The physicochemical characterisation of pepsin degraded pig gastric mucin

Atiga Abodinar\textsuperscript{1,2}, Kristoffer Tømmeraas\textsuperscript{3}, Elena Ronander\textsuperscript{3}, Alan M. Smith\textsuperscript{2} and Gordon A. Morris\textsuperscript{1,✉}

\textsuperscript{1}Department of Chemical Sciences, School of Applied Sciences, University of Huddersfield, Huddersfield, HD1 3DH, UK;
\textsuperscript{2}Department of Pharmacy, School of Applied Sciences, University of Huddersfield, Huddersfield, HD1 3DH, UK;
\textsuperscript{3}Biofac A/S, Englandsvej 350-356 DK-2770 Kastrup, Denmark

✉Corresponding author

Tel: +44 (0) 1484 473871
Fax: +44 (0) 1484 472182
Email: g.morris@hud.ac.uk
Abstract

Mucins are the main macromolecular components of the mucus secretions that cover the oral cavity, gastrointestinal and urogenital tracts of animals. The properties of the mucus secretions are therefore directly correlated with the physicochemical properties of mucin glycoproteins. In this study, mucins were obtained from pig gastric mucus after digestion with pepsin at 37 °C for 4 hours, these mucins were characterised in terms of compositional and hydrodynamic properties.

Compositional analysis showed that this mucin contains protein (15%), carbohydrates (55%) of which the constituents are: fucose (4%), galactose (9%), glucosamine (55%), glucosamine (33%) and sialic acid (2%). The latter component gives the mucin polymer a pH-dependant negative charge, with a \( \zeta \)-potential of -3 mV at pH 1.2 up to -11 mV at pH 7.4. The weight average molar mass was \( \sim 1 \times 10^6 \) g/mol and intrinsic viscosity was \( \sim 0.42 \) dL/g although there was a small pH dependency due to the polyelectrolyte behaviour of the polymer. The measurements of viscosity versus shear rate showed shear thinning behaviour and the critical overlap concentration was determined to be 10-11% w/v indicating a compact structure. Knowledge of these properties is fundamental to the understanding interactions of mucins, with for example, novel drug delivery systems.

Keywords: pepsin degraded mucin; physicochemical properties; compact conformation
Highlights:

- The physicochemical properties of extensively degraded mucin were investigated
- Mucin consisted of fucose, galactose, glucosamine, glucosamine and sialic acid
- Weight average molar mass was $1 \times 10^6$ g/mol and intrinsic viscosity was $\sim 0.42$ dL/g
- Critical overlap concentration was determined to be 10-11 % w/v
- Data is consistent with a weak polyelectrolyte behaviour and compact conformation
1. Introduction

Mucins are the main macromolecular components of the mucus secretions that cover the oral cavity and the respiratory, gastrointestinal and urogenital tracts of animals. Moreover, they provide protection for the delicate exposed epithelial surfaces and are responsible for the viscoelastic properties of the mucosal secretions [1]. The polymeric structure of the component mucins are directly correlated with the protective properties of the mucus gel [2]. Mucins are large, extracellular, abundant, filamentous molecules [3] with the molecular weight range from $5 \times 10^5$ up to $2 \times 10^7$ g/mol [4]. Mucin structures are stabilized by inter-chain disulphide bonds [5, 6]. The mucin protein core contains highly glycosylated regions comprising of 80% carbohydrates primarily of $N$-acetylgalactosamine (GalNac), $N$-acetylglucosamine (GlcNac), galactose (Gal), fucose (Fuc) and sialic acid (N-acetylneuraminic acid, Neu5Ac) and traces of sulphate ($SO_4^{2-}$) and mannose (Man) (Figure 1) [7] which are therefore highly resistant to proteolysis and whereas the regions which are sparsely glycosylated or non-glycosylated regions are subsequently much more susceptible to proteolysis [5, 8, 9]. Mucin is negatively charged due to the presence of sulphate esters and sialic acid. The oligosaccharide chains consisting of 5–15 units show moderate branching and are attached to the protein core by O-glycosidic linkages to the hydroxyl side chains of serine and threonines and arranged in a “bottle brush” shape about the protein core [7, 10]. Colonic mucin in either its polymeric, reduced (with mercaptoethanol) or digested (with papain) forms have been reported to adopt random coil conformations [11-13] as was proposed by the general model [14].
Figure 1. (a) A schematic drawing of the pig gastric mucin monomer consisting of glycosylated regions flanked by regions with relatively little glycosylation. (b) The symbols indicate the different domains in the sketch in (a). (This representation is based in part on Figures 1 and 2 [3]. The cysteine rich regions contain domains that are similar to von Willebrand factor (vWF) C and D domains, and C-terminal cysteine knot domains which have been shown to be involved in dimerization and subsequent polymerisation to form larger multimers. The bottom of the figure shows (c) a dimer formed by two monomeric subunits linked via disulfide bonds in the non-glycosylated regions and in (d) dimers that are further disulfide linked to form higher multimers. This gives rise to the high molecular weight and polydispersity of secretory mucins. Polymers of greater than 16-mers have been described in MUC5AC from human airway secretions by [15]. (The bottom part of the figure is adapted from Figure 8 in [15]. Figure reprinted with permission from [7].)

As the rheological interactions of mucoadhesive polymers will be affected not only by the chemical structure of mucins but also by the way in which the mucin has been prepared [16]. The aim of this article is to fully characterise extensively degraded pig gastric mucin with the respect to compositional and hydrodynamic properties to underpin the understanding of mucin interactions with polysaccharide based drug delivery systems. Furthermore, any information about this material could open up opportunities for novel application areas of digested mucins.

Materials and methods

Glucose, sodium tetraborate (borax), sodium acetate, phenyl phenol, glacial acetic acid, sodium acetate trihydrate, trifluoroacetic acid, sialic acid, periodic acid, sodium arsenite, bovine serum albumin (BSA), Bradford reagent, n-butanol, hydrochloric acid, sodium hydroxide, sulphuric acid, thiobarbituric acid and sodium chloride were all obtained from Sigma-Aldrich (Gillingham, UK). Extensively degraded pig gastric mucin was obtained from Biofac A/S (Kastrup, Denmark). All materials were used without any further purification.

2.2. Preparation and purification of digested gastric mucins

The mucins were prepared as a by-product from large scale preparation of pharmaceutical quality pepsin at Orthana Kemiske Fabrik A/S (part of the Biofac group) in Copenhagen, Denmark. Red linings from porcine stomachs were obtained from abattoirs in the US (Farmland). These were kept frozen (-18 °C) until they were taken into use in the production area. First, approx. 1000 kg of
Frozen linings were minced in a large meat mincer (screen 18 mm). The minced raw material was transferred into a stirred tank before adding 100 kg of RO water. Then, the pH was adjusted to 2.0 using concentrated HCl before heating to 38 °C. After 4.5 h, the pH was adjusted to 2.8 using concentrated NaOH. The process liquid was transferred to a precipitation tank and cooled down to -5 °C. The crude mucin was then precipitated with 97 % acetone added slowly until 61 % w/w. The precipitation liquid was held at -5 °C and mixed using mild agitation for 30 minutes. The process liquid was then separated on a Flotweg decanter (1500 rpm inner speed, 6000 rpm outer speed) into liquid and solid phases where the latter contained fat and mucins. The precipitate was solubilized by adding approx. 5 volumes of water. Remnants of acetone were evaporated off at 40 °C under vacuum. Subsequently, the liquid was left to sediment for 3 days before pumping the top phase (clear liquid) out. The crude mucin was then filtered on a Seitz Orion plate and frame filter press three times using cellulose and filter aid based filter plates (first T2600, T1000 and finally K250, all from Seitz, Pall Corporation, New York, USA) coated with filter aid (Hyflo Super Cel). The mucin was then concentrated to 5 % solid content and washed with 3 volumes of RO water before pH adjustment to 3-4 and subsequently frozen at -18 °C and lyophilized.

2.3. Chemical characterisation of gastric mucin

2.3.1. Determination of total carbohydrate using a phenol sulphuric acid assay [17]

Total carbohydrates in the mucin sample were colorimetrically determined by m-hydroxydiphenyl method. Firstly, a stock solution of glucose (200 mg/L) was prepared and from this stock solution, standard solutions with concentrations of 0 - 100 mg/L were prepared, then the glucose test was performed by taking 400 µL from the standard solutions. Two ml of 0.5 % borax in concentrated sulphuric acid was added and then incubated at 100 °C in water bath for 5 min to which 40 µL of 0.15 % 3-phenylphenol (in 1 M sodium hydroxide) was added and incubated for 5 min. The absorbance for each standard and the sample was measured at 520 nm using Shimadzu UV-160A UV-vis spectrophotometer. The blank for the sample was prepared by taking 400µL of the sample, 2 mL of deionised water and 40 µL of 0.15 % 3-phenylphenol while the blank for the standard was prepared by taking 400 µL of deionised water, 2 mL of 0.5 % borax in concentrated sulphuric acid and 40 µL of 0.15 % 3-phenylphenol.
2.3.2. Determination of total protein using Bradford assay

Five dilutions of Bovine Serum Albumin (BSA) standard with a range of 5 to 100 mg/L were prepared. 30 µL of each mucin solution (250 mg/L) and the standard solutions were added to separate test tubes. The blank was prepared using 30 µL ultrapure water instead of standard solution or mucin sample. Bradford reagent (1.5 mL) was added to each tube and mixed well. The samples were incubated at room temperature for 10 min. The absorbance measurements of the mucin samples were recorded at 595 nm and the concentration of protein was calculated from a standard curve and expressed as a percentage by weight of mucin.

2.3.3. Determination of the constituent sugars by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD)

Mucin (2.0 mg in duplicate) was dissolved in 2 mL of deionized water in separate pressure tubes. Concentrated trifluoroacetic acid (0.85 mL) was then added to each sample solution using a micropipette. The pressure tubes were then placed in a heating block for 2 hours at 120 °C. After 2 hours the samples were evaporated to dryness under a stream of nitrogen gas at 65 °C for 1 hour. The dried samples were reconstituted with 2 mL of deionized water and the sample diluted 10 times prior to HPAEC-PAD analysis. Neutral sugars, amino sugars and sialic acid composition were analysed using a Dionex ICS-5000 HPAEC-PAD system (Thermo Fisher, Loughborough, UK). A 0.5 mL/min flow rate was used the first 12 minutes at a concentration of 10 mM NaOH this was then followed by a 0.05 minute step to change from 0-17 % 1 M sodium acetate in 150 mM NaOH and the remainder of the run was continued at 17 % 1 M sodium acetate in 150 mM NaOH to elute any uronic acids present. A pre-run equilibration step of 10 minutes using 200 mM NaOH followed by 20 minutes of 10 mM NaOH was used to regenerate the column prior to each injection.

2.3.4. Determination of sialic acid using sialic acid assay

Sialic acid determination was achieved by using the method of [18]. 10 mg of mucin was hydrolysed in 2 mL 100 mM H₂SO₄ at 80 °C for 1 h to release sialic acids (in duplicate), then neutralised with 1M NaOH (45 µL). The samples were incubated with 250 µL periodic acid solution (25 mM in 62.5 mM H₂SO₄) at 37 °C for 30 min. The reaction was concluded by adding 0.2 mL sodium arsenite (2 % in 0.5 M HCl), left for 3 min before adding 2 mL thiobarbituric acid (0.1 M, pH 9.0). The solutions were heated in a boiling water bath for 7.5 min then cooled in ice water and mixed with 5 mL of n-butanol /concentrated HCl solution (95:5, v/v), shaken and the absorbance of the butanol layer was measured at 550 nm. The concentration of sialic acids was
calculated from a standard curve constructed with N-acetyl neuraminic acid (1–500 µg/mL) and expressed as a percentage by weight of mucin.

2.4. Physical characterisation of gastric mucin

2.4.1. Determination of weight-average molecular weight by size-exclusion chromatography coupled to multi-angle laser light scattering (SEC-MALS)

A 0.5 % w/v of mucin was analysed by size exclusion chromatography which was carried out at ambient room temperature on a PL aquagel guard column (Polymer Labs, Amherst, U.S.A.) which was linked in series with PL aquagel-OH 60, PL aquagel-OH 50 and PL aquagel-OH 40 (Polymer Labs, Amherst, U.S.A.) and was eluted with distilled water at a flow rate of 0.7 mL/min. The eluent was then detected online firstly by a DAWN EOS light scattering detector (Wyatt Technology, Santa Barbara, U.S.A.) and a REX differential refractometer (Wyatt Technology, Santa Barbara, U.S.A.). The refractive index increment, \(dn/dc\) was taken to be 0.150 mL/g.

2.4.2. Determination of intrinsic viscosity

Appropriate concentrations of mucin were prepared (0.025 – 0.2 % w/v) at pH 1.2, 4.4 and 7.4, respectively. The measurements were performed with a Cannon capillary viscometer size 50 at 25 °C. The relative (\(\eta_{rel}\)) and specific viscosities (\(\eta_{sp}\)) were calculated as described in equations 1 and 2, respectively:

\[
\eta_{rel} = \left( \frac{t}{t_0} \right) 
\]

(1)

\[
\eta_{sp} = \eta_{rel} - 1 
\]

(2)

where \(t\) is the average flow time of the solutions at each concentration, \(t_0\) is the flow time for the appropriate solvent [19]. Measurements were made at different concentrations and extrapolated to infinite dilution using both the Huggins and Kraemer approaches [20, 21]:

\[
\frac{\eta_w}{c} = [\eta](1 + K_H [\eta]c) 
\]

(3)
\[
\frac{\ln(\eta_{rel})}{c} = [\eta](1 - K_h [\eta] k) 
\]

(4)

where the intrinsic viscosity \([\eta]\) is taken as the is the mean of the intercepts from equations (3) and (4) and \(K_h\) and \(K_K\) are the Huggins [20] and Kraemer [21] constants respectively.

### 2.4.3. Determination of the critical coil overlap (\(c^*\))

A stock solution mucin (40 w/v %) was prepared by dissolving 40 g of mucin in 100 mL of deionized water. Once fully dissolved, the stock solution was diluted to appropriate range of concentrations (1 – 40 %). Mucin solutions of the same concentrations were also prepared at pH 1.2 and 7.4 pH by adjusting the pH with 0.1 M HCl and 0.1 M NaOH respectively. The viscosities at 130 s\(^{-1}\) were measured using cone plate 55 mm geometry on a Bohlin Gemini HR Nano Rheometer at 37 °C.

### 2.4.4. Determination zeta potential, \(\zeta\)

A solution of mucin (0.5 % w/v at pH 1.2, 4.4 and 7.4) was prepared by dissolving 0.5 g of mucin in 100 mL of deionized water and the pH was adjusted accordingly with 0.1 M HCl or 0.1 M NaOH. The zeta potential of the three samples was determined using Malvern Zetasizer NANO-Z (Malvern Instruments Limited, Malvern, UK). Measurements in triplicate were performed by using a folded capillary cell at 25.0 ± 0.1 °C and refractive index was set at 1.450.

### 2.4.5. Rheological study

Measurements of viscosity vs. shear rate were performed at 37 °C on 7 % and 15 % w/v mucin samples prepared at pH 1.2, 4.4 and 7.4 across shear rates ranging from 1 s\(^{-1}\) to 1000 s\(^{-1}\) using cone and plate 55 mm geometry fitted to a Bohlin Gemini Rheometer (Malvern Instruments, UK). Small deformation oscillatory measurements were also performed on these solutions (7 % and 15 % at pH 1.2, 4.4, and 7.4) to monitor the viscoelastic behaviour of the mucin using the same rheometer as in the viscosity measurements but using a double gap geometry to minimise signal to noise ratio. Measurements of storage modulus (\(G'\)) and loss modulus (\(G''\)) were taken at frequencies from 0.1 rad/s to 10 rad/s to ascertain mechanical spectra of the gels at an isothermal temperature of 37 °C and at a fixed strain of 2 %. Measurements were performed in triplicate and mean values plotted.
3. Results and discussion

3.1. Chemical characterisation of gastric mucin

A phenol sulphuric acid assay was used to determine the total carbohydrate of the mucin samples relative to glucose standards. The mucin had a total carbohydrate content of 55 % as glucose equivalents and a total protein content of 15 % when using bovine serum albumin as a standard (Table 1). It is noted that the recovery for total protein and total carbohydrate does not equate to 100 % this may be due to the use of glucose as standards, as the response to the assay varies with different monosaccharides [17]. The mucin also contains ~ 10 % moisture.

Table 1: Some physicochemical properties of the gastric mucin

<table>
<thead>
<tr>
<th>Property</th>
<th>Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total carbohydrate, % (as glucose equivalents)</td>
<td>55 ± 1</td>
</tr>
<tr>
<td>Fucose, mol%</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>Galactose, mol%</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>N-acetylgalactosamine, mol%</td>
<td>55 ± 1</td>
</tr>
<tr>
<td>N-acetylglucosamine, mol%</td>
<td>33 ± 1</td>
</tr>
<tr>
<td>Sialic acid, %</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>Total protein, % (relative to BSA standards)</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>$M_w$, $10^6$ g/mol</td>
<td>1.04 ± 0.05</td>
</tr>
<tr>
<td>$M_w/M_n$</td>
<td>5.5 ± 0.5</td>
</tr>
<tr>
<td>$r_{g,z}$, nm</td>
<td>31 ± 6</td>
</tr>
</tbody>
</table>

Constituent sugar analysis using HPAEC revealed the presence of Fuc, Gal, GalN and GlcN (Table 1) which are consistent with previous results [22]. We were unable detect any sialic acid using this method, but it has been determined by an alternative method (sialic acid assay – section 2.3.4) to be 1.7 %.

3.2. Molecular weight

The weight-average molecular weight as measured by size-exclusion chromatography coupled to multi-angle laser light scattering (SEC-MALS) was found to be $1.04 \times 10^6$ g/mol which is in general agreement with previous estimates [12] and demonstrates that the enzymatic digestion has resulted in a large reduction in molecular weight as typically non-degraded pig gastric mucin has a weight-average molecular weight of $5 – 9 \times 10^6$ [11, 12, 23]. MALS can also give an approximation...
of the radius of gyration \( r_g(z) \), which was estimated to be 31 nm. This is indicative of compact structure and is of the size of typical T-domains [24].

### 3.3. Zeta potential

Measurements of \( \zeta \)-potential were taken as an indirect measurement of surface charge and were performed on the samples at pH 1.2, 4.4 and 7.4. Figure 2 shows a negative charge for all the samples tested with a progressive negative charge increase with increasing pH. This may be attributed to the presence of the carboxylic acid group in sialic acid. Studies on native pig gastric mucin have previously shown an isoelectric point at ~ pH 2-2.5 [25] and sialic acid has a pK\(_a\) of 2.6 [26].

![Figure 2. Zeta potential of samples of gastric mucin (0.5 % w/v) prepared in deionised water and pH adjusted to pH 1.2, 4.4 and 7.4.](image)

### 3.4. Intrinsic viscosity

The weight-average intrinsic viscosity, \([\eta]_w\) was found to be 0.42 – 0.44 dL/g which is in general agreement with previous estimates [27] and is also consistent with the reduction in molecular weight. A weight-average intrinsic viscosity of 0.42 – 0.44 dL/g coupled with a weight-average molecular weight of 1.04 x 10\(^6\) g/mol suggests a compact conformation [28, 29].

### 3.5. Critical overlap concentration \( (c^*) \)

In a dilute solution, random coils of polymer are spaced from each other. With increasing the concentration of polymer solution, the distance between the coils become smaller and coils starts to overlap and entangle. The concentration at which the individual polymer coils starts to overlap and entangle is termed overlap concentration \( (c^*) \) [30]. Above \( c^* \), viscosity increases rapidly with increasing concentration [31] as the chains of polymer interpenetrate with each other. This leads to difficulty in studying the characteristics of individual chains in solution [30]. Entanglement
characteristic is affected by the concentration of the solution and the hydrodynamic radius of the polymer, which for polyelectrolytes is dependent on pH and ionic strength [32]. As the entangling of polymer coils depend on their molecular size (hydrodynamic volume), chain stiffness and excluded volume effects [32]. Where the latter is probably very important for branched mucins. Therefore a decrease in molecular weight would be expected to have high impact on the viscoelastic properties of degraded mucin solutions [31].

It has been found that, at a mucin concentration of ~11 % (w/v) the mucin chains start to overlap (Figure 3) which agrees with the generalised theory where log c*[η] ~ 0.6 and log ηsp ~ 1 [33]. The relatively high c* is consistent with the molecular weight of the mucin being relatively low (compared with native mucins) and in this case adopting a compact conformation (Table 2) for example pullulan (a random coil type polysaccharide) of the same molar mass would be expected to have an intrinsic viscosity of ~ 2 dL/g [34] under similar conditions and a polyanion like pectin (semi-flexible coil) would be expected to be ~ 20 dL/g [35]. There is little influence of the pH change on either intrinsic viscosity or c*, probably due to excluded volume effects between the different branches on each mucin molecule forcing the chains into an expanded conformation giving less possibility for relaxation of the chain stiffness even when electrostatic repulsion along the chains decreases with lower pH due to fewer of the carboxylic acid moieties of sialic acid being deprotonated [32]. Pepsin degraded pig gastric mucin therefore appears to be similar hydrodynamic size to the T-domains produced using trypsin digestion [14]. The values of c* measured here are higher than what has previously been suggested 0.2-0.4 % [31], 2.5 % for Muc5ac and 3 % for Muc2 [36] and again, this is probably due to the specific pepsin degradation during processing.
Figure 3. Intersection of two curves of log concentration*[$\eta$] versus log specific viscosity. The means slopes of the plots are 1.4 and 3.2 for the dilute and concentrated regimes, respectively.

Table 2. The effect of pH on some physical properties of digested porcine gastric mucin

<table>
<thead>
<tr>
<th>Property</th>
<th>pH</th>
<th>pH 1.2</th>
<th>pH 4.4</th>
<th>pH 7.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>[$\eta$], dL/g</td>
<td>pH 1.2</td>
<td>0.416 ± 0.003</td>
<td>0.426 ± 0.004</td>
<td>0.443 ± 0.012</td>
</tr>
<tr>
<td>c*, g/dL (%)</td>
<td>pH 4.4</td>
<td>11.0 ± 0.1</td>
<td>11.1 ± 0.1</td>
<td>10.3 ± 0.3</td>
</tr>
<tr>
<td>$\zeta$-potential, mV</td>
<td>pH 7.4</td>
<td>-3.4 ± 0.2</td>
<td>-7.8 ± 0.3</td>
<td>-11.4 ± 1</td>
</tr>
</tbody>
</table>

3.6. Rheological study

All mucin samples showed typical shear-thinning behaviour with viscosity decreasing with increasing shear rate (Figure 4). The 7 % w/v sample (below c*) at pH 7.4 showed a distinctly higher viscosity compared with the samples at acidic pH. This can be explained by the mucin molecules becoming more extended at a higher pH causing an increase in entanglement and hence viscosity. Zeta potential measurements showed that the charge increased with increasing pH which would likely be the cause of a more extended conformation due to an increased intra molecular
repulsion which is consistent with increased viscosity. This difference is not apparent at 15 % w/v (above c*) due to the increase in polymer concentration, the intermolecular entanglements increase and dominate the viscosity effect of intra molecular repulsion. The relatively low viscosity suggests that the hydrodynamic size of the mucins is likely to be relatively small due to compact structure and/ or branching.

**Figure 4.** Viscosity vs. shear rate of 7 % (w/v) and 15 % (w/v) mucin samples at varying pH measured at 37 °C.

### 3.7. Dynamic mechanical measurements

Small deformation oscillatory measurements of elastic (G') and viscous modulus (G'') were undertaken to monitor the viscoelastic behaviour of the mucin using a Bohlin Gemini rheometer fitted with a double gap geometry. Amplitude sweeps were performed to ascertain the linear viscoelastic region of the samples. To reveal the mechanical spectra of the mucin, measurements were taken over a frequency range of 0.1 to 10 rad/s at 2 % strain at 37 °C. **Figure 5** highlights the difference in mechanical spectra of 7 % w/v mucin at pH 1.2, 4.4 and 7.4. These results show a slight increase in moduli at pH 4.4 and 7.4 compared with the values obtained at pH 1.2. Interestingly this contradicts the results on *native* pig gastric mucin which exhibits a pH dependent sol-gel transition when pH is reduced to ≤ pH 4 [37], although this would also be expected to be concentration dependent [36]. Again this is attributed to the polymer extending as the pH increases allowing a higher degree of polymer entanglement.
4. Conclusions

In conclusion the physicochemical properties of extensively degraded mucin were studied and revealed that this type of mucin contains: protein, carbohydrate (Fuc, Gal, GalN, GlcN) and sialic acid, which provides the negative charges that becomes progressively stronger with increasing pH. The measurements of viscosity vs. shear rate showed that mucin has a shear thinning behaviour and a relatively low viscosity which is consistent with a high critical overlap concentration (c*), small hydrodynamic size and hence compact structure (high molecular weight coupled with low intrinsic viscosity). This is further supported by the weak pH dependency of the mechanical spectra. Knowledge of the physicochemical properties of this low molecular weight, pepsin degraded mucin could lead to new applications of this material, and in addition, is fundamental to understanding interactions of mucins with other macromolecules.

5. Acknowledgements

The authors would like to thank the University of Huddersfield and the Libyan Government for studentship of Atiga Abodinar.

6. Conflict of interest statement

Drs. Tømmeraas and Ronander are, or were at the time of the study, employees of Biofac A/S.
7. References


