linear dynamics. Macromolecules 31, 1386–1397.

Rueb, C.J., Zukoski, C.F., 1997. Viscoelastic properties of colloidal gels. J. Rheol. 41, 197–218.

Shojaei, A.H., Li, X., 1997. Mechanisms of buccal mucoadhesion of novel copolymers of acrylic acid and polyethylene glycol monomethylether monomethacrylate. J. Contr. Rel. 47, 151–161.

Singla, A.K., Chawla, M., Singh, A., 2000. Potential applications of carbomer in oral mucoadhesive controlled drug delivery system: a review. Drug Dev. Ind. Pharm. 26, 913–924.

Smart, J.D., 1999. The role of water movement and polymer hydration in mucoadhesion. In: Mathiowitz, E., Chickering, D.E., Lehr, C.-M. (Eds.), Bioadhesive Drug Delivery Systems. Fundamentals, Novel Approaches, and Development. Marcel Dekker, New York, pp. 11–23.

Tamburic, S., Craig, D.Q.M., 1995. An investigation into the rheological, dielectric and mucoadhesive properties of poly(acrylic acid) gel systems. J. Contr. Rel. 37, 59–68.

Tamburic, S., Craig, D.Q.M., 1997. A comparison of different in vitro methods for measuring mucoadhesive performance. Eur. J. Pharm. Biopharm. 44, 159–167.

Tirosh, B., Rubinstein, A., 1998. Migration of adhesive and nonadhesive particles in the rat intestine under altered mucus secretion conditions. J. Pharm. Sci. 87, 453–456.

Wang, C., Tam, K.C., Jenkins, R.D., Bassett, D.R., 2000. Potentiometric titration and dynamic light scattering of hydrophobically modified alkali soluble emulsion (HASE) polymer solutions. Phys. Chem. Chem. Phys. 2, 1967–1972.

Wientjes, R.H.W., Duits, M.H.G., Jongschaap, R.J.J., Mellema, J., 2000. Linear rheology of guar gum solutions. Macromolecules 33, 9594–9605.