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TEMPO-Mediated Oxidation of Pullulan and Influence of Ionic Strength and Linear Charge Density on the Dimensions of the Obtained Polyelectrolyte Chains

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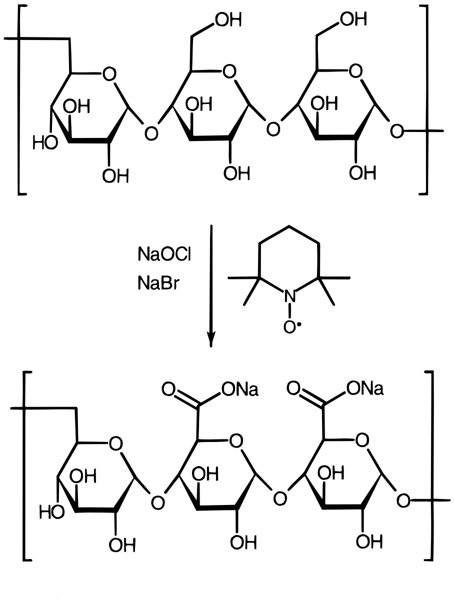
ABSTRACT: The applied pH during the TEMPO-mediated (2,2,6,6-tetramethylpiperidine-1-oxyl) oxidation of the polysaccharide pullulan has a substantial influence on the molar mass distribution of the products. The least degradation was found in the pH range 9.2−9.7 and especially at higher pH, significant degradation occurs due to β-elimination. Four samples were prepared at pH 9.4 with different degrees of oxidation and the influence of the charge parameter ξ on the rms radius of gyration at various ionic strengths (C_s) was determined with size exclusion chromatography (SEC) using a multiangle laser light scattering (MALLS) detection system. Experimental values were compared to values obtained with the wormlike chain model for polyelectrolytes in combination with excluded-volume theory. The ionic strength−linear charge density dependence of the rms radius of gyration is quite accurately described, whereas the prediction of its absolute value is more problematic for lack of a quantitative description of solvent quality. However, very reasonable values for the rms radii of gyration are obtained using eq 15, originally derived as a scaling relation. Furthermore, it is shown that there is no discrepancy between the apparent persistence length, proportional to $\xi/C_s^{1/2}$, and the real persistence length, proportional to ξ^2/C_s, when excluded-volume effects are accounted for.

Introduction

Modification of polysaccharides may yield commercially interesting products, as is evidenced by the application of cellulose and starch derivatives in various industries.[1] A promising route to obtain new polymers is oxidation of the hydroxyl groups present in carbohydrate polymers.[2] We recently described a method for the oxidation of polysaccharides with hypochlorite, using catalytic amounts of TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) and sodium bromide (Scheme 1).[3,4] The reaction is performed under homogeneous conditions in water and primary alcohol groups are selectively oxidized to yield carboxylates. The regioselectivity of the reaction is essentially complete, that is, we were not able to detect any other products than uronates from the oxidation of various unprotected monomeric and polymeric carbohydrates.[3,4] Natural polyuronates like alginate and pectate are widely used in food as well as nonfood industries.[1]

Scheme 1



Although the TEMPO-mediated oxidation of polysaccharides has been optimized with respect to the reaction rate,[4] the influence of the reaction conditions on the molar mass distribution has not been studied. Here we report such a study, with the linear, water-soluble polysaccharide pullulan as the substrate. Pullulan consists of a repeating trimer, having two primary alcohol groups per three monomers (Scheme 1). Under conditions with the least degradation, four samples were prepared with different degrees of oxidation. The radii of gyration of these samples were determined at several ionic strengths with size exclusion chromatography (SEC) multiangle laser light scattering (MALLS) and compared to the calculated radii of gyration according to the wormlike chain model for polyelectrolytes[5,6] in combination with excluded-volume theory.

Theoretical Approach

A basic relation of the wormlike chain model expresses the unperturbed mean-square radius of gyration

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$\langle S^2\rangle_0$ of the polymer in terms of its persistence length L_P and its contour length L:[7]

$$\langle S^2\rangle_0 = LL_P/3 - L_P^2 + 2L_P^3/L - 2L_P^4/L^2[1 - \exp(-L/L_P)] \quad (1)$$

which correctly describes the two limiting cases of random coils and rodlike polymers. Odijk,[5] and Skolnick and Fixman[6] showed that in the case of polyelectrolytes, the stiffening of the polymer chain due to electrostatic repulsion could be described by adding an electrostatic persistence length L_E to the intrinsic persistence length, giving

$$L_T = L_P + L_E \quad (2)$$

where L_T is the total persistence length. This gives for the unperturbed mean-square radius of gyration of polyelectrolytes in the random coil limit

$$\langle S^2\rangle_0 = LL_T/3 \quad (3)$$

The electrostatic term in eq 2 is expressed as[5,6]

$$L_E = \xi^2/(4Q\kappa^2) \quad (4)$$

where Q is the Bjerrum length, κ^{-1} the Debye screening length, and ξ the charge parameter, the number of charges per Bjerrum length. In water at 25 °C, the Bjerrum length Q is about 0.715 nm and the Debye screening length is given by κ^{-1} (nm) $= 0.304C_s^{-1/2}$, where C_s is the concentration of added 1:1 electrolyte in mol/L.

Equation 3 is valid only under θ conditions, when excluded-volume effects are absent. A number of theories regarding excluded-volume effects for polymers have been developed, of which no single one is significantly better than others.[8] We follow the line of reasoning by Odijk and Houwaart,[9] including the calculation of the effective diameter of the polyelectrolyte according to Fixman and Skolnick.[10]

Deviations from the unperturbed state are expressed by the expansion factor α_s, giving for the perturbed mean-square radius of gyration $\langle S^2\rangle$

$$\langle S^2\rangle = \alpha_s^2\langle S^2\rangle_0 \quad (5)$$

where α_s is solely a function of the excluded volume parameter Z. The relation between α_s and Z can be approximated by the Yamakawa–Tanaka equation:[7]

$$\alpha_s^2 = 0.541 + 0.459(1 + 6.04Z)^{0.46} \quad (6)$$

It is assumed that, at moderate ionic strength, excluded-volume effects are dominated by electrostatic interactions and $Z = Z_{el}$, with

$$Z_{el} = [3^{3/2}/(32\pi^{3/2})]\beta_{el}L^{1/2}L_T^{-7/2} \quad (7)$$

where β_{el} is the effective volume excluded from one Kuhn segment, equal to $2L_T$ in the wormlike chain model, by the presence of another. The electrostatic excluded volume is given by

$$\beta_{el} = 2\pi L_T^2 d_{eff} \quad (8)$$

where d_{eff} is the effective diameter of the wormlike chain, which was derived by Fixman and Skolnick[10] and may be approximated by

$$d_{eff} = \kappa^{-1}(-\ln Q\kappa + 2\ln\xi + 2.61) \quad (9)$$

When the effective diameter, calculated according to this equation, becomes unrealistically low (at high ionic strength and/or a low linear charge density), it is simply set at 0.6 nm as a minimum hard-core diameter for the oxidized pullulans.

We are well aware of the fact that the application of this theoretical treatment is subject to several restrictions. The most important, regarding this paper, is probably the local stiffness approximation, $L_T \gg \kappa^{-1}$, which should be satisfied. In this work, L_T is generally in the order of κ^{-1}, and we assume that the calculations are at least good first-order approximations.

Experimental Section

Materials. Pullulan was obtained from Hayashibara (Okayama, Japan), and dextrans were obtained from Pharmacia (Uppsala, Sweden). TEMPO was acquired from Sigma (St. Louis). A ~15% sodium hypochlorite solution was a gift from AKZO-Nobel (Arnhem, The Netherlands). All other chemicals were analytical-grade commercial products and were used without prior purification.

Sample Preparation. (Highest degree of oxidation: pullox4). Pullulan (2.0 g, 7% water, 11.5 mmol anhydroglucose units) was dissolved in 250 mL of demineralized water. TEMPO (0.040 g, 0.26 mmol) and NaBr (0.20 g, 1.9 mmol) were added, and the solution was cooled in an ice bath. A ~15% sodium hypochlorite solution (12 mL, ~25 mmol) was brought to pH 9.4 by adding 4 M aqueous HCl and also cooled in an ice bath. The solutions were mixed, and the pH was maintained at 9.4 during the oxidation by adding 0.5 M aqueous NaOH with an automatic buret. An ice bath sufficed to maintain the temperature at ~1 °C. When addition of sodium hydroxide became very slow (after 110 min and after addition of 15.0 mL of 0.5 M NaOH), the reaction was quenched by adding methanol (5 mL) and neutralized by adding 4 M HCl. Then excess sodium borohydride (0.5 g, 13 mmol) was added and the solution was stirred overnight at room temperature. The reaction mixture was brought to pH 6 by adding 4 M HCl, and the oxidized polymer was desalted by ultrafiltration, after which the solution was freeze-dried and the polyelectrolyte isolated in a yield of 95% (based on the ideal molar mass of completely oxidized pullulan).

The three polymers with lower degrees of oxidation, pullox1–3, were prepared in an analogous manner. Here sodium hypochloride was slowly added until the desired amount of sodium hydroxide (3.75, 7.5, and 11.25 mL of 0.5 M NaOH, respectively) was consumed.

Analysis. The selectivity of the oxidation was reported before,[4] and here only the degree of oxidation of the isolated products was determined with a colouric assay for uronic acids.[11] This proved to be satisfactory, with a disagreement in the degree of oxidation of less than 5% with respect to the values obtained from the consumption of hydroxide during the oxidation. For optimization of the reaction with respect to the molar mass distribution of the oxidized polymers, a relative determination of the molar mass distribution was done during the reaction with SEC (TSK G5000PW-column) in combination with RI (refractive index) detection. Eluent was a 0.1 M phosphate buffer at pH 7. Figure 1 shows a normal calibration of the column with known

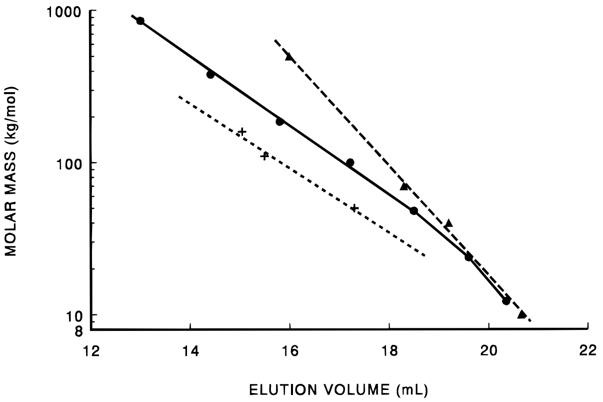


Figure 1. Calibration of the TSK G5000PW column with known molar mass pullulans (●), dextrans (▲) and completely oxidized pullulans (+).

molar mass (obtained by light-scattering) pullulan, dextran, and completely oxidized pullulan samples. The difference between the slopes of the calibration lines of pullulan and dextran is due to the branching structure of the latter.[1,12] The calibration line of oxidized pullulan shows a parallel shift to smaller elution volumes with respect to the corresponding line of the native polysaccharide. At the same molar mass, the oxidized sample is larger due to stiffening of the chain by electrostatic repulsion, and it will elute earlier than the native polymer. Consequently, only samples with equal oxidation grades can be compared.

SEC-MALLS. Pullulan samples were dissolved in 0.01−1 M NaNO$_3$ aqueous solutions and were fractionated with three PW TSK columns (G6000PW-G5000PW-G3000PW, Toyo Soda) in series. Absolute molar masses and radii of gyration were determined on-line with a DAWN-DSP-F (Wyatt Technology Co.) MALLS detector. Since the solutions were very diluted, both data were calculated ignoring an effect of the second virial coefficient A_2. An interferometric refractive index detector (Optilab, Wyatt Technology Co.) was used as the concentration detector. The refractive index increment, dn/dc, was measured with the Optilab refractometer. For pullulan a value of 0.152 mL/g, and for all oxidized pullulan samples a value of 0.143 mL/g was found.

Results and Discussion

1. Optimization. Influence of pH. The adjustable variables in the reaction system are the concentrations of the reactants, the pH, and the temperature. It can be expected that an increase of the temperature will cause more degradation of the polymer chain during the reaction, and the influence of this variable was not investigated. Initial experiments showed that a variation in the concentration of TEMPO or bromide did not have much influence on the molar mass distribution of the products. Both catalytic concentrations were kept constant throughout this investigation as described in the Experimental Section. On the other hand, the pH was found to substantially influence degradation during the oxidation. Previously, we reported that the highest reaction rate occurs at a pH 10−11.[4] Here, we find that the least degradation occurs at a lower pH, despite the fact that longer reaction times are necessary (Figure 2). Apparently, hydroxide causes degradation of the polymer chain during the oxidation, which leads one to suspect β-elimination[13] for this undesired side reaction. Electron-withdrawing groups such as aldehydes and ketones induce β-elimination under alkaline conditions when a good leaving group is present in the β-position (Scheme 2). The alkoxyl group in polysaccharides is a relatively good leaving group compared to a hydroxyl function of monomeric substrates[13] and even at pH 11, no β-elimination is observed with monomeric substrates. On the other hand, this is not surprising since at pH 11 in the order of only 1% of the polymeric links in pullulan is broken.

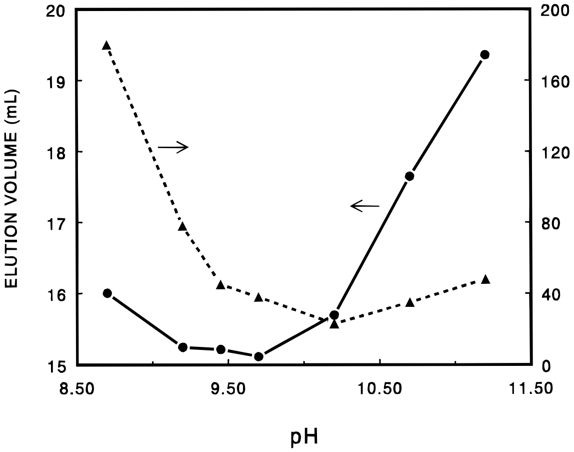
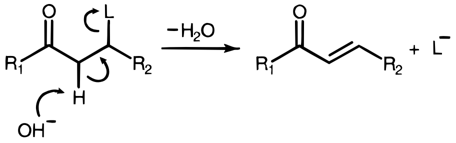


Figure 2. Dependence of the elution volume (●) and reaction time (▲) on the pH during the oxidation of pullulan. Values are taken after approximately 80% of the primary alcohols are oxidized.

Scheme 2



During the TEMPO-mediated oxidation of methyl α-D-glucopyranoside, up to 10% intermediate C-6 aldehyde with respect to the initial concentration substrate is present.[14] To study the influence of intermediate C-6 aldehydes on the molar mass distribution, the oxidation of pullulan was compared to that of dextran at pH 9.5 and 10.5. Dextran has a low proportion of primary alcohols (~3%), consequently degradation *via the C-6 aldehyde* will not play an important role. Under the applied conditions, the TEMPO-mediated oxidation of primary alcohol groups is almost complete after half an hour, implying that degradation via the C-6 aldehyde should occur during this period. This is indeed found (Figure 3); at pH 10.5 degradation of pullulan is substantial during the first half hour (compare the elution volumes of oxidized pullulan after half an hour at pH 10.5 and 9.5), whereas degradation of dextran at this pH is much slower. However, degradation of dextran at this pH, which cannot proceed via the C-6 aldehyde, is still more rapid than at pH 9.5, where degradation is negligible. It may be noted that hypohalite under alkaline conditions is known to be an oxidant for secondary alcohols.[15] Although this competing oxidation is very slow compared to the TEMPO-mediated oxidation,[4] intermediate ketones, which also undergo β-elimination, will be present. Uronates have a lower affinity toward hypophalite oxidation,[16] which is thought to be the reason that no more degradation is observed after pullulan is oxidized.

Lowering the pH below ~9 also has a negative influence on the molar mass distribution (Figure 2). The rate-determining variable of the TEMPO-mediated oxidation in this pH region seems to be the hydroxide

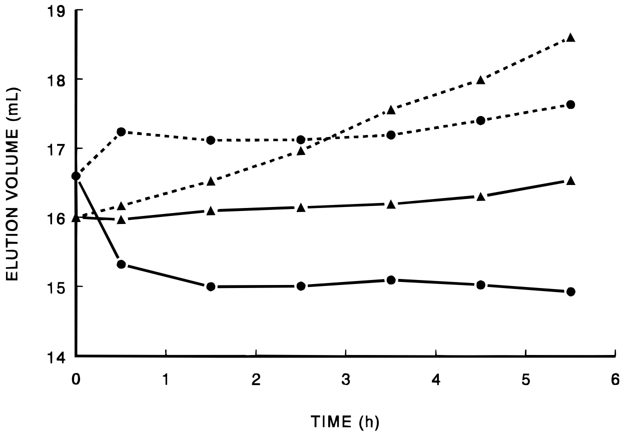


Figure 3. Comparison of the influence of the pH and substrate on the elution volume during the oxidation. Pullulan (●) and dextran (▲) at pH 9.5 (—) and at pH 10.5 (- - -).

Table 1

polymer	DO (%)[a]	charge parameter ξ	$\langle M_w\rangle / \langle M_n\rangle$[b]	M (kg/mol)	M_0 (g/mol)	L (nm)
pullulan			3.5	400	162	1180
pullox1	24	0.24	2.5	400	164	1170
pullox2	49	0.49	2.3	401	167	1155
pullox3	73	0.73	2.3	402	169	1140
pullox4	97	0.96	2.2	402	171	1130

[a] Degree of oxidation: percentage of primary alcohol groups oxidized. [b] Referring only to the main peaks of Figure 4.

concentration, as was also found earlier.[4] Thus, at lower pH much longer reaction times are necessary, which generally has a negative influence on the selectivity of a reaction. Furthermore, hypohalite becomes more active and less selective when lowering the pH.[15] Hypohalite is most active around pH 6−8, where excessive degradation occurs.[15] At pH < 9, oxidative degradation by hypohalite is thought to be responsible for the decreasing molar mass.

2. Oxidized Samples. For the preparation of pullulan samples with various linear charge densities, the oxidation at pH 9.4 was followed by a sodium borohydride reduction to remove carbonyl groups present in the polymer. This was especially important for the three samples that were not completely oxidized, where intermediate aldehydes are still present. With SEC-RI it was found that the reduction did not influence the molar mass distribution of the samples. Furthermore, the reduction of ketones in glucans with sodium borohydride has been shown to proceed with considerable stereoselectivity,[17] implying that ketones are mainly reduced to the natural *gluco* configuration. From the degree of oxidation (DO, Table 1), the charge parameter ξ was calculated with a monomeric unit length of 0.479 nm[18] (Table 1). Since the reaction is performed under homogeneous conditions, it is assumed that the charges due to the oxidation are randomly distributed in the polymer with the structural condition that every third monomer is without charge. Due to this structural aspect, block copolymers cannot be formed and a strong negative influence of neighboring carboxylates on the reaction rate would show in the kinetics of the reaction. This is not found; the reaction could be completed without excessive reaction times and the assumption of random oxidation seems to be gratified.

The molar mass distribution of the native polymer and the four oxidized samples is shown in Figure 4. From a qualitative view, these plots are in agreement with the expected results. It is clear that the average molar mass slowly diminishes during the oxidation. In fact, degradation is slightly underestimated, since the molar mass per monomer increases with the degree of oxidation. The polydispersity decreases during the oxidation (Table 1). In view of the chance of breaking a macromolecule of a highly polydisperse distribution, this is expected since larger molecules are more likely to be ruptured than smaller ones. This effect is especially clear when the molar mass distribution of the native polymer is compared to the sample with the lowest degree of oxidation. The molar mass distribution of the polymers is still quite broad, which is an advantage because the molecular weights and radii of gyration are obtained over a wide range of fractions with SEC on-line MALLS. Thus, a double-logarithmic plot of the radius of gyration vs the contour length can be extracted for each sample (Figure 5a−d). The slope of the curves is equal to ν in

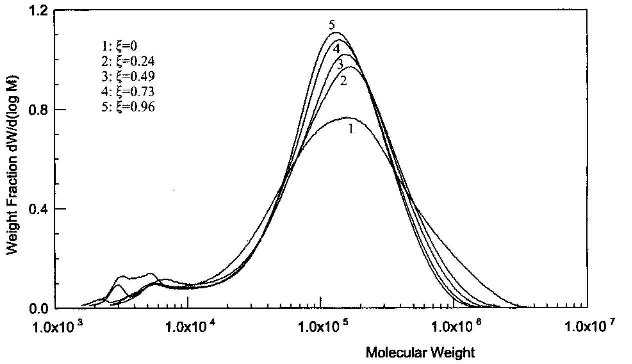


Figure 4. Differential molar mass distribution of pullulan (1) and pullox1−4 (2−5, respectively).

$$\langle S^2\rangle^{1/2} \sim L^{\nu} \tag{10}$$

In a good solvent, with $L \gg L_T$, ν should approximate the Flory value $^3/_5$, which indeed was found for pullulan as well as for the oxidized pullulans (Table 2), except at 1 M $NaNO_3$, where the value of the exponent of the oxidized pullulans was lower. This may possibly be attributed to a decrease of the solvent quality due to the added salt. The oxidized polymers may thus be treated as swollen coils in the good solvent limit at the lower ionic strengths. Furthermore, in Figure 5a−d typical polyelectrolyte behavior is seen: increasing the ionic strength has a similar effect as decreasing the linear charge density on the polymer backbone. At an ionic strength of 1 M, the charges are nearly completely shielded and the curves remain near the curve of uncharged pullulan. Finally, the experimentally found values ν for aqueous pullulan agree well with reported values,[18,19] implying that SEC-MALLS can be succesfully applied to determine these exponents.

3. Radius of Gyration, Comparison Experiment and Theory. For all four oxidized polymers, the calculated charge parameters ξ (Table 1) are smaller than unity; therefore, counterion condensation is neglected.[20,21] Furthermore, for comparison of experimental and calculated rms radii of gyration, one specific molar mass fraction is chosen (Table 1) from the distribution to circumvent the effect of polydispersity. We are aware of the fact that this choice may be of importance for a quantitative comparison. For example, major deviations are expected for relatively long polymers when the exponent ν is not exactly predicted. However, with these substrates in the range of $M_w \sim$

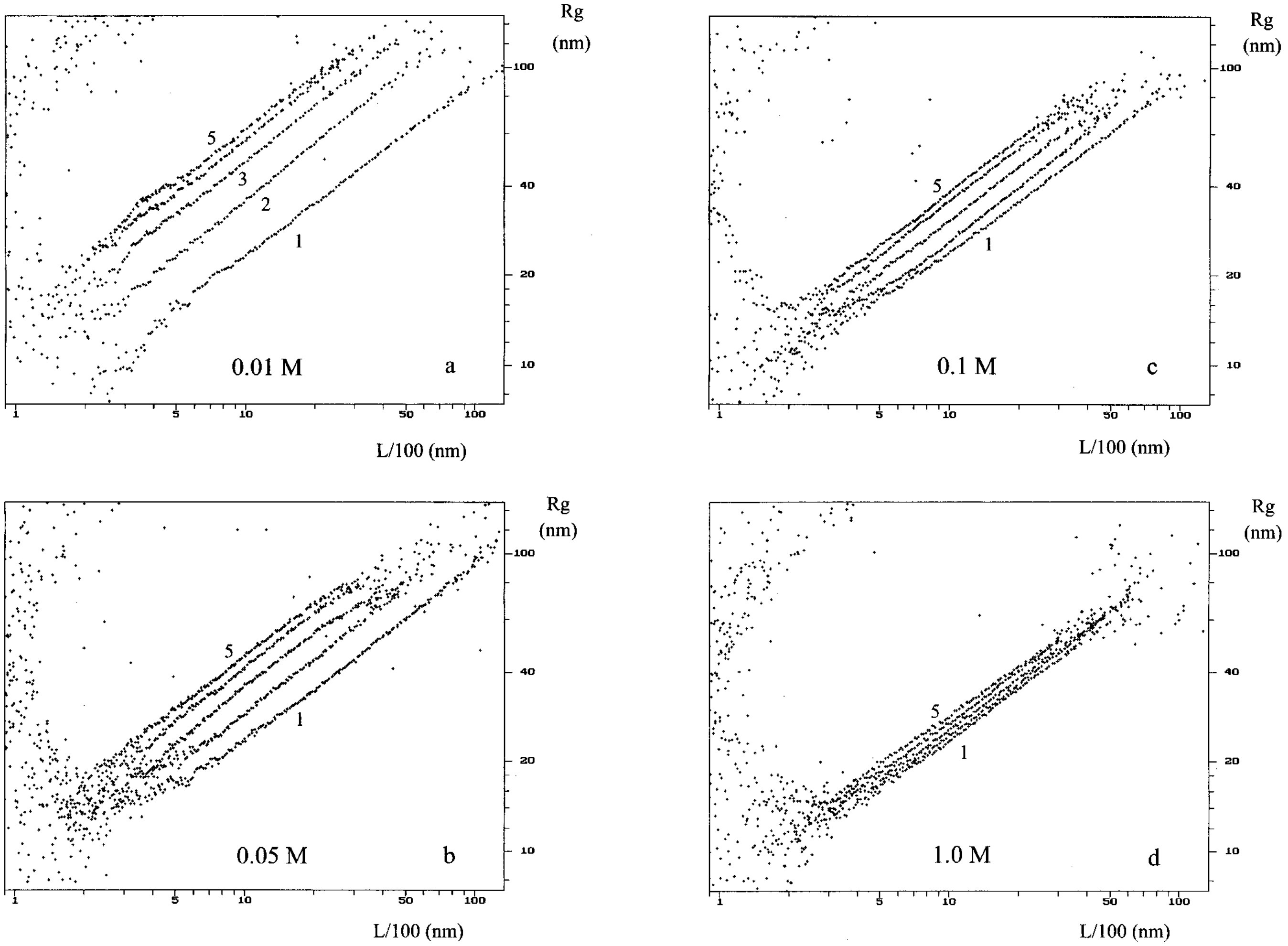


Figure 5. Double-logarithmic plots of the rms radius of gyration vs the contour length of pullulan and pullox1−4 at different ionic strengths: 5a, 0.01 M; 5b, 0.05 M; 5c, 0.1 M; and 5d, 1 M.

Table 2

polymer	C_s (M)	ν	κ^{-1} (nm)	L_E (nm)	L_T (nm)	d_{eff} (nm)	α_s^2	$\langle S^2\rangle^{1/2}cal1^a$ (nm)	$\langle S^2\rangle^{1/2}cal2^b$ (nm)	$\langle S^2\rangle^{1/2}exp$ (nm)
pullulan	0.01	0.57								26.1
	0.05	0.58								26.2
	0.1	0.58								26.2
	1	0.58								26.5
pullox1	0.01	0.63	3.04	0.19	1.49	3.66	3.93	47.8	44.4	39.3
	0.05	0.62	1.36	0.04	1.34	0.6	2.17	33.7	30.2	32.0
	0.1	0.59	0.961	0.02	1.32	0.6	2.18	33.5	30.2	28.5
	1	0.57	0.304	0	1.30	0.6	2.20	33.4	30.1	26.0
pullox2	0.01	0.58	3.04	0.78	2.08	8.00	4.38	59.2	55.1	53.1
	0.05	0.60	1.36	0.15	1.45	2.48	3.43	43.8	40.6	39.2
	0.1	0.59	0.961	0.08	1.38	1.42	2.87	39.0	35.9	33.7
	1	0.56	0.304	0.01	1.31	0.6	2.19	33.2	29.9	27.9
pullox3	0.01	0.58	3.04	1.72	3.02	10.4	3.89	66.8	62.1	62.0
	0.05	0.61	1.36	0.34	1.64	3.57	3.66	47.8	44.4	45.2
	0.1	0.59	0.961	0.17	1.47	2.19	3.24	42.5	39.4	38.9
	1	0.55	0.304	0.02	1.32	0.6	2.18	33.1	29.7	29.1
pullox4	0.01	0.57	3.04	2.98	4.28	12.1	3.36	73.8	68.2	66.4
	0.05	0.59	1.36	0.59	1.89	4.32	3.63	50.8	47.2	48.9
	0.1	0.60	0.961	0.30	1.60	2.72	3.35	44.9	41.5	41.4
	1	0.54	0.304	0.03	1.33	0.6	2.17	33.0	29.6	30.6

[a] Calculated according to eqs 1−9. [b] Calculated according to eq 15.

100−500 kg/mol, the choice of the molar mass fraction is of minor importance. The contour length L (Table 1) is calculated from the chosen molar mass, the molar mass per unit (M_0, Table 1) and the length per unit (0.479 nm). The experimental rms radii of gyration of these fractions at ionic strengths ranging from 0.01 to 1 M are given in the last column of Table 2. In this table also the calculated rms radii of gyration values due to eqs 1−9 are presented ($\langle S^2\rangle^{1/2}cal1$), with L_P taken as 1.3 nm.[22] It is clear that the calculated values are 10−20% higher than the experimental ones, whereas the ionic strength and linear charge density dependence is predicted quite well. The higher values obtained are thought to be due to the use of the Yamakawa−Tanaka eq 6, which results in an overall exponent ν in relation 10 of approximately 0.615 (depending on Z). This has a substantial effect on the final calculated value of $\langle S^2\rangle^{1/2}$, despite the lower overall exponent of the other

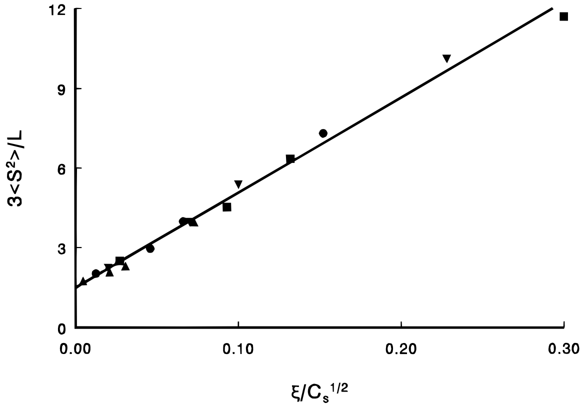


Figure 6. Plot according to eq 11 with an apparent persistence length proportional to $\xi/C_s^{1/2}$. Pullox1 (▲), pullox2 (●), pullox3 (▼), and pullox4 (■) at ionic strengths 0.01, 0.05, 0.1, and 1 going from right to left for each polymer. The best linear fit gives an intercept of 1.38 and a slope of 35.8.

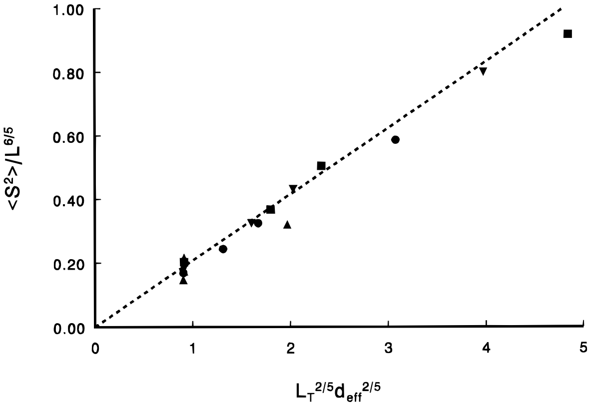


Figure 7. Comparison between experimental values and theory (eq 15, dashed line). Symbols have the same meaning as in Figure 6.

length scales (naturally, for the calculation of $\langle S^2\rangle^{1/2}$, the sum of the exponents of the length scales should be unity). Since we found an average value of ν of approximately 0.59 for the oxidized polymers (the 1 M salt measurements not included), an overestimation of the rms radius of gyration using eq 6 is not surprising. Moreover, recently Li and Witten[23] showed that in the case of charged flexible polymers, fluctuations at length scales smaller than the screening length modify the persistence length to a slightly smaller value, which may also be a cause for overestimation of the radius of gyration.

For many flexible polyelectrolyte chains, an empirical power law of $\langle S^2\rangle \sim \xi/C_s^{1/2}$ is found,[24] which has in fact also been found with Monte Carlo simulations.[24] Consequently, the ionic strength and linear charge density dependence of the extension of polyelectrolytes in solution has been described using an *apparent* persistence length L_{TA}:[25]

$$\langle S^2\rangle = LL_{TA}/3 \qquad (11)$$

where L_{TA} is proportional to $\xi/C_s^{1/2}$. For the oxidized pullulans, we also found a linear relation between $\langle S^2\rangle$ and $\xi/C_s^{1/2}$ (Figure 6)

$$\langle S^2\rangle\ (\mathrm{nm}^2) \approx L(1.38 + 35.8\xi/C_s^{1/2})/3 \qquad (12)$$

The apparent intrinsic persistence length L_{PA} of 1.38 nm obtained here is close to the theoretical persistence of pullulan (1.3 nm[22]) and in agreement with the experimental value of 1.2–1.9 nm obtained from small-angle X-ray scattering measurements.[26] Furthermore, expression 12 is remarkably similar to the empirical expression obtained by Reed et al.[27] for polyacrylamide–polyacrylate polymers with different linear charge densities:

$$\langle S^2\rangle\ (\mathrm{nm}^2) \approx L(2.7 + 35.0\xi/C_s^{1/2})/3 \qquad (13)$$

Although an apparent persistence length has no physical meaning, expressions such as 12 and 13 are interesting since no adjustable parameters are used and the ionic strength–linear charge density dependence of the polyelectrolyte extension is quite accurately described, as is shown in Figure 6. This experimental ionic strength–linear charge density dependence of L_{TA} is clearly different from the dependence of the real persistence length L_T, which is proportional to ξ^2/C_s. L_{TA} is an upper limit value of L_T, due to the fact that excluded-volume effects are incorporated in L_{TA}; consequently, excluded-volume theory should account for this different proportionality. However, eqs 5–9 are not very transparent in this respect. A more clear expression can be obtained by assuming large Z and applying the following relation:[7]

$$\lim_{Z\to\infty}\alpha_s^{\,5} = 1.67Z \qquad (14)$$

From eqs 7–9 one sees that this relation may be used only for $L \gg L_T$, not too weakly charged polymers, and moderate ionic strength. Consequently, the prediction of the rms radius of gyration using eq 14 may be doubtful in the case of C_s is 1 M or $\xi = 0.24$. For the other experiments, eq 14 seems to be more appropriate than eq 6, since the Flory value for ν is obtained by combination of relations 3, 5, 7, 8, and 14, which gives for the mean-square radius of gyration[28]

$$\langle S^2\rangle \approx 0.208L^{6/5}L_T^{\,2/5}d_{\mathrm{eff}}^{\,2/5} \qquad (15)$$

For the calculation of the effective diameter eq 9 is used, which is seen to depend on the ionic strength as well as on the linear charge density. In contrast to eq 11, all parameters in relation 15 have a definite physical meaning. A plot of the data analogous to Figure 6 but now according to eq 15 is shown in Figure 7 ($L_P = 1.3$ nm). The prediction of the rms radius of gyration is quite good, which relates the real and the apparent persistence length via excluded-volume theory, as was of course expected from the correct behavior as predicted with eqs 5–9. In Table 2 we also give the values of the rms radius of gyration as calculated with eq 15 ($\langle S^2\rangle^{1/2}$*cal2*). These values are in good agreement with the experimental data, especially when compared to $\langle S^2\rangle^{1/2}$*cal1*, which is thought to be due to the different exponent ν as discussed above.

Finally, we want to deduce the intrinsic diameter d_0, the effective diameter when the charges are completely shielded. This value should be obtainable from the data of the uncharged pullulans, with eq 15 modified to

$$\langle S^2\rangle \approx 0.208L^{6/5}L_P^{\,2/5}d_0^{\,2/5} \qquad (16)$$

where L_P is taken again as 1.3 nm. We found that the dimensions of pullulan do not depend on C_s in the concentration range we used (Table 2). For a molar mass fraction of 400 kg/mol, a rms radius of gyration of

26.2 nm was obtained and from eq 16 an effective diameter of 0.3 nm is calculated. This seems a too low value for these polymers, implying that the excluded volume is overestimated. An experimental value for the exponent ν of 0.58 was obtained from the average of 12 measurements at different ionic strengths with values ranging from 0.56 to 0.59. This can explain the underestimation of the effective diameter using eq 16. Furthermore, the proportionality constant 0.208 as results from eq 14 is a matter of debate[7] and may give rise to errors. In this light, the good prediction of the rms radius of gyration using eq 15 is partly fortuitious.

Concluding Remarks

The TEMPO-mediated oxidation of primary alcohol groups in water-soluble polysaccharides is a reliable method for the introduction of negative charges with variable charge densities. The optimum pH range with respect to the least degradation was found to be pH 9.2−9.7. The primary alcohols of pullulan $\langle M_w \rangle \approx 170$ kg/mol could be completely and selectively oxidized at pH 9.4 to give the corresponding polyelectrolyte with $\langle M_w \rangle \approx 120$ kg/mol. At higher pH, β-elimination increases more rapidly than the reaction rate, which can be useful when low molecular weight fractions are desired. At lower pH, the reaction becomes very slow and probably oxidative cleavage due to hypohalite comes to the fore.

Experimental values of the rms radius of gyration were obtained from SEC-MALLS data of the samples, which directly gives a value of the polymer size exponent without polydispersity effects. For moderate ionic strengths, the exponent of the polymers used in this investigation was close to the Flory value of $^3/_5$, implying that pullulan as well as the oxidized pullulans may be treated as swollen coils in the good solvent limit. As with many other polyelectrolyte coils, the apparent persistence length was proportional to $\xi/C_s^{1/2}$ for ξ varying from 0.24 to 0.96 and C_s varying from 0.01 to 1 M. Since the real persistence length is proportional to ξ^2/C_s, excluded-volume theory should account for this different proportionality. This was shown to be the case, both through calculations according to the Odijk−Skolnick−Fixman theory in combination with excluded volume theory as well as through the use of eq 15. In this study, both approaches predict the correct ionic strength−linear charge density dependence of $\langle S^2 \rangle^{1/2}$, at least within the limits of the accuracy of the measurements. The absolute values of the experimental $\langle S^2 \rangle^{1/2}$ are best predicted with eq 15, which is thought to arise from the difference of the exponent of L, which is exactly $^3/_5$ in eq 15 whereas this value is approximately 0.615 when $\langle S^2 \rangle^{1/2}$ is calculated through eqs 5−9.

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