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Compositional heterogeneity of alginates through NMR analysis

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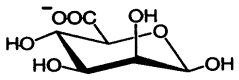
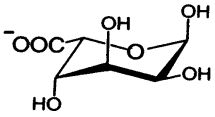
Summary
Two analytical methodologies are used for the analysis of the NMR data of alginates. The samples are shown to be compositionally heterogeneous, and the NMR data can be treated with either a discrete or a continuous statistical model. The unfractionated alginate is found to contain at least four components: two mostly homopolymer blocks, one somewhat alternating copolymer block, and one or more random copolymer blocks. Other information available includes chemical composition distribution, component statistics and reaction probabilities, and average M and G block lengths.

Introduction

Alginates are a family of polysaccharides extracted from brown algae and used commercially as thickeners, binders, encapsulants, stabilizers, film-forming agents, and suspending agents (1-4). Structurally, they are unbranched copolymers of (1-4)-linked ß-D-mannuronic acid (M) and α-L-guluronic acid (G) residues.

M = 

G = 

These comonomers are known to form both blocky and alternating sequences in alginates, and the microstructure is essential for their properties. For example, the selectivity for cations (and thereby the gel-forming properties) has been correlated with the content of the G-blocks. In contrast, chain flexibility is enhanced with increased M content (1,2).

The commonly accepted technique to study polymer microstructure is NMR (5-8). Alginates have been extensively studied by NMR (9-20). Grasdalen, Larsen, Smidsrod, Skjak-Braek, and their coworkers have done most of the work in this area (10-15). They have provided the spectral assignments and also recommended a computational procedure to extract structural information. Thus, ^{1}H NMR can be used to compute composition, diad sequences, and G-centered triads, and ^{13}C NMR to calculate both M-and G-centered triads. The calculation procedure involves the use of Bemoullian, first- and second-order Markovian models (1,2,15).

Recently, several novel models have been developed for compositionally heterogeneous systems, primarily vinyl polymers (21-29). In this work these alternative models are used to study the compositional heterogeneity of alginates.

Statistical Models

The application of Bernoullian and Markovian models to NMR data is well documented

(5,6,21). Whereas mechanistically the polymer propagation models may not be suitable for alginates (15,30), such models are useful as an analytical framework to delineate polymer microstructure. The Bernoullian (**B**) model corresponds to random placement of M and G residues along the polymer chain and is characterized by one parameter (e.g., P_M, the probability of M placement on the chain). The first-order Markovian (**M1**) model posits that the nearest neighbor has an effect on monomer placement and is characterized by two parameters (P_{MG} and P_{GM}, corresponding to the placement of G next to M, and the placement of M next to G, respectively). The second-order Markovian (**M2**) model assumes that the next two neighbors have an effect on the placement and is characterized by four parameters. It has been reported that in general the distribution of M and G along the polymer chains for alginates cannot be described by Bernoullian statistics except in some fractionated samples (1,2,12). The first-order Markovian model has been found to be valid in many cases (12,18,19). For a wide range of alginates, the second-order Markovian model was reported to produce the best fit to the observed NMR data (15).

In recent years, there has been an increasing awareness of the effects of compositional heterogeneity on the NMR data for many polymeric systems (21-29). At least four types of compositional heterogeneity have been identified (29): (1) statistical heterogeneity, arising from the statistical fluctuations of copolymer composition; (2) conversion heterogeneity, which may result when the comonomers have different reactivity ratios, thereby producing different compositions at different conversions; (3) multi-state heterogeneity, where the polymer is composed of several polymer components; and (4) process heterogeneity, which may come about through variations in reaction process conditions, e.g., temperature fluctuations, inadequate stirring, dead volume in reactor, and gel effect.

The theory and the methodologies involved with each type of heterogeneity have been developed, and many polymers have been studied, demonstrating their utility in the analysis of NMR data (21-29). These methodologies should be applicable to alginates as well. For alginates, these treatments can be grouped into two kinds, depending on the manner in which the chemical composition distribution (CCD) is represented: continuous functions and discrete components.

In the case of continuous function treatments, the perturbed Markovian models have been developed, using symmetric function (26), non-symmetric functions (27), or a function-free approach (27). For convenience, the exponentially modified Gaussian (EMG) function will be used in this work. Thus, the Bernoullian probability is represented not by one value (P_M) but by a distribution:

$$f(z) = \frac{N}{\tau\sigma\sqrt{(2\pi)}} \int_0^\infty \exp\left[-\frac{(z-P_M-z')^2}{2\sigma^2} - \frac{z'}{\tau}\right] dz' \qquad (1)$$

where z is the Bernoullian probability, N the area under the Gaussian, σ the standard deviation, τ the skew factor, z' the dummy variable of integration, and P_M the average value of Bernoullian probability without the exponential modification.

For the EMG models, the equations for polymer composition, diad, triad, and higher n-ad sequences have been previously derived (27). The experimentally observed sequence intensities can be fitted to the theoretical sequence intensities to obtain P_M, σ, and τ. Although the Bernoullian probability is shown in Equation 1, the EMG for the first-order Markovian probabilities can be similarly expressed (27).

In the discrete component approaches (23-25), the polymer is considered to be the

mixture of two or more discrete components. No assumption is made of the nature of the components: they may be separate chains or joined together as block copolymers. In this case, each experimentally observed sequence (composition, diad, triad, or higher n-ad) is the weighted average of the corresponding sequences of all the components.

$$I_i = \sum_{\alpha} w_\alpha \, I_{i\alpha} \qquad (2)$$

where I_i is the total intensity for sequence i, w_α is the weight fraction for component α, and $I_{i\alpha}$ is the intensity for sequence i and component α. Several computational methodologies have been developed for this analysis (22-25). In this work, both Bernoullian and the first-order Markovian models will be used.

Results and discussion

From previous studies of heterogeneous polymers (21,27), it is known that the higher the n-ad sequences being examined, the more discriminating are the data towards the models. Grasdalen et al. (12,13) have published detailed NMR triad data for an alginate extracted from *Laminaria digitata* and for four fractions obtained from it. These data (summarized in Table 1) are highly suited as a test case for analysis. In this work MG and GM diads are combined and called simply MG. Likewise MMG denotes both MMG and GMM triads, and GGM denotes both GGM and MGG triads.

Whole Polymers. For the purpose of illustration, the analysis of the NMR data of the whole polymer is given in detail here. The observed data are first fitted to the Bernoullian and the first-order Markovian models (Table 2, columns 3 and 4). The mean deviations are large, suggesting that these models are not really appropriate for these data.

The use of discrete two-component models gives much lower mean deviations. The two-component **B**/**B** model provides a good fit to the data (Table 2, column 5). As expected, the two-component (**M1**/**M1**) model gives an even better agreement with the observed data than the two-component (**B**/**B**) model. The continuous EMG functions also produce acceptable goodness-of-fit (mean deviation ≤ 1.0%, comparable to experimental precision). Although the EMG/**M1** function introduces one additional parameter, the improvements in the fit over EMG/**B** function is only marginal (mean deviation 0.8 versus 0.9).

Using the values of the EMG/**B** function, we can plot the chemical composition distribution (CCD) curve for the whole polymer (Figure 1a). The composition heterogeneity may also be represented in Figure 1c by two discrete **M1**/**M1** components. It is important to note that the same data can be represented in two different ways. For a

Table 1. Triad sequences for alginates obtained from *Laminaria digitata* and four fractions

sample	MMM	MMG	GMG	MGM	GGM	GGG	refs.
whole polymer	38	16	6	10	8	22	12
fraction $L_{1,4}$	22	28	11	11	28	0	12
fraction MG	12	22	22	27	12	5	12
fraction M	61	22	0	10	2	5	12
fraction G	2.5	5	2.5	0	4	86	12,13

Table 2. Fitting of the whole polymer NMR data to different models

triad	I_{obsd}	I_{calc}(B)	I_{calc}(M1)	I_{calc}(B/B)	I_{calc}(M1/M1)	I_{calc}(EMG/B)	I_{calc}(EMG/M1)
MMM	38	38.0	38.0	38.0	38.0	38.0	35.7
MMG	16	28.9	18.3	16.4	16.0	18.1	16.0
GMG	6	5.5	2.2	5.1	6.0	4.3	6.0
MGM	10	14.5	3.1	8.2	10.0	9.1	9.8
GGM	8	11.0	16.5	10.3	8.0	8.5	8.3
GGG	22	2.1	22.0	22.0	22.0	22.0	24.2
mean dev.		6.8	3.6	0.9	0.0	0.9	0.8
		P_M=0.724	P_{GM}=0.272	comp.1	comp.1	P_M=0.643	P_{GM}=0.695
			P_{MG}=0.194	w_1=0.344	w_1= 0.344	σ =0.294	P_{MG}=0.437
				P_M=0.143	P_{GM}=0.144	τ = -0.039	σ = 0.367
					P_{MG}=0.996		τ = -0.042
				comp.2	comp.2		
				w_2=0.656	w_2= 0.656		
				P_M=0.833	P_{GM}=0.970		
					P_{MG}=0.174		

Table 3. Fitting of the NMR data of polymer fractions to different models

	Fraction $L_{1,4}$				Fraction MG			
triad	I_{obsd}	I_{calc}M1	I_{calc}M1/M1	I_{calc}EMG/B	I_{obsd}	I_{calc}M1	I_{calc}M1/M1	I_{calc}EMG/B
MMM	22	22.0	22.0	22.0	12	7.5	12.0	14.7
MMG	28	28.0	27.9	24.0	22	25.6	22.0	22.0
GMG	11	8.9	9.9	14.0	22	22.0	22.0	15.8
MGM	11	12.8	14.2	12.0	27	27.0	27.0	11.0
GGM	28	20.3	19.4	28.0	12	15.6	12.0	31.5
GGG	0	8.0	6.6	0.0	5	2.3	5.0	5.0
mean dev.		3.3	3.3	1.3		2.4	0.0	7.4
		P_{GM}=0.558	comp.1	P_M=0.555		P_{GM}=0.776	comp.1	P_M=0.485
		P_{MG}=0.389	w_1=0.978	σ =0.010		P_{MG}=0.632	w_1=0.801	σ =0.010
			P_{GM}=0.594	τ =0.045			P_{GM}=0.856	τ =0.040
			P_{MG}=0.414				P_{MG}=0.700	
			comp.2				comp.2	
			w_2= 0.022				w_2= 0.199	
			P_{GM}=0.984				P_{GM}=0.268	
			P_{MG}=0.000				P_{MG}=0.179	

	Fraction M				Fraction G			
triad	I_{obsd}	I_{calc}M1	I_{calc}M1/M1	I_{calc}EMG/B	I_{obsd}	I_{calc}M1	I_{calc}M1/M1	I_{calc}EMG/B
MMM	61	61.0	61.0	61.0	2.5	2.5	2.5	0.6
MMG	22	22.0	19.8	21.6	5.0	3.7	4.0	4.9
GMG	0	2.0	1.6	0.6	2.5	1.4	1.7	2.0
MGM	10	11.2	10.5	10.8	0	0.1	1.7	2.5
GGM	2	3.5	2.0	1.2	4	6.3	4.0	4.0
GGG	5	0.3	5.0	4.8	86	86.0	86.0	86.0
mean dev.		1.6	0.7	0.5		0.8	0.6	0.8
		P_{GM}=0.865	comp.1	P_M=0.955		P_{GM}=0.035	comp.1	P_M=0.072
		P_{MG}=0.153	w_1=0.931	σ =0.006		P_{MG}=0.427	w_1=0.861	σ =0.160
			P_{GM}=0.921	τ = -0.123			P_{GM}=0.005	τ =0.003
			P_{MG}=0.142				P_{MG}=0.699	
			comp.2				comp.2	
			w_2= 0.069				w_2= 0.138	
			P_{GM}=0.019				P_{GM}=0.528	
			P_{MG}=0.056				P_{MG}=0.434	

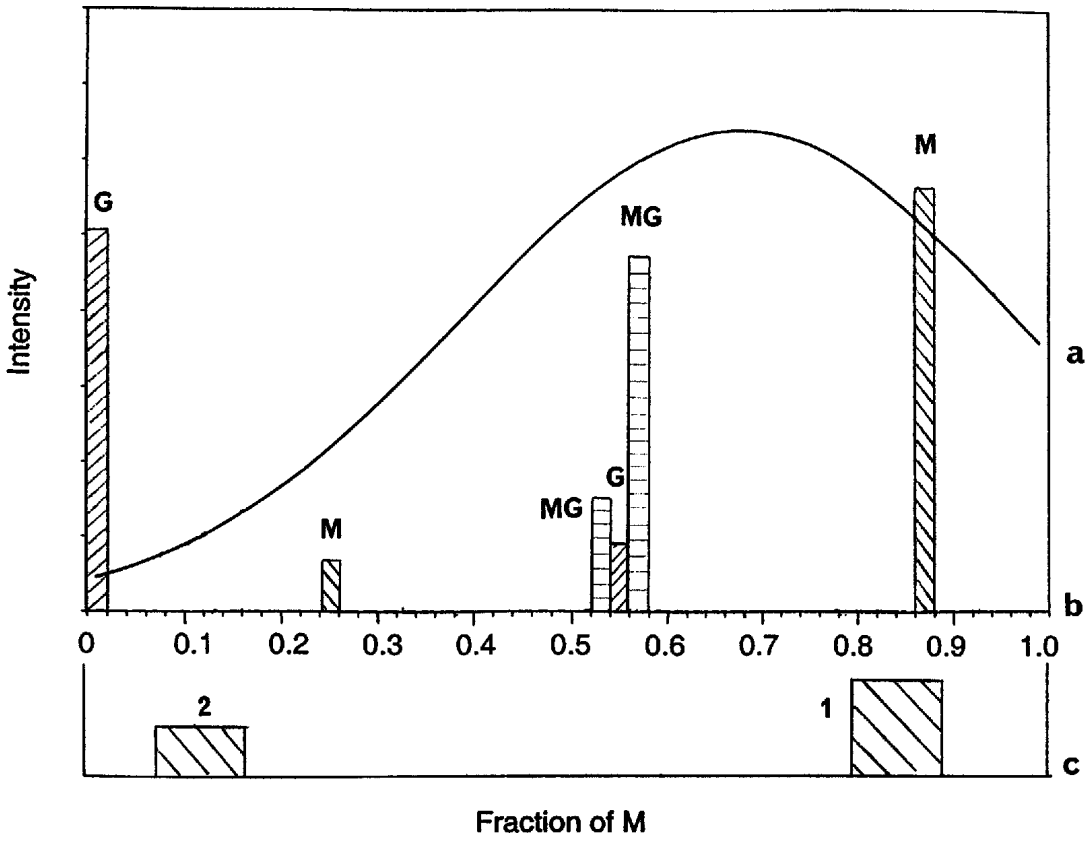


Figure 1. Calculated chemical composition distribution (CCD) for alginate isolated from *Laminaria digitata*: (a) continuous function approach, (b) results from fractionation/NMR analysis, (c) two-component approximation (discrete model). For ease of presentation, each component is shown as separate polymer chains. The bar widths are arbitrarily set.

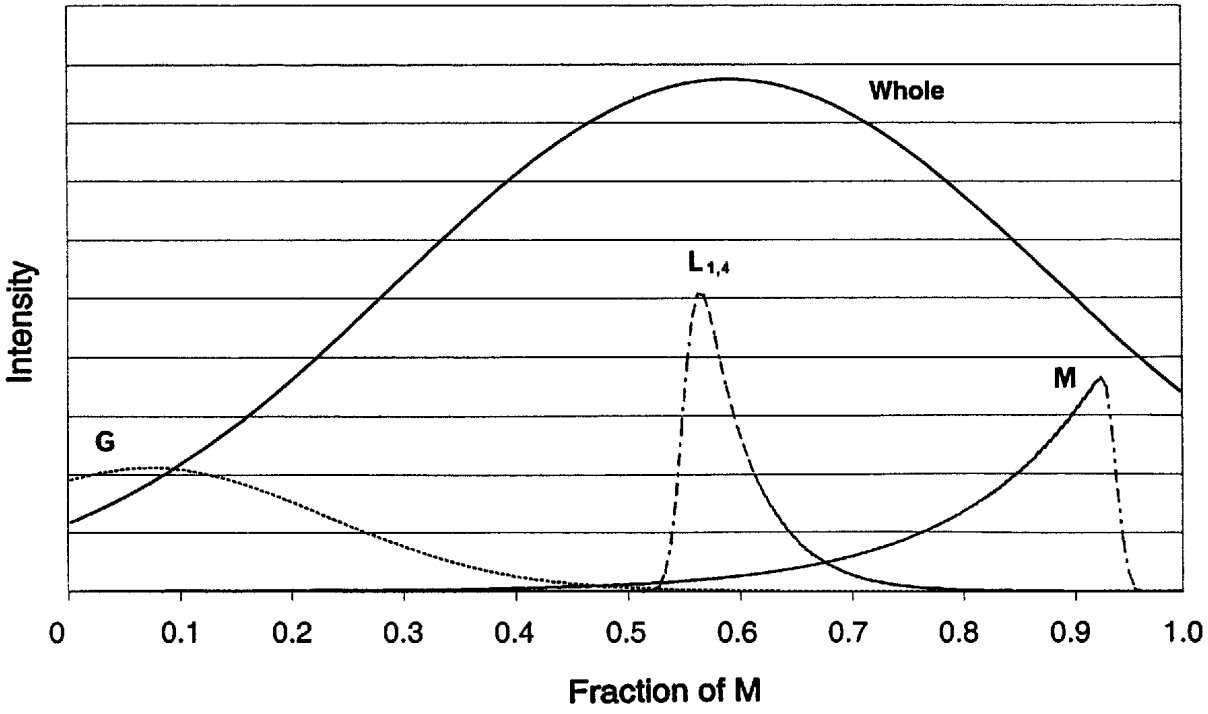


Figure 2. Calculated chemical composition distribution (CCD) functions for alginate isolated from *Laminaria digitata* and its fractions.

Table 4. Summary of the parameters for the exponentially modified Gaussian (EMG/**B**) Bernoullian model

sample	P_M	σ	τ
whole polymer	0.643	0.294	-0.039
fraction $L_{1,4}$	0.555	0.010	0.045
fraction MG	--	--	--
fraction M	0.955	0.006	-0.123
fraction G	0.072	0.160	0.003

polymer with a complex microstructure, often the NMR data on the whole polymer alone do not enable us to tell if one representation is preferred over another. Additional data are needed; one possibility is to examine the polymer fractions.

Polymer Fractions. Grasdalen's data (12,13) on the alginate fractions can be subjected to a similar analysis for all six models. In order to save space, only the results of the **M1** model, the two-component **M1**/**M1** model, and the EMG/**B** model are shown in Table 3. It is of interest to note that Fraction $L_{1,4}$ cannot be fitted satisfactorily to a discrete two-component model; only the continuous EMG/**B** function provides a fair agreement with observed data. In contrast, Fraction MG cannot be fitted to the continuous EMG functions; however, the two-component discrete models give excellent agreements. Fraction M and Fraction G can be fitted to either a discrete or a continuous model.

A summary of the parameters for the continuous models is shown in Table 4. These parameters can be used to plot the calculated CCD curves (Figure 2). As expected, the G fraction is centered at high G composition with a tail toward the low G end. The M fraction is centered at the high M composition, tailing towards the low M. Fraction $L_{1,4}$ has a composition distribution near the middle with a skew. Also shown in Figure 2 is the computed CCD of the whole polymer.

As instructive as the CCD curves are, they do not provide detailed information on the blocky and the alternating structures expected for alginates. Such information can be obtained from the discrete-component analysis. The results of the two-complement **M1**/**M1** models are summarized in Table 5. Note that for each component,

$P_{GM} + P_{MG} < 1$, tendency to form blocks;
$P_{GM} + P_{MG} \sim 1$, nearly random comonomer placement;
$P_{GM} + P_{MG} > 1$, tendency to alternate.

Thus, each of the Fractions M and G consists of an almost random M/G polymer component and an almost blocky M/G component. In contrast, Fraction MG contains a blocky component (with M and G blocks), as well as a component that shows a tendency to alternate. The whole polymer, being a mixture of all these fractions, then contains at least four components: a predominantly M block, a predominantly G block, a somewhat alternating M/G component, and one or more random M/G components (Figure 1b). This finding is consistent with the broad CCD displayed in Figure 1a.

Note that the triad data for the whole polymer also produced a good fit with the discrete two-component model (Table 2). Since we now know that the whole polymer contains at least four components covering a broad compositional range, the two-component model for the whole polymer is clearly inadequate. The observed triad data for the whole polymer are the averages of all the components. When only two components are used to fit the data, some smearing of the information becomes

unavoidable. Thus, although a good fit is achieved, the Markovian probabilities (P_{MG} and P_{GM}) are averages and do not necessarily provide information on microstructure. In this connection, the NMR analysis of unfractionated alginate using simple one-or two-component Bernoullian or Markovian models should be carried out with care.

The discrete model does permit us to estimate the block lengths for the M and the G sequences (n_M and n_G, respectively). The average block lengths are (6,12):

$$\overline{n_G} = \frac{2\,(G)}{(MG)}, \qquad \overline{n_M} = \frac{2\,(M)}{(MG)} \tag{3}$$

For the **M1** model, these correspond to

$$\overline{n_G} = \frac{1}{P_{GM}}, \qquad \overline{n_M} = \frac{1}{P_{MG}} \tag{4}$$

The results are shown in Table 6. The block length is another measure of the alternating or blocky tendency of the components. Thus, when n ~ 1, the component is alternating, and when n >> 1, it is blocky. The overall block length for each sample is obtained by taking the weighted average of the two components: $\langle n_G \rangle = \Sigma w_i P_{MGi} / \Sigma w_i P_{MGi} P_{GMi}$, and $\langle n_M \rangle = \Sigma w_i P_{GMi} / \Sigma w_i P_{MGi} P_{GMi}$. The calculated overall block lengths are in good agreement with the observed block lengths, as reported by Grasdalen, et al (12).

Experimental

The NMR triads were analyzed using computerized analytical approaches described previously (23-27). The fitting of the data to the **B** and the **M1** models was achieved with program TRIAD. The two-component models were fitted with the program TRIADX (23). The continuous EMG functions were fitted with the program PERTEMG (27). All programs were written in QuickBASIC and run on a personal computer.

A referee pointed out that for some alginate samples the experimental data may contain errors due to inherent limitations in quantitative NMR measurements. Indeed, when errors are present it is important to include more data (e.g., from polymer fractions) in the analysis in order to minimize uncertainties and maximize information content.

Table 5. Summary of the parameters for the two-component **M1/M1** model

	component 1				component 2			
sample	w_1	$P_{GM}(1)$	$P_{MG}(1)$	tendency	w_2	$P_{GM}(2)$	$P_{MG}(2)$	tendency
whole polymer	0.656	0.970	0.174	mixture	0.344	0.144	0.996	mixture
fraction $L_{1,4}$	--	--	--		--	--	--	
fraction MG	0.801	0.856	0.700	alternating	0.199	0.208	0.179	blocky
fraction M	0.931	0.921	0.142	near random	0.069	0.019	0.056	blocky
fraction G	0.139	0.528	0.434	near random	0.861	0.005	0.699	blocky

Table 6. Average block lengths for the individual components and for the whole samples

	component 1			component 2			overall sample (calc)	
sample	w_1	$\bar{n}_G$	$\bar{n}_M$	w_2	$\bar{n}_G$	$\bar{n}_M$	$\langle n_G \rangle$	$\langle n_M \rangle$
whole polym	0.656	1.03	5.75	0.344	6.94	1.00	2.86	4.29
frac. MG	0.801	1.17	1.43	0.199	4.81	5.59	1.22	1.49
frac. M	0.931	1.09	7.04	0.069	52.6	17.9	1.12	7.05
frac. G	0.139	1.89	2.30	0.861	200	1.43	19.0	2.23

Conclusions

In this work two analytical methodologies are proposed to treat the NMR data of alginates. The chemical composition distribution is approximated by either discrete components or exponentially modified Gaussian functions. The methodologies give complementary information on the microstructure of the polysaccharide. With these methodologies, one type of alginate was shown to be compositionally heterogeneous with at least four separate polymeric components. Caution is advised when the unfractionated alginate is analyzed by NMR and statistical models. It is preferable to fractionate the polymer and include the NMR data of the fractions in the analysis.

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