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Boronate-containing polymers form affinity complexes with mucin and enable tight and reversible occlusion of mucosal lumen by poly(vinyl alcohol) gel

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ABSTRACT

Copolymers of *N*-acryloyl-*m*-aminophenylboronic acid (NAAPBA) with *N*,*N*-dimethylacrylamide (DMAA) formed insoluble interpolymer complexes with mucin from porcine stomach at pH 9.0. The complex formation based on boronate–sugar interactions took place between the similarly charged macromolecules and resulted in coacervate particles formation, which depended both on pH and ionic strength of the solution. The coacervation rate displayed a maximum at the intermediate DMAA-NAAPBA copolymer: mucin weight ratio, that is a pattern typical of interpolymer complex formation. The effective hydrodynamic particle diameter of the coacervates monotonously grew from 155 ± 20 nm up to 730 ± 120 nm in 2 days in 0.1 M sodium bicarbonate buffer solution, pH 9.0. Electrophoretic mobility of the resultant nanoparticles was intermediate between those of individual polymers, whereas the particles zeta-potential was -7.5 ± 0.4 mV in the above buffer solution. Pre-treatment of the inner mucosal epithelium of excised male pig urethras with 5% (w/v) solutions of acrylamide-NAAPBA or DMAA-NAAPBA copolymers at pH 8.8 allowed for tight occlusion of the lumen by poly(vinyl alcohol) – borax gel injected via a two-way catheter. Leakage of 0.15 M NaCl solution through the thus occluded organs could be prevented, while the leakage through the organs occluded by the gel without the pre-treatment was unavoidable. The gel plug could be quickly dissolved on demand after injection of 5% (w/v) aqueous fructose solution into the lumen. The described technique may be useful for temporal occlusion of mucosal lumens in living organisms. In contrast to the conventional mucoadhesive polymers like polyacrylic acid or chitosan, the boronate-containing copolymers display their mucoadhesivity at weakly alkaline pH of 8–9 and physiological ionic strength.

1. Introduction

The gels of synthetic and natural polymers are increasingly used in medicine as tissue sealants, implants and drug delivery agents. Fibrin, gelatin and poly(cyanoacrylates) have been widely studied and used as tissue sealants in a number of surgical procedures (Morikawa, 2001). Poly(vinyl alcohol) (PVA) and cellulose triacetate were successfully used for filling large and giant aneurism cavities and suggested as a new method of endovascular treatment (Piotin et al., 2001; Mawad et al., 2002). Many synthetic non-specific bioadhesives based on polyacrylic acid (Smart et al., 1984; Ahn et al., 2002) hydroxyalkyl cellulose (Taylan et al., 1996) or chitosan (van der Lubben et al., 2001) displayed satisfactory performance as materials for transmucosal drug delivery or vaccination. The polymer-based hydrogels are well suited for bioadhesion due to their flexibility and nonabrasive characteristics in the partially swollen state, which reduced damaging attrition to the epithelial tissues in contact (Ahn et al., 2002). In particular, PVA membranes and hydrogels were used to prevent abnormal joining of anatomic structures after abdominal and pelvic surgery (Weis et al., 2004). PVA hydrogels enforced with cellulose fibers were proposed as a material for cardiovascular soft tissue replacement applications (Millon and Wan, 2006).

Adhesion of polymeric gels to the adjacent biological tissues can be enhanced by incorporation of specific cell adhesion peptides into the polymers. The conjugation of ArgGlyAsp (RGD) cell adhesion peptides with a vinyl alcohol copolymer allowed

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for attachment of endothelial cells to the polymer clots formed within the aneurism cavities (Ohyama et al., 2004). The RGD-grafted polymers assisted reorganizing the cavities better than the non-grafted polymers. Another type of bioaffinity adhesion is presented by sugar-specific proteins, lectins, situated on bioadhesive microparticles and liposomes. These particles were shown to provide highly selective targeting of human or mouse epithelial cells (Lehr, 2000; Ann Clark et al., 2000). Sugar-specific interactions offer wide prospects for directed drug delivery and tissue sealing because both the epithelial and endothelial glycocalix contain many mucins or mucin-like glycoproteins exhibiting numerous oligosaccharides (Lasky, 1995). Thus, sugar-specific bioadhesivity was imparted to microfabricated poly(methyl metacrylate) (PMMA) microdevices (150 μm × 150 μm) by means of chemical attachment of tomato lectin capable of targeting cells in the gastrointestinal tract. The *in vitro* studies performed with Caco-2 cell cultures demonstrated a several fold increase in quantity of the cell-bound microdevices compared with the PMMA microdevices containing no lectins (Tao et al., 2003). These findings confirm a high potential of sugar-specific interactions for chemical design of new bioadhesive materials and devices.

Incorporation of lectins into macro dosage forms and tissue sealants, as a mean to increase their bioadhesivity, can face, however, some limitations. The surface area of a macro dosage is many times larger than the surface of a particular cell in contact. Real mucosal surfaces containing different cell types as well as insoluble layer of mucus would exhibit many different end-group saccharides besides those complementary to the lectin. Broadly speaking, the overall strength of bioadhesion can be expected to suffer from the high selectivity of the saccharide–lectin interactions. High cost of lectins is also a limiting factor.

One might anticipate synthetic polymers with lectin-like binding specificity to carbohydrates as ideal mucoadhesives. Such polymers might be used as components of macro dosage forms with increased and/or controlled mucoadhesivity. Lectin-like binding activity is a known feature of water-soluble polymers containing phenylboronate (PBA) functional groups (Uchimura et al., 2001; Winblade et al., 2002; Kuzimenkova et al., 2006). Ability of these polymers to coat cell surfaces and to block cell–cell adhesion was confirmed in an *in vitro* model relevant to peritoneal adhesion formation using a monolayer of RM4 mesothelial cells (Winblade et al., 2002). The high viability of cells demonstrated in these experiments has proven non-toxicity of the boronate-containing copolymers (BCC). Non-toxicity and mitogenic properties of DMAA-NAAPBA copolymers have been independently demonstrated by Uchimura et al. (2001).

Recently, we have observed a spontaneous sugar-specific adsorption of water-soluble BCC onto the cross-linked polysaccharide gel (Kuzimenkova et al., 2006). Further, the BCC spontaneously formed insoluble complexes with mucin from porcine stomach in aqueous solution, due to boronate–sugar interactions (Ivanov et al., 2006). It seems challenging to investigate whether BCC can enhance mucoadhesivity of polymeric gels, in particular, those intended to plug the corporal cavities and lumen-containing organs. A temporal plugging of animal organs followed by restoration of their patency may have applications in surgery (Schmitt, 1994; Naughton et al., 2004). Dissolution of the plugs, even by means of organic solvents like dimethylsulfoxide, still faces, however, serious difficulties (Naughton et al., 2004). The goals of the present study are to investigate the complex formation between the BCC and mucin and to evaluate possibility of tight occlusion of a mucosal lumen by means of PVA gel. Another goal of the study is to show a possibility of the gel-plug dissolution under mild, physiologically acceptable conditions.

2. Experimental

2.1. Materials

Sodium hydrogen carbonate, disodium hydrogen phosphate, di-sodium tetraborate (borax) and sodium hydroxide, were products of Merck KGaA (Darmstadt, Germany). Acrylamide (AA), *N*,*N*-dimethylacrylamide (DMAA) and neutral aluminium oxide, type 507C, were products of Aldrich (Steinheim, Germany). 2,2′-Azobis(2-methylpropionitrile) (AMPN) was purchased from ACROS (Geel, Belgium). 1,4-Dioxane-D8 99 atom% D and deuterium oxide 99.95 atom% D were from Dr.Glaser AG (Basel, Switzerland). *N*-Acryloyl-*m*-aminophenylboronic acid (NAAPBA) was prepared as described by Ivanov et al. (2004). PVA, Mowiol 20–98, M_w = 125000 g mol^{-1}, was purchased from Clariant GmbH (Frankfurt, Germany). Freshly excised male pig urethras were excessively rinsed with 0.15 M NaCl containing 0.02% (w/v) sodium azide to remove blood both from the outside of the organ and from the lumen. The rinsed organs were frozen and kept at −18 °C.

2.2. Synthesis of DMAA-NAAPBA and AA-NAAPBA copolymers

Synthesis of the copolymers was performed according to the earlier described method of Ivanov et al. (2004), the molar proportion of the monomers being varied from 97.5:2.5 to 90:10. Briefly, DMAA (18–19.5 mmol), NAAPBA (0.5–2 mmol) and AMPN (10 mg) were dissolved in 20 mL of ethanol. Free radical polymerization was started by heating the reaction mixture to 70 °C under nitrogen bubbling and carried out for 6 h. The thus obtained solution of copolymer was added drop-wise to 200 mL diethyl ether for precipitation of the copolymer and its separation from the monomers. The precipitate was collected by filtration on Munktell No. 3 filter paper, washed with diethyl ether and dried in air and under vacuum. The yields of the copolymers were in the range of 75–85%. Synthesis of the AA-NAAPBA copolymer was performed as described by Kuzimenkova et al. (2006). Designations and characteristics of the copolymers are listed in Table 1.

2.3. Molecular weight determination

Weight-average molecular weight (M_w) of DMAA-NAAPBA copolymers with boronate units molar percentage up to 8.8 was calculated from their intrinsic viscosity using the formula for poly-DMAA: $[\eta] = 17.5 \times 10^{-5} \times M_w^{0.68}$ (Polymer Handbook, 1989). An Ubbelohde viscosimeter was used to measure intrinsic viscosity of the copolymer in methanol at 25 °C.

2.4. Turbidity of the mucin-copolymer coacervates

DMAA-NAAPBA copolymers were dissolved in 0.1 M sodium bicarbonate buffer solution (pH 9.0) at concentration of 8 mg mL^{-1}. Mucin from porcine stomach was dissolved in the buffer solution at concentration of 4 mg mL^{-1}. The solutions of polymers were filtered through a Minisart® filter with pore size of 0.45 μm. The solution of mucin (0.5 mL) was combined with the solution of the copoly-

Table 1
Characteristics of polymers

Polymer sample	NAAPBA mol% taken for synthesis	NAAPBA mol% in the copolymer	M_w (g mol^{-1})
polyDMAA	0	0	7000
DMAA-NAAPBA(2.5)	2.5	2.7	N.D.
DMAA-NAAPBA(5)	5	5.2	22000
DMAA-NAAPBA(10)	10	8.8	19000
AA-NAAPBA(15)	15	13	6700

N.D. = not determined.

mer (from 0.02 to 3 mL) and diluted to 4 mL by the buffer solution. The reaction mixtures were kept at room temperature (22 °C) in the vials with screwed caps. Turbidity of the formed coacervates was measured as optical density at λ = 400 nm at certain time intervals. Turbidity of the coacervates formed in 0.1 mM sodium phosphate buffer solution, pH 8.0, 10 mM sodium bicarbonate solution, pH 9.0 or 10 mM sodium bicarbonate solution, containing 0.15 M NaCl, pH 9.0, were prepared by combination of 4 mg mL^{-1} mucin (0.5 mL) and 8 mg mL^{-1} copolymer (1 mL) in the corresponding buffer solution, diluted to 4 mL, and studied in the similar manner.

2.5. Measurments of light scattering intensity, coacervate particle size and electrophoretic mobility

The measurements were performed using a Malvern Zetasizer 4 (United Kingdom). Decimolar sodium bicarbonate buffer solution (pH 9.0) was filtered through a Minisart® filter with pore size of 0.45 μm. The solutions of DMAA-NAAPBA copolymers (1 ml) and mucin (0.5 mL) were prepared and combined as described in Section 2.4. The solutions were thermostated at 25 °C before mixing and the mixture was injected into the measuring cell of Zetasizer 4 thermostated at the same temperature. Four to five measurements of particle size were performed for each of the reaction times of 35 ± 15 min, 19 h and 42 h. Correctness of the particle size measurements was verified using polystyrene Nanosphere™ Size Standards of 105 ± 3 and 304 ± 6 nm, Duke Scientific Corporation (USA). Electrophoretic mobility of the individual mucin and DMAA-NAAPBA(10) was measured at concentrations of 0.5 and 1 mg mL^{-1}, respectively, in 0.1 M sodium bicarbonate buffer, pH 9.0 or 10 mM sodium bicarbonate buffer, pH 9.0, at 25 °C. Each sample was injected twice; four subsequent measurements were made after each injection and the obtained values were averaged. Electrophoretic mobilities of the coacervate particles were studied for the reaction time of 42 h, when the average amount of registered light scattering events (counts per second) was ca. 20-fold higher than those produced by the solutions of individual polymers.

2.6. Injection of the gelling system into pig urethra or silicon tubing and the leakage test

A freshly defrosted urethra was rinsed through the lumen with 0.15 M NaCl containing 0.02% (w/v) sodium azide. Aqueous solutions of PVA (0.5 mL, 5%, w/v) and 0.1 M $Na_2B_4O_7$ (0.5 mL) were simultaneously injected through a two-way catheter into the open end of the lumen of an excised urethra (see Fig. 1), the pressure valve being closed. The injection was also performed into a silicon tubing (5 mm i.d.) situated in the same manner, as a control. In 5 min after the injection, a pressure of ca. 40 mPa created with the sodium chloride solution was applied by opening the pressure valve connected to the upper end of the lumen and the flow rate of 0.15 M NaCl was measured by collecting the effluent into a cylinder for certain times. The flow rate through the unsealed organs (see Fig. 1a) was measured in the same way. All the experiments were performed at room temperature (22 °C).

2.7. Treatment of the urethra lumen by aqueous solutions of boronate-containing polymers

The copolymers were dissolved in deionized water at concentrations of 5% (w/v) and pH of the solutions was adjusted to 8.8 by a drop-wise addition of 1 M NaOH. The prepared solutions (0.4 ml) were injected with a syringe into the lower end of the urethra lumen to the depth of ca. 8–10 cm, to wet and coat the inner surface of the organ with the copolymers. After 5 min, the injection of the binary 5% PVA–0.1 M $Na_2B_4O_7$ system to the treated part of the organ was performed as described in Section 2.6.

2.8. Dissolution of PVA-borax gels

2.8.1. Dissolution in the excess of aqueous phase

A PVA-borax gel was prepared by mixing 5% (w/v) PVA (0.5 mL) and 0.1 M $Na_2B_4O_7$ (0.5 mL) in a test-tube. A piece of the gel (0.6–0.7 g) was placed in a beaker with 10 mL of 0.15 M NaCl or 0.15 M NaCl containing fructose (5%, w/v) and slowly (30 cycles/min) swung on an eccentric rocking table. From time to time, the gel was mechanically withdrawn by spatula, weighed and returned in the beaker.

2.8.2. Dissolution of the gels situated within the urethra lumen

A fine polyethylene tubing (i.d. 0.28 mm, o.d. 0.61 mm, Intramedic, Becton Dickinson and Co., USA) sealed by melting from one end was inserted to the lumen via its lower end to the depth of ca. 10 cm before injection of the gelling PVA-borax system (see Section 2.6 and Fig. 1b). In 30 min after the formation of stable gel the outer, sealed end of the tubing was opened and 5% (w/v) fructose solution in 0.15 M NaCl (5 mL) was slowly injected through it to

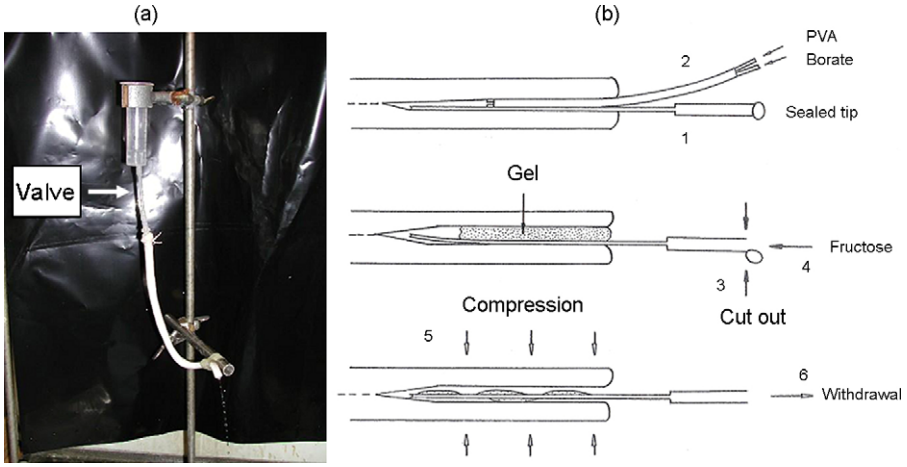

Fig. 1. General setup of the occlusion experiments. (a) Flow of 0.15 M NaCl goes through the lumen of excised pig urethra. Arrow indicates the position of the pressure valve (a tubing clamp; not shown in the figure). (b) Sequence of the steps enabling the gel plug formation and dissolution: (1) one end-sealed fine tubing is placed into the lumen; (2) mixture of PVA and borate solutions is injected through the two-way catheter; (3) the seal is cut out; (4) fructose is injected; (5) urethra is gently compressed; (6) pressure valve is opened and remains of the gel are withdrawn.

the lumen, the pressure valve being kept open. After the injection the pressure valve was closed. The occluded part of the organ was gently compressed by fingers 2 min after the injection to promote the gel-plug dissolution. After another 2 min the valve was opened again to withdraw the detached and partially dissolved gel plug.

3. Results and discussion

3.1. Characterization of boronate-containing copolymers

Copolymers of NAAPBA with AA and DMAA were prepared and characterized by ^{1}H NMR and viscometry. The characteristics are listed in Table 1. Molar percentage of NAAPBA in the copolymers m/(m + n) was calculated from the areas of resonance peaks in the ^{1}H NMR spectra (see Fig. 2) as (d + e)/(c + d + e) or 3(d + e)/a and averaged. DMAA-NAAPBA copolymer could be easily dissolved in water at high concentration of 50 mg mL^{-1}. The AA-NAAPBA copolymer was less soluble in water. It could be completely dissolved, however, in 50 mM NaOH, the pH of the prepared solution being immediately adjusted to the required value of 8.8 by adding 2 M HCl. The freshly prepared solutions were used for the treatment of the urethra lumen (see Section 3.4).

3.2. Interaction of the boronate-containing copolymers with mucin in solution

Interaction between DMAA-NAAPBA(10) and mucin from porcine stomach in 0.1 M sodium bicarbonate buffer, pH 9.0 (I = 0.12 M) resulted in the formation of fine coacervate with gradually increasing turbidity, see Fig. 3, lines 1–3. Intermolecular cross-linking of the mucin macromolecules by the multiple boronate functions of the copolymer is the most probable reason, as the homopolymer of DMAA produced almost no effect on the turbidity of the mucin solution (Fig. 3, line 5). Indeed, the coacervates obtained at all the studied copolymer: mucin ratios could be easily dissolved on the addition of 1 M fructose, a sugar strongly and competitively binding to borate and boronate (van den Berg et al., 1994), see Fig. 3, line 1. This confirmed the specific character of the complex formation.

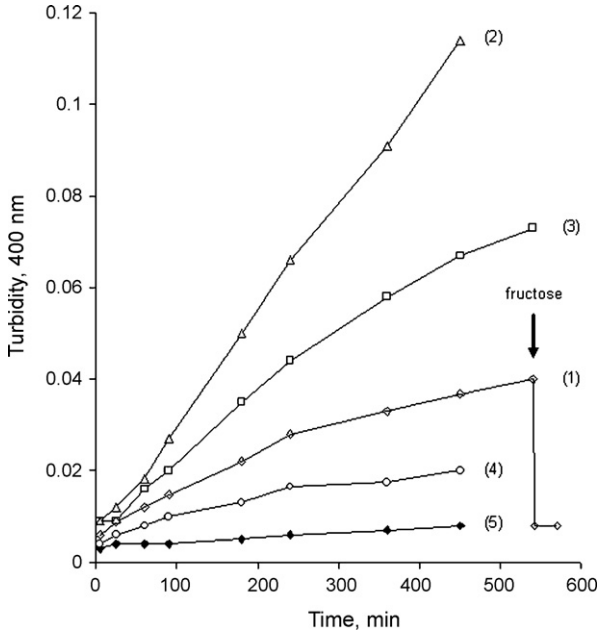

Fig. 3. Changes in turbidity resulting from the interpolymeric complex formation between the boronate-containing copolymers and mucin from porcine stomach in 0.1 M sodium bicarbonate buffer, pH 9.0. Concentration of mucin 0.5 mg mL^{-1}. Concentration of DMAA-NAAPBA(10): 0.1 mg mL^{-1} (◊), 1 mg mL^{-1} (△), 4 mg mL^{-1} (□). Concentration of DMAA-NAAPBA (2.5): 1 mg mL^{-1} (○). Concentration of polyDMAA: 1 mg mL^{-1} (♦). The arrow indicates addition of 1 M fructose (0.1 mL) to the reaction mixture (3 mL).

Development of the coacervate turbidity with time (see Fig. 3) strongly depended on the weight proportion of the polymeric counterparts. Interestingly, the highest rate of coacervation was registered at the intermediate copolymer: mucin weight ratio of 2 (Fig. 3, line 2), whereas the reaction mixtures with the higher (8, line 3) or lower (0.2, line 1) weight ratios displayed a slower increase in turbidity of the coacervates. The observed phenomenon is typical of polyelectrolyte complex formation, where the highest coacerva-

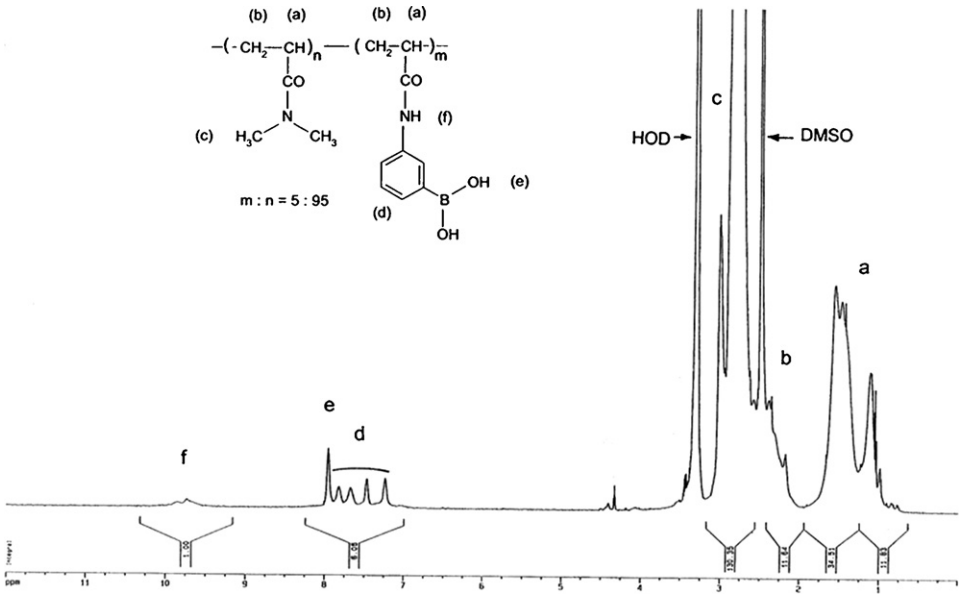

Fig. 2. ^{1}H NMR spectrum of DMAA-NAAPBA(5) copolymer was recorded on a Bruker DRX400 spectrometer operating at 400.1 MHz at 21 °C. The dry copolymer was dissolved in dimethylsulphoxide-D6 at 5 mg mL^{-1}. Chemical shifts are reported as ppm downfield from tetramethylsilane.

tion intensity takes place at the point of stoichiometric equivalency between the oppositely charged groups (Smid and Fish, 1988; Izumrudov, 2002). A similar dependence of coacervation rate on the ratio of interacting counterparts was observed for affinity binding of concanavalin A to the pendant groups of α-D-glucopyranoside chemically attached to Eudragit S-100, a water-soluble synthetic polymer (Linné Larsson et al., 1996). The decrease of coacervation rate in response to the increasing concentration of the polymer above its equivalence concentration was interpreted by the authors as product inhibition. Recently, similar maxima of coacervation intensity as a function of polymer concentration were reported by Fefelova et al. (2007) who studied reversibility of intermolecular interactions between mucin and amphiphilic cationic copolymers. We presume that a large excess of DMAA-NAAPBA(10) over mucin taken in the present study, resulted in a dense population of a mucin macromolecule with the copolymer chains, which resisted the bridging between the neighboring coacervate particles and, therefore, inhibited the coacervation process. The inhibition was reflected by the sigmoid shape of the kinetic curve (Fig. 3, line 3). It is worth noting that the reaction of 1 mg mL^{-1} DMAA-NAAPBA(2.5), a copolymer with low content of PBA groups, with mucin, resulted in the lowest rate of coacervation (Fig. 3, line 4), though the latter reaction mixture contained a higher overall concentration of PBA compared to that containing DMAA-NAAPBA(10) at 0.1 mg mL^{-1} concentration, see Fig. 3, line 1. Obviously, the high molar percentage of the boronate monomer units in the copolymer contributes to the multipoint character of the copolymer binding to mucin and results in a faster formation of the coacervate particles.

Formation and growth of coacervate particles could be detected by dynamic light scattering as soon as in 5 min after mixing the ingredients. The solutions of individual polymers: DMAA-NAAPBA(10) and mucin taken at the same concentrations as in the reaction mixture (1 and 0.5 mg mL^{-1}, respectively), did not produce a sufficiently high count rate for particle size determination, under the experimental conditions. The effective hydrodynamic particle diameter remained in the range from 135 to 175 nm during the first 50 min of measurement and exhibited a polydispersity index of 0.4 ± 0.1. The particle diameter increased further with time and reached the values of 520 ± 100 nm in 19 h, and 730 ± 120 nm in 42 h. The profiles of light scattering intensity as functions of particle diameter, obtained at different reaction times are illustrated in Fig. 4. It is relevant to note that polydispersity index of the coacervate particles did not increase with time, but remained in the range of 0.3–0.4. Most likely, affinity association of the polymer chains took place on the surface of the existing particles rather than resulted in the formation of new particles. If the new small particles appeared at the later stages of the process, they would have caused an increasing polydispersity index. Monotonous growth of nanoparticles formed by polymeric glycoconjugates and complementary lectins has been registered earlier by Linné Larsson et al. (1996).

The reaction of DMAA-NAAPBA copolymers with mucin was carried out at pH 9.0, corresponding to nearly equal fractions of negatively charged and neutral groups of phenylboronic acid in the copolymers exhibiting the apparent pK_a = 9.0 ± 0.2 (Kuzimenkova et al., 2006). It is worth noting that formation of the affinity intermolecular complexes takes place between two negatively charged polymers. Mucin from porcine stomach is negatively charged at pH 6 (Hong et al., 2005) and, therefore, should bear a negative charge under the conditions of experiment (pH 8.0 and 9.0). Indeed, electrophoretic mobilities of the copolymer and mucin in 0.1 M sodium bicarbonate buffer, pH 9.0, were found to be $-(7.3 \pm 1.1) \times 10^{-5}$ and $-(4.9 \pm 1.1) \times 10^{-5}$ cm^2 s^{-1} V^{-1}, respectively. At low ionic strength of 10 mM sodium bicarbonate buffer, pH 9.0 (I = 0.012 M), electrophoretic mobility of mucin was higher: $-(11.2 \pm 1.7) \times 10^{-5}$ cm^2 s^{-1} V^{-1} because the lower fraction of the polymer charge was compensated by the counterions. Hence, electrostatic repulsion, opposing affinity binding, is a likely explanation for the low coacervation intensity (Fig. 5, line 3). Similarly, electrostatic repulsion between negatively charged carboxylic groups was shown to prevent coacervation of immune complexes, if both the antigen and the antibody were each conjugated with strongly ionized polymethacrylic acid, as reported by Muronetz et al. (2000).

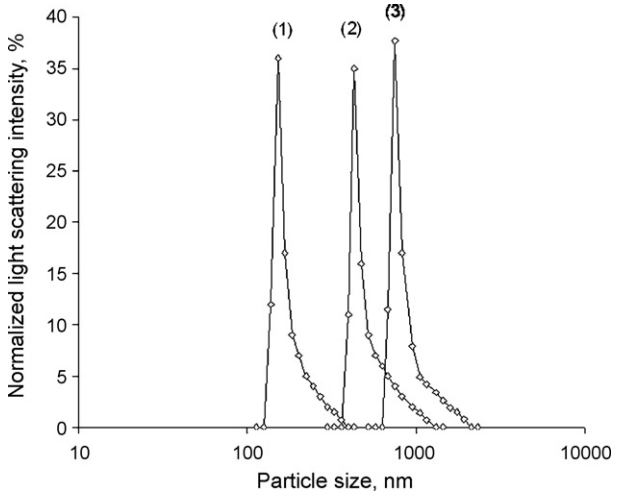

Fig. 4. Normalized light scattering intensity as a function of the particle size of DMAA-NAAPBA(10) – mucin coacervates obtained in 50 min (1), 19 h (2) and 42 h (3) after the reaction start, in 0.1 M sodium bicarbonate buffer, pH 9.0.

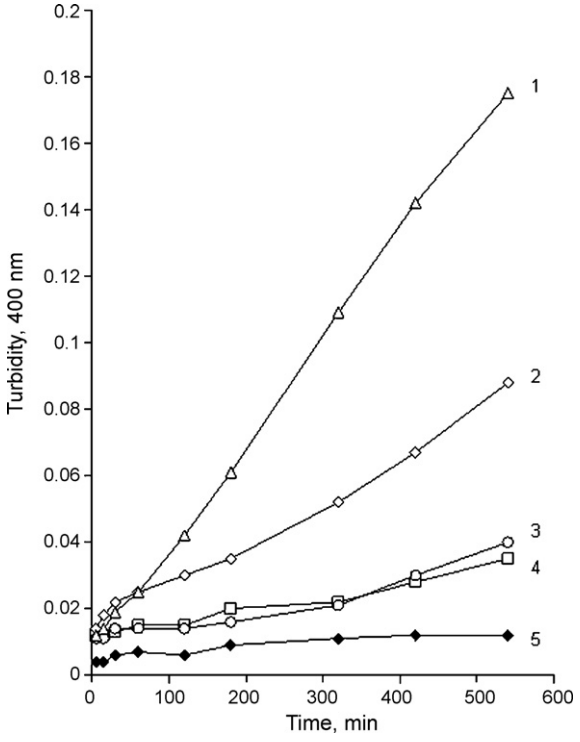

Fig. 5. Changes in turbidity resulting from the complex formation between DMAA-NAAPBA(10) and mucin from porcine stomach at different pH and ionic strength. (1) 10 mM sodium bicarbonate buffer, containing 0.15 M NaCl, pH = 9.0, I = 0.16 M; (2) 0.1 M sodium bicarbonate buffer, pH = 9.0, I = 0.12 M; (3) 10 mM sodium bicarbonate buffer, pH = 9.0, I = 0.012 M; (4) 0.1 M sodium phosphate buffer, pH = 8.0, I = 0.19 M; (5) polyDMAA, 0.1 M sodium bicarbonate buffer, pH = 9.0, I = 0.12 M. Concentration of mucin 0.5 mg mL^{-1}. Concentration of DMAA-NAAPBA(10) or polyDMAA: 1 mg mL^{-1}.

The rate of mucin-copolymer coacervation increased with increasing ionic strength at pH 9.0 (see Fig. 5, lines 1 and 2). At pH 8.0 the coacervation intensity was low (see Fig. 5, line 4), in spite of the relatively high ionic strength of 0.19 M. The low fraction of the anionic PBA groups in the copolymer at pH 8.0 (ionization degree $\alpha = 0.2$, according to Kuzimenkova et al., 2006) was a probable reason for the low reactivity. Apparently, formation of the coacervate particles required a high fraction of the negatively charged PBA groups (i.e. a relatively high ionization degree of the copolymer determined by pH) as well as a high ionic strength needed to diminish the electrostatic repulsion between the similarly charged macromolecules of DMAA-NAAPBA copolymer and mucin.

Mutual repellency of the negatively charged polyelectrolytes within the affinity interpolymer complexes is a probable reason for their facile disintegration in the presence of fructose, in 0.1 M sodium bicarbonate buffer (see Fig. 3). Electrophoretic mobility of the coacervate particles could be estimated directly in the mixture of the polymeric reagents, due to a large excess of the counting events registered at long reaction times (42 h) over those produced by the solutions of individual polymers. The electrophoretic mobility of $-(5.9 \pm 0.3) \times 10^{-5}\ cm^2\ s^{-1}\ V^{-1}$ was intermediate between those of DMAA-NAAPBA(10) and mucin in 0.1 M sodium bicarbonate buffer, pH 9.0. The corresponding zeta-potential of the particles was calculated according to the Helmholtz–Smoluchowsky equation as -7.5 ± 0.4 mV.

Mucoadhesive properties of cationic polymers like chitosan (He et al., 1998) or synthetic methacrylate copolymers (Fefelova et al., 2007) are based on multiple electrostatic interactions between the oppositely charged functional groups of the polymers and mucin. Stability of their interpolymer complexes was maximal at low ionic strengths and decreased at the higher ionic strength of 0.2 M (He et al., 1998). Mucoadhesivity of the PBA-containing polymers exhibited the opposite pattern with well-exhibited coacervation at $I = 0.16$ M, see Fig. 5, that is close to physiological values. This property may be considered as an advantage of PBA-containing polymers as mucoadhesive reagents.

3.3. Occlusion of lumen in silicon rubber tubing and pig urethra by poly(vinyl alcohol) gel

Poly(vinyl alcohol) readily forms gels with borate ions in alkaline (Shibayama et al., 1988; Wise and Weber, 1995), weak alkaline and neutral (Shibayama et al., 1988; Ivanov et al., 2004) aqueous media. These gels are clear, elastic and thermoreversible (Shibayama et al., 1988). Their elastic modulus and the melting points increase with pH due to the increasing number of chemical cross-links in the gel network (Ivanov et al., 2004). In the present study we have occluded the lumens of silicon tubing and excised urethra organs with the gel prepared by mixing of 0.1 M $Na_2B_4O_7$ (pH 9.3) and 5% (w/v) PVA taken in equal volumes. The gels formed immediately on mixing of the components and after some rest exhibited an elastic modulus of ca. 2 kPa (Ivanov et al., 2004).

Injection of the PVA-borax system was performed as illustrated in Fig. 1b (step 2) and resulted in the formation of a gel plug, which enabled a tight and well reproducible sealing of the lumen in silicon tubing (see Section 2.6). The plug withstood easily the pressure of 4 mPa and allowed no leakage of 0.15 M NaCl for at least 1 h. The attempts to achieve the same tight plugging in the urethra lumen were unsuccessful. Injection of the PVA-borax gelling system typically decreased the flow rate of 0.15 M NaCl through the lumen 30–100-fold, compared to the unsealed organ, but could not prevent the leakage completely (see Fig. 6, line 2). The gel plug mechanically extracted from the lumen is shown in Fig. 7. Obviously, the tight lining of mucosa with the gel was not attained, although the gelation within the lumen was successful.

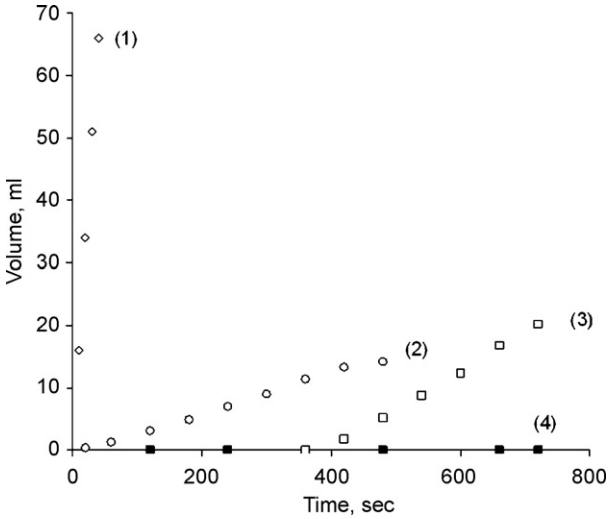

Fig. 6. Patterns of flow resistance exerted by the PVA-borax gels occluding the lumen of urethra: (1) no gel plug; (2) immediate leakage; (3) tight sealing followed by a breakthrough; (4) stable tight sealing.

The weak adhesion of the gel to the mucosal tissue most probably originated in the low effective area of their contact, high elasticity of mucosa and a low friction between the two highly hydrated counterparts. Mucosal tissue is covered by a layer of mucus gel situated above the elastic villous epithelium (Ann Clark et al., 2000). Due to a quick formation of the PVA-borax gel, the individual PVA macromolecules did not have enough time to diffuse into the mucus. Further, their diffusion could be hindered by the exclusion volume effects exerted by the swollen mucus gel. To attain a more intimate contact between the mucosal tissue and the PVA gel, a substance capable of specific binding both to the carbohydrates of mucus and the diols of PVA, was needed. This was the reason why the effect of BCC on the plugging properties of PVA gels has been studied.

3.4. Effect of the boronate-containing copolymers on the occlusion of urethra lumen

The aqueous solutions of the copolymers (5%, w/v, pH 8.8) were injected into the urethra lumen before the injection of PVA-borax system (see Section 2.7). Then the gel plug was formed as described above. The treatment of the mucosal lumen by the solutions of BCC

Fig. 7. The PVA-borax gel plug (1.5–3 mm in thickness) mechanically extracted from pig urethra and placed on a 9 cm (Ø) Petri dish.

Table 2
Effect of polymers on the occlusion provided by PVA-borax gel: number of cases exhibiting different patterns of the flow resistance observed with different polymer samples[a]

Polymer sample	Immediate leakage	Breakthrough within 20 min	No leakage after 30 min	Number of trials
polyDMAA	5	0	0	5
DMAA-NAAPBA(2.5)	5	0	0	5
DMAA-NAAPBA(5)	3	4	3	10
DMAA-NAAPBA(10)	0	5	5	10
AA-NAAPBA(15)	0	3	9	12

[a] There were no leakages observed in the time interval between 20 and 30 min.

exerted a clear effect on the plugging capabilities of the PVA gel. In many cases the lumen could be occluded by the PVA gel tight enough to completely prevent the leakage of 0.15 M NaCl aqueous solution through the organ (Table 2). Fig. 6 illustrates the different patterns of resistance exerted to the flow by the gel plugs. One can distinguish between the immediate leakage (Fig. 6, line 2), tight sealing followed by a breakthrough (line 3) and a stable tight sealing (line 4). The plugs that withstood the pressure for more than 30 min were considered as stable ones. The success of sealing depended, however, on the type of copolymer used for the treatment. The results obtained with different samples of the BCC are summarized in Table 2. Obviously, the success of sealing increased with increasing molar percentage of NAAPBA units in the copolymers. The homopolymer of DMAA produced no effect on sealing, neither did the copolymer with low NAAPBA molar percentage (DMAA-NAAPBA(2.5)). The strongest effect on the efficiency of sealing was produced by AA-NAAPBA copolymer, having the highest amount of NAAPBA units among the copolymers studied, see Table 2. Preparation of the BCC with molar percentage of NAAPBA above 15% is not, however, reasonable as it results in the limited copolymer solubility at pH 7–9. The copolymer AA-NAAPBA(15) exhibited a pH-dependent solubility discussed in detail by Kuzimenkova et al. (2006).

The increased stability of the gel plugs achieved in the presence of BCC can originate in two independent phenomena: first, the sugar-specific adsorption of the copolymers on the mucus gel and, second, much higher relaxation times of the PVA-BCC gels compared to those of PVA-borate gels, prepared under similar conditions such as pH and PVA concentration (Ivanov et al., 2004). The sugar-specific adsorption of BCC at high concentration of the copolymer results in the formation of loop-and-tail structure (Kuzimenkova et al., 2006) capable of the further complex formation with PVA. Most probably, the layers of PVA-BCC gels were situated in between the mucosal tissue and PVA-borax gel. Due to their inherent high relaxation times the intermediate layers might have higher shape stability than that of PVA-borax gel itself.

It is known that the complex formation of borax with PVA as well as with other polyols and sugars shifts pH of the reaction medium to lower values (Lorand and Edwards, 1959). In particular, we have observed that mixing of 40 mM sodium borate buffer (pH 8.6) with 5% (w/v) PVA resulted in the formation of a viscous polymer complex solution with pH 7.4. Though the true pH within a gel is difficult to measure, one may suppose that the pH value in the gel plugs prepared from borax solution of pH 9.3 was below 9. As follows from the literature, the tissues such as nasal mucosal epithelium or corneal epithelium were not affected by treatment with buffers of pH 9 and higher, including borate buffers *in vivo* (Pujara et al., 1995; Tamai et al., 2002). Phosphate buffer solutions within the pH range of 3–10 caused minimal release of the biochemical markers such as lactate dehydrogenase and 5′-nucleotidase from the nasal mucosa. Neither membrane no intracellular damage was caused, therefore, by the treatment with the buffer solutions (Pujara et al., 1995). At first approximation, these observations suggest no adverse effects of PVA-borax gels on mucosal tissues due to the gel's pH, which can be higher than the physiologic one.

It is known from the literature that polyacrylic acid (PAA) displayed the rheological synergy on mixing with solutions of mucin at relatively low pH of 3–4 (Riley et al., 2001). Under these conditions PAA contains mainly uncharged carboxylic groups available for hydrogen bond formation with mucin. Thiolated dervatives of PAA exhibited the maximum total work of adhesion to mucosa at pH 5 (Bernkop-Schnurch and Steininger, 2000). Conversely, the interaction of PBA-containing copolymers with mucin, took place in weakly alkaline media of pH 8–9. In this respect, the mucoadhesives containing borate or boronate functions are complementary to the conventional mucoadhesive polymers. Besides of the occlusion of mucosal lumens, the BCC may have advantages for the treatment procedures demanding for slightly alkaline media. For example, enhanced permeability of buccal mucosa for some drugs at pH > 8 (Shojaei et al., 1998) might be achieved using the BCC as a drug deliverer. At the same time, the sensitivity of gels to food sugars like fructose or glucose may oppose their functions in the sugar-containing environments, such as that of the oral cavity. The suggested applications may be limited to the short (few hours) treatments, in the above environment.

3.5. Dissolution of the gel plugs in vitro and within the mucosal lumen

Fructose forms relatively stable complexes with borate: the association constant K_{ass} = 1700 M^{-1} (van den Berg et al., 1994) is incomparably higher than the association constant of borate with

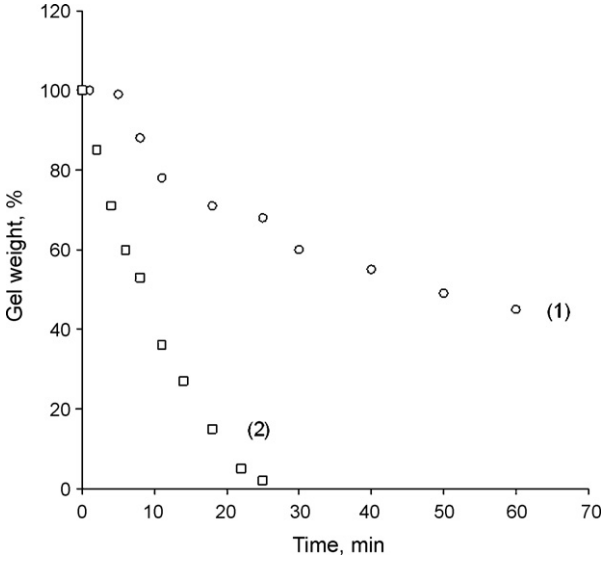

Fig. 8. Dissolution of PVA-borax gel (0.7 g) in 10 mL of 0.15 M NaCl (1) or 1% (w/v) solution of fructose in 0.15 M NaCl (2). The graph depicts a wet gel weight as a function of time.

PVA diols: K_{ass} = 2.8 M^{-1} (Wise and Weber, 1995). This is the reason why fructose strongly competes with PVA for binding to borate. Practically, the PVA-borax gels can be dissolved in 5% (w/v) fructose solution in 0.15 M NaCl, but can not be dissolved completely in 0.15 M NaCl (see Fig. 8), because of the gel shrinking in the presence of salt (Keita et al., 1995). Injection of 5% (w/v) aqueous fructose solutions was used for dissolution of the PVA-borax gel plugs as described in Section 2.8.2. After opening the pressure valve (see Fig. 1a), the slimy remains of the PVA-borax gel together with the fine injection tubing were withdrawn from the lumen by the flow of 0.15 M NaCl and by gravity. Dissolution of the gel plug was attained in relatively short time (4–5 min) in physiologically acceptable conditions. The facile disintegration of PVA-borate and PVA-boronate gels in the presence of high affinity sugars provides a good basis for temporal occlusion of mucosal lumens or cavities in humans.

4. Conclusions

We have developed a gel-based system for tight and reversible occlusion of mucosal lumen assisted by boronate-containing copolymers (BCC). The copolymers were shown to form interpolymer complexes with soluble mucin at pH 9.0 and ionic strength of the solution above 0.1 M. The complexes could be easily disintegrated in the presence of fructose, a sugar capable of strong binding to boronate groups. Similarly, dissolution of the gel plugs formed by poly(vinyl alcohol) and borax, chemically attached to the mucosal surface by means of BCC, could be easily attained. The described technique may be useful for temporal occlusion of mucosal lumens in living organisms. In contrast to the conventional mucoadhesive polymers like polyacrylic acid, its thiolated derivatives or chitosan, the BCC display their mucoadhesivity at weakly alkaline pH of 8–9 and physiological ionic strength. This may create possibilities for new approaches to the controlled mucosal drug delivery.

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