Reply to Comment on "Conformational Changes and Aggregation of Alginic Acid as Determined By Fluorescence Correlation Spectroscopy"

M_n/M_w/Polydispersity. We agree that our observed polydispersity was atypical, and Christensen et al.[1] are, of course, correct in pointing out the uncertainty that may affect size exclusion chromatography refractive index (SEC-RI) determinations performed using pullulan standards. However, in this paper, this point is somewhat of a red herring. We provided polydispersity and M_w values of an *unpurified* alginate for information purposes only. Diffusion coefficients were obtained on a *purified* alginate sample, and only M_n values were employed in our calculations. Although we cannot comment precisely on the authors' own data suggesting a large overestimation of the molar masses for their alginate and pullulan samples, subsequent confirmation of our SEC system using a multiple-angle laser light scattering (MALLS) detector has shown that our number average molar mass values were certainly not overestimated to the extent suggested by the authors. Furthermore, upon the basis of the labeling procedure that we employed, the diffusion coefficient D obtained from fluorescence correlation spectroscopy (FCS) is a number average value, D_n,[2] (as stated in the original paper[3]). For consistency, number-average values were thus employed in all calculations, and number-average L_c and L_p values were generated throughout the paper.

Calculation of R_g from D. More details regarding the use of eq 1 can be found in ref 5. It is valid for linear polymers in good solvents, which we contend will apply to the dilute aqueous solutions of alginic acid under the investigated pH range. In eq 1, the constant (0.0829) results from an estimation of $(R_h/R_g) = 0.64$, which is valid for a self-avoiding walk.[5] Admittedly, the evolution of R_h/R_g is very difficult to evaluate as it continuously changes with conformation. In our paper, conformational changes were only qualitatively discussed in Figure 7, where for pH 4 and lower a nonambiguous and significant increase in the hydrodynamic radius was observed (upon the basis of the direct observation of increased diffusion times and the corresponding smaller diffusion coefficients).

Influence of pH. Upon the basis of eq 2, a change in R_g necessarily results in a change in either L_p or L_c. We agree with Christensen et al.[1] that in basic solutions, where depolymerization occurs, L_c is likely the affected parameter rather than L_p. The decrease in L_p in Figure 7 (zone iii) was due to the use of a constant L_c for the calculations based upon eq 2. The assumption of a constant L_c is unlikely at the high pH values that are likely to cause polymer degradation.

L_p Values. We disagree with the authors' contention that our value is "well below that... found by others". Our determinations of L_p fall in the 4.3−6.1 nm range, below the value of 12 nm found in the authors' recent paper[6] but clearly in line with the literature, including values that the authors themselves have published previously (5 nm[7]). As the authors are well aware, one cannot directly compare samples that come from different sources with different M/G ratios nor those performed under different physicochemical conditions. It is unfortunate that we were unable to take into account the work done by Vold et al.[6] in our discussion; however, their paper was submitted nearly at the same time as our own.

Environmental Relevance. Finally, Christensen et al.[1] criticize the absence of Ca^{2+} in our study conditions, even though it is abundant in seawater. We agree that Ca^{2+} will likely influence the aggregation and indeed gelation of alginic acid; however, this point was outside the scope of this study, which was limited to investigating the influence of ionic strength and pH.

In conclusion, we admit that some uncertainty necessarily arises from the determination of M_n (and subsequently L_p) from the SEC-RI measurement. Nonetheless, we strongly believe that the trends discussed in the paper provide valuable and novel insights into the physicochemistry of alginates. We have described a novel approach for evaluating the properties of alginic acid as a function of pH and ionic strength by combining FCS, SEC, and molecular modeling. Although improvements in the determination of M_n would necessarily increase the precision of the L_p values, values produced in this paper are generally consistent with literature values for alginates and are unique for this given alginate. We welcome the interest Christensen et al.[1] have shown for this paper; however, we reject their main criticisms as being not critical to the main points of the study.

References and Notes

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BM078005C

10.1021/bm078005c CCC: $37.00