Mucin/Poly(acrylic acid) Interactions: A Spectroscopic Investigation of Mucoadhesion

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Studies using infrared, ^{1}H and ^{13}C nuclear magnetic resonance, and X-ray photoelectron spectroscopies and differential scanning calorimetry support the hypothesis that hydrogen bonds, formed between the carboxylic acid functionality of the mucoadhesive material poly(acrylic acid) and the glycoprotein component of mucus, play a significant role in the process of mucoadhesion. There are fewer H-bonded interactions between the components than within the bulk of the pure mucoadhesive agent. The pH of the medium influences the structures of both the poly(acrylic acid) and the mucus, which, in turn, determine the nature and the extent of mucoadhesive interactions.

Introduction

Mucus is an insoluble viscoelastic aqueous (≥95% w/w) gel whose main components are glycoproteins, inorganic salts, proteins, lipids, and mucopolysaccharides.[1,2] Gastric mucus contains electrolytes (1%), proteins (0.5−1%), lipids, and glycoproteins (0.5−1%); in nasal mucus these concentrations are higher (electrolytes ≤2%, glycoproteins ≤3%).[3] Rheological studies have shown that the gel properties of mucus are attributable to the glycoprotein component.[4−6] The mucus gel can be disrupted physically (e.g., low shear force, denaturing medium); its structure is a highly entangled system of macromolecular chains, stabilized by a combination of hydrogen bonding, electrostatic interactions, and disulfide linkages.[3,7]

The mucus glycoprotein (mucin) macromolecule consists of four[8,9] or five[10,11] protein subunits (M_r 380−720 kDa[6] (predominant residues for porcine gastric mucus: glutamate, glycine, proline, serine, threonine, and valine[10]) sections of which are covalently bonded with carbohydrate side-chains. Branched oligosaccharides (160−200 per subunit, 50−80% of dry weight; monomers: D-galactose, *N*-acetylgalactosamine, *N*-acetylglucosamine, and terminal L-fucose and *N*-acetylneuraminic acid[1,10,12]) are attached to the polypeptide backbone predominantly by *O*-glycosidic linkages between *N*-acetylgalactosamine (α-1) and serine or threonine.[10] *N*-Acetylneuraminic acid and sulfate groups, the relative proportions of which increase progressively in mucins found along the length of the gastrointestinal tract,[13] confer on them negative charge. The subunits are linked by terminal disulfide bridges, one for each unit with the "bottle-brush" structure (one glycosylated section with a short nonglycosylated protein section, susceptible to enzymatic proteolysis,[8] at one end) and two for "rolling pin" structures which have nonglycosylated sections at both ends.[10] Bottle-brush subunits may also be linked by peptides.

Mucoadhesion, the reversible/partial retention of some viscous liquids by mucus, involves adsorption, wetting, diffusion, and fracture interactions.[3,12,14−18] The development of mucoadhesives, e.g., for drug delivery, has focused on water-soluble polymers containing groups capable of forming polar or hydrogen bonds.[12] Strong adhesion is obtained by the transfer of such a polymer into the crevices of the tissue, i.e., the entanglement of mucus glycoprotein strands with flexible polymer segments and/or the interpenetration of the bioadhesive polymer chains and the glycoproteinic network.[14,19] Interactions are pH-dependent, because of the involvement of ionizable groups.[20] Tobyn et al.[21] have shown that, at low pH, hydrogen bonding between mucus glycoprotein and mucoadhesive poly(carboxylic acid)s is a significant feature of the interaction: at pH ≤ 4.0, the majority of the carboxylic acid groups are available for the formation of conventional head-to-head dimers, whereas at pH > 4, the majority of the carboxylic acid groups of both poly(acrylic acid) and the mucus glycoprotein are ionized. However, from rheological studies, mixtures of mucus with poly(acrylic acid)s exhibit optimum gel strength in the pH range 4−6.[22,23] In connection with the fouling-release properties of mucin, surfactants have also received attention.[24,25] However, H-bonding appears to be less significant in this context.

Although mechanical testing and rheology have been used to classify polymers as mucoadhesives, spectroscopic techniques may provide further insights into the mechanism of bioadhesion. Here we describe the use of infrared (IR), nuclear magnetic resonance (NMR), and X-ray photoelectron (XPS) spectroscopies and differential scanning calorimetry (DSC) to investigate molecular-level interactions between mucin and a pharmaceutically approved poly(acrylic acid), Carbopol 934P. It has been suggested that hydrogen bonding contributes significantly to mucoadhesion. [26]

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Experimental Section

Materials. Carbopol 934P was obtained from B.F. Goodrich, Hounslow, U.K. Phenylmethylsulfonyl fluoride in isotonic aqueous solution (PMSF) was prepared by stirring an ethanolic solution (0.0175% w/v in ethanol; 2 mL) into sodium azide (0.04 g), EDTA (0.372 g), sodium chloride (1.8 g), and potassium thiocyanate (8.552 g) in water (150 mL) and adjusting the total volume to 200 mL.

Mucus and Mucin. Crude mucus (20 porcine stomachs) was stirred with an equal volume of the PMSF solution (4 °C, 24 h) and centrifuged (4 °C, 1.4×10^4 g, 1 h). For homogenized mucus, the separated gel layer was dialyzed (36 h, 4 °C) and stirred (24 h, 4 °C; storage at −20 °C). Alternatively, for mucin, unbound protein was removed[27] by adding aqueous cesium chloride (40% w/w, to density 1.40 g/mL) and centrifuging (12 × 15 mL portions, 1.5×10^5 g; 69 h). Subsequently, each gradient was unloaded in 1 mL fractions from the bottom of the tube; densities and absorbances (280 nm) of each fraction were determined. Fractions containing glycoprotein (Schiff colorimetric assay) were dialyzed (distilled water 2 × 1.25 L; each 24 h, 4 °C; final density 1.0 g/mL), apportioned, and freeze-dried (total mucin 2.95 g, storage −20 °C).

Instrumental Methods. For IR spectroscopy, mixed samples were prepared by combining homogenized mucus or mucin (thawed, pH 4.45, 1.00 or 2.00 g) with aqueous Carbopol 934P (0.5% w/v, pH 2.95, 0.50 or 1.00 g), adjusting the pH (2.1−8.0) and adding water to 4.0 g. Single components and mixed samples were stirred (4 °C, 16 h), freeze-dried (−20 °C, 16 h), and, finally, dried over phosphorus pentoxide. IR spectra (4000−650 cm^{-1}, resolution 4 cm^{-1}) were obtained using KBr disks (2% w/w).

DSC. Samples (10 mg × 3, as prepared for IR spectroscopy, encapsulated) were examined using a Perkin-Elmer DSC-7 instrument (−10 to +150 °C, 10 K min^{-1}, glass transition temperature T_g determined from the second heating run).

NMR. Samples in D_2O (2 mL) were prepared of Carbopol 934P (20 mg, pH = 2.95), mucus (40 mg, pH = 5.45), mucin (20 mg; pH = 6.1), and mixtures of Carbopol 934P (10 mg/mL) with respectively mucus (20 mg/mL, pH = 4.5) and mucin (10 mg/mL, pH = 3.6). To allow for a downshift of the carbonyl resonance (179−185 ppm) with increasing pH,[22] solutions (2 mL) of Carbopol 934P in D_2O adjusted with NaOD (0.1 M) to pH 3.6 and 4.5 were also prepared. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra (37°C) were recorded using a JEOL Eclipse + Spectrometer. A ^{13}C NMR spectrum of the mucus sample could not be obtained.

XPS. Samples (200 mg, as prepared for IR studies) were compressed (1 tonne, 30 s) to form disks (diameter 13 mm). XPS experiments were carried out using a VG Scientific ESCALAB Mk.II instrument (source: nonmonochromatized Al Kα, 1486.6 eV, 250 W; detector pass energy: 20 eV). Atomic proportions of bonded carbon, nitrogen, and oxygen were obtained from the relative areas of the peaks for C1s, N1s, and O1s, using line-shape analysis and standard atomic sensitivity factors.[28] The depth of sampling was less than 10 nm.

Results and Discussion

DSC. For Carbopol 934P and homogenized mucus, T_g values were respectively 130.8 ± 1.0 and 45.3 ± 1.0 °C. The value for Carbopol 934P reflects a relatively high degree of cross-linking in the material used; a value of 24.8 °C has been reported for porcine gastric mucin.[29] For mucus/Carbopol 934P mixtures (ratios 1:1, 1:2, and 1:4; unadjusted pH = 3.6), glass transitions were not detected for the 1:1 and 1:2 combinations, indicating the formation of blended structures. The 1:4 mixture, however, showed a glass transition at 130.0 ± 1.0 °C, indicating that this mixture contained distinct domains of the poly(acrylic acid).

IR spectra were measured of the individual components, Carbopol 934P, homogenized mucus and mucin, and of binary mixtures, both as formed (pH 3.6) and at controlled pH (2.1−8.0). Of the components' spectra, Table 1, the bands for Carbopol 934P are consistent with the macromolecular repeating unit $-(CH_2CH(CO_2H))-$ and those for mucus and mucin are attributable to amino acids or to oligosaccharide groups. Although in the mucus and mucin spectra (Figure 1b,c) the broad band at 3400−3100 cm^{-1} masks many stretching vibrations (O−H, amino N−H, and amide N−H) and the primary amine deformation (expected ≈1600 cm^{-1}) is masked by the stronger amide C═O stretch (1658 cm^{-1}), narrow bands at 1076 and 1038 cm^{-1} are assigned to the C−N stretching vibrations for primary and secondary α-carbons of primary amines. Significant features (3000−2800 cm^{-1} and <2000 cm^{-1}) differentiating the mucin and mucus spectra include more intense alcohol and *p*-amine bands, less intense carboxylic acid bands, and less well-defined methylene bands. The greater proportion of alcohol groups in glycoproteins is indicated by the relative intensities for C−O (primary alcohols, 1319 cm^{-1}), O−H (primary and tertiary alcohols, 1058 and 1155 cm^{-1}), and, less prominently, O−H (secondary alcohols, 1118 cm^{-1}). Also in the mucin spectra, the intensities of the narrow primary-amines bands (1076 and 1038 cm^{-1}) are double that of the amide I band (1650 cm^{-1}), whereas fewer carboxylic acid functional groups are indicated, and CH_2 bands (1454 and 2935 cm^{-1}) are also relatively weak. Thus, compared with homogenized mucus, mucin is characterized by more amine and alcohol groups, fewer carboxylic acid functionalities, and a lower proportion of long methylene chains. This reflects the removal of the lipophilic component of mucus in the isolation of the glycoprotein. Varying the pH (2.1−8.0) gives trends associated with acid−base dissociation, Figure 2. Mainly affected are the carboxylic acid bands of poly(acrylic acid). As the pH is increased (2.1−6.0), the strong C═O band (1710 cm^{-1}) is progressively replaced by the CO_2^- band (1556 cm^{-1}). Similar spectral changes occur for mucus and glycoprotein, but they are less well defined because of the lower proportion of carboxylic acid groups. In particular, the CO_2^- bands (1402 and 1556 cm^{-1}) prominent in homogenized mucus are no stronger in mucin samples at pH 6.0. However, increasing the pH does enhance the intensity of the broad primary amine (N−H) band (900−650 cm^{-1}).

For mixtures of Carbopol 934P with homogenized mucus and with mucin, altered (nonadditive) spectral features are

Table 1. Band Positions and Assignments for Mucus, Mucus Glycoprotein, Carbopol 934P, and Their Mixtures

band (cm^{-1})	assignment	comments
≈ 3384	N−H	primary amine, asymmetric stretch; mucus/ glycoprotein
≈ 3300	N−H	primary amine, symmetric stretch; mucus/ glycoprotein
3000−3100	O−H	enol, H-bonded, stretch; mucus/ glycoprotein
2956	C−H	$>CH_2$, asymmetric stretch; polymer and mucus/glycoprotein
2923	C−H	$>CH_2$,, symmetric stretch; mucus
2871−3	C−H	$-CH_3$, symmetric stretch, mucus/glycoprotein
2852	C−H	$>CH_2$, out-of-phase vibrations of hydrogen atom, mucus/glycoprotein
2500−2700	O−H	-CO.O−H, stretch, broad band: strong H-bonding; polymer
≈ 2120	CO_2^-	combination band involving $-CO_2^-$ stretch, zwitterionic structure in the amino acid
	NH_3^+	
1710	C═O	carbonyl, stretch; carboxylic acid of polymer (high intensity, suppressed by increasing pH)
1658	C═O	carbonyl, stretch, amide I band; mucus/ glycoprotein (high intensity, unaffected by pH change)
1642	N−H	primary amine, deformation; mucus/ glycoprotein
1556	CO_2^-	carboxylate, anti-symmetric stretch; polymer (enhanced by increasing pH)
1540	N−H	amide II band, N−H deformation; mucus/ glycoprotein (unaffected by pH change)
1454	C−H	$>CH_2$, deformation; polymer and mucus/glycoprotein
1402−1415	CO_2^-	carboxylate, symmetric stretch, polymer (enhanced by increasing pH) and mucus/glycoprotein
1384	C−H	$>CH_2$, deformation influenced by electronegative groups; polymer and mucus/glycoprotein,
1340	C−H	>CH− deformation; polymer (low intensity)
1324	CO_2^-	carboxylate, asymmetric stretch weakened by H-bonding; polymer + mucin mixtures
1313	C−O	C−O stretch, primary alcohols; mucus/ glycoprotein
1245	C−O	C−O stretch, carboxylic acid; polymer (suppressed by increase in pH)
1236−1240	N−H	amide III band secondary amides; mucus/ glycoprotein
1166−1170	C−C	skeletal; polymer (decreases with increasing pH)
1155	O−H	tertiary alcohol, deformation; mucus/ glycoprotein
1118	O−H	secondary alcohol, deformation; glycoprotein
1076	C−N	primary amine, stretch, primary α-carbon of amino acids; mucus/ glycoprotein
1058	O−H	primary alcohols deformation band; mucus/ glycoprotein
1038	C−N	primary amine, stretching, secondary α-carbon of amino acids; mucus/ glycoprotein
910	O−H	-CO.O−H out-of-plane deformation; polymer
796 (−863*)	C−H	$H-C(CO_2H)$, out-of-plane deformation; polymer (strengthened by increasing pH, *at pH 8.0)
650−900	N−H	amine, unprotonated, broad band due to H-bonding;; mucus/ glycoprotein (alkaline pH)

shown (Figures 1d−f and 3): a new band at 798 cm^{-1}, enhancement (relative to mucus) of the band at 1238−1240 cm^{-1}, and loss of the bands for Carbopol 934 at 906 and 802 cm^{-1}. Again at higher pH, the C═O stretch (1712 cm^{-1}) is weakened and the CO_2^- band (1556 cm^{-1}) emerges. The lost bands could be due to, either, molecular interactions between the mucus and polymer, or, masking by the added mucus. Spectra for mixtures of different proportions (pH 3.5 and 6.0, Figures 1 and 3) show that all bands are observed with appropriate relative intensities and the systematic spectral changes are most pronounced for mixtures Carbopol 934P + mucus 2:1 v/v, which therefore are close to being equimolar in IR-active groups. The effects on the infrared spectra obtained from mixtures (Figure 3) are analogous to those seen in the spectra of the individual components, although the overlap of certain bands impedes spectral interpretation. For example, the strong amide II band at ≈1540 cm^{-1} is seen under acidic conditions but, with

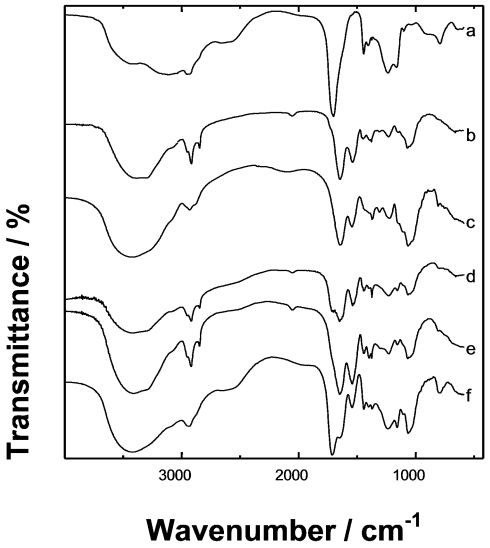

Figure 1. IR spectra (3500−600 cm^{-1}, KBr disks). (a) Carbopol 934P, (b) homogenized porcine gastric mucus, (c) derived glycoprotein (pH 3.5), (d) Carbopol 934P + mucus (1:1 by volume, pH 3.7), (e) Carbopol 934P + mucus (1:1 by volume, pH 6.0), (f) Carbopol 934P + glycoprotein (1:1 by volume, pH 3.5).

increasing pH, becomes masked by the CO_2^- asymmetric stretch (1556 cm^{-1}). Correspondingly, the C−O symmetric stretch (1245 cm^{-1}) is hidden for pH ≥ 8.0.

Additional absorptions and significant shifts (>5 cm^{-1}) in the CO_2^-, COOH, and OH bands, in mixtures of mucus or glycoprotein with poly(acrylic acid) compared with the separate components, reflect differences in hydrogen bonding. With the CO_2^- group, this leads (at pH > 6.0) to lower wavenumbers for the asymmetric stretch vibration: 1556 cm^{-1} in mucus + Carbopol 934P and 1562 cm^{-1} in glycoprotein + Carbopol 934P, compared with 1573 cm^{-1} for dissociated poly(acrylic acid). The carbonyl absorption of COOH, 1730 cm^{-1} in Carbopol 934P at pH < 4.5, is displaced to 1712 cm^{-1} in glycoprotein + Carbopol 934P and becomes two bands, 1716 and 1697 cm^{-1}, in mucus + Carbopol 934P. The broad low-intensity band at 2120 cm^{-1} in mucus and glycoprotein, a combination band for zwitterionic structures $CO_2^-.C(R_1R_2).NH_3^+$, is hardly influenced by pH, but a considerable effect is observed for both mixtures with Carbopol 934P (2180 cm^{-1} at pH 8.0 to 1950 cm^{-1} at pH ≤ 4.5). This trend is consistent with pH-dependent intercomponent H-bonding, which displaces the contributing vibrations to lower wavenumbers. Under slightly acid conditions, undissociated CO_2H groups (poly(acrylic acid)) form H bonds with N lone electron pairs (unprotonated amine groups, mucus or mucin), which, however, are protonated at lower pH. Under alkaline conditions, the $-CO_2^-$ groups do not H-bond with N lone pairs, so that there is a considerable upward shift in the combination band wavenumber. The O−H deformation bands of primary (1058 cm^{-1}) and secondary alcohols (1118 cm^{-1}), from the carbohydrate structures in mucus, are not influenced by pH. For mixtures however, and with the greater effect for mucin + Carbopol 934P, these bands are well defined at pH ≤ 3.5 but become less intense with increasing pH (Figure 3a,c). Tobyn et al.[21] have suggested that the enhancement of the 1058 cm^{-1} band with the cumulative addition to glycoprotein

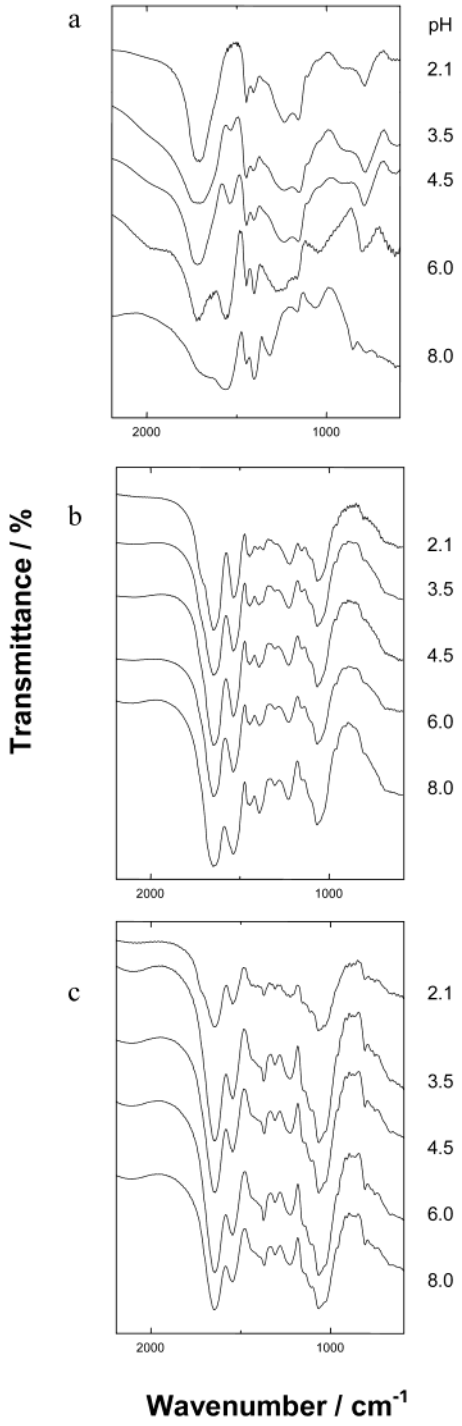

Figure 2. IR spectra (2000−600 cm^{-1}, KBr disks) of components at controlled pH (2.1−8.0). (a) Carbopol 934P, (b) homogenized porcine gastric mucus, (c) derived glycoprotein.

of a mucoadhesive agent is evidence for hydrogen bond formation. This effect is also seen for polymer + mucus (1:1, 2:1, and 4:1; Figures 1b,d and 3a,b): by comparing the relative intensities of the bands at 1058 and 1078 cm^{-1} (C−N, unaffected). Further, as the alkalinity is increased (pH 4.5−8.0), both of the alcohol band intensities (1058 and 1118 cm^{-1}) are reduced, because of (i) the lower relative proportion of un-ionized carboxylic acid available for hydrogen bonding and (ii) the deprotonation of alcohol OH.

NMR. ^1H- and ^{13}C NMR spectra of Carbopol 934P, separately and mixed with mucus or mucin, have been obtained at pH ≤ 5.0, above which the polymer tends to

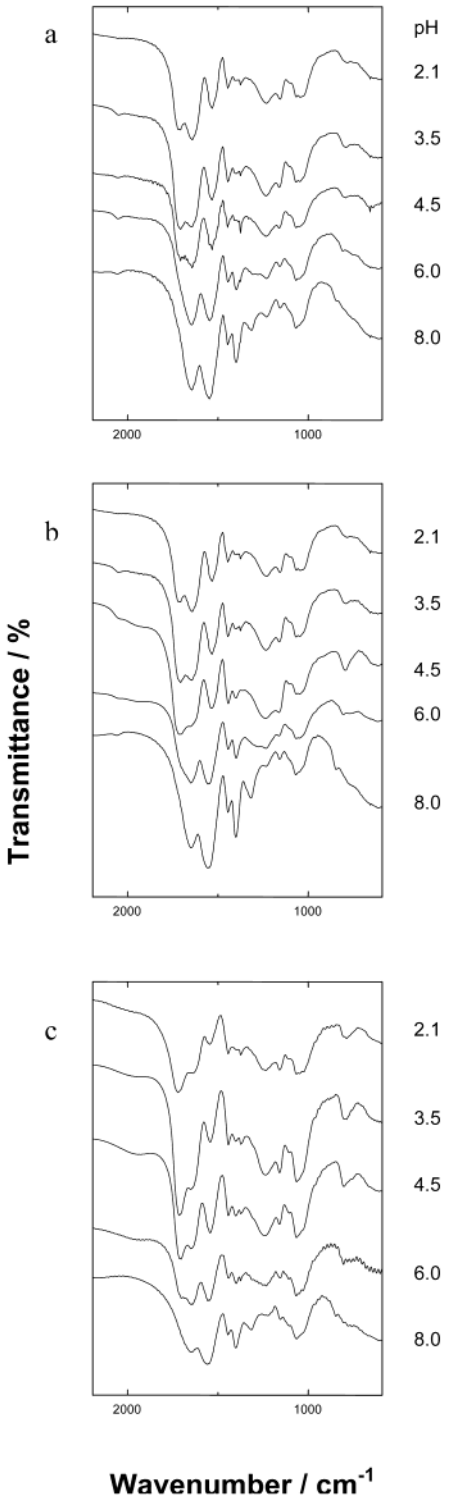

Figure 3. IR spectra (2000−600 cm^{-1}, KBr disks) of mixtures at controlled pH (2.1−8.0). (a) Carbopol 934P + mucus (2:1 by volume), (b) Carbopol 934P + mucus (4:1 by volume), (c) Carbopol 934P + glycoprotein (2:1 by volume).

gel. Three ^{13}C resonances were seen for Carbopol 934P, both separately and in mixtures, Table 2; both mucus and mucin showed only very weak resonances. Raising the pH for the separate polymer significantly increased the three chemical shifts, by most for the carbonyl carbon atom; this enhanced shielding reflects the increased electron density accompanying ionization and hence also reduced H bonding. The addition of glycoprotein partially reversed these displacements; this is consistent with some withdrawal of electron density from the carbon atoms because of the formation of intermolecular H bonds involving the remaining un-ionized CO_2H groups on the polymer. In contrast, no changes were detected in the ^1H chemical shifts of Carbopol 934P with addition of the mucus, and added mucin had only slight effects, Table 3, probably because the backbone protons are sufficiently removed from the carboxylic acid groups not to be influenced by changes in the pH or hydrogen bonding. Glycoprotein protons, however, were affected considerably by mixing with the polymer, with protons adjacent to electronegative groups being influenced by intercomponent H-bonding. The ^1H spectrum of a fresh homogenized mucus sample was complex. The main functional groups identified, Table 4, were consistent with the structure of glycoprotein.

A mucus sample allowed to age (ambient temperature, under moist air, 9 weeks) showed significant ^1H spectral changes. Resonances associated with sp^2 carbons (≈5.5 ppm), and the CH−OH signal (≈3.8 ppm), decreased relative to resonances at ≈7.5 ppm. Signals emerged of a possible ketone functionality (≈2.17 ppm, 2.8−3.0 ppm) and of aliphatic hydrocarbon chains (≈1.44 ppm). These changes are consistent with oxidative degradation.

Table 2. Chemical Shifts for ^{13}C Atoms in Carbopol (Cp) 934P under Different Conditions; $[-C^1H_2.C^2H(C^3O.OH)-]_n$

	chemical shift/ppm		
	C^1	C^2	C^3
Cp934P (pH = 2.95)	34.45	41.9	179.1
Cp934P (pH = 3.60)	34.66	42.11	179.37
Cp934P + glycoprotein (1:1 by mass, pH = 3.60)	34.52	41.96	179.19

Table 3. ^1H Chemical Shifts (δ) for Carbopol (Cp) 934P, Mucin and a Mixture (1:1 by mass, pH 3.6)

	δ/ppm		
Cp934P	mucin	mixture	assignment
	1.25	1.13	C**H₃**.CH_2.
1.73		1.64	.C(**H^A**,**H^B**)
	2.06	1.95	C**H₃**.CO.OR
2.37		2.36	.C**H**(CO.OH).
	3.84	3.66	.C**H**(OH).
	4.24	4.07	.C**H₂**.O.,.C**H₂**.N:

Table 4. ^1H Chemical Shifts (δ), and Some Possible Assignments, Observed for Homogenized Mucus

δ/ppm	assignments
1.07	methyl (C**H₃**−C−N)
1.44	methylene (−C**H₂**-)
1.59	methyl (C**H₃**-X-)
1.83	methine (−C**H**(R)-)
2.17	methyl (C**H₃**−CO-)
2.68	methine (−C**H**(CO.R)-)
3.32	methylene (−C**H₂**−OH)
3.38	methylene (−C**H₂**−O−R)
3.79	methine (−C**H**-OH)
3.97	methine (−C**H**(R)-)
5.35−5.5	R**H**C=
6.4−7.75	Ar-**H**

Table 5. XPS 1s Band Centers and Corresponding Atomic Proportions for Carbopol 934P, homogenized Mucus, and the Mixture Carbopol 934P:Homogenized Mucus = 1:2 (w/w)

*C*934P		homogenized mucus		mixture		
BE/eV	at. %	BE/eV	at. %	BE/eV	at. %	assignment
284.85	22.6			284.85	17.2	adventitious carbon
285.0	14.4			285.0	3.2	-***C***H_2−CH<
		285.0	34	285.0	34	>***C***H−CH<
285.6	14.3			286.1	3.1	>***C***H−COOH
		286.55	12.8	286.6	10.3	>***C***H−O-
		288.15	5.8	288.15	3	>***C***=O
289.2	15.2			289.25	2.9	-***C***OOH
		531.75	6.5	531.7	5.6	***O***=C<
532.65	15.5			532.55	2.6	***O***=C<
		533.1	11.6	533.05	10.5	H***O***-C
533.9	17.3			533.85	2.1	H***O***-C
		398.25	0.6	398.25	0.3	N
400.25	0.4			400.2	0.4	N
		400.25	3.9	400.25	2.3	N
402.9	0.2	402.5	0.7	402.5	0.7	N
		406.45	0.3	406.45	0.2	N

XPS. The spectrum of Carbopol 934P in the C1s region showed a complex peak (envelope) at 284−286 eV and a single emission, ***C***OOH,[28] centered at 289.20 eV, Table 5. Deconvolution was performed on the basis of similar peak areas respectively for ***C***OOH, ***C***H_2, and ***C***H(CO_2H). The contribution thereby attributed to adventitious carbon (284.85 eV) includes the cross-linker (pentaerythritol). For the mucus sample, a good fit for the C1s emissions was obtained by positioning peaks at 285.00 eV (***C***−C), at ca. 286.5 eV (***C***−O), and at ca. 288.0 eV (***C***=O). For the polymer/mucus mixture, a deconvolution procedure based on the polymer ***C***OOH peak was again used, as the mucus sample did not have any interfering peaks close to 289.2 eV. Peaks due to O1s were fully resolved and showed significant differences between the samples of polymer and mucus. Nitrogen detected with the polymer sample is probably due to surface adsorption/impurity. In the spectrum of the mucus sample, the main nitrogen peak is associated with the glycoprotein component. For the mixture of Carbopol 934P with homogenized mucus, the total areas of C1s and O1s peaks assigned for the two components indicated a mucus:polymer ratio in excess of 2:1; if adventitious carbon is discounted the ratio is higher (Table 5). This is inconsistent with both the prepared composition (1:2) and the relative intensities of the IR bands. Because XP spectra reflect concentrations within a surface layer (<10 nm), these results indicate that in mixtures the mucus tends to encapsulate the polymer.

Differences are observed with respect to the atomic percentages of carbon and oxygen functional groups in both the polymer and mucus components in the mixture. For the polymer (with equal proportions of all atom types), the peaks for ***C***O.OH, C=***O***, and CO.***O***H were all slightly higher than those for ***C***H_2C and H***C***COOH, whereas in the mixture, this inequality was reversed. For the mucus sample (for which the expected relative areas were not known), the ***C***−O, ***O***=C, and H***O***−C proportional peak areas were all 10−20% lower in mixtures, but those for ***C***−C, ***C***=O, and ***N*** were reduced by 40−48%. Because the signals are most sensitive to surface atom concentrations, the observations indicate that in the single polymer COOH groups tend to project from the surface whereas in mixtures with mucus they are more directed into the bulk. These changes, and similar displacements from surface to bulk for ***C***=O and ***N*** in mucus, are consistent with the formation of H bonds between the amide groups in glycoproteins and nondissociated carboxylic acid groups in the polymer (although the observation for ***O***=C in mucus is anomalous).

Conclusions

The formation of hydrogen bonds between mucus and the mucoadhesive agent poly(acrylic acid) has been shown by the displacement of IR absorption bands and by NMR resonances. XPS results indicate that, in mixtures, the mucus tends to encapsulate the polymer. In addition, differences in surface atom concentrations, between mixtures and separate components, are consistent with H-bonding between the components.

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