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Characterization of polyelectrolyte features in polysaccharide systems and mucin

Bo Nyström a,⁎, Anna-Lena Kjøniksen a, Neda Beheshti a, Atoosa Maleki a, Kaizheng Zhu a, Kenneth D. Knudsen b, Ramón Pamies a,c, José G. Hernández Cifre c, José García de la Torre c

a Department of Chemistry, University of Oslo, P.O. Box 1033, Blindern, N-0315 Oslo, Norway
b Department of Physics, Institute for Energy Technology, P.O. Box 40, N-2027 Kjeller, Norway
c Departamento de Química Física, Facultad de Química, Universidad de Murcia, 30071 Murcia, Spain

A B S T R A C T

This review elucidates several aspects on the behavior of charged polysaccharides and mucin. Viscosification of dilute aqueous solutions of hyaluronan (HA) occurs in the course of time at low shear flow, whereas shear thinning as time evolves is found at moderate shear rates. Hydrogen bonds and electrostatic interaction play an important role for the emergence of these features. No time effect of the viscosity is observed for semidilute HA solutions. A degradation of HA is observed at low and high pH and this effect continues over long times, and it is only in the approximate interval 5<pH<10 that HA is stable. Small angle neutron scattering (SANS) measurements on semidilute aqueous solutions of mucin at pH=7 reveal a fractal dimension of 1.4, and the effect of temperature is insignificant on the fractal structure. This suggests that the mucin chains on a semi-local dimensional scale are rod-like. From various experimental methods on solutions of mucin it was found that at pH values around 2 (uncharged polymer), the intensive hydrophobic interactions lead to large association complexes, whereas at pH>2 the negative charges suppress the tendency of forming associations. At pH<2, the mucin chains are compressed and they are decorated by some positive charges. In the semidilute regime, a fragmented network is developed. The intense association in semidilute solutions of mucin at pH=2 is further supported by the results from rheo-small angle light scattering measurements. Effects of ionic strength on the radius of gyration (Rg) for dilute solutions of HA (pH=7) and positively charged hydroxyethylcellulose (HEC(+)) are studied with the aid of Monte Carlo simulations, and essential features of the polyelectrolyte effect on Rg are captured in the computer simulation. Strong interactions are observed in aqueous mixtures of an anionic polysaccharide (HEC(−)) and an oppositely charged surfactant (cetyltrimethylammonium bromide; CTAB); this gives rise to extensive associations and macroscopic phase separation is approached. The massive association complexes are disclosed in the SANS experiments by a pronounced upturn in the scattered intensity at low values of the wave vector.

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A R T I C L E   I N F O

Available online 15 May 2009

Keywords:
Polyelectrolytes
Hyaluronan
Polysaccharides
Charged polysaccharide and oppositely charged surfactant
Structure
Viscosity
Monte Carlo simulation

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⁎ Corresponding author.
E-mail address: bo.nystrom@kjemi.uio.no (B. Nyström).

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doi:10.1016/j.cis.2009.05.003
1. Introduction

Polyelectrolytes and ion-containing polymers are an intriguing class of macromolecules with special features and with broad scientific and technological appeal [1–3]. Polyelectrolytes contain polymer chains carrying ionisable groups, which under appropriate conditions dissociate, leaving charges on the chain (polyion) and counterions in the solution. The characteristic properties of amphiphilic polyelectrolytes in water are governed by a delicate interplay between associative interactions (e.g., hydrophobic interactions) and electrostatic repulsive forces. Many biopolymers are amphiphilic and equipped with ionisable groups, and the charge density of the polyion can be tuned by changing pH of the solution. Two very important biopolymers of this category are the anionic polysaccharide hyaluronan (sodium hyaluronate, hyaluronic acid, HA) and the charged high molecular weight glycoprotein mucin, which both are biocompatible and biodegradable and used in various medical applications [4,5]. Effects of pH on structural, dynamical, and rheological features of hyaluronic acid and mucin are examined in this work to gain a better understanding of the behaviors of these complex biopolymers.

A typical polyelectrolyte effect is the extension of polymer chains due to electrostatic repulsive forces, and by adding salt, the electrostatic interactions are screened and this leads to contraction of polymer coils. The effect of ionic strength on the chain dimensions of dilute aqueous solutions of charged polysaccharides is studied by means of light scattering, and the results are compared with those obtained by utilizing the Monte Carlo simulation technique [6] in this review.

Interactions between a charged polymer and an oppositely charged surfactant [7–11] or an oppositely charged polymer [12–16] have attracted a great deal of interest in recent years, because of their importance in both fundamental research and in biological and technological applications [17,18]. The association complexes formed between oppositely charged polymer–surfactant pairs or oppositely charged polymers can be soluble or insoluble, depending on the properties of the system, which can be tuned by many variables such as hydrophobicity and the charge density of the polyion, and the stoichiometry of the mixture [7,18,19]. The attractive association between the polyion and the oppositely charged surfactant or between oppositely charged polyions can lead to macroscopic phase separation, and this behavior is usually accompanied by intriguing structural and dynamical features of the association complexes.

When two oppositely charged polymers are mixed in a solvent, they interact spontaneously and form what is called a polyelectrolyte complex (PEC) [20,21]. It has been argued [22] that the entropy gain associated with the release of counter ions is one of the important driving forces for PEC formation. The properties of the PECs depend on factors such as the nature of the polymer, hydrophobicity of the polycations and, the charge densities of the polymers.

With the intention to limit the scope of this work, most of our attention is focused on interactions in aqueous mixtures of charged polysaccharides and an oppositely charged surfactant. These special features of various polysaccharide systems and mucin are examined in the present review by analyzing results obtained from inter alia turbidimetry, light scattering, small angle neutron scattering (SANS), shear viscosity, and rheo-small angle light scattering (rheo-SALS). Interaction in systems of charged polysaccharides is a vast research area, and it is only possible in this short review to address a few issues in this fast expanding field. Most of the illustrations are taken from our own research.

The paper is organized as follows. In Section 2, we will present results and discuss effects of pH on the viscosity of dilute and semidilute solutions of HA and shear-induced viscosifications of dilute HA solutions. Influence of pH on the structure and association behavior of solutions of mucin is analyzed and structural alterations of the mucin network under the influence of shear flow are scrutinized. Computer simulations are performed in Section 3 to capture changes of the size of the polyion as the ionic strength is increased. The results from the Monte Carlo simulations are compared with light scattering measurements on some charged polysaccharides. Section 4 presents some structural and dynamical results from studies of a charged polysaccharide and an oppositely charged surfactant to illustrate the mechanism behind the formation of large association complexes. Finally, in Section 5 we summarize the main conclusions.

2. Dynamics and structure of polyelectrolyte solutions

Biopolymers that are charged, usually exhibit complex structural and dynamical features. Hyaluronic acid [3] and mucin [5] belong to this category, they exist abundantly in mammalian, and they have been the subjects of intense research in recent years. Below some special properties of these polymers are examined.

2.1. Effects of shear flow and pH on the associating power of hyaluronan

Hyaluronan is a linear anionic polysaccharide [23] consisting of repeating disaccharide units of D-glucuronic and 2-acetamido-2-deoxy-D-glucose that are bound together by a β-(1→3)-type glucosidic bond (see Fig. 1). HA is one of the essential components of the extracellular matrix of the connective tissues, and it is found in different places such as synovial fluid, skin, and umbilical cords [3,24]. HA is known to be degraded in vivo by a specific enzyme called hyaluronidases [25].

HA is an attractive component in the preparation of novel biocompatible and biodegradable biopolymers with possible applications in drug delivery, viscosurgery, viscosupplementation, ophthalmic surgery, and tissue engineering [26–30]. In view of these functions, the rheological features of aqueous solutions of the high molecular weight HA are of special interest. It has been found [31] that dilute solutions of HA exhibit interesting shear-induced viscoelastic effects. Time evolution of the viscosity at two fixed shear rates for a dilute HA solution is depicted in Fig. 2. At the lowest shear rate, a time evolved viscoelasticity of the solution occurs and this behavior is ascribed to shear-induced alignment of the semirigid HA chains and additional stretching of the chains may take place. Because of these effects, more sites on the polymer chains may become accessible for intermolecular interactions through hydrogen bonds and thereby forming interchain complexes. This process may be described in terms of staggered ladder-like structures [31,32]. The fundamental conditions for the formation of this type of arrangements are cooperative mobility, orientation effects, and cooperative zipping of stretched chains through hydrogen bonds. Recent SANS studies [33,34] of aqueous hyaluronan solutions at low ionic strength have revealed the semirigid structure of this polyelectrolyte polysaccharide and interpolymer associations were detected at low scattering wavevectors. Shear-induced viscoelasticity or even gelation has recently been observed [35,36] in aqueous solutions of pectin.

When a higher shear rate is applied, the viscosity drops in the course of time and this indicates that the chains are gradually aligned in the flow field, but the shear rate is too high to promote growth of interchain complexes. At higher polymer concentrations (1 wt.%) in the semidilute regime, the viscosity is virtually independent of time.
In connection with the use of HA in the formulation of drugs and medical applications, the value of pH plays an essential role. For this purpose, it is important to investigate the effect of pH on the rheological properties of HA. Evidence has been provided [37] that HA is degraded at pH values below 1.5 and above 11. In another study, a minor degradation of HA was reported [38] at acid conditions (pH = 1.6) and in a strongly alkali medium (pH = 12.6), but only a minor influence of pH on the rheological features was detected.

A systematic investigation of the effects of pH, time, and shear flow on the degradation of HA in buffered aqueous solutions has recently been conducted [39]. Fig. 3a shows shear rate dependence of the viscosity for buffered semidilute HA solutions at different pH values. The results suggest that strong association networks are formed at intermediate pH values, and at these conditions pronounced shear thinning is observed at high shear rates, which is attributed to shear-induced disruption of interchain associations and entanglements. In contrast, at pH = 13 the viscosity is very low and a virtually Newtonian behavior is registered. At low shear rates (Fig. 3b), a marked decrease of the viscosity is found at low and high pH values for the semidilute system, and this trend is ascribed to disruption of the network structure because of pH-induced degradation of the polymer. A similar trend is found for the dilute solutions of HA, but in this case, the effect is much less pronounced than for the corresponding semidilute HA solutions (cf. Fig. 3b). This is expected because intramolecular cleavage of chains in dilute solutions should have a less impact on the viscosity than network breakup in the semidilute regime.

This picture of polymer degradation is strengthened by the fact that the molecular weight of HA drops at low and high pH values. In Fig. 3c, the weight-average molecular weight ($M_w$) of HA, measured (see the inset of Fig. 2) because the entanglements at these low shear rates resist the breaking up of the network structures.

Fig. 2. Time evolution of the shear viscosity at a fixed shear rate ($0.001\text{ s}^{-1}$ or $0.1\text{ s}^{-1}$) for 0.1 wt.% and 1 wt.% HA solution. Data taken from ref. [31].

Fig. 3. (a) Viscosity as a function of shear rate for semidilute-buffered (0.1 M) systems of HA ($M_w = 1.9 \times 10^6$ and $M_w/M_n = 1.1$) at the pH values indicated. Effects of pH on the zero-shear viscosity for buffered dilute and semidilute samples (b) and on the weight-average molecular weight (c) in dilute HA solutions (AffFF results). (d) Time evaluation of the degradation of 0.5 wt.% HA solutions in terms of molecular weight from AffFF at the indicated pH values. The dissolving of HA in solvents at pH 1 and 7 required longer time than at pH 13. This is the reason why the data points for these two pH values are missing at early times. Data taken from ref. [39].
with the aid of asymmetric flow field-flow fractionation (AFFFF) methods [39] after the same time of preparation of the samples as for the rheological experiments, is plotted as a function of pH for dilute solutions of HA. It has previously been argued [40] that intramolecular cleavage (hydrolysis) of glycosidic bonds occurs at strongly basic conditions. In the investigation referred to earlier [38], a minor degradation of the polymer was reported in the range 1.6< pH<12, but the rheology of the system was almost unchanged. In the cited study, a decrease of the viscosity was found at pH>12, but this observation was related to a situation of reduced stiffness of the polymer backbone under alkaline conditions due to partial breakage of the H-bond network. The results presented above have clearly established that HA is degraded at low and high pH values, and it is only in the approximate pH interval 5<pH<10 that the polymer can be considered to be stable and it does not undergo a time-induced degradation.

To obtain some information about the time window for the degradation of HA, Fig. 3d shows molecular weight results from AFFFF measurements conducted at various times on dilute solutions of HA at different pH. The results clearly show that the degradation rate is faster at pH=13 than at pH=1. At pH=7, the molecular weight is practically unaffected by time. Over a period of 50 h, the molecular weight of HA at pH=13 decreases by approximately a factor of 10, while at pH=1 the degradation of the polymer is slower, but still important. These findings show that the degradation of HA at low and high pH values is significant and continuous over a long time. This problem is also important when HA chains are chemically cross-linked to form hydrogels at acid or alkaline conditions [28].

2.2. Effect of pH on the structure, dynamics, and viscosity of mucin

Mucin is a charged polymer with a broad molecular weight distribution, and it consists of high molecular weight (M>10^6) D-linked glycoproteins, composed of a polypeptide backbone and covalently linked oligosaccharide side chains [5,41]. Mucin is a very complex polymer [42] with monomers consisting of glycosylated and non-glycosylated peptide blocks linked by intramolecular disulphide bridges. Mucin contains a small number of amino acids that build up the protein backbone and sugar residues, mainly galactose, fucose, N-acetylgalactosamine, N-acetylgalactosamine, and sialic acid that form the oligosaccharide chains. The electrostatic character of mucin is generated by charges both within the polypeptide backbone, for example, glutamic acid and aspartic acid residues (pK_a≈4) [43] as well as in the oligosaccharide side chains, for example, sialic acid residues (pK_a≈2.6), together with the sulfide residues (pK_a≈1) [44]. At physiological pH, the mucin chains are negatively charged, whereas at very low pH they carry positive charges. Mucin is a highly ordered molecule, whose molecular assemblies produce structures exhibiting length scales from a few hundred nanometers up to several microns [45], and a schematic view of the mucin structure is displayed in Fig. 4. The sample utilized in the experiments presented below, is a noncommercial purified pig gastric mucin with a high molecular weight (M_w≈1.5×10^7) and a broad (M_w/M_n≈3) molecular weight distribution [46].

Small-angle neutron scattering is an ideal tool to investigate structures in the size range of 5 Å to several hundred angstroms. In scattering experiments the scattering vector is a central quantity and is given by q=(4π/λ) sin(θ/2), where λ is the wavelength of the incident beam, θ is the scattering angle, and n is the refractive index of the medium (for SANS, n is set to 1). Fig. 5 shows the variation of the SANS scattering intensity as a function of q (log-log plot) for a buffered (pH≈7) semidilute (5 wt%) aqueous solution of noncommercial pig gastric mucin [41] at 25 °C and 37 °C (body temperature). The scattered intensity is linear over a large q-range (more than one decade) and no sign of a Guinier regime (plateau in the scattered intensity) in the low-q-range domain can be detected. This extended power-law regime is not a typical behavior for semidilute solutions of charged biopolymers, where the SANS scattered intensity profile usually consists of regions with different q dependence [9,33,34,47-49], and in some cases a structure peak [48,49] appears at intermediate q values that accounts for interparticle correlations in charged systems. The absence of a correlation peak for the present system indicates that the polyelectrolyte effect is weak. The power-law appearance seen in Fig. 5 is typically that of a fractal-like system.

In semidilute solutions, i.e., above the overlap concentration, the characteristic length is the correlation or screening length ξ that decreases with increasing polymer concentration. In this concentration regime the solution can be visualized as a transient network with a certain average mesh size ξ [50]. The quantity ξ is frequently used as a measure to decide whether global or local features of the network are probed. In the Guinier region (ξ < 1), the mesh size of the network can be estimated, whereas in the region ξ > 1 a more local dimension scale is monitored.

In the regime ξ > 1, the length scale q^{-1} is associated with more local properties of the system, and the scattered intensity depends strongly on the length scale. The conjecture is that the present SANS results cover this regime, and in this region the semidilute solution can be viewed as an irregular fractal network [51], formed by more or less interpenetrating clusters. In this case, the scattered intensity I(q) decays with the wave vector as I(q)∝q^{-Df}, where the slope of the scattered intensity in the power-law regime yields the fractal dimension d_f (0<d_f≤3). The results suggest that d_f =1.38±0.01 at 25 °C and d_f =1.35±0.01 at 37 °C. The values are quite close to each other, and values around 1 are indicative of an interconnected open network consisting of locally stretched structures. The structure is very little affected by temperature in this temperature interval. This

Fig. 4. Schematic view of the mucin molecule. Native mucins isolated from body secretions or tissues are always heavily glycosylated.
When pH is varied in the interval 1–4, dilute concentration regime, entanglements will also come into play as the charges is changed and the charge density is altered. Hydrophobic interactions will play an important role, promoting the formation of large interchain complexes \( R_{h,app} \leq 600 \text{ nm} \). It has previously been observed \[53\] that huge aggregates are formed in the semidilute concentration regime, a heterogeneous network with a large average mesh size is formed consisting of bundles of interlacing chains. This is compatible with the results from a recent study \[45\] on mucin gels at pH = 2, where it was observed that the gel is highly heterogeneous, containing a wide distribution of pore sizes. In most of the cited studies above, the importance of hydrophobic interactions in the formation of multi-chain complexes has been emphasized, and it seems to be consensus of its vital role for associations and gelation in mucin systems at pH around 2.

In dilute solutions at pH = 1, the polyions are weakly positively charged \[56\] and a situation emerges where the aggregates are compressed and probably stabilized by positive charges on the surfaces of the moieties. In semidilute solutions at this pH, we note that the average mesh size is small and the viscosity has dropped strongly (almost 4 decades). In view of the low viscosity, the conjecture is that the combined effect of association and repulsive forces has given rise to a state of a fragmented network, with patches of close-packed chains. It is difficult to imagine a fully connected network when the viscosity is so low.

At pH > 2, the density of negative charges of the polymer chains rises gradually as pH of the system increases \[57\]. An increase of pH in the dilute regime will repress the tendency of forming large interchain species (see the inset pictures in Fig. 6) because of electrostatic interactions. At pH = 7, the average size of the entities is about 75 nm and probably most of the chains are molecularly dispersed. This value is higher than that \( R_{h,app} = 44 \text{ nm} \) reported \[43\] for gastric mucin at pH = 2. This is probably due to the very high molecular weight of the present sample. In the semidilute regime, the inclination of forming
close-packed chains is reduced at higher pH and a more homogeneous and regular network evolves as the pH rises (see the inset pictures in Fig. 6).

These results can be rationalized in a framework of a model [58,59] that was developed to describe association and gelation [60] in semidilute aqueous mixtures of ethyl(hydroxyethyl)cellulose and an ionic surfactant. In this model, the connectivity of the network is provided by polymer “lumps” (hydrophobic interchain associations) and entanglements, and swelling of the network is generated by the electrostatic repulsions of the ionic surfactant. Using this scenario in the interpretation of the results for the mucin system, the swelling dominates at pH=7 and this yields a homogeneous and loose network. As the pH decreases, the hydrophobic associations become progressively more important and the lumps grow and at pH=2 a strong heterogeneous network is evolved. At pH=2, compaction of the chains continuous and a fragmented network is formed with substructures that are decorated with some charges.

Rheo-small angle light scattering (rheo-SALS) is a powerful method to obtain information about shear-induced structural changes of polymer systems. Light scattering is used to monitor morphological alterations of the samples under the influence of shear flow. Fig. 7 shows typical SALS patterns obtained from 1 wt.% solutions of mucin at different pH values and shear rates [46]. The 2D images reveal that scattered intensity profiles are strongest at pH=2 and weakest at pH=7. This behavior substantiates the hypothesis launched above that huge interchain complexes are formed at pH=2, and the growth of multichain structures is inhibited at pH=7. We note that at pH=2 at high shear rates, the scattered intensity profile exhibits an anisotropic pattern with an elliptical image with a major axis parallel to the flow direction. This type of feature is usually a signal of strong concentration fluctuations, which are coupled to the applied mechanical stresses [61,62]. In the present system this behavior is most likely due to a shear-induced deformation of the mucin complexes, followed by a preferred orientation of the major axis along the flow-field. An inspection of the patterns at pH=7 discloses that augmentation of the structure is accentuated at high shear rate. This announces that shear stresses promote the growth of association structures. At pH=1, the scattered intensity profiles are weaker than those at pH=2, but stronger than those at pH=7. These findings support the results presented above.

A direct comparison of the rheo-SALS 2D patterns with the turbidity results for 1 wt.% mucin at various pH values is depicted in Fig. 8. The turbidity ($\tau$) values of the samples are determined from the relation $\tau = (-1/L)\ln(I/I_0)$, where $L$ is the light path length of the measuring cell, $I$ is the transmitted light intensity, and $I_0$ is the incident light intensity. The turbidity shows that large “lumps” are formed at pH=2 and 4, whereas the interchain association is reduced at pH=1 and pH=7. The trends of the intensities of the scattered patterns (insets in Fig. 8) are consistent with the turbidity results and the other experimental data presented above.

3. Monte Carlo simulation of the effect of ionic strength on the size of charged polysaccharides

Charged hydroxyethylcellulose (HEC (+)) and HA belong to a class of water-soluble linear polysaccharides bearing charges in water, and they belong to the class of semirigid polyelectrolytes. The idea behind this concept is that apart from electrostatic interactions, the backbone of the chains are locally stiff enough to yield an intrinsic persistence length $L_0$ that is much larger than the length of one monomer, and $L_0$ can be measured when the electrostatic interactions are screened, e.g., by addition of salt. The size of the polion can be estimated from the root mean squared radius of gyration $R_g$ obtained from intensity light scattering (ILS) measurements. Alterations of $R_g$ with increasing ionic strength (raising the salt concentration) are here monitored by ILS for dilute solutions of HA and charged HEC, and the results are compared with computer simulations.

In the case when the repulsive forces between the charges along the chains are not screened, they will tend to strengthen the local rigidity of the chains and the global sizes of the polions will increase. As a result, the total persistence length $L_0$ increases. $L_0$ consists of an electrostatic persistence length $L_e$, due to Coulombic repulsive forces, and an effective persistence length $L_0$, i.e. the total persistence length can be written as [63] $L_0 = L_e + L_0$. Thus $L_0$ represents the effective rigidity of the polion in terms of the sum of the intrinsic persistence length of the corresponding uncharged chain and the electrostatic persistence length that depends on the screening of the electrostatic interactions through, e.g., addition of salt. For semirigid polyelectrolyte polysaccharides, $L_0$ is usually longer than $L_e$, especially when the salt concentration is higher than 0.01 M.

3.1. Simulation methodology

As a complement to experimental results, we have carried out computer simulations that are able to capture the conformational behavior of flexible and semiflexible polyelectrolytes at different ionic strength. For that purpose, we have employed the Monte Carlo simulation technique, which is most appropriate for obtaining equilibrium conformational properties of macromolecules [6], together with a coarse-grained (non-atomicistic) model of the flexible linear polyelectrolyte. The coarse-grained model used, consists of a linear chain of $N$ beads connected by $N−1$ FENE (finite extensible non-linear elongational) springs [6]. The mathematical expression for the FENE potential is

$$V^{\text{FENE}}(Q) = -\frac{1}{2}H\sigma_{\text{max}}^2 \ln \left(1 - \frac{Q^2}{Q_{\text{max}}^2}\right)$$ (1)

where $Q$ is the spring vector, $Q_{\text{max}}$ is the maximum spring length and $H = 3k_bT/B^2$ is the spring constant, $B^2$ is the equilibrium mean squared length of a Hookean spring, $k_b$ the Boltzmann constant, and $T$ the absolute temperature.

Good solvent (excluded volume effects) conditions were introduced by means of the following Lennard–Jones potential between non-bonded beads $i,j$

$$V^{\text{LJ}}(r_{ij}) = 4\sigma_{ij}^6 \left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - 2\sigma_{ij}^6 \left(\frac{\sigma_{ij}}{r_{ij}}\right)^6$$ (2)
where \( r_{ij} \) is the distance between beads \( i \) and \( j \) and the potential parameters take the values \( \alpha_{ij} = 0.8b \) and \( \bar{\epsilon}_{ij} = 0.1 \) \( k_B T \) [64].

Following previous simulation studies by Pamies et al. [65,66], electrostatic interactions were taken into account by using the Debye–Hückel potential that reads

\[
V^{\text{DH}}(r_{ij}) = \frac{A}{r_{ij}} \exp(-\kappa r_{ij})
\]

(3)

In the above expression

\[
A = \frac{\varepsilon_0}{4\pi\sigma_{\text{LJ}}} n^2
\]

(4)

where \( \varepsilon_0 \) is the permittivity of vacuum and \( \sigma_{\text{LJ}} \) is the distance between beads. The total charge of the polymer chain is considered to be uniformly distributed along the chain and \( \bar{\epsilon}_0 \) are the vacuum permittivity and the dielectric constant of the solvent, respectively. On the other hand, the screening parameter \( \kappa \) is related to the ionic strength of the medium \( I \). At room temperature, for a 1:1 electrolyte such as \( \text{NaCl} \), which are the conditions employed in our simulations, the screening constant in \( \text{nm}^{-1} \) was computed through the equation [67]

\[
\kappa = \frac{\sqrt{\varepsilon_{\text{NaCl}}}}{0.304}
\]

(5)

where \( \varepsilon_{\text{NaCl}} \) is the concentration (\( M \)) of \( \text{NaCl} \) in the bulk.

As mentioned before, we use the Monte Carlo simulation technique according to which an ensemble of conformations of the bead-and-spring model is generated by an “importance sampling” Monte Carlo simulation as implemented in our public domain program MONTEHYDRO [68]. In this way, five independent polymer trajectories are generated. Steady-state properties are computed by averaging over each trajectory, after discounting the initial part for equilibration purposes. Then, the mean and standard deviation over the five trajectories are taken to characterize the actual property values.

A simulation technique useful to study non-Newtonian performance of polymers under flow is the Brownian dynamics that consists of solving the stochastic equation of motion of the polymer chain. Here we do not present simulations about the non-Newtonian behavior, but we made some Brownian dynamics simulations at equilibrium by employing a predictor-corrector method proposed by Iniesta and García de la Torre [69]. As expected, they yielded the same values as the Monte Carlo technique.

3.2 Estimate of the model parameter values and comparison with experimental data

All the parameters involved in the bead-and-spring model described above can be related to polymer structural and electrostatic features, leaving the number of beads \( N \) as the only free parameter, which can have any arbitrary sufficiently high value. In order to obtain the value of the parameters defining the FENE springs, we follow the parameterization procedure described in [70], which is based on the quantity \( E \) that gauges the extensibility of the polymer chain, \( E = L/R_g \), where \( L \) is the contour length of the polymer. Provided with the experimental value of \( E \), and for a given \( N \) we can obtain \( H \) and \( Q_{\text{max}} \).

The Debye–Hückel interaction energy parameters can be estimated once we know the total charge of the polymer \( Q \), the ionic strength of the solvent \( I \), and the permittivity of the medium, \( \varepsilon = \varepsilon_0 \varepsilon_r \) or alternatively the Bjerrum length \( \lambda_B \). We consider that the charges of the polymer are uniformly distributed among the beads of the chain, and therefore the charge on bead \( i \) will be \( q_i = Q/N \). In terms of electrostatic valences, \( Z_i = q_i/e = Q/(eN) \) (\( e \) being the electron charge). The parameter \( A \) refers to the Debye–Hückel potential and it is computed from the polyelectrolyte charge, which can be obtained if we know the polymer degree of ionization or substitution. In our case, we took \( A \) as a free parameter in order to probe the effect of this parameter when comparing experiment and simulated data. For the dilute aqueous hyaluronan solutions, we can assume that the HA chains are charged because the \( pK_a \) value is 2.9 [71] and our ILS experiments were carried out at \( pH = 5 \). The parameter \( \kappa \) is computed from the salt concentration as mentioned before Eq. (3). A value of \( R_{\text{app}} = 114 \text{ nm} \) was determined with the aid of ILS measurements on a dilute \( (0.01 \text{ wt.}\%) \) solution of HA at \( pH = 5 \) and at an ionic strength \( I = 0.1 \text{ M} \). At this high ionic strength, a total screening of the electrostatic interactions can be assumed [34]. We calculated the value\( L = M_w/M_0 \), where \( M_0 \) is the average molecular weight determined by Asymmetric Flow-Field Flow Fractionation (AFFFF) methods [39] and \( M_0 = \text{392 Da/nm} \). This yields a value of \( E = 30.4 \). Taking an arbitrary number of beads \( N = 20 \), a value of \( Q_{\text{max}} = 186 \text{ nm} \) (Eq. (1)) is calculated. We assigned a value of \( A = 2 \times 10^{-16} \text{ erg cm}^{-1} \).

In Fig. 9, effect of ionic strength (addition of salt) on the apparent radius of gyration \( R_{\text{app}} \) obtained from ILS experiments, for a 0.01 wt. % buffered HA solution at \( pH = 5 \) is illustrated, together with results from computer simulations by using different values of the Debye–Hückel potential \( A \), with \( A = 8.7 \times 10^{-19}, 2 \times 10^{-16}, 4 \times 10^{-16}, 10^{-18}, 2 \times 10^{-15} \).

![Fig. 9. Radius of gyration as a function of ionic strength for a 0.01 wt.% buffered HA solution at pH=5. Both experimental ILS data and computer simulation results are shown. The error bars of the experimental data are of approximately the same size as the symbols and the errors in the simulation data are ±1 standard deviation.](image)

Fig. 9. Radius of gyration as a function of ionic strength for a 0.01 wt.% buffered HA solution at \( pH = 5 \). Both experimental ILS data and computer simulation results are shown. The error bars of the experimental data are of approximately the same size as the symbols and the errors in the simulation data are ±1 standard deviation.
the polyelectrolyte effect is very strong. In the computer simulations of HEC(+), we considered a HEC chain with the parameters employed in a recent Brownian dynamics simulation of HEC [73]. For the HEC (+) sample with low charge density, a suitable value of $A = 4 \times 10^{-17}$ erg·cm was observed to be $4 \times 10^{-17}$ erg·cm and HEC with 60 mol% charges a value of $A = 2 \times 10^{-16}$ erg·cm was utilized. The experimental ILS data for the HEC(+) sample with a low amount of charges are well portrayed by the simulation curve. However, the experimental data for the polymer with high charge density display a polyelectrolyte transition of $R_g$ at a much higher ($I_{0.05}$) ionic strength than the corresponding simulation data ($I_{0.01}$). The reason for this divergence can probably be ascribed to a higher local stiffness of the HEC chains at this high charge density (60 mol% charges). The simulation model is developed for fairly flexible chains, and the approach is not capable to address the effect of strong chain rigidity. Nevertheless, it is interesting to note that this quite unsophisticated model works pretty well for the semi-rigid polymers considered above.

4. Interactions between charged polysaccharide and an oppositely charged surfactant

The investigation of polyelectrolyte–surfactant complexes is an area of considerable interest due to the establishment of systems of novel properties with their high potential for applications. Aqueous mixtures of polyelectrolytes and oppositely charged surfactants associate strongly due to the favorable electrostatic interactions and the aggregation of hydrophobic moieties between the species. These types of interactions have been studied widely over the last decades using various experimental techniques by scientists all over the world regarding various aspects of these interactions [74–78]. To advance the understanding of the physics governing these interactions it is scientifically challenging since the structural and dynamical changes of both the polymer and the surfactant affect their interactions and the properties of the mixed system. It is well established that the onset of binding of the surfactant to the polymer occurs at a concentration several orders of magnitude below the critical micelle concentration (CMC) of the surfactant, which is referred to as the critical aggregation concentration (CAC) [18,77]. In this section, we focus on association behavior in aqueous mixtures of anionic hydroxyethylcellulose HEC (−) in the presence of the cationic surfactant CTAB. In order to compare the results, similar measurements in the presence of a surfactant (SDS) with charges of the same sign as the polymer have also been conducted [9].

4.1. Characterization of polymer–surfactant interactions by DLS

Because of its advantageous properties like biodegradability and biocompatibility, hydroxyethylcellulose is one of the most commonly used cellulose ethers in various industrial fields such as cosmetics, personal care products, and pharmacy. An illustration of the chemical structure of HEC with incorporated negative charges is displayed in Fig. 11.

To gain insight into dynamical changes of the system, dynamic light scattering measurements were performed for the semidilute aqueous solutions of HEC(−) in the presence of CTAB or SDS (see Fig 12a). The time correlation functions at all conditions have been fitted with the aid of Eq. (6) (see below). The formation of a plateau-
The strong attractive electrostatic forces between negatively charged polymer and the cationic surfactant lead to the evolution of large association complexes in the system and the following expression with two stretched exponentials was found [9] to describe the correlation function data well

$$g^s(t) = A_t \exp[-(t/\tau_{fe})^{\alpha_t}] + A_s \exp[-(t/\tau_{se})^{\beta_s}]$$

(6)

with $A_t + A_s = 1$. The parameters $A_t$ and $A_s$ are the amplitudes for the fast and the slow relaxation modes, respectively. The variable $\tau_{fe}$ represents the fast effective relaxation time and this quantity in the semidilute concentration regime is related to a cooperative diffusion coefficient $D_c$ ($\tau_{fe} = D_c q^2$). The parameter $\tau_{se}$ refers to the slow effective relaxation time, and this mode is associated with disengagement of individual chains or cluster relaxation [9]. The parameters $0 < \alpha \leq 1$ and $0 < \beta \leq 1$ measure the widths of the distributions of relaxation times for the fast and the slow mode, respectively. The mean relaxation modes are given by

$$\tau_{fe} = \int_0^\infty \exp[-(t/\tau_{fe})^{\alpha_t}] \, \frac{dt}{t} = (\Gamma/\alpha_t)^{1/\alpha_t}$$

(7a)

and

$$\tau_{se} = \int_0^\infty \exp[-(t/\tau_{se})^{\beta_s}] \, \frac{dt}{t} = (\Gamma/\beta_s)^{1/\beta_s}$$

(7b)

where $\Gamma$ is the gamma function.

Fig. 12b illustrates the effects of surfactant addition and the type of surfactant on the reduced intensity $I_{\text{red}}$ for semidilute aqueous mixtures of HEC(-)/SDS or HEC(-)/CTAB systems. The reduced intensity rises strongly with surfactant concentration for the HEC(-)/CTAB system. This is another indication of formation of large aggregates in oppositely charged HEC(-)/CTAB mixtures, which is driven by attractive Coulombic interactions. In contrast, the addition of like charge surfactant SDS to a semidilute aqueous solution of HEC(-) leads to another pattern of behavior. Since both species carry charges of similar sign, the repulsive electrostatic forces limit the evolution of large clusters. The enhanced repulsive forces between the HEC(-) and the like charge surfactant at sufficiently high surfactant concentrations will break up the associations and result in a moderate decrease in the reduced intensity.

It is worth noting that the associative phase separation phenomena [78], which generally take place in oppositely charged systems lead to the formation of insoluble complexes. This feature usually occurs above a certain level of surfactant addition, where the charge neutralization point between the polyelectrolyte and the oppositely charged surfactant [79] is located. This region is not addressed in this review.

The effects of surfactant concentration and the type of surfactant on the fast and the slow relaxation time are depicted in Fig. 13. The slow relaxation time that is ascribed to disengagement of individual chains or clusters, displays a pronounced upturn for the HEC(-)/CTAB systems as the level of surfactant addition increases, whereas for the HEC(-)/SDS system and for the mixture of uncharged and unmodified HEC(0) with SDS or CTAB, only a slight change of the slow relaxation time with surfactant concentration can be traced. This upturn of $\tau_s$ for the HEC(-)/CTAB system suggests that the relaxation of clusters or individual chains is strongly impeded when complexes of oppositely charged polymer and surfactant are formed. The fast relaxation time, which reflects a concerted motion of polymer chains in a transient network relative to the solvent, reveals a similar pattern of behavior as for the slow relaxation time (see the inset plot of Fig. 13). The marked increase of $\tau_s$ is attributed to the development of large polymer–surfactant aggregates for HEC(-)/CTAB mixture as the CAC concentration is approached and the relaxation of the transient network is slowed down. These findings clearly demonstrate that the progressive addition of the cationic surfactant CTAB to the semidilute aqueous solutions of oppositely charged HEC(-) leads to the evolution of large clusters in the system as a result of the strong attractive electrostatic interactions between the species, while in the case of similarly charged HEC(-)/SDS mixtures, or for the unmodified HEC(0) solutions in the presence of SDS or CTAB, no significant change of the values of $\tau_f$ and $\tau_s$ can be observed, suggesting that there is no strong tendency to create large polymer–surfactant complexes.
4.2. Investigation of polymer–surfactant structural features by SANS

SANS measurements allow investigation of the structure of the polymer network on a mesoscopic scale. This powerful technique makes us enable to probe the structure of the polymer solutions on a more local scale. Fig. 14 elucidates the SANS intensity profiles on a log–log plot for the systems indicated in the figure. As is evident, there is a strong upturn of the scattered intensity at low q values for the HEC(−) in the presence of 2 mm CTAB (above its CAC value). This observation is associated with the presence of large-scale structures from the polymer–surfactant complexes. Similar behavior has been observed for associative polymer systems of various natures [80–82]. A scattering maximum or a correlation peak at intermediate values of q is found for the HEC(−)/CTAB mixture. This type of behavior is frequently observed for various polyelectrolytes [83–85] accounting for interparticle correlations in charged systems.

The SANS scattering profile for the HEC(−)/2 mm SDS mixture and for the aqueous solutions of HEC(0) and HEC(−) without surfactant exhibit a similar trend with much less pronounced upturn at low q values compared to that for HEC(−) in the presence of 2 mm CTAB. This findings are in concordance with the results obtained by the DLS experiments and provide another evidence for the existence of large associations in the solutions of HEC(−) and the oppositely charged surfactant (CTAB).

5. Conclusions

The present review elucidates special features of some important charged polysaccharide systems and mucin. In dilute aqueous solutions of hyaluronan, some interesting time-dependent shear thickening and shear thinning features were observed under the influence of constant shear flows. In semidilute solutions of HA, no time-dependent effect was detected. HA is a polysaccharide that is sensitive to environmental conditions. A pH-induced degradation of HA is registered at acid (pH<5) and at basic (pH>10) conditions, and this scission of the chains continues over a long period of time. In the approximate interval 5<pH<10, no degradation of the polymer is found.

Mucin is a complex biopolymer with many intriguing features. The structure of mucin in the semidilute concentration regime (pH≈7) was investigated by SANS, and the fractal dimension d_f is close to 1.4, which suggests a rather extended local conformation of mucin. The effect of temperature on d_f is very modest. At pH≈2, mucin is practically uncharged and the intense hydrophobic interactions give rise to the formation of interchain complexes in dilute solutions, and a heterogeneous network with polymer “lumps” in the semidilute concentration regime. At higher pH, the polymer becomes progressively more negatively charged as the pH increases and the repulsive forces counteract the hydrophobic interactions; the arrangement of interchain complexes is compressed and a more homogeneous network evolves. At pH<2, the polymer chains are contracted and they carry positive charges that stabilize the species and a fragmented network is formed. The immense multichain association at pH around 2 is supported by rheo-SALS experiments and turbidity measurements.

Effects of ionic strength on the radius of gyration for dilute solutions of HA and positively charged hydroxyethylcellulose (HEC (+)) were studied by intensity light scattering, and the results were compared with Monte Carlo simulations. Most of the features were well portrayed by the computer simulation showing a good agreement with the experimental data. The unsophisticated simulation model that was developed for flexible chains worked satisfactorily also for these semirigid polymers. It was only for the highest charge density (60 mol% charges) of HEC(+) that a significant deviation occurred, due to the increment of the rigidity when a larger number of charges are present in the chain. We proved that the presence of salt, i.e. high ionic strength, provokes screening of the charges in the chain leading to a contraction of the polymer.

Formation of interchain complexes were detected in mixtures of negatively charged HEC and an oppositely charged surfactant (CTAB). These interaction effects lead to high turbidity values and the pronounced upturn of the scattered intensity at low q values from the SANS experiments signalizes the formation of large association structures.

Acknowledgments

We gratefully acknowledge support from the Norwegian Research Council for the project (177665/V30) and the Fundación Séneca-C.A.R. M for the grant 04531/GERM/06. R.P. acknowledges a postdoctoral fellowship from the Fundación Séneca-CARM, J.G.T. thanks M.E.C. for financial support provided by grant CTQ-2006-06831, including FEDER funds. A.M. and B.N. thank for the financial support provided by a FUNMAT project (Novel functional polymer materials for drug delivery applications).

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