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Comment and Reply

Comment on “Conformational Changes and Aggregation of Alginic Acid as Determined By Fluorescence Correlation Spectroscopy”

In a recent paper, Avaltroni et al.[1] provide estimates of the chain stiffness of alginate (“alginic acid”) based on measurements of the molecular weight and the diffusion coefficient. The estimates and some of the methodologies can in our opinion be questioned. First, the work is based on a single sample only (alginic acid from *Macrocystis pyrifera*, A2033 supplied by Sigma). The authors report a M_w of 989 kDa and a polydispersity (M_w/M_n) of 51.4. M_n is the critical parameter entering subsequent calculations. We question this estimate based on our own experience with hundreds of alginates, where M_w/M_n seldom goes above 3 and never above 10, irrespective of molecular weight range. Indeed, it appears that the same alginate batch has been analyzed in our laboratory using size exclusion chromatography multiple-angle laser light scattering (SEC-MALLS) (SEC combined with light scattering (MALLS) and concentration-sensitive (refraction index (RI)) detectors). It is part of a larger study[2] where it is reported as the first entry in Table 1, with M_w of 210 kDa and M_w/M_n of 1.9. A re-examination of the data files reveals no anomalies compared to other alginates. M_n depends somewhat on the fitting procedure of log M versus elution volume. M_w/M_n of 1.9 refers to no fitting, whereas a linear fit yields M_w/M_n of 2.6. Under no circumstance can the values reported by Avaltroni et al. be obtained. Their estimates of the molecular weight (both M_w and M_n) were obtained by SEC using pullulan standards for calibration. It is generally known that the elution properties of pullulans and alginates differ considerably because of widely different chain extensions. This can be shown clearly using SEC-MALLS, which is a calibration-independent method. As an example, Figure 1 shows results obtained for polydisperse pullulan ($M_w = 1300$ kDa) and a polydisperse alginate ($M_w = 200$ kDa). In this case the “calibration curve” is obtained by calculating the molar mass for each elution slice. The data show that the calibration curve for pullulan is shifted by a factor 4−6 toward higher molar masses as compared to alginate. Data were obtained at an ionic strength of 0.17 M, which is slightly different from that in ref 1 (0.1 M). Although this difference may affect chain expansion and, hence, the elution behavior, the effect is marginal compared to the large elution differences between alginate and pullulan. This implies that using a calibration curve based on pullulan standards leads to a correspondingly large overestimation of the molar mass of the alginate. The difference in M_w obtained in refs 1 and 2 is in excellent agreement with this explanation. It may be noted that since investigations are carried out at pH 6 (neutralized by NaOH) the material is sodium alginate not alginic acid.

Avaltroni et al.[1] further state that their estimate of polydispersity is “in agreement with other studies”, referring to an article by Lee et al.[3] In examining this article we find no mention of such extreme polydispersites. On the contrary, it describes low molecular weight alginates with polydispersities below 2.0.

To obtain estimates of the persistence lengths Avaltroni et al.[1] use the Benoit−Doty equation for wormlike chains. Estimates for R_g are then needed, and the authors apply a direct relationship with the diffusion coefficient (eq 1 of ref 1). The equation is ascribed to de Gennes (group) renormalization theory but is in reality the Stokes−Einstein equation with R_g replacing the Stokes radius, R_h, and using the value of R_g/R_h for excluded volume chains. R_g/R_h increases with chain stiffness, so the constant will not be correct for alginates.[4] The diffusion coefficient is further obtained by fluorescence correlation spectroscopy measurements. In a polydisperse system a correct average is needed to match the corresponding molecular weight average. It is unclear why the number average is preferred in the present case. Would the estimates of the persistence length differ if the weight average chain lengths and diffusion coefficients were used in calculations? In their Materials and Methods section Avaltroni et al.[1] do not explain in sufficient detail how the number average diffusion coefficient is obtained, and critical evaluation of the methodology is strictly not possible.

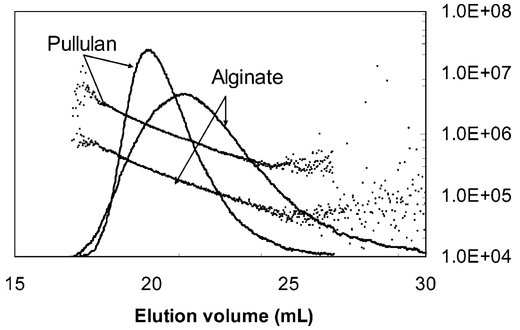

Figure 1. SEC-MALLS analysis[6] of polydisperse alginate and pullulan.

Avaltroni et al.[1] also observe a decrease in the estimated L_p when measurements are performed at high pH, where M decreases because of alkaline degradation. The lability at high pH is well-known,[6] but L_p should not change as indicated in Figure 7B, only the molar mass and R_g. This strongly indicates problems with the methodologies and/or the applicability of the underlying theories. Their estimate of L_p (5.4 nm) is well below that recently found by others. For instance, ref 6 reports 12 nm (extrapolated to infinite ionic strength) based on a wide selection of alginates.

In their Introduction Avaltroni et al.[1] state that “few studies have been performed under the dilute conditions that are representative of its concentration in natural water”. Their measurements are carried out in pure NaCl or $MgCl_2$ solutions. In seawater, with its high content of calcium ions, alginates will certainly behave differently due to their high selectivity for calcium. Most likely, they will aggregate and form microgels, analogous to the calcium-induced gelation seen at higher concentrations.

In conclusion, the SEC method and anomalous sample behavior presented by Avaltroni et al. is clearly questionable as a basis for estimating L_p, and the estimates are most likely erroneous unless verified by independent measurements and better characterized samples.

References and Notes

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