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Enhanced nasal retention of hydrophobically modified polyelectrolytes

L. E. Bromberg

Abstract

Hydrophobically modified polyelectrolytes (HMP) are polymers with a high content of ionizable groups bonded to hydrophobic groups. Copolymers of poly(acrylic acid) and Pluronic surfactants constitute a special class of HMP whereby poly(propylene oxide) segments act as hydrophobes. The poly(propylene oxide) segments possess temperature-dependent aqueous solubility and the solutions of the Pluronic-poly(acrylic acid) copolymers (MW $>$ 3000 000) undergo a sol-gel transition when kept at body temperature. Due to the presence of the poly(acrylic acid) segments, the Pluronic-poly(acrylic acid) copolymers are bioadhesive. We have examined the hypothesis that the in-situ gelling polymer formulations of Pluronic-poly(acrylic acid) copolymers may have an enhanced retention in the nasal cavity. The effects of putative bioadhesive (Carbomer 934P) and thermogelling (Pluronic F127) polymers on nasal clearance were compared with Pluronic-poly(acrylic acid) copolymers using a rat model.

The enhancement of the residence time of fluorescent labels by the Pluronic-poly(acrylic acid) copolymers was shown to be 5–8-fold that of Carbomer, and 3–6-fold that of Pluronic F127. The results unequivocally demonstrate the superior retention of the HMP that combines bioadhesive and thermogelling capabilities over either a bioadhesive polyelectrolyte or a polymer of a low molecular weight that undergoes a sol-gel transition.

Introduction

The use of bioadhesive polymers such as poly(acrylic acid), carboxymethylcellulose and chitosan can lengthen the residence time of formulations administered to the nasal cavity and enhance bioavailability of the delivered drugs (Donovan & Zhou 1995; Zhou & Donovan 1996; Illum 1999). However, application of the viscous solutions to the nasal cavity by a common spray device is unlikely. Therefore, application of in-situ gelling solutions of low molecular weight triblock copolymers of poly(ethylene oxide) and poly(propylene oxide) (Pluronics) have been proposed to lower the viscosity of the nasal formulations below the body temperature (Illum 1999). When administered, formulations based on concentrated solutions of Pluronics undergo a sol-gel transition and the resulting gels show an enhancement of the residence time in the nasal cavity (Zhou & Donovan 1996). Gelation of concentrated solutions of Pluronics is a well-documented phenomenon related to the appearance of tightly-packed cubic liquid crystalline micellar phases (Bromberg & Ron 1998). It should be noted, however, that due to a relatively low molecular weight of the Pluronics, high polymer concentrations are required for their solutions to gel. For instance, solutions of Pluronic F127 undergo significant sol-gel transitions only at concentrations above 16 wt% (Bromberg & Ron 1998).

Department of Physics and Center for Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

L. E. Bromberg

Correspondence: 15 Sherwood Road, Swampscott, MA 01907, USA. Email: cpbrolev@msn.com or cpbrolev@mit.edu

Concerns over application of Pluronics as in-situ drug delivery vehicles (high polymer concentrations, low gelation temperatures, and lack of bioadhesion) can be allayed by utilization of the copolymers of Pluronics with a bioadhesive polymer. We have recently introduced a novel class of copolymers whereby poly(acrylic acid) is attached to the Pluronic backbone via C—C bonding (Bromberg 1998a, b, c). Semidilute solutions (up to 2%) of the Pluronic-poly(acrylic acid) copolymers undergo clear sol-gel transitions and yield gels with significant viscoelastic moduli (Bromberg 1998d). Bioadhesive properties of the Pluronic-poly(acrylic acid) copolymers have been demonstrated (Bromberg et al 1997; Bromberg 1999). It was thus of interest to test the enhancement of the retention and clearance of these copolymers in the nasal cavity. This work is the first study of the clearance of the Pluronic-poly(acrylic acid) copolymers in-vivo.

Materials and Methods

Materials

Poly(acrylic acid) (average MW approximately 2.5×10^5) and fluoresceinamine (isomer II) were obtained from Aldrich Chemical Co. (Milwaukee, WI). Water-soluble condensing agent, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDA) was obtained from Pierce (Rockford, IL). Pluronic F127 NF was kindly donated by BASF Corp. (Parsippany, NJ) and used without further treatment. Poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-(polyethylene oxide)-*g*-poly(acrylic acid) (CAS #186810-81-1) was synthesized by dispersion/emulsion polymerization of acrylic acid along with simultaneous grafting of poly(acrylic acid) onto Pluronic backbone as described by Bromberg (1998a). In this work we have referred to the ensuing copolymer as Pluronic-poly(acrylic acid). The polymer had a weight-average molecular mass of 3.5×10^6 Da and consisted of 45% Pluronic F127 and 55% poly(acrylic acid) as measured by FTIR and NMR. The residual concentration of monomers in Pluronic-poly(acrylic acid) was below 10 ppm (Bromberg 1998a). Carbomer 934P NF was obtained from B. F. Goodrich (Brecksville, OH). Orange FluoSphere latex beads (effective size, 0.1 μm; fluorescein equivalents per microsphere, 7400; λ_{ex} 540 nm, λ_{em} 560 nm) were obtained from Molecular Probes, Inc. (Eugene, OR). All other chemicals, gases and organic solvents of the highest purity available were obtained from commercial sources.

Procedures

To prepare formulations the corresponding polymer samples were dispersed in 10 mM phosphate buffer and gently stirred at 4°C for 48 h. The pH was adjusted to 6.5 ± 0.1 with 5 M NaOH as needed. The solutions were filtered through nylon filters (Gelman Sciences) with pore diameters of 0.8 μm, deoxygenated by nitrogen flow and stored at 4°C. FluoSphere latex suspension was added to the polymer solution under stirring, and the pH was readjusted to 6.5. Fluorescent polymer solutions were allowed to equilibrate in the dark for at least 48 h.

In a separate series of experiments, the Pluronic-poly(acrylic acid) copolymers were tagged by a fluorescent label via conjugation by simultaneous activation and coupling with EDA (Bromberg & Salvati 1999). The Pluronic-poly(acrylic acid), fluoresceinamine, and EDA components were dissolved in 10 mM phosphate buffer (pH 7.4) at 4°C to result in final concentrations of 10, 2, and 2 mg mL^{-1}, respectively. The mixture was then gently shaken for 24 h at 4°C. The resulting Pluronic-poly(acrylic acid)-Fl conjugate was precipitated by addition of 1 M HCl at pH 4.8, quickly filtered off and redissolved in chilled 10 mM phosphate buffer. The conjugate was then dialysed against phosphate buffer at 4°C for 24 h using Spectra/Por cellulose ester membrane (MW cut-off 50 000; Spectrum, Laguna Hills, CA), and lyophilized. The Pluronic-poly(acrylic acid)-Fl solutions were assayed fluorometrically (typical λ_{ex} 490 nm, λ_{em} 510 nm). In experiments with Pluronic-poly(acrylic acid)-Fl, the tagged polymer was mixed with the Pluronic-poly(acrylic acid) to arrive at a proper fluorescence intensity of the resulting polymer solutions, without compromising their rheological characteristics. Fluorescence spectra were recorded using a 10-mm path-length quartz cell in a thermostatted cuvette holder using a Shimadzu Model RF-5301 PC spectrofluorophotometer with a UV/vis polarizer under controlled temperature conditions (slit widths 5.0 or 10 nm) at right-angle geometry. Rheological measurements were performed using a controlled stress Rheolyst Series AR1000 Rheometer (TA Instruments, 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.

Animal studies

The clearance rates of polymer formulations in the rat model were assessed by the method described by Donovan & Zhou (1995) and Zhou & Donovan (1996).

Male Sprague-Dawley rats (250–300 g) were housed in a contract laboratory and the experiments were conducted under controlled temperature and humidity conditions. Three animals were exposed to each polymer or control formulation. Throughout the experiments, the rats were sedated by 25-μL injections of 10% ketamine every 30 min. The polymer formulations or a control suspension of FluoSpheres (25 μL) that had been kept at 4°C were instilled into the left nostril of a rat with a pipette, and the experiment commenced. The FluoSpheres or Pluronic-poly(acrylic acid)-Fl exiting the nasal cavity were collected in the oral cavity by swabbing with moistened cotton-tipped applicators. Samples were taken every 1 min for the first 30 min and then every 5 min for the next 60 min (Zhou & Donovan 1996). The FluoSpheres or the Pluronic-poly(acrylic acid)-Fl were extracted from the applicators by the known volume of deionized water, and the intensity of the fluorescence emission was measured. Concentration of the fluorescent species was calculated using separate calibration curves for FluoSpheres and the Pluronic-poly(acrylic acid)-Fl samples.

Clearance was expressed via erosion (E) of the sample from the nasal cavity:

$$E\% = 100 \times (\text{cumulative probe concentration in samples collected in oral cavity by time t}/\text{initial probe concentration in the sample instilled into nasal cavity})$$

where probe stands for the fluorescent species, and concentration is calculated from the relative fluorescence intensity.

The E data obtained during the first 30 min of the clearance experiment were fitted to the first-order decomposition equation $\ln(100\text{-}E) = \text{const} - kt$ (Irwin 1990) and the rate constant k obtained from the linear regression fit (r^2 exceeded 0.96 in all cases) was taken as an initial clearance rate (Zhou & Donovan 1996). The time of reaching 80% of the fluorescence probe recovered (t80) was used, as the more traditional parameter (t90) could not be determined with formulations based on Pluronic-poly(acrylic acid). The area under the curve (AUC) was measured from the 100-E vs t plots using the trapezoidal rule (Welling 1986). The mean values for each of these parameters for each group of rats receiving separate formulation were compared with the observed mean control values found for that same group using the Student's *t*-test (the results reported herein had only $P \leqslant 0.05$). The control experiments with the FluoSpheres in the buffer solution had been conducted with the same animals that were subsequently subjected to the polymer formulations. The interval between the control and polymer instillation was set at two days (Zhou & Donovan 1996).

To visualize the retention of the Pluronic-poly(acrylic acid) in the nasal cavity over the 2-h period, scanning electron microscopy (SEM) was applied. Some animals were sacrificed by rapid intravenous injection of sodium pentobarbital overdose. The nasal chamber was opened and the anterior surface of the nasal ciliated epithelium along with the remaining gel was gently removed by dissecting microprobe. The sample was immediately snap-frozen in liquid nitrogen and lyophilized. The dried samples were sputter-coated with gold 200–300 Å and microscopy was performed with a dual-stage electron microscope DS-701 (Topcon Co., Tokyo, Japan).

Results and Discussion

Retention of a formulation in the nasal cavity could be prolonged by an altered clearance due to increased viscosity and/or through bioadhesion (Zhou & Donovan 1996). Rheological parameters of the thermogelling Pluronic-poly(acrylic acid) are presented in Figure 1, along with the temperature dependencies of the storage moduli (G′) of the solutions of Pluronic F127 and a physical blend of Pluronic F127 and poly(acrylic acid). While the physical blend did not show any sign of gelation, the concentrated solution of Pluronic transitions from a near-Newtonian liquid to a strong viscoelastic gel with a gelation threshold temperature (T_{gel}) at approximately 9°C. Dissociation of the micellar structures above 45–50°C led to the weakening of the gel elasticity, as reflected in the decline of G′. Solution of Pluronic-poly(acrylic acid) copolymer underwent the transition from a Newtonian liquid (phase angle δ close to 90°) to a viscoelastic gel ($\delta = 2.5°$) at T_{gel} of approximately 18–20°C. Although the storage modulus of the 2% Pluronic-poly(acrylic acid) solution was approximately 160-times lower at body temperature than that of 20% Pluronic F127 (Figure 1), the rheological parameters of the Pluronic solution may not be advantageous with regard to the rate of mucociliary clearance, due to the lack of bioadhesion. On the other hand, it is apparent that the Pluronic and Pluronic-poly(acrylic acid) solutions would be cleared more slowly than the non-gelling blend of Pluronic and poly(acrylic acid). Further experiments were designed to compare clearance rates of Pluronic-poly(acrylic acid) with those of the solutions of the parent Pluronic and bioadhesive polymer, such as Carbomer (lightly cross-linked poly(acrylic acid)). The

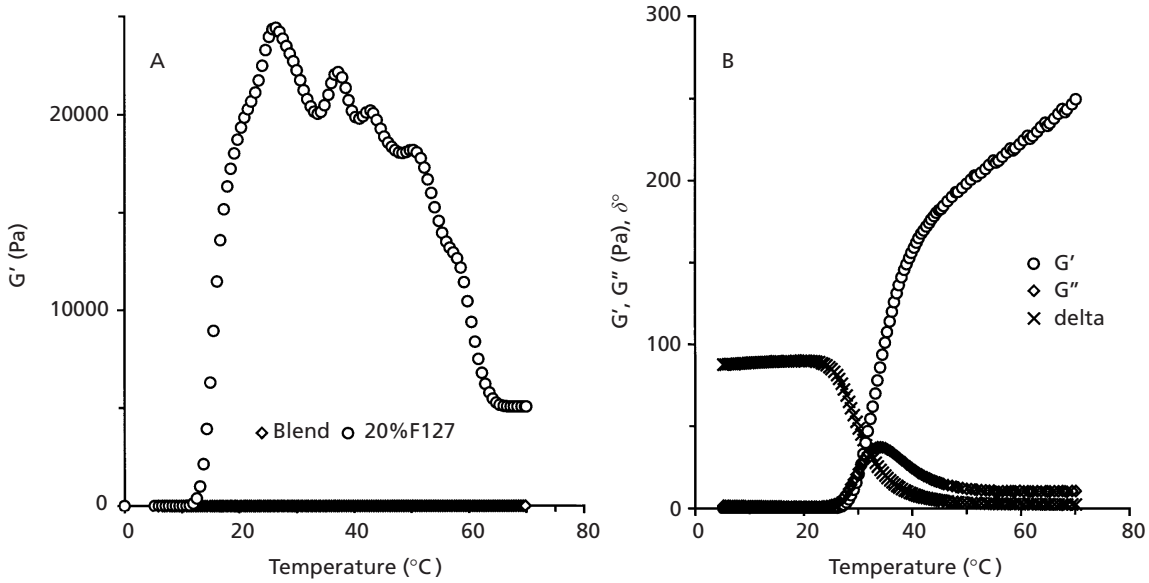

Figure 1 Temperature dependence of rheological parameters of Pluronic-poly(acrylic acid), Pluronic F127, and Pluronic/poly(acrylic acid) physical blend solutions. A. Temperature dependence of the storage moduli (G′) of 20 wt% Pluronic F127 and a blend (5 wt% total, 1:1 weight ratio of polymers) of Pluronic F127 and poly(acrylic acid) (M_r 120000) is depicted. B. Storage (G′) and loss (G″) moduli and phase angle ($\delta°$) of 2% Pluronic-poly(acrylic acid) solution are shown. Oscillatory stress of 60 mPa, frequency of oscillatory shear of 1 Hz, and pH 6.5 are applied throughout.

Carbomer 934P concentration of 0.4% used herein for the purpose of comparison with Pluronic-poly(acrylic acid) corresponded to the bioadhesive formulation used by Zhou & Donovan (1996). It is interesting to note that viscosity of a gel formulation alone cannot explain the variations in clearance from the nasal cavity. For instance, Zhou & Donovan (1996) observed no correlation between the complex viscosities of gels such as 5% poly(ethylene oxide), 3% carboxymethylcellulose, 3% methylcellulose, 3% hydroxypropylcellulose, 3% chitosan glutamate, 0.2% Carbomer, and 25% Pluronic F127, and the clearance rates of the corresponding gels from the nasal cavity of rats. Formulation based on 3% methylcellulose, while less viscous than most of the other formulations, showed the slowest clearance rate (Zhou & Donovan 1996). However, the rheological parameters of such a formulation are temperature-independent, and thus methylcellulose formulations cannot be applied as in-situ gelling agents.

Representative SEM micrographs of the nasal epithelium obtained at the end of the 2-h collection interval after the instillation of 2% solution of Pluronic-poly(acrylic acid) into the nostril of a rat are shown in Figure 2. The nasal mucus appeared to be covered by the film-forming gel. These observations illustrated significant retention of the gelling solution in the nasal cavity even after the 2-h period. For comparison, Zhou & Donovan (1996) observed that 90% of the fluorescently-labelled microspheres loaded into 0.4% Carbomer or 25% Pluronic F127 solutions cleared out of the nasal cavity of rats within 0.5–1 h, which suggested faster clearance rates of these solutions.

To directly compare clearance rates of the Pluronic-poly(acrylic acid), Pluronic, and Carbomer solutions, formulations loaded with fluorescent microspheres and fluorescently-labelled copolymers (Pluronic-poly(acrylic acid)-Fl) were instilled and their clearance was measured. The initial clearance profiles of the FluoSpheres and Pluronic-poly(acrylic acid)-Fl (Figure 3) proved that erosion of the Pluronic-poly(acrylic acid) from the nasal cavity was distinctly slower than any other formulation. Comparison of the initial clearance rate constant (k), AUC, and t80 (Table 1) shows that the enhancement of the residence time of FluoSpheres by the Pluronic-poly(acrylic acid) copolymers was 5–8-fold that by 0.4% Carbomer, and 3–6-fold that by 20% Pluronic F127. The capability of Pluronic-poly(acrylic acid) copolymers to solubilize hydrophobic drugs such as steroid hormones (Bromberg & Barr 1999; Bromberg & Temchenko 1999) and enhance their bioavailability in

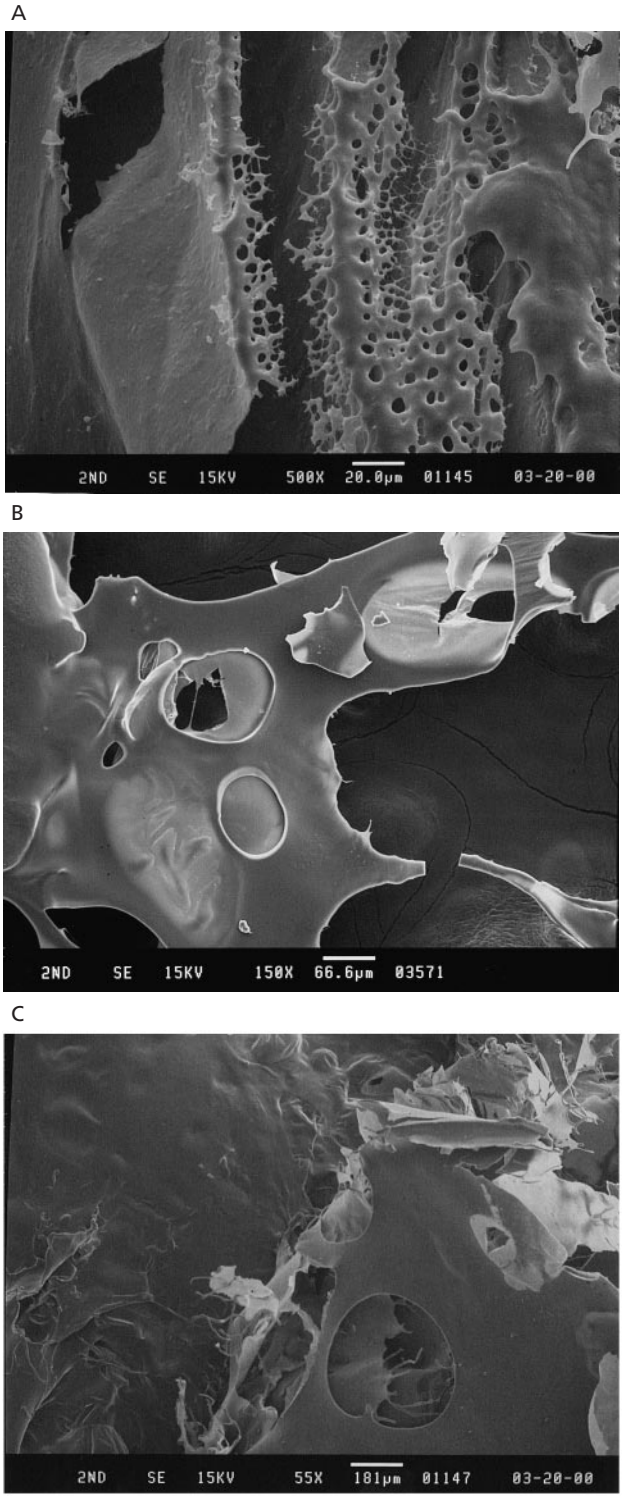

Figure 2 SEM micrographs of the surface of the nasal mucosa covered by the gel-forming solution of Pluronic-poly(acrylic acid) 2 h after instillation. A. Mucus removed from the nostril. B. Gel film removed from the nostril. C. Mucosa covered with the film (the latter seen in the lower right part of the image).

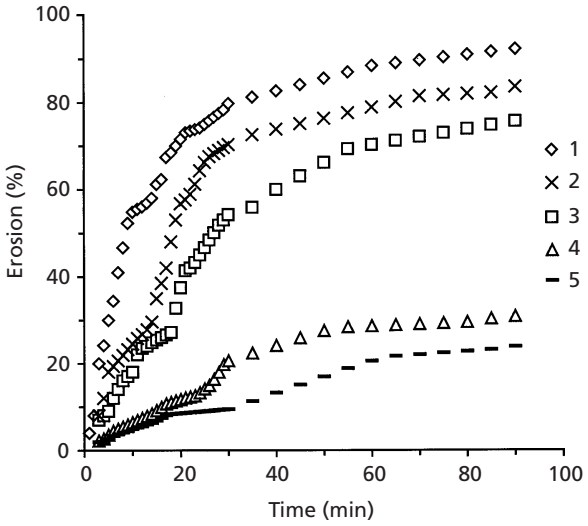

Figure 3 Intranasal clearance profiles for the buffer solution (1), 20 wt% Pluronic F127 (2), 0.4% Carbomer (3), 2% Pluronic-poly(acrylic acid) (4), and 2% Pluronic-poly(acrylic acid)-Fl (5). Curves 1–4 were obtained using fluorescence of FluoSpheres loaded into corresponding formulation; curve 5 was obtained using fluorescence of the polymer. At least three animals were used to obtain each curve. Error bars are not shown for clarity. Maximum standard deviations were 9%, 18%, 33%, 32%, and 34% for data points in curves 1, 2, 3, 4, and 5, respectively.

Table 1 Initial clearance rate (k), time for 80% FluoSphere clearance (t80), and area under the curve (AUC) for intranasal clearance in rat model.

System	$[k] \times 10^3$ (1000 min^{-1})	t80 (h)	AUC (% min)
Control	51 ± 7.2	0.55 ± 0.11	2330 ± 120
0.4% Carbomer	46 ± 5.3	1.1 ± 0.29	4490 ± 250
20% Pluronic	27 ± 3.5	1.6 ± 0.54	6140 ± 520
2% Pluronic-poly(acrylic acid)	6.0 ± 0.79	9.0 ± 4.3	22890 ± 2560
2% Pluronic-poly(acrylic acid)-Fl	3.1 ± 0.46	8.0 ± 3.6	30280 ± 4300

Values are mean ± s.e.m.

vaginal applications (Bromberg 2001), as well as stabilize peptides and proteins (Bromberg & Ron 1998), has been demonstrated. Combined with a long residence time of such gelling compositions as demonstrated here, these properties indicate that the Pluronic-poly(acrylic acid) copolymers can be used advantageously in the nasal delivery of a variety of drugs.

References

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

Bromberg, L. (1998b) Self-assembly in aqueous solutions of polyether-modified poly(acrylic acid). *Langmuir* **14**: 5806–5812

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. (2001) Hydrophobically modified polyelectrolytes and polyelectrolyte block-copolymers. In: Nalwa, H. S. (ed.) *Handbook of Surfaces and Interfaces of Materials*. Academic Press, In press

Bromberg, L., Salvati, L. (1999) Bioactive surfaces via immobilization of self-assembling polymers onto hydrophobic materials. *Bioconjug. Chem.* **10**: 678–686

Bromberg, L., Temchenko, M. (1999) Loading of hydrophobic compounds into miceller solutions of hydrophobically modified polyelectrolytes. *Langmuir* **15**: 8627–8632

Bromberg, L. E. (1999) Interactions between hydrophobically modified polyelectrolytes and mucin. *Polym. Prep.* **40**: 616–617

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

Bromberg, L. E., Barr, D. P. (1999) Aggregation phenomena in aqueous solutions of hydrophobically modified polyelectrolytes. A probe solubilization study. *Macromolecules* **32**: 3649–3657

Bromberg, L. E., Orkisz, M. J., Ron, E. S. (1997) Bioadhesive properties of polyoxyethylene-b-polyoxypropylene-b-polyoxyethylene-g-poly(acrylic acid) polymers. *Polym. Prep.* **38**: 626–627

Donovan, M. D., Zhou, M. (1995) Drug effects on in vivo nasal clearance in rats. *Int. J. Pharm.* **116**: 77–86

Illum, L. (1999) Bioadhesive formulations for nasal peptide delivery. In: Mathiowitz, E., Chickering, D. E., Lehr, C.-M. (eds) *Bioadhesive Drug Delivery Systems*. Marcel Dekker, New York, pp 507–562

Irwin, W. J. (1990) *Kinetics of Drug Decomposition*. Elsevier, Amsterdam, pp 114–116

Welling, P. G. (1986) *Pharmacokinetics. Processes and Mathematics*. ACS Monograph 185, American Chemical Society, Washington, DC, pp 145–149

Zhou, M., Donovan, M. D. (1996) Intranasal mucociliary clearance of putative bioadhesive polymer gels. *Int. J. Pharm.* **135**: 115–125