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Reaggregation of Sodium Alginate Microgel Structures After Shear-Induced Deaggregation at Filtering

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Summary

The time dependence of reaggregation of sodium alginate (SA) after following the shear-induced deaggregation of the aggregates ('egg-box' like microgel structures) at filtering through neutral 0.20 μm filters has been found. The aggregation of SA in solution prior to filtering was induced by addition 0.1 mM Ca^{2+} ions. For SA at concentration of 1mg/ml and room temperature, the reaggregation process is finished after four minutes from the end of the filtration of 3.5 cm^3 of the solution with rate of filtration of 1 cm^3/min. The reaggregation phenomenon is simply explained by diffusional driven processes of calcium ion – SA electrostatic interactions.

Introduction

The ionotropic gelation of alginates induced by addition of calcium divalent ions in aqueous solution of sodium alginate (SA) is well known phenomena described by the "egg-box" model of gelation [1]. Here, when Ca^{2+} ions are found in excess of sodium ions in solution [2], electrostatic interactions between Ca^{2+} ions and the guluronic acid monomer residues from different polymer chains of SA take place and infinite network or gel is formed. In gelation processes of polysaccharides and other polymers, carrageenans from example [3], it has been found that final gel properties are predetermined by the presence of small amount of metal cations in the so call pre-gel solution which always induce aggregation and association of the polyelectrolyte molecules. Those aggregates and associates determine the gel properties of the polysaccharide after finishing the gelation depending on the type and concentration of the metal cations [2,3,4]. Filtration is necessary procedure for preparation of SA solution for characterization or some production technology such as gelation. When filtering SA solution full of aggregates it is of great importance to know at what time after filtration to apply some characterization technique, for example, size exclusion chromatography or UV spectroscopy. The same is valid when high shear flow exists in some technological processes, for example, preparation of microparticles of calcium alginate gel by spray drying. This knowledge is based on the fact that time dependence of reaggregation after following the shear-induced deaggregation of the

aggregates occurs at filtering through neutral filters with pore size comparable to the dimension of the aggregates (Figure 1) or at flowing of sodium alginate solution through small nozzle when spray drying is applied. The technique of electric birefringence has been already found useful when dealing with aggregates and we decided to apply it [5,6]. The main efforts in this work were put on analysis of decay or rotational relaxation of SA solutions with connection to reaggregation kinetics after filtering. Light scattering was applied only as a technique for checking for aggregates and for determination of the mass and dimension of SA macromolecules (aggregated and unaggregated).

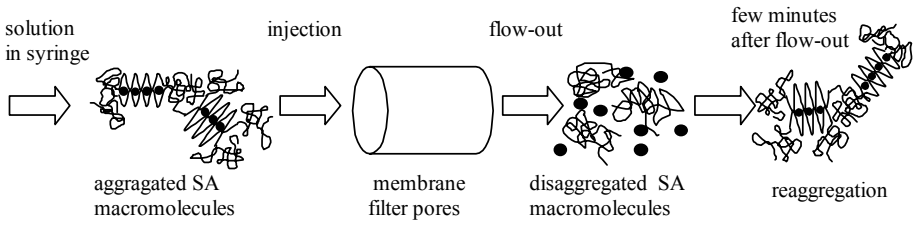


Figure 1. The scheme of reaggregation of calcium ion induced SA aggregates ('egg-box' like microgel structures) after filtering through membrane filter with pore size comparable to the dimension of the aggregates. The dark spots represent calcium ions.

Materials and Solution Preparation

Sodium alginate (SA) was purchased from FMC, Norway. Calcium chloride dyhydrate was purchased from Sigma, USA. The filter used for filtration was Anotop^{TM} inorganic aluminium oxide membrane filter. The gulluronic-to-mannuronic ratio (G/M) of 45:55 and GG block fraction in the polymer chain of approximately $F_{GG} = 0.20$ has been found on ^{13}C-NMR at the supplier. The SA sample was used as received. Deionized and double distilled water with conductance of 2 - 4 $M\Omega^{-1}cm^{-1}$ was used for solution preparation. The sodium alginate (SA) solution at concentration of 1 mg/mL was prepared by adding appropriate amount of SA in water as solvent and agitated the solutions on magnetic stirrer at 600 rpm at room temperature. For preparation of SA solution with Ca^{2+} ions, appropriate amount of $CaCl_2 \cdot 2H_2O$ was added to the water solvent prior to SA. The solutions were stirred for two hours than sealed and allowed to equilibrate for 24 hours in a refrigerator at + 4 ^{0}C. After that, the solutions were centrifuged at 3,700*g* and again sealed and hold in the refrigerator prior to use for measurements. Sodium salt was not added in SA solution in order not to change the ionic strength of the solution. The pH of the two SA solutions was 6.4 ± 0.1. Using the value of 198 g/mol for the molecular weight of the monomer unit, the SA solution with concentration of 1 g/L will contain 5 mM Na^+ ions at full dissociation. A large fraction of them is supposed to be fixed to the SA polyanions [7]. The concentration of free Na^+ counterions in solutions increase as SA concentration increase. We have worked with SA concentrations lower than 1.00 mg/mL which means that Na^+ counterions concentration is lower than 5.4 mM when having in mind Na^+ ions from the SA sample. Choosing these concentrations of SA we were confined that the main differences in the SA solution properties came from the presence of 0.1 mM Ca^{2+} ions as an initiator of microgel structures (aggregates).

Static Light Scattering Measurements

Static light scattering measurements were made on Brice-Phoenix BP 2000 Light Scattering Photometer with unpolarized incident light of wavelength 546 nm supplied from a high-pressure mercury lamp. The BP 2000 light scattering photometer measures the intensity (in arbitrary units) of light scattered at angles between 30° - 150° from cylindrical glass cell, 12.5 mm in diameter with solution holding volume of 30 ml, supplied from Hellma, Germany. We choose to measure the intensity of light scattered at angles between 45° – 135° with an increment of 10^0. The solution temperature in the glass cell was held at 298.2 K ± 0.1 K. The refractive index of solution was measured on Pulfrich refractometer at investigated temperature (298.2 K) and practically same values 1.3344 ± 0.0006 were found for the two sample solutions. The RI increment of alginate solution was measured on Brice-Phoenix Differential Refractometer and, when analyzing the uncertainties of the measurements, no changes of RI increment were found by adding calcium ions in the solutions. RI increment value of $dn/dC = 0.165 \pm 0.005$ mL/mg have been determined and taken in calculating the optical constant of light scattering. Each sample solution was filtered through 0.20 μm Anotop filter directly in the light scattering cell on the beginning of each measurement. The correction on sodium alginate concentration in solution after filtering was made by the method of evaporation to constant weight. The same was done for solutions prepared for EB measurements. Prior to the static light scattering measurements, the BP light scattering instrument was calibrated using double distilled benzene and polystyrene standards with $\langle M_W \rangle$ of 70,000 g/mol and 990,000 g/mol (Pressure Chemicals, USA) dissolved in benzene. Thus, the performance of BP instrument for work on samples with wide range of molecular weights was checked and discrepancies of ± 5 % have been found satisfying.

Electric Birefringence Measurements

Electric birefringence measurements were made on facility organized according operation scheme already described [5,8,12]. We used He-Ne laser operated at λ = 632.8 nm (Uniphase, Germany) emitting linear polarized laser light with polarization plane fixed at + 45^0 to direction of electric field applied between chromated plain stainless steel electrodes in the Kerr cell, 72.0 mm in length and 1.5 mm in gap fixed in teflon holder with dimensions appropriate to fit quadratic glass-quartz cell from Hellma, Germany. Total cell's hold volume was approximately 3.5 cm^3. Laser light emerging the glass cell pass through quartz waveplate compensator of λ/20 and Glan-Thompson polarizer of extintion ratio of 10^{-6} set at - 45^0 to the direction of the applied electric field (i.e., crossed to polarization plane of the incident light). Electric birefringence (EB) effects on solutions filling the cell were obtained applying single rectangular voltage pulse of 500 V between the electrodes with regulated duration from 50 μs to 5 ms produced by self-made low voltage generator connected to high voltage amplifier from Cober Co., USA. The EB effects were detected with silicon Hamamatzu photodiode connected to the oscilloscope HP 54600B, USA, capable to transfer the effects to the computer for further procession and storing. The EB measurements were made at temperature of 298.2 K. One typical EB signal captured by the oscilloscope is shown on Figure 2. The rise time and fall time of single rectangular voltage pulse and the silicon photodiode were ≈ 0.3 μs which is twenty times below shortest measured relaxation times of SA obtained in this work thus

making applied pulses and the photodiode suitable. Determination of steady state EB, Δn_0, and the decay of the electric birefringence, $\Delta n(t)$, were done applying the Brice method [9]. The Brice method demands precise determination of linear relation between the intensity of the photodiode signal of laser light traveled through the investigated solution and the angle of rotation of the λ/20 compensator which was done at the beginning of EB measurements on each sample solution (calibration of the compensator). Positioning the compensator at fixed angle α_0 during the work which corresponds to the beginning of the linear part of calibration curve give us a possibility to determine Δn (and $\Delta n(t)$) from vicinity of photodiode trigger-timed signal output of EB effect, ΔI (and $\Delta I(t)$), according the equation

$$\Delta n = \frac{\Gamma}{L}\mathrm{Sin}\left[2(\alpha - \alpha_0)\right], \qquad (1)$$

where L is the length of electrodes; Γ is a calibration constant and α is angle read from the calibration curve which corresponds to ΔI (or ($\Delta I(t)$).

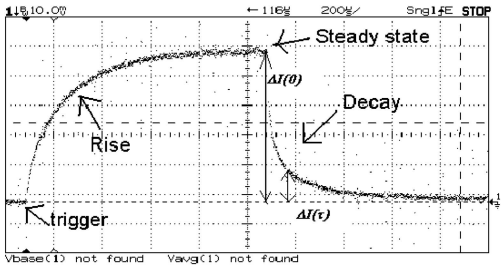


Figure 2. EB signal on SA solution obtained on the oscilloscope (10 mV/cm) at applied electric field strength of $4.2 \cdot 10^5$ V/m in single square pulse with duration of 960 μs. The most important regions, trigger point, rise, steady-state and decay, are pointed out on the picture. $\Delta I(t)$ (and $I(0)$) from the oscilloscope (in cm) is easily converted in α and then in $\Delta n(t)$ after using the calibration curve of the compensator and Equation 1.

Results and Discussions

The data from the static light scattering were analyzed by using the Zimm method [10]. On Figure 3, the Zimm plot of static light scattering measurements on SA solution in double distilled water is shown. The Zimm plot is best described by the equation

$$\frac{Kc}{R_\theta} = \frac{1}{< M_w >}\left(1 + \frac{q^2 < R_G^2 >_z}{3}\right) + 2A_2 \cdot c\,, \qquad (2)$$

where, K is the optical constant, q is the amplitude of the scattering vector ($|\vec{q}| = q = (4\pi n/\lambda_0)\sin(\theta/2)$, θ - scattering angle, n - index of refraction of the solvent, λ_0 – wavelength of the light in a vacuum), c is the concentration of solution, R_θ is the Rayleigh ratio, $<M_W>$ is the weight average molecular weight of the solute macromolecules, $<(R^2_G)^{1/2}>$ is the radius of gyration of the solutes, A_2 is the second virial coefficient. The extrapolation lines to $c = 0$ and $\theta = 0$ from the Zimm plot of SA in pure water are shown on Figure 3 with dot lines. From there we obtained: $<M_W> = 85{,}000 \pm 10{,}000$ g/mol; $A_2 = 0{,}4 \cdot 10^{-6} \pm 0{,}1 \cdot 10^{-6}$ cm^3·mol/g and $<(R_G^2)^{1/2}> = 22 \pm 4$ nm. The Zimm plot of light scattering data for SA dissolved in water with added 0.1 mM Ca^{2+} is distorted (Figure 4) in comparison to Zimm plot for SA without calcium ions (Figure 3). This indicates presence of large structures in solution than only single SA

chains. From Figure 4 it is impossible to get real values for $<M_W>$, A_2 and $<(R_G^2)^{1/2}>$ of the aggregates but only approximate ones. Only the weight-average molecular weight and radius of gyration were approximated by single extrapolation at zero concentration and taking data only for scattering angles between 45 and 90 degrees. The obtained molecular weight was $<M_W>$ = 333,000 ± 45,000 g/mol and the radius of gyration $<(R_G^2)^{1/2}>$ = 215 ± 25 nm. By comparing values for $<M_W>$ and $<(R_G^2)^{1/2}>$ for these two different solutions, it is noticeable that $<M_W>$ and $<(R_G^2)^{1/2}>$ for SA with added calcium ions is much greater than for SA in pure water, which is an evidence that small quantities of calcium ions in SA solution led to formation of aggregates that probably are to be 'egg-box' like microgel structures of calcium alginate in SA solution.

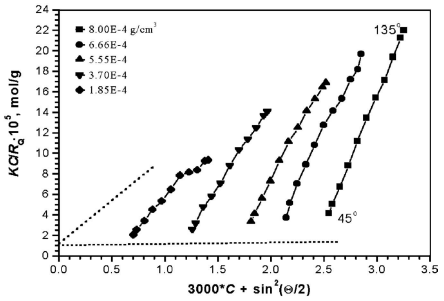


Figure 3. The Zimm plot for SA solution in pure water. The concentration of the solutions subjected to static light scattering measurements is shown in the legend. The increase of the scattering angle from 45° to 135° is indicated. Dot lines represent double extrapolation to zero concentration and zero angle.

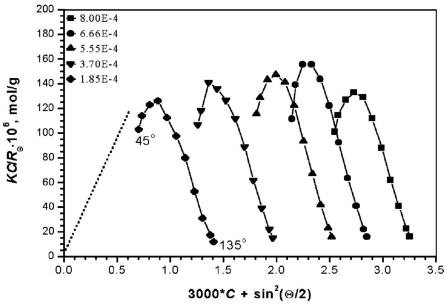


Figure 4. The Zimm plot for SA solution with added 0.1 mM calcium ions. The concentration of the solutions subjected to static light scattering measurements is shown in the legend. The increase of the scattering angle from 45° to 135° is indicated. Dot line represents extrapolation to zero concentration.

The electric birefringence decay following the removal of the electric field is exponential and usually, in the case of polydispersity on forms and dimensions of the particles in solution, is best described by multiexponential function [5,8]

$$\Delta n(t) = \sum_{i=1}^{n} \Delta n_{oi} \cdot \exp(-t/\tau_i), \qquad (3)$$

where, Δn_{0i} are the steady-state values of electric birefringence for every fraction of the polydisperse particles and τ_i is the respective rotational relaxation time. When assuming that in solution exist only single strands of monodisperse particles and large aggregates of the same particles associated in microgel structures, Equation 3 can be rewritten as

$$\Delta n(t) = \Delta n_{01} \exp(-t/\tau_1) + \Delta n_{02} \exp(-t/\tau_2). \qquad (4)$$

In our case of polydisperse particles, the same Equation 4 can be used but then $\Delta n_{01,}$ $\Delta n_{02,}$ τ_1 and τ_2 represent averaged values. Information for absolute values of the

electrical and optical parameters of the particle are usually provided through the Δn_0 dependence on the square of the electric field strength E,

$$\Delta n_0 = \frac{2\pi C_V}{n}(g_1 - g_2)\cdot\Phi(\rho_s, \rho, \gamma'), \qquad (5)$$

where C_V is the volume concentration of the solutes, $(g_1 - g_2)$ is the optical anisotropy factor, n is the refractive index of the solution, and Φ is the orientation function.
In order to explain the field strength dependence of the steady-state electric birefringence of SA, the SUSID orientation function Φ derived by Yamaoka and Fukudome [11] on the basis of the saturable and unsaturable induced dipole moment mechanism can be found useful. We didn't apply SUSID analysis because all decays of the EB were taken at same electric filed strength of $4.2\cdot10^5$ V/m. The optical anisotropy factor and the orientation function of aggregates depend on the form, size and some electrical properties of the aggregates but it is fixed value for single macromolecules of SA when working at constant electric field strength. Thus, the change of the measured steady-state value of EB of SA solution could be caused by change of the concentration of non-aggregated and aggregated particles, C_{V1} and C_{V2}, and the change of the optical anisotropy $(g_1 - g_2)_2$ and the orientation function Φ_2 of the aggregates. The steady-state EB of aggregated solution can be expressed by steady-state values of single macromolecules found in solution Δn_{01} and steady-state value of the aggregates $\Delta n_{02,}$

$$\Delta n_0 = \Delta n_{01} + \Delta n_{02} = \frac{2\pi}{n}\left[C_{V1}(g_1 - g_2)_1\cdot\Phi_1\right] + \frac{2\pi}{n}\left[C_{V2}(g_1 - g_2)_2\cdot\Phi_2\right]. \qquad (6)$$

When measuring the time-dependence of decay of the EB of reaggregated SA solution after following the shear-induced deaggregation of the aggregates at filtering through neutral 0.20 μm filters, it is possible to distinguish n_{01} from n_{02}. The change on n_{01} is caused by the change of the concentration of single macromolecules C_{V1} and the percentage of n_{01} to total n_0 is the same as the percentage of the C_{V1} to total volume concentration of the SA sample in solution, C_V. Taking that $C_{V1} + C_{V2} = C_V$ it is possible to find the percentage of the volume concentration of aggregates to total SA concentration without knowing the exact values of their Φ_2 and $(g_1 - g_2)_2$.
On Figure 5 the decay profile of EB on SA solution without added calcium ions is shown. Short average rotational relaxation time of 6.8 μs was found associated to them. When solution was filtered through the membrane filter directly in the Kerr cell and subjected after one minute to electric birefringence, only small changes were observed. The pause of one minute after filtration is necessary solution to get equilibrium free of convections. The same was found when electric birefringence was applied after five minutes. This was expected result because the size of the macromolecules is small ($<(R_G^2)^{1/2}>$ = 22 nm) and pass well the filter pores (size of 200 nm). The long rotational relaxation time of 130 μs is attributed to interparticle interactions because of relatively higher concentration of the SA macromolecules (1 mg/mL). From static light scattering it was proved that there are no aggregates in the solution and this long relaxation time cannot be connected to existence of aggregates of larger size than single macromolecules.
On Figure 6 the decay profile of EB on SA solution with added 0.1 mM calcium ions is shown. This is the case for the solution full of aggregates in syring prior to

filtration. When fitting the decay profile of EB to Equation 4, short and long relaxation times were observed. Long rotational relaxation time of 2.20 ms was found for the aggregates. Although there are single macromolecules of SA in the solution, because the decay dynamics of the aggregated structures cover relaxation time up to few milliseconds, the rotational relaxation time of 6.8 microseconds for single macromolecules is masked. The short relaxation time of 280 μs was found which is close to that found for SA solution without added calcium ions. This time is also attributed to interparaticle interactions of single SA macromolecules (unaggregated).

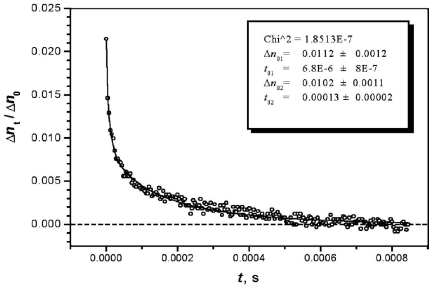


Figure 5. The digitalized decay profile of EB on SA solution in pure water.

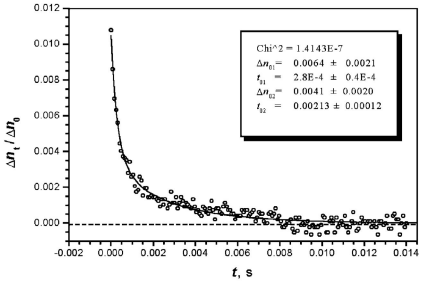
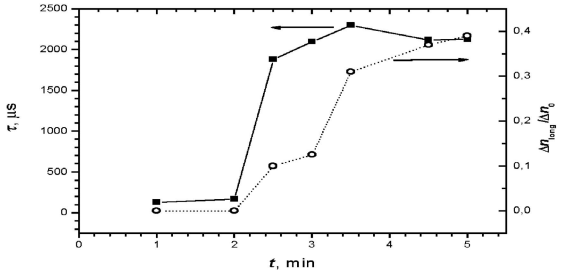


Figure 6. The digitalized decay profile of EB on SA solution with added 0.1 mM calcium ions.

Figure 7. Left axis: time dependence after filtration of average value of the rotational relaxation time of the aggregates. Right axis: the ratio between steady-state value of EB of the aggregates to total steady-state EB from SA solution with added 0.1 mM calcium ions (times 100 in percentage).



When solution was filtered through the membrane filter directly in the Kerr cell and subjected after to electric birefringence, significant changes in the long rotational relaxation time were observed. The results for the rotational relaxation time of the aggregates measured at several times after the end of filtration are shown on Figure 7 (left axis). It is noticeable that after 4 minutes the long relaxation time of the aggregates gets constant value of 2.12 ms, which practically is the same as that found in the same solution prior to filtration (2.20 ms). This indicates that reaggragation process takes place on time scale of several minutes at this concentration of SA and calcium ions. The time dependence of the reaggregation is driven by the diffusional dependence of calcium ion – SA electrostatic interactions in solution. Another information was obtained when the decay profiles of EB on SA solutions with added calcium ions were analyzed. The steady-state value of EB of aggregates, Δn_{02} (or Δn_{long}), approached saturation after 4 minutes, but the percentage value from the total steady-state value n_0 was 39 % and that for the solution in the siring (prior to filtration) was 48 % (Figure 7, right axis). This indicate that there was loss of mass of the aggregates up to 19 % at filtration because sticking of large sized aggregates in the

pores of the membrane filter. By using the values of the long rotational relaxation time from electric birefringence measurements, τ, and the Stokes-Einstein equation for rotational relaxation,

$$a = (3kT\tau / 4\pi\eta_0)^{1/3}, \qquad (7)$$

the radius of the aggregates a approximated as spheres was calculated, a = 134 nm. In Equation 7, k is the Bolzman constant, T is the temperature and η_0 is the viscosity of the solvent ($\eta_0 = 0.898 \cdot 10^{-3}$ Pa·s). The value for a is not the same as the radius of gyration of the aggregates found from static light scattering ($<(R_G^2)^{1/2}> = 215$ nm). Altough the difference could lay in the assumption for spherical form of the aggregated particles, the main cause for the difference is the imprecise estimation of $<(R_G^2)^{1/2}>$ from the distorted Zimm plot (Figure 4).

Conclusions

Zimm plot from static light scattering measurements from SA solution with added 0.1 mM calcium ions is distorted which indicate presence of aggregates (more like 'egg-box') micogel structures. The average value of the radius of gyration of the aggregates was estimated to be 215 nm, which is close to the pore size of the membrane filter used for filtration of SA solutions. At this dimension it is obvious that shear-induced deaggregation take place in the filter followed by reaggregation after passing it. Analyzing the decay profiles of electric birefringence effects obtained at various times after filtration, the reaggregation process has been found to be finished after four minutes after the end of the filtration. The average rotational relaxation time of the aggregates, and thus the dimension, have been found of same value as those for SA solution subjected to filtration which indicate that same structures are reorganized in average after filtration as those found prior to it.

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