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Mucoadhesive interactions of amphiphilic cationic copolymers based on [2-(methacryloyloxy)ethyl]trimethylammonium chloride

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Abstract

Three series of water-soluble cationic copolymers have been synthesised by free-radical copolymerisation of [2-(methacryloyloxy)ethyl]-trimethylammonium chloride (MADQUAT) with methyl acrylate (MA), butyl acrylate (BA) and butyl methacrylate (BMA). The interactions between these copolymers and porcine stomach mucin have been studied in aqueous solutions using dynamic light scattering, zeta-potential measurements, turbidimetric titration and transmission electron microscopy (TEM). It was demonstrated that mixing aqueous dispersions of mucin with solutions of the cationic copolymers results in significant changes in size distribution and zeta-potential of its particles. It was found that an increase in the content of hydrophobic groups in copolymers leads to more efficient adsorption of macromolecules on the surface of mucin particles, which evidences the importance of hydrophobic effects in mucoadhesion. The efficiency of mucoadhesive interactions was found to be significantly dependent on pH, which affects the surface charge and aggregation stability of mucin.

Keywords: Cationic polymers; Copolymerisation; Mucins; Mucoadhesion; Hydrophobic effects; Drug delivery

1. Introduction

Over the last decade, mucoadhesive polymers have been widely used in designing drug delivery systems to improve buccal, nasal, oral, ocular and vaginal administration of drugs, and considerable attention has been paid to the development of novel mucoadhesive materials (Lee et al., 2000; Edsman and Hagerstrom, 2005; Grabovac et al., 2005).

Mucus is a fully hydrated viscoelastic gel that covers the surface of the eye, nose, mouth, respiratory tract, cervix and gastrointestinal tract. The most important functions of mucus are to protect epithelial cells from physical and chemical destruction, provide lubrication, act as a wetting agent and modulate water content in the underlying tissue (Yang and Robinson, 1998). Mucins are the major components responsible for the gel-like structure of the mucus. They are divided into two classes, membrane bound and secretory forms, and each type has characteristic structure-function relationships at specific tissue locations. Mucins are large molecules with molecular weights ranging from 0.5 to over 20 MDa. Most mucins carry a net negative charge due to the presence of sialic acids and ester sulphates at the terminus of some sugar units (Harding, 2003).

Mucoadhesion is a complex phenomenon that is not fully understood (Edsman and Hagerstrom, 2005; Smart, 2005). There are several general theories that have been used to explain mucoadhesion phenomena and it is believed that specific interactions between macromolecules of a dosage form and mucins play an important role.

Typical polymers that exhibit good mucoadhesive properties are poly(acrylic acid) (PAA) and its weakly cross-linked derivatives such as Carbopol® and Noveon AA-1 (Polycarbophil), sodium salt of carboxymethylcellulose (NaCMC) and chitosan. PAA and NaCMC are believed to have excellent mucoadhesive characteristics due to strong hydrogen bonding with mucins (Park and Robinson, 1987; Mortazavi et al., 1992; Patel et al., 2003). Good mucosal adhesion of chitosan was reported to be due to its cationic nature, which facilitates electrostatic attraction with negatively charged mucins (Rossi et al., 2000, 2001). However, taking into consideration the carbohydrate nature of chitosan and the presence of a number of hydroxyl groups in its

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structure one can expect a significant contribution of hydrogen bonding into mucoadhesive interactions. The role of non-ionic interactions in mucosal adhesion of chitosan is also confirmed by the findings of Snyman et al. (2003), who reported that quaternisation of amino-groups decreased its mucoadhesive properties. If it would be just electrostatic attraction between chitosan and mucins the quaternisation should have resulted in better mucoadhesive properties.

Recently Keely et al. (2005) have studied the mucosal adhesion of synthetic cationic polymer–poly[2-(dimethylamino-ethyl)methacrylate] (PDMAEMA) with varied levels of quaternisation (10, 24 and 32%) towards human mucus-secreting and non-mucus secreting intestinal cell monolayers. It was demonstrated that in the case of unquaternised and 24% quaternised derivatives, the polymers were significantly more mucoadhesive than N-trimethylated chitosan. The presence of non-ionic interactions between mucins and both N-trimethylated chitosan and partially quaternised PDMAEMA cannot be completely ruled out because these polymers have functional groups able to form hydrogen bonds (primary amino- and hydroxyl groups for chitosan and dimethylamino-groups for PDMAEMA).

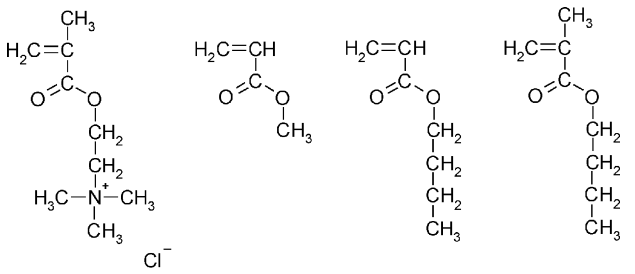
In the present study, we have attempted to synthesise cationic copolymers, for which hydrogen bonding can be excluded completely and only electrostatic forces and/or hydrophobic effects can be expected to contribute to polymer–mucin interactions. Three series of amphiphilic cationic copolymers were synthesised by free-radical copolymerisation of [2-(methacryloyloxy)ethyl]trimethylammonium chloride (MADQUAT) with methyl acrylate (MA), butyl acrylate (BA) and butyl methacrylate (BMA). MADQUAT is a cationic monomer, whose charge does not depend on the environmental pH.

2. Materials and methods

2.1. Materials

MADQUAT was purchased from Aldrich, Ltd. (USA) in the form of 75% aqueous solution and was purified twice by precipitation into acetone. The precipitate was dried in a vacuum oven at 45 °C until a constant weight was obtained, and it was stored in a desiccator at 5 °C.

MA, BA and BMA were purchased from Aldrich, Ltd. and were purified from stabiliser by distillation under vacuum.



MADQUAT MA BA BMA

Azoisobutyronitrile (AIBN) was purchased from Aldrich, Ltd. and was re-crystallized from ethanol before use.

Mucin from porcine stomach (type III) was purchased from Sigma–Aldrich (UK) and used as received. It was stored at −20 °C prior to use.

2.2. Copolymerisation

The copolymers of MADQUAT with MA, BA and BMA were synthesised by free-radical copolymerisation in water–ethanol solutions (30:70 vol.%) in the presence of AIBN at 60 °C for 3 h. The concentration of AIBN was 5×10^{-3} mol/L. Before the copolymerisation the monomer mixtures with AIBN were bubbled with argon for 10 min and sealed in glass ampoules. The concentration of the monomers in the feed mixture was 10%. All copolymers were purified by double re-precipitation into ethyl acetate with subsequent drying under vacuum until a constant weight was achieved. In order to remove the traces of monomers and solvents the copolymers were re-dissolved in water and then dialysed against 5 L of distilled water (5 changes over 48 h). The dialysis membranes with molecular weight cut-off 12–14 kDa purchased from Medicell International Ltd. (UK) were used for this purpose. The purity of the copolymers was assessed using ^{1}H NMR spectroscopy. The composition of the copolymers was calculated based on the nitrogen content, determined using Elemental Analyzer CE-440 (Exeter Analytical Ltd., USA).

2.3. Determination of copolymer molecular weights

The molecular weights of selected BA–MADQUAT samples were determined by static light scattering using Malvern Zetasizer Nano-S (Malvern Instruments, UK). These experiments were carried out in 0.5 M NaCl solutions. The refractive index increments (dn/dc) of these copolymers in solutions were determined using thermostated Abbe refractometer at 25 °C.

2.4. Preparation of solutions

Deionised water was used for preparation of all solutions. The pH of solutions was adjusted by addition of small amounts of 0.5 M HCl and measured with a pH meter (Metrohm, Switzerland). The solutions of mucin were prepared by dispersing the required amounts of mucin in deionised water (pH 6.8) and acidified water (pH 2.0) to form stable colloidal suspensions (1 mg/mL), which were subsequently sonicated for 20 min and centrifuged at 300 rpm for 5 min. The supernatant solutions were retained and used for further experiments. Freshly prepared mucin dispersions were used in all experiments.

2.5. Viscosity of polymer solutions

The viscosity of copolymer solutions in water was measured with an Ubbelohde type viscometer at 25 ± 0.2 °C with a flow time for distilled water of 105 s. For each solution three consecutive measurements were performed and the average values were then calculated and reported.

2.6. Transmission electron microscopy (TEM)

TEM images of mucin and mucin–copolymer mixtures were acquired using Philips CM20 Analytical TEM. For sample preparation the cooper grids were brought in 30 s contact with aqueous dispersions of pure mucin and mucin mixed with copolymers, and then they were treated with 2% of phosphotungstic acid for 10 s and finally dried off with a filter paper.

2.7. Size- and zeta-potential measurements

Particle size measurements of mucin and mucin-polymer solution mixtures were performed using Malvern Zetasizer Nano-S (Malvern Instruments). Zeta-potential of mucin and its mixtures with polymers was determined using Malvern Zetasizer 3000HS (Malvern Instruments). All experiments were performed at 25 °C and every measurement has been repeated at least three times and the averaged titration curves are presented.

2.8. Turbidity measurements

Turbidity of mucin and mucin-polymer solutions was measured at 400 nm using Perkin-Elmer Lambda 25 UV/vis spectrometer. Every titration was repeated at least three times and the averaged results are reported.

3. Results

3.1. Synthesis and characterisation of amphiphilic copolymers

The copolymers of MADQUAT with MA, BA and BMA were synthesised by free-radical copolymerisation initiated by decomposition of AIBN for 3 h at 60 °C. A mixture of ethanol and water (70:30 vol.%) was chosen as a reaction medium to provide a complete solubility of both water-soluble MADQUAT and water-insoluble MA, BA and BMA. The resulting copolymers were also fully soluble in this solvent mixture. The copolymerisation was terminated by cooling down the reaction mixture and its immediate precipitation into ethyl acetate. After complete purification of the copolymers their compositions were analysed by determination of nitrogen content. The data on copolymers compositions are listed in Table 1. The analysis of the compositions of the copolymers reveals that MADQUAT undergoes completely random and azeotropic copolymerisation with MA. With the exception of 10:90 mol.% ratio, the copolymers of MADQUAT with BA are slightly enriched with MADQUAT. However, the copolymers of MADQUAT with BMA have excessive amount of the hydrophobic monomer compared to the feed mixture. The difference in reactivity of MA, BA and BMA towards MADQUAT may be related to different affinity of these monomers to the solvent medium.

A visual examination of the solubility of the synthesised copolymers was performed by placing small amounts of each sample (0.1–0.2 mg) in 10 mL of water and *n*-butanol and observation whether the polymers form a clear solution or remain insoluble or swollen within 24 h. The data obtained show that irrespectively on the copolymer composition all MADQUAT–MA samples are soluble in water and insoluble in *n*-butanol. The copolymers based on MADQUAT and BA remain water-soluble when BA content is less than 60 mol.% and the samples containing more BA can be dissolved in *n*-butanol only. The aqueous solubility of MADQUAT–BMA is even lower: only samples with less than 65 mol.% of BMA can be fully dissolved in water, the rest samples are soluble in *n*-butanol. These observations can be logically explained by the fact that the hydrophobicity of the co-monomers increases in the following order: MA > BA > BMA.

In order to estimate the macromolecular dimensions of these copolymers in aqueous solutions we have determined the intrinsic viscosity values for all water-soluble samples. Since all the synthesised copolymers are polyelectrolytes the determination of intrinsic viscosity can be performed only in aqueous solutions containing inorganic ions. The presence of inorganic ions in solutions results in a suppression of the polyelectrolyte effect, which leads to additional swelling of macromolecular coils upon dilution and does not allow extrapolation of reduced viscosity values to zero concentration in order to get the intrinsic viscosity. A complete suppression of the polyelectrolyte effect for MADQUAT–MA copolymers was observed in 0.1 M NaCl solutions; however the copolymers MADQUAT–BA and MADQUAT–BMA required higher salt concentrations (0.5 M NaCl). It can be seen from the data obtained that an increase in MADQUAT content in copolymers always leads to higher values of intrinsic viscosity, which is likely related to more unfolded macromolecular conformations due to the higher charge density. Similar trend was previously reported for the copolymers of MADQUAT with 2-hydroxyethylacrylate (Nurkeeva et al., 2006).

The molecular weights of selected copolymer samples were determined by static light scattering technique. It was found that the weight-average molecular weight of MADQUAT–BA copolymer (57:43 mol.%) is 282 kDa, whereas the molecular weight of MADQUAT–BA (73:27 mol.%) is 229 kDa. The copolymer MADQUAT–BA (73:27 mol.%) with a lower molecular weight (229 kDa) exhibits higher intrinsic viscosity in aqueous solution (0.144 dL/g) compared to MADQUAT–BA (57:43 mol.%), which molecular weight is higher (282 kDa), but viscosity is lower (0.125 dL/g). Hence the growth in solution viscosity upon increase in MADQUAT content in copolymers is indeed due to the higher charge density and not molecular weight changes.

3.2. Interactions between the copolymers and mucin in solution

A commercial sample of lyophilised porcine mucin (type III) has been chosen for the study of the mucoadhesive interactions with the MADQUAT copolymers. This product may differ slightly from the native porcine mucus gel because the purification and storage may result in a partial degradation of glycoproteins and also in formation of disulphide bridges due to the oxidation of thiol-groups in cystein-rich sub-domains. Nevertheless, the commercial mucin is often used in the studies of

Table 1
Compositions of copolymers and their solution characteristics

Feed mixture (mol.%)		Composition of copolymers (mol.%)		Solubility of copolymers		Intrinsic viscosity (η, dL/g)
MADQUAT	MA	MADQUAT	MA	Water	*n*-Butanol	In 0.1 M NaCl
10	90	9	91	s[a]	i[a]	0.150
20	80	18	82	s	i	0.160
30	70	27	73	s	i	0.162
40	60	37	63	s	i	0.170
50	50	47	53	s	i	0.175
60	40	57	43	s	i	0.185
70	30	67	33	s	i	0.180
80	20	77	23	s	i	0.190
90	10	87	13	s	i	0.200
Feed mixture (mol.%)		Composition of copolymers (mol.%)		Solubility of copolymers		Intrinsic viscosity (η, dL/g)
MADQUAT	BA	MADQUAT	BA	Water	*n*-Butanol	In 0.5 M NaCl
10	90	8	92	i	s	–
20	80	25	75	i	s	–
30	70	40	60	s	i	0.115
40	60	50	50	s	i	0.120
50	50	57	43	s	i	0.125
60	40	63	37	s	i	0.127
70	30	73	27	s	i	0.144
80	20	83	17	s	i	0.150
90	10	93	7	s	i	0.152
Feed mixture (mol.%)		Composition of copolymers (mol.%)		Solubility of copolymers		Intrinsic viscosity (η, dL/g)
MADQUAT	BMA	MADQUAT	BMA	Water	*n*-Butanol	In 0.5 M NaCl
10	90	2	98	i	s	–
20	80	8	92	i	s	–
30	70	15	85	i	s	–
40	60	18	82	i	s	–
50	50	25	75	i	s	–
60	40	35	65	s	i	0.115
70	30	45	55	s	i	0.120
80	20	55	45	s	i	0.125
90	10	60	40	s	i	0.127

[a] s, Soluble; I, insoluble.

mucoadhesive dosage forms because its particles have the same functionality (Rossi et al., 2001; Leitner et al., 2003; Takeuchi et al., 2005). One of the advantages of using commercial mucins is a lower batch-to-batch variability compared to those freshly prepared (Rossi et al., 1995).

The particle size of mucin dispersions was determined by dynamic light scattering and the results are presented in Fig. 1(a and b). It can be clearly seen that in both deionised water and acidified water the dispersions of mucin show bimodal size distribution. In water the smaller particles have a mean diameter 133 ± 30 nm and the larger particles 464 ± 61 nm. At pH 2.0 the mucin tends to aggregate further and form particles having the mean sizes 368 ± 27 nm and 2167 ± 929 nm, respectively. According to the mucin manufacturer it contains about 1% of bound sialic acids, which ionization is pH-dependent. Under acidic conditions the ionization of sialic acids is partially suppressed, which decreases the colloidal stability of mucin and promotes its further aggregation into larger particles.

In order to get a deeper insight into the structure of mucin particles we have examined their aqueous dispersions by TEM (Fig. 2). The TEM data confirm the polydisperse nature of mucin, which is represented by larger and smaller particle populations. It is likely that larger particles result from the aggregation of smaller particles. The sizes of both particle populations obtained by TEM method are in a good agreement with the results of dynamic light scattering. Previously different researchers have also reported the polydisperse nature of mucins, their tendency to aggregation and pH-dependent sol–gel transitions (Cao et al., 1999; Waigh et al., 2002; Bansil and Turner, 2006). The structure of freshly isolated porcine gastric mucin has also been previously studied by Fiebrig et al. (1995) using TEM. They reported the existence of mucins in the form of highly swollen regions (50–150 nm), joined by nearly linear linker chains (200–400 nm). In our study, we observed quite different structures, which may be due to the difference in mucins origin (freshly isolated against commercial sample) and techniques used for preparation of specimens for TEM imaging. It has also been emphasised by Fiebrig et al. (1995) that the techniques used for isolation of mucins can affect their 3-dimensional architecture significantly.

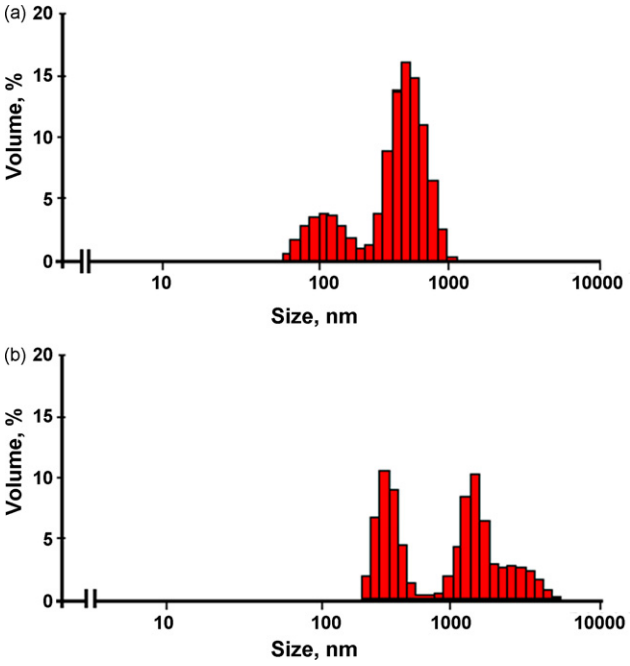


Fig. 1. Size-distributions of 1 mg/mL mucin aqueous dispersions at pH 6.8 (a) and pH 2.0 (b).

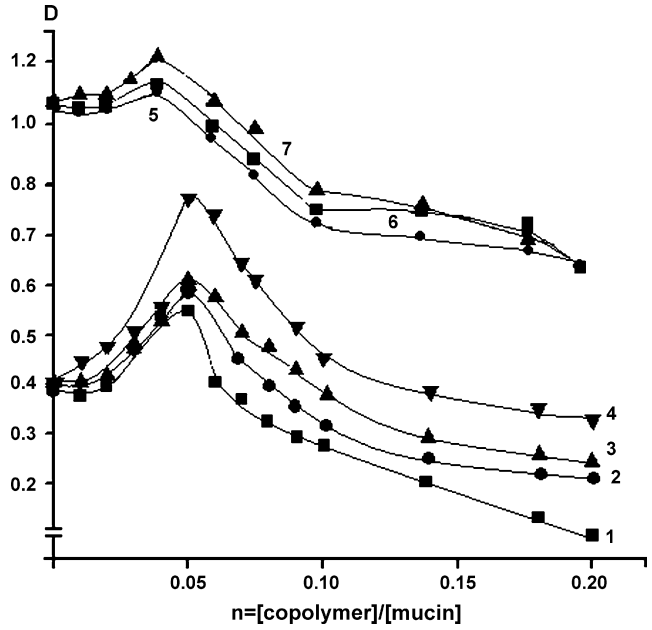


Fig. 3. Turbidimetric titration of 1 mg/mL mucin by 0.5 mg/mL MADQUAT–BA copolymers at pH 6.8 (1–4) and pH 2.0 (5–7). BA content in the copolymers: 7 (1, 7), 17 (2), 27 (3, 6) and 43 mol.% (4, 5).

The interactions between mucin and cationic copolymers were initially studied by turbidimetric titration (Fig. 3). The starting mucin solutions are cloudy at both pH 6.8 and 2.0, but the turbidity of the latter system is higher, which reflects the larger sizes of the aggregates and confirms the sizing results. An addition of MADQUAT–BA copolymers to mucin solutions at pH 6.8 leads to increase in turbidity until the [copolymer]/[mucin] ratio (n) reaches 0.05 and then the D values decrease gradually. An increase in turbidity upon mixing of mucin with the copolymers as well as the shape of the titration curves confirms the presence of specific interactions between the components of the binary system. When the titrations were performed at pH 2.0 similar curves with maxima are observed but their position is slightly shifted to the left, which means that the interaction still exist but a smaller amount of copolymer is needed to saturate the surface of mucin particles. It is interesting to note that when the mucin was titrated by the copolymers at pH 6.8 the higher turbidity values were observed for the copolymers containing more butyl acrylate in the structure, but at pH 2.0 this trend is reverted. Similar trend was found for the titration of mucin by MADQUAT–MA copolymers at pH 6.8, when the higher turbidity values are observed for the copolymers having more MA (data not shown).

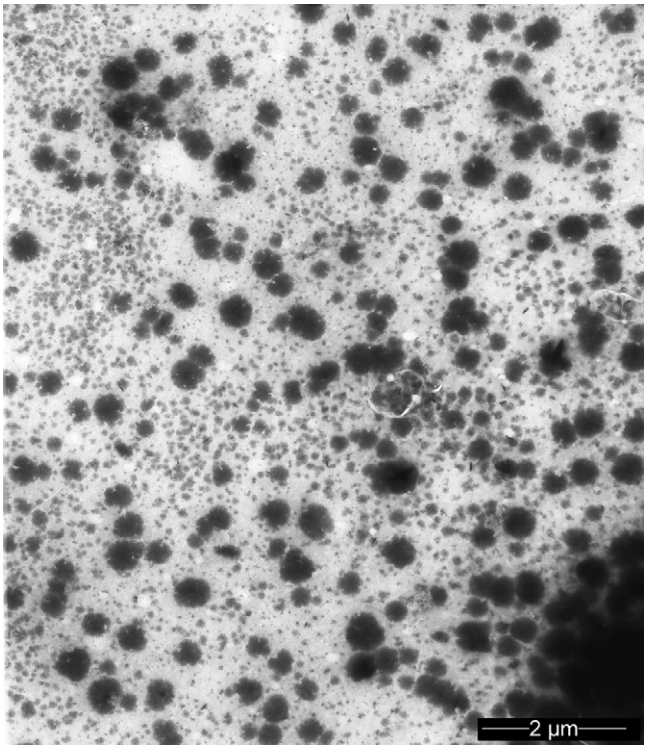


Fig. 2. TEM image of 1 mg/mL mucin aqueous dispersion at pH 6.8.

The effect of addition of MADQUAT–BA (63:37 mol.%) on the particle size distribution of mucin at pH 6.8 is shown in Fig. 4(a–c). The initial bimodal particle size distribution observed for pure mucin becomes more complex upon addition of MADQUAT–BA copolymer. In the mixture, which components ratio corresponds to the maximum on turbidimetric titration curve ($n = 0.056$), we can clearly see the presence of three populations of particles with the size distributions within 20–60 nm, 70–1990 nm and 3580–6440 nm, respectively. Even though the accuracy of the particle size measurements for this highly polydisperse system is expected to be very low, the dramatic change in the particle size distribution is a strong evidence for the existence of specific interactions between the mucin and macromolecules of the copolymer. The further addition of the copolymer to mucin results in the growth of smaller particles population and some narrowing of the size distributions. When the ratio [copolymer]/[mucin] reaches 0.21, which corresponds

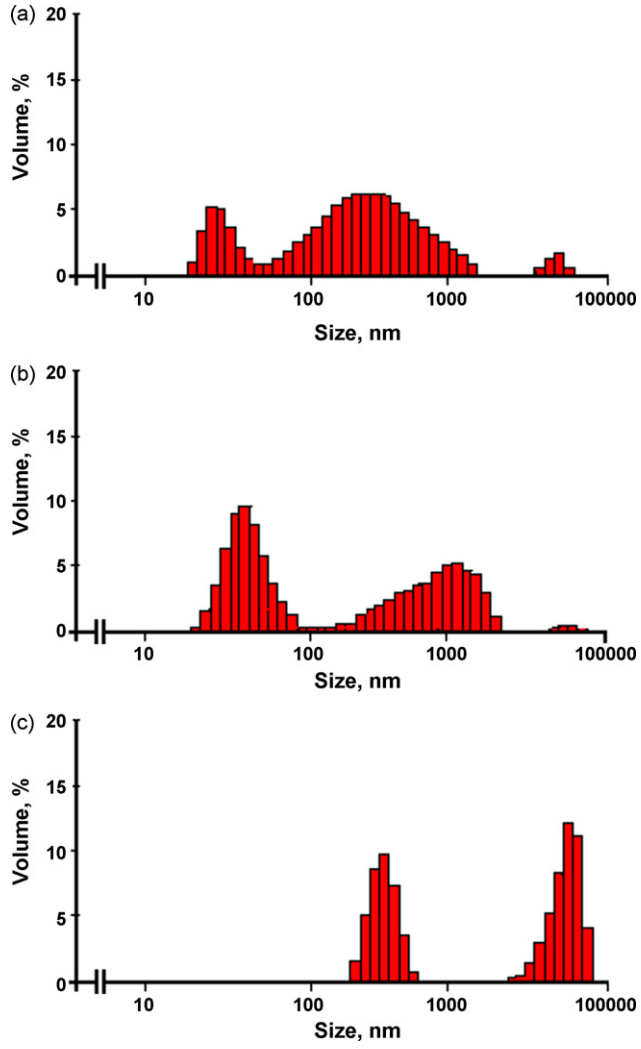


Fig. 4. Size-distributions in mucin–MADQUAT–BA (63:37 mol.%) mixtures with n = [copolymer]/[mucin] = 0.056 (a), 0.125 (b) and 0.21 (c) at pH 6.8. Initial concentrations of mucin and copolymer are 1 mg/mL and 0.5 mg/mL, respectively.

to the turbidity values lower than those of mucin alone, the size distribution becomes bimodal again with occurrence of nanoparticles and microparticles having the mean diameters 288 ± 5 nm and 4267 ± 379 nm, respectively. Significant changes in particle size distribution were observed for mucin/copolymer mixtures at pH 2.0 and also for copolymers of MADQUAT with MA and BMA (data not shown), confirming the existence of specific interactions. The TEM image of the sample obtained by mixing the interacting components at the ratio [copolymer]/[mucin] = 0.125 confirms a more significant proportion of smaller particles over larger aggregates (Fig. 5).

The aggregation/de-aggregation phenomena occurring in mucin/copolymer mixtures can be explained by specific interactions and adsorption of macromolecules on the mucin surfaces (Fig. 6). The mucin alone can be considered as a mixture of particles having different aggregation state. There are smaller particles and larger aggregates. When copolymer is added to mucin until the [copolymer]/[mucin] ratio reaches 0.05 the macromolecules adsorb on mucin surface and cause further

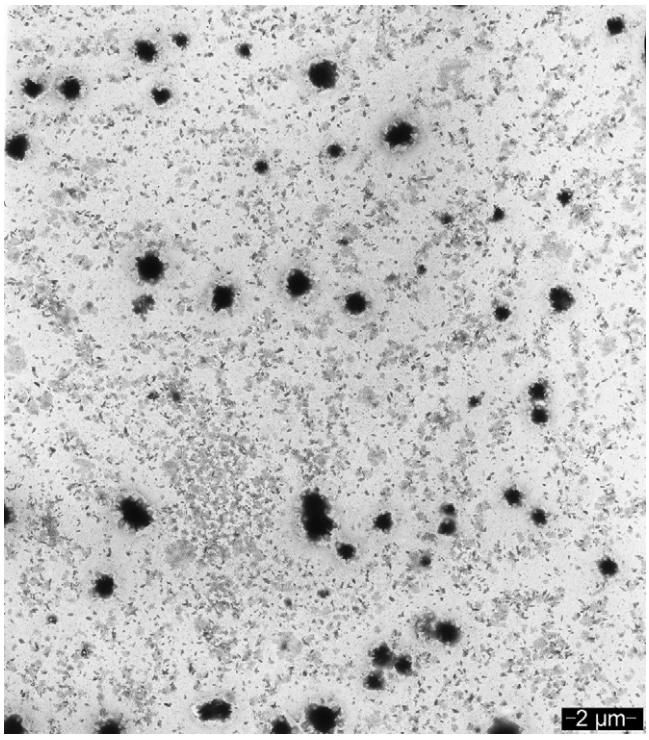


Fig. 5. TEM image of mucin–MADQUAT–BA 63:37 mol.% mixture (n = [copolymer]/[mucin] = 0.125) at pH 6.8. Initial concentrations of mucin and copolymer are 1 mg/mL and 0.5 mg/mL, respectively.

aggregation due to formation of polymeric bridges between smaller particles. In this case, we observe an overall increase in a sample particle size and broadening of the size distribution. At the same time the mucin particles, which have more complete coverage by macromolecules tend to de-aggregate due to electrostatic repulsion. When the [copolymer]/[mucin] ratio exceeds 0.05 we have more mucin particles fully covered by macromolecules, which leads to de-aggregation of larger particles.

In order to confirm the above-mentioned model we have studied the interactions between mucin and the copolymers using zeta-potential measurements. Previously Takeuchi et al. (2005) have reported the possibility of using this method to investigate the interactions between mucins and polymers. Fig. 7(a and b) shows the changes in zeta-potential of mucin particles upon addition of MADQUAT–BA copolymers at pH 6.8 and 2.0, respectively. The zeta-potential of mucin alone is

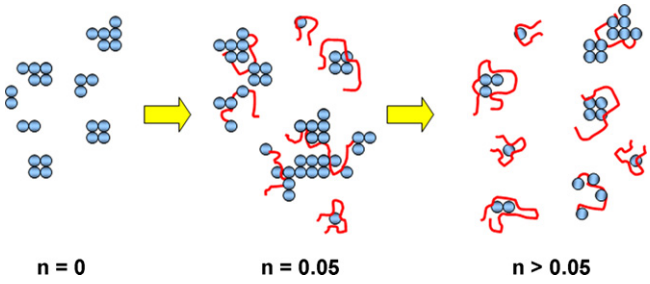


Fig. 6. Aggregation/de-aggregation phenomena in mucin–copolymer mixtures at different ratios n = [copolymer]/[mucin].

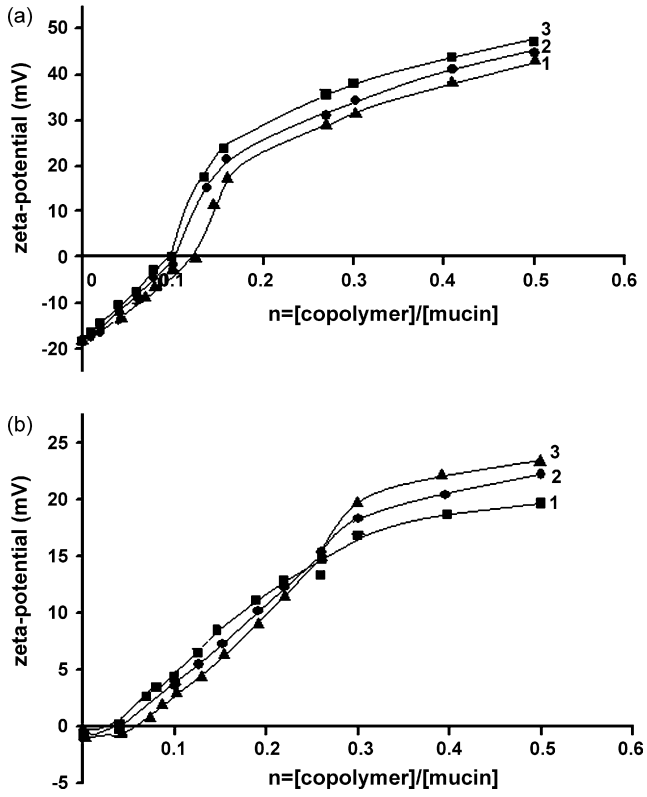


Fig. 7. Zeta-potential of copolymer–mucin mixtures at pH 6.8 (a) and pH 2.0 (b). Initial concentrations of mucin and copolymer are 1 mg/mL and 0.5 mg/mL, respectively. BA content in the copolymers: 7 (1), 27 (2) and 43 mol.% (3).

-18.7 ± 0.6 mV, which is in a good agreement with the data, previously reported by Takeuchi et al. (2005). The negative charge of mucin particles can be explained by the presence of 1% of bound sialic acid. At pH 2.0 the ionisation of sialic acid is suppressed and the zeta-potential of mucin approaches zero (-0.6 ± 0.2 mV). An addition of the copolymers to mucin at pH 6.8 results in increase of its zeta-potential and when the [copolymer]/[mucin] ratio exceeds 0.10–0.13 the particles become positively charged. It should be noted that the position of isoelectric point of copolymer–mucin mixture is higher than the position of the maxima on turbidimetric titration curves, which confirms our hypothesis about the bridging effect as a reason of mucin particles aggregation. At maximal turbidity the adsorbed macromolecules form bridges between mucin particles causing their aggregation, but at the same time the total zeta-potential of the aggregates is still negative because of incomplete coverage of their surface with copolymers. The further addition of the copolymers leads to more macromolecules adsorbed on mucin surface, which finally results in recharging. The effect of the content of BA in copolymers on the turbidimetric results can also be observed from zeta-potential data: higher BA content in the copolymers results in faster recharging of mucin particles, which can only be explained by better adsorption of more hydrophobic macromolecules on the particle surfaces. The role of non-ionic interactions in mucin–copolymer interaction is also clearly seen from the data of zeta-potential obtained at pH 2.0. The initially non-charged mucin particles acquire positive charge upon addition of the copolymers.

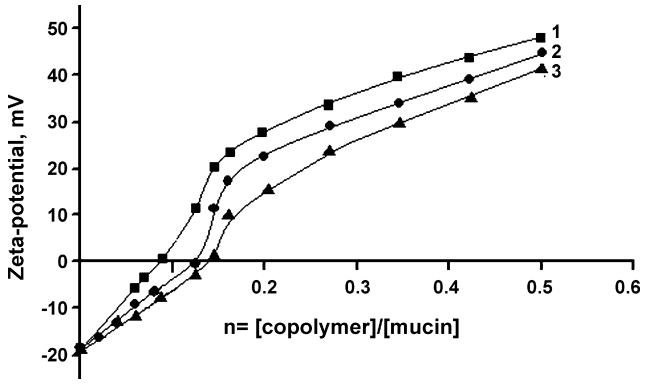


Fig. 8. Zeta-potential of copolymer–mucin mixtures at pH 6.8. Initial concentrations of mucin and copolymer are 1 mg/mL and 0.5 mg/mL, respectively. Copolymers: MADQUAT–MA 47:53 mol.% (1), MADQUAT–BA 57:43 mol.% (2) and MADQUAT–BMA 55:45 mol.% (3).

A comparison of the efficiency of mucoadhesive interactions by the copolymers composed of different hydrophobic co-monomers is shown in Fig. 8. Even though the strict comparison between the copolymers having different hydrophobic co-monomers is complicated because of the difference in copolymers composition we can arrange them in the following order: MADQUAT–MA > MADQUAT–BA > MADQUAT–BMA by their ability to recharge mucin. The copolymers based on less hydrophobic co-monomers in this case recharge mucin surfaces more efficiently. These findings indicate on the high complexity of the interactions between mucins and polymers. One of the possible reasons of this opposite trend can be the flexibility of macromolecules. It is known that the better chain flexibility favors mucoadhesion (Lee et al., 2000; Edsman and Hagerstrom, 2005; Grabovac et al., 2005). Indeed the glass transition temperatures, determined for these copolymers by differential scanning calorimetry, are the following: 114, 117 and 158 °C for MADQUAT–MA, MADQUAT–BA and MADQUAT–BMA, respectively. These glass transition temperatures can serve as an indication of chain flexibility: more flexible polymers have lower glass transition temperatures.

4. Conclusions

Specific interactions between aqueous dispersions of porcine stomach mucin and cationic copolymers of amphiphilic nature result in occurrence of aggregation/de-aggregation phenomena, which may be easily monitored by a number of physicochemical methods traditionally used for investigation of polymer–polymer or polymer–colloid interactions. In the present work, we have demonstrated the presence of these interactions using turbidimetric titration, dynamic light scattering, zeta-potential measurements and transmission electron microscopy.

The mechanism of interactions between mucin and these copolymers involves both electrostatic attraction and hydrophobic adsorption. However, due to a soft matter nature of mucin particles the possibility of macromolecules deeper penetration cannot be completely ruled out. In this connection the difference

in chain flexibility of the synthesised copolymers may play a certain role in mucoadhesion.

The amphiphilic copolymers synthesised in the present work may be potentially used for the design of mucoadhesive dosage forms and the properties of tablets based on MADQUAT copolymers will be reported in our subsequent publications.

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