Correlation between Chemical Structure and Physical Properties of Alginates

ARNE HAUG, SVERRE MYKLESTAD, BJØRN LARSEN and OLAV SMIDSROD

Norwegian Institute of Seaweed Research, N.T.H., Trondheim, Norway

Alginic acid from Ascophyllum nodosum and Laminaria hyperborea stipes was subjected to partial acid hydrolysis, and the products were examined by free boundary electrophoresis. The various alginate fragments were isolated by fractional precipitation and analysed. The same types of building elements as previously found in L. digitata alginate were present, and the differences between the three alginates were caused by the building elements occurring in different proportions. The solubility of the alginate fragments in acidic solutions and in the presence of calcium ions was examined and correlated with the properties and composition of the alginates. The higher solubility of A. nodosum alginates in acidic solutions was caused by a smaller proportion of homopolymer blocks in this alginate than in alginates from the Laminaria species. A fraction of the A. nodosum alginate, with a particularly low content of homopolymer blocks, was found to be completely soluble in acidic solutions at low ionic strength.

Until 1955, alginic acids from different brown algae were supposed to be chemically identical and only to differ in physical properties due to different molecular weights. The discovery of the presence of L-guluronic acid residues in addition to the wellknown D-mannuronic acid residues in alginic acid has made it necessary to recognize that the term "alginic acid" may cover chemically different compounds. Different brown algae contain chemically different alginates\(^1,2\) and a corresponding difference was also found in commercial alginates.\(^2\)

The correlation between the chemical composition of alginates and their physical properties is, therefore, of both practical and theoretical interest. It has been established that there is a close correlation between the mannuronic-guluronic acid ratio of the alginates and their ion exchange properties,\(^3-4\) acid dissociation,\(^2,3,6\) and gel-forming ability.\(^2,7\) The solubility of alginates in acidic solutions has also been shown to depend on the uronic acid composition of the alginate,\(^2,7\) but marked differences were also observed between samples with nearly identical uronic acid composition. It was suggested that these differences might be due to the presence of small amounts of the sulfated
polysaccharide ascophyllan in alginate samples prepared from Ascophyllum nodosum.  

Recent work \(^8,^9\) has shown that alginate from Laminaria digitata is built as a block polymer, containing long sequences of only guluronic or only mannanuronic acid residues, and also sequences with a predominantly alternating structure. It is well known from the field of synthetic high polymers \(^10\) that the properties of a copolymer is determined not only by the proportion between the two monomers, but also to a large extent by the sequence of the two monomers along the polymer chain. It is reasonable to assume that if the monomer sequence is different for alginates from different sources, the properties of the alginates may also differ, even for alginates of the same overall composition.

In the present work some properties of alginates from different raw materials are compared with the properties of fragments with different uronic acid compositions prepared from L. digitata. By means of heterogeneous hydrolysis, free boundary electrophoresis, and fractionation of the hydrolytic products, the amounts of the corresponding fragments in alginates from A. nodosum and L. hyperborea stipes are estimated. On this background, the correlation between some alginate properties and uronic acid composition and sequence is discussed.

**EXPERIMENTAL**

_Materials._ Alginates were prepared from Ascophyllum nodosum, Være 3/5—63 (65% mannanuronic acid), Laminaria digitata, Svellingen 15/1—60 (59% mannanuronic acid) and L. hyperborea stipes, Hustad 4/5 (27.5% mannanuronic acid). Fractions with different uronic acid compositions were prepared from a commercial L. digitata alginate as described in the preceding paper.\(^5\)

_Preparation of alginate._ The alginate was extracted as described elsewhere.\(^5\) In order to reduce the amount of ascophyllan and other fucose-containing polysaccharides in the product, alginic acid was precipitated by addition of acid to the extract. Alginic acid from L. digitata was precipitated at pH 1.85 in the presence of 0.02 N sodium chloride, while alginic acid from A. nodosum was precipitated at pH 2.15 in the presence of 0.5 N sodium chloride.

_Determination of precipitation curves._ The solubility of alginates and alginate fragments at low pH was determined by mixing equal volumes of 0.5% alginate in 0.04 N and 2.0 N potassium chloride, respectively, and dilute hydrochloric acid. The pH of the mixture was determined, and the mixture left for 30 min and centrifuged. The precipitate was suspended in water and dissolved by neutralization, and the amounts of carbohydrate in the two fractions determined by the phenol-sulfuric acid reaction.

Precipitation of alginate fragments by calcium ions was determined by mixing a 0.5% solution in 0.2 N sodium chloride with equal volumes of calcium chloride solution. The mixture was centrifuged, the precipitate dissolved by suspension in a solution containing ethylenediaminetetraacetic acid (EDTA), and the amounts of carbohydrate in the two fractions were determined by the phenol-sulfuric acid reaction.

_Fractionation of A. nodosum alginate._ Alginate from A. nodosum, prepared as described above, was dissolved in water (0.5%), potassium chloride added to 2 N and the solution mixed with an approximately equal volume of 0.13 N hydrochloric acid to obtain a pH of 1.4. The mixture was centrifuged and the insoluble fraction suspended in water and dissolved by the addition of dilute alkali. The fraction was isolated in the usual way by precipitation with ethanol, washing with ethanol and ether and drying. The soluble phase was mixed with 1 N potassium hydroxide in an amount sufficient to increase the pH to 2.2. The precipitate which was formed was removed by centrifugation, suspended in water and dissolved by neutralization with alkali and isolated as usual.
Hydrolysis of alginic acid. The rates of hydrolysis of alginic acid from *A. nodosum* and *L. hyperborea* stipes and of fractions from the former were determined by suspending 5 parts of sodium alginate in 100 parts of 1 M oxalic acid, and heating the suspension in a boiling water bath. A stream of nitrogen was passed through the suspension, and samples were removed at intervals for analysis. The samples were centrifuged and the soluble and insoluble phases analysed separately.

The procedure described in the preceding paper was used to examine the electrophoretic patterns obtained after different degrees of hydrolysis.

Methods for fractionation of degraded alginate, electrophoresis, and analysis for carbohydrates, reducing power, and uronic acid composition were those described in the preceding paper. Fucose was determined by the method of Dische and nitrogen by the micro-Kjeldahl procedure of Hiller, Plazin and van Slyke. Schöninger-combustion of the samples followed by precipitation with BaCl₂ and complexometric titration of excess Ba²⁺ was used for the determination of sulfate. Experimental details have been published previously. O-Acetyl groups were determined according to Kunz and Hudson.

RESULTS

a) Solubility of alginates from *L. digitata* and *A. nodosum* at low pH. Previously it was found that alginates from *A. nodosum* are more soluble at pH values below 2.5 than *L. digitata* alginates with the same uronic acid composition. The presence of ascoplyllan may be expected to influence the solubility in acid medium and care has been taken to prepare the *A. nodosum* alginate in a way which reduces the ascoplyllan content of the product to a minimum (see Experimental). The amounts of fucose in the alginate preparations were in all cases well below 1%. A preliminary report of the properties of this preparation has been given.

The solubility in acidic solution was determined in the presence of 0.02 N and 1.0 N potassium chloride for alginate samples prepared from *A. nodosum* and *L. digitata*. The results are given in Fig. 1. The intrinsic viscosity of both samples was approximately 8 (100 ml/g). In agreement with previous results, the *A. nodosum* alginate was significantly more soluble than the *L. digitata* preparation at low salt concentration (0.02 N potassium chloride). In 1.0 N

![Fig. 1. Solubility of alginic acid as a function of pH in the presence of potassium chloride. 

--- = *A. nodosum* alginate 

--- = *L. digitata* alginate.](image)

![Fig. 2. Solubility of alginic acid fractions prepared from *Ascophyllum nodosum*. 

--- = Fraction soluble at pH 1.4. 

--- = Fraction insoluble at pH 1.4.](image)
potassium chloride, however, the main difference between the two samples was the amount of alginate which became soluble at pH values below 2.

b) Fractionation of alginate from A. nodosum. The precipitation curve given in Fig. 1 indicated that a fraction of the *A. nodosum* alginate was soluble at pH values below 2. Alginate from *A. nodosum* was, therefore, fractionated by precipitation at pH 1.4 in 1 N potassium chloride. The soluble fraction was precipitated by increasing the pH to 2.2. About 25% of the total alginate sample was recovered in the acid soluble fraction (Fraction S₁₄).

Precipitation curves for the two fractions in acidic media were determined in 0.02 and 1.0 N potassium chloride. The results given in Fig. 2 clearly demonstrate that alginate from *A. nodosum* contains two fractions with very different solubilities in acidic solutions. The two fractions were analysed and the intrinsic viscosity determined. The results, given in Table 1, give no indication of the chemical basis for the remarkable solubility differences.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Decarb. matter</th>
<th>% mannnuronic acid</th>
<th>[η]</th>
<th>% fucose</th>
<th>% SO₃Na</th>
<th>% N</th>
<th>Acetyl %</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₁₄</td>
<td>96</td>
<td>65.5</td>
<td>3.3</td>
<td>0.3</td>
<td>&lt;0.5</td>
<td>0.01</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>I₁₄</td>
<td>94</td>
<td>63.5</td>
<td>4.1</td>
<td>0.4</td>
<td>&lt;0.5</td>
<td>0.06</td>
<td>&lt;0.5</td>
</tr>
</tbody>
</table>

The two fractions were also examined by free boundary electrophoresis in glycine buffer at pH 3.1 and phosphate buffer at pH 6.0. In both buffers the fractions had the same mobility and migrated as one peak.

c) Solubility of alginate fractions. The preparation of three alginate fragments with different uronic acid composition and approximately the same number average degree of polymerization (Pₙ ≈ 20) was described in the preceding paper. Preparation A contained mainly alternating mannuronic-guluronic acid residues (65% mannuronic acid), while preparation B and C contained 92 and 13% mannuronic acid, respectively.

![Fig. 3. Solubility of alginate fragments (Preparations A, B, and C) as a function of pH.](image)

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Some solubility properties of the three fractions were investigated. Each of the three fractions were subjected to acid precipitation in the presence of 0.02 and 1.0 N potassium chloride. The results are given in Fig. 3. At low ionic strength preparation A was completely soluble at all pH values, while the solubility of preparation B and C decreased with increasing acidity. In 1.0 N potassium chloride both preparations A and B exhibited a marked solubility minimum around pH 2 in the strong potassium chloride solution.

The three fractions were also precipitated with calcium ions in the presence of 0.1 N sodium chloride. The results are given in Fig. 4. In this case, the fraction with an intermediate uronic acid composition also exhibited an intermediate solubility.

d) Acid hydrolysis of alginate prepared from A. nodosum and L. hyperborea stipes. The course of the heterogeneous acid hydrolysis of alginate from L. digitata has been reported previously.\textsuperscript{8,9} Similar experiments were performed with alginate samples prepared from A. nodosum and L. hyperborea stipes. The hydrolysis was carried out in 1 M oxalic acid at 100°C and the amount of material dissolved and the reducing power of the hydrolysate are shown in Figs. 5 and 6. The amount of material dissolved in the hydrolysate approached a limit. In the case of L. digitata alginate, this limit was about 30% of the

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**Fig. 4.** Solubility of alginate fragments (Preparations A, B, and C) in the presence of calcium ions.

**Fig. 5.** Hydrolysis of alginic acid from Ascophyllum nodosum in 1.0 M oxalic acid. 1: Soluble material. 2: Reducing power of the hydrolysate.

**Fig. 6.** Hydrolysis of alginic acid from L. hyperborea stipes in 1.0 M oxalic acid. Legend as Fig. 5.

Fig. 7. Hydrolysis of a fraction (I\textsubscript{1,4}) of alginic acid from \textit{A. nodosum}. Legend as Fig. 5.

Fig. 8. Ascending electrophoretic patterns of alginate from \textit{A. nodosum}, hydrolysed in 0.3 N HCl at 100°C.

total alginate, while the limit for alginate from \textit{A. nodosum} was as high as 55%, and that for alginate from \textit{L. hyperborea} stipes was 23%. In all cases the number average degree of polymerization of the insoluble fraction approached 20—25.

The two fractions of \textit{A. nodosum} alginate, I\textsubscript{1,4} and S\textsubscript{1,4}, were also examined separately. Fig. 7 gives the results of the heterogeneous hydrolysis of fraction I\textsubscript{1,4}. In this case, the limit of soluble material was about 30%; the same as that obtained for \textit{L. digitata} alginate. Fraction S\textsubscript{1,4} was not precipitated from a 2% solution by the addition of oxalic acid to 1 M. On the other hand, only 65% of the sample was dissolved by shaking in 20 parts of 1 M oxalic acid or 0.3 N hydrochloric acid. After hydrolysis for 20 min at 100°C, however, an amount corresponding to 25—30% of the sample was insoluble in all cases.

e) Electrophoretic examination of hydrolytic products of alginate from \textit{A. nodosum} and \textit{L. hyperborea} stipes. An examination of the hydrolytic products obtained from alginate from \textit{L. digitata} was reported in the preceding paper. Alginates from \textit{A. nodosum} and \textit{L. hyperborea} stipes were investigated by a similar method. The hydrolysis was carried out in 0.3 N hydrochloric acid at 100°C. The non-dialysable material of the soluble and insoluble phases were examined after 20 min, while after 2, 10, and \(10 + 10\ h\) only the non-dialysable part of the insoluble phase was examined. The ascending electrophoretic patterns and the number average degree of polymerization are given in Figs. 8 and 9.

The fraction S\textsubscript{1,4} of the \textit{A. nodosum} alginate was hydrolysed for 20 min at 100°C and the soluble and insoluble phases examined separately. The ascending patterns and the number average degree of polymerization are given in Fig. 10.

f) Fractionation of partially hydrolysed alginate from \textit{A. nodosum} and \textit{L. hyperborea} stipes. The results given in Fig. 8 and 9 show that alginates from \textit{A. nodosum} and \textit{L. hyperborea} stipes were split into components with different

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electrophoretic mobilities. If the electrophoretic patterns are compared with the results obtained with *L. digitata* alginate, given in the previous paper, the various peaks may be identified as indicated by the numbers in Figs. 8 and 9, where peak 1 corresponds to the mannuronic acid rich component and peak 2 and 3 correspond to the two guluronic rich components with the lowest and the highest degree of polymerization, respectively. In order to confirm this, the insoluble parts of *A. nodosum* and *L. hyperborea* stipe alginates were fractionated after 2 h of hydrolysis. Two methods, described in the preceding paper, were used for the fractionation; precipitation in acid medium and precipitation with calcium ions in the presence of magnesium chloride. The ascending patterns of the fractions are given in Figs. 11 and 12.

The uronic acid composition, the number average degree of polymerization and the approximate yields of the fractions are given in Table 2. For comparison the results previously obtained for *L. digitata* alginate are included in the table.

The material which was dissolved in the hydrolysate after 20 min at 100°C gave one main peak and a small, slower moving peak. By fractionation with calcium ions in the presence of magnesium chloride, the components corresponding to the main peak was obtained in electrophoretically pure form. The component giving rise to the small peak was not obtained pure, but corresponded in all cases to a very small fraction of the total alginate. The results are given in Table 3.

**Table 2. Fractionation of three different types of alginites, degraded for 2 h at 100°C.**

<table>
<thead>
<tr>
<th>Soluble material</th>
<th>L. digitata alginate</th>
<th>A. nodosum alginate</th>
<th>L. hyperborea stipes alginate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield, % of total</td>
<td>27.2</td>
<td>47</td>
<td>15</td>
</tr>
<tr>
<td>% mannnuronic acid</td>
<td>67</td>
<td>60</td>
<td>65</td>
</tr>
<tr>
<td>$P_n$</td>
<td>4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Insoluble, non-dialysable</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak I, Yield, % of total</td>
<td>34.4</td>
<td>31.5</td>
<td>0</td>
</tr>
<tr>
<td>% mannnuronic acid</td>
<td>86.5</td>
<td>81.5</td>
<td></td>
</tr>
<tr>
<td>$P_n$</td>
<td>35</td>
<td>33</td>
<td></td>
</tr>
</tbody>
</table>

| Peak II, Yield, % of total | 7.4                      | 6.4                 | 42.8                         |
| % mannnuronic acid         | 21.8                     | 18                  | 18                           |
| $P_n$                      | 20.0                     | 21                  | 27.5                         |

| Peak III, Yield, % of total| 11.6                     | 3.2                 | 31                           |
| % mannnuronic acid         | 12.3                     | 8.5                 | 6.8                          |
| $P_n$                      | 70                       | 60                  | 75                           |

**Table 3. Fractionation of the non-dialysable material soluble by hydrolysis for 20 min at 100°C.**

<table>
<thead>
<tr>
<th>Soluble by Ca²⁺ precipitation (main peak)</th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield, % of total</td>
<td>7.9</td>
<td>18</td>
<td>4.5</td>
</tr>
<tr>
<td>% mannnuronic acid</td>
<td>60.5</td>
<td>63</td>
<td>70.5</td>
</tr>
<tr>
<td>$P_n$</td>
<td>15</td>
<td>39</td>
<td>20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Insoluble</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield, % of total</td>
<td>1.0</td>
<td>2.7</td>
<td>0.9</td>
</tr>
<tr>
<td>% mannnuronic acid</td>
<td>58.5</td>
<td>42</td>
<td>77</td>
</tr>
<tr>
<td>$P_n$</td>
<td>40</td>
<td>74</td>
<td>33</td>
</tr>
</tbody>
</table>

DISCUSSION

Even though the complete constitution of alginic acid is not yet known, the results described previously \(^8,^9\) indicated that alginic acid may be regarded as composed of three different building elements: blocks of mannuronic acid residues ("M-blocks"), blocks of guluronic acid residues ("G-blocks") and fragments with predominantly alternating mannuronic and guluronic acid residues. The latter parts of the alginic acid molecule are dissolved in the hydrolysate by heterogeneous acid hydrolysis and rapidly broken down. Even if the absolute amounts of this component in an alginate sample are not known, it seems reasonable to assume that the relative abundance of this component may be estimated by determining the amounts dissolved in the hydrolysate after a certain time of hydrolysis.

Free boundary electrophoresis and fractionation of hydrolytic products have shown that the same type of building elements occur in all the three types of alginates investigated. Both uronic acid composition and degree of polymerization of the components giving the various electrophoretic peaks were remarkably similar in all three alginates, and the marked difference between the alginates was only caused by different proportion between the various types of fragments (Table 2). In the Ascochyta nodosum alginate a very large proportion of the sample was dissolved in the hydrolysate, and correspondingly a relatively large fraction has been isolated which has an intermediate uronic acid composition and is electrophoretically homogeneous (Table 3). The amount of "M-blocks" is approximately the same as for L. digitata alginate, while the amount of "G-blocks" is much lower. Particularly, the amount of "G-blocks" with a high degree of polymerization (Peak 3) is very low, and the peak corresponding to this component could not be detected after \(10 + 10\) h of hydrolysis. The alginate from L. hyperborea stipes had, as usual for alginates from this raw material,\(^2\) a high content of guluronic acid. The amount of material which was dissolved in the hydrolysate was lower than for the other two species, and the composition was approximately the same as for the corresponding fraction from the other alginates. The amount of "M-blocks" was very low, and this component has only been detected in the electrophoretic patterns and has not been isolated from this alginate sample. The "G-blocks" are the dominating elements in this type of alginate.

We have in the preceding discussion assumed that there exists a sharp distinction between the fragments with a predominantly alternating sequence and the "M"- and "G-blocks". It is possible that the "alternating" fragments should be regarded as composed of short blocks, and that significant amounts of blocks of intermediate size also exist in the molecule. This point was discussed in the preceding paper. If the latter alternative is true, it would be more correct to consider the difference between A. nodosum alginate and L. digitata alginate as a difference in average block size. However, we have no method for determining such an average, and for the present purpose, the characterization of the alginates in terms of different amounts of the various fractions should be permissible.

The precipitation curves of the three types of alginate fragments at low pH are strikingly different. The importance of the sequence of the uronic
acid residues is clearly demonstrated by the curves in 0.02 N potassium chloride, where the fragments with intermediate composition are completely soluble, while the two fractions with more extreme uronic acid composition exhibit precipitation curves of the type well-known for undegraded alginate. This observation indicates that the existence of homopolymer blocks in the alginate is essential for acid precipitation. In accordance with this, the acid soluble fraction of the \( A. \ nodosum \) alginate (\( S_{1.4} \)) had a very high percentage of material (70 \%) which was found in the hydrolysate after 20 min at 100°C. This material was electrophoretically homogeneous and with an intermediate uronic acid composition (Fig. 10). However, even if the percentage of “M- and G-blocks” was low, the electrophoretic pattern of the insoluble fraction of \( S_{1.4} \) (30 \%) indicated that these components were present also in this fraction of the \( A. \ nodosum \) alginate. As shown by Table 1, no other chemical difference has been observed between the two fractions of \( A. \ nodosum \) alginate, and it is assumed that the different solubility of the two fractions is solely due to different amounts of homopolymer blocks.

The fraction \( S_{1.4} \) of the \( A. \ nodosum \) alginate shows a marked minimum in solubility at intermediate pH-values in the presence of 1 N potassium chloride (Fig. 2). A similar minimum, but less pronounced, was observed both for the “M-blocks” and the fragments with intermediate composition. A comparison of the curves giving the solubility of the “M-blocks” in 0.02 N and 1 N potassium chloride, shows a marked salting-in of this alginate component at low pH with potassium chloride. Further studies in this field are in progress, and these results will not be discussed in the present paper.

Fig. 3 gives the precipitation curves for the three types of alginate fragments in the presence of calcium and sodium ions. In this case, the fraction with intermediate composition was precipitated at higher calcium ion concentrations than the guluronic acid rich fraction and at lower calcium concentrations than the mannuronic acid rich fraction. For this type of precipitation, the ratio between mannuronic and guluronic acid seems to determine the precipitation behaviour, while for the solubility in acidic media, the sequence of the two monomers along the polymer chain is of prime importance. This is in agreement with previous observations on the ion-exchange properties of alginites, where no difference has been found between alginites from \( A. \ nodosum \) and \( L. \ digitata \) of the same composition.

Considering the properties of alginites from different species, it should be expected (Table 2) that the properties of \( A. \ nodosum \) alginate would be characterized by the presence of a large proportion of fragments with alternating structure or short blocks, and very small amounts of “G-blocks”. The properties of \( L. \ hyperborea \) stipes alginate should be mainly determined by the “G-blocks”, while alginate from \( L. \ digitata \) should be in an intermediate position. This seems to be in good agreement with what is known about properties like solubility in acidic solutions and in the presence of divalent ions.

It is well established that alginate samples are chemically heterogeneous, \( i.e. \) they may be separated into fractions with different chemical compositions by precipitation methods. In the present paper a new type of chemical heterogeneity has been reported; the separation of \( A. \ nodosum \) alginate into fractions with different amounts of homopolymer blocks. Acid precipitation

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curves and electrophoretic examination of the products formed in the first stages of the acid hydrolysis have yielded strong evidence for "G-blocks" and "M-blocks" being in the same molecules. The results given in the present paper indicate that the fragments with a predominantly alternating structure also are present in the same molecules as the "M" and "G-blocks". The high solubility (in acidic solutions) of the S<sub>1,4</sub> fraction of <i>A. nodosum</i> alginate, containing a significant amount of blocks, indicates that molecules containing mainly alternating mannuronic-guluronic acid residues would be completely soluble, even with a high molecular weight. The fact that alginites and alginate fractions (I<sub>1,4</sub>) contain considerable amounts of fragments with predominantly alternating structure without containing a correspondingly large acid soluble fraction before degradation, strongly supports the assumption that considerable amounts of homopolymer blocks are present in all alginate molecules. The chemical heterogeneity observed may, therefore, be assumed only to be due to varying proportion of the different building elements in the molecules.

REFERENCES


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