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# Alkaline Degradation of Alginate

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The rate of depolymerization of alginate at pH values above 11 was found to depend on the ionic strength, the nature of the cations present, and the concentration of hydroxyl ions and other basic anions like the carbonate and the phosphate anions. The results indicate that the depolymerization is a general base catalysed reaction.

The correlation between random depolymerization, measured by the viscosity decrease, and the formation of unsaturated derivatives, measured by the thiobarbituric acid (TBA) assay, is independent of the reaction rate, provided the pH is 11 or above. It is concluded that the viscosity decrease and the colour in the TBA assay must be caused by the same reaction; a β-alkoxy-elimination reaction.

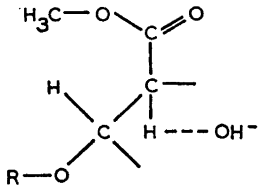
The rate of this reaction for alginic acid methyl ester is $10^4-10^5$ times that of the unesterified alginate.

It is well known that pectin is rapidly degraded in alkaline solutions.[1,2] The mechanism responsible for this degradation is an alkali-catalysed β-alkoxy-elimination, which leads to the formation of unsaturated uronic acid derivatives. A similar rapid degradation has been observed for alginic acid methyl ester.[1]

Whether this elimination reaction also takes place when the uronic acid groups are not esterified is less clear.[3] However, in a recent review,[4] the β-elimination reaction is described as also taking place with the uronic acid residue in its ionized form. The present authors [5] have reported on a study of the alkaline degradation of alginate where the decrease of viscosity was compared with the formation of a derivative giving colour in the thiobarbituric acid (TBA) assay.[6] Assuming this derivative to be the unsaturated compound formed by the β-elimination reaction, it was concluded that β-elimination also took place with the unesterified alginate. The reaction was compared with the enzymic degradation of alginate, which is an elimination reaction giving rise to the same unsaturated derivatives.[7] The rate of the degradation increased rapidly when the pH increased above 10; judging from the correlation between the viscosity decrease and the TBA assay, the elimination was the main degradation reaction taking place. When the pH decreased, the rate of the elimination reaction decreased rapidly, and other slow degradation

reactions, not giving rise to the unsaturated derivatives, played an increasingly significant role.

The first step in the β-elimination reaction is supposed to be the nucleophilic attack of the hydroxyl ion on the hydrogen atom attached to C-5 in the uronic acid residue (I). The electron-attracting effect of the carbonyl group at C-6 is considered essential for the removal of the hydrogen atom at C-5. When the carbonyl group is replaced by an ionized carboxyl group, as in alginate (II), the group at C-6 is no longer electronattracting, hence removal of the C-5 hydrogen atom by nucleophilic attack is not facilitated.



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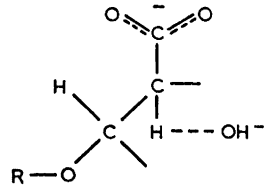

II

A further study of the alkaline degradation of alginate was therefore indicated in order to establish: 1) whether an elimination reaction really takes place when the carboxyl group is not esterified; 2) eventually, to study the influence of different conditions on the rate of the reaction; 3) and to compare the rates of degradation of alginate and alginic acid methyl ester.

## RESULTS

1. *Rate of alkaline degradation of alginate determined by viscosity measurements.* The rate of alkaline degradation of alginate was followed by boiling alginate solutions (0.5 %) in glycine buffers (0.025 M) of varying pH. Samples were removed at intervals for viscosity determinations. The viscosity decrease was expressed as

$$\Delta \frac{1}{[\eta]} = \frac{1}{[\eta]_t} - \frac{1}{[\eta]_0}$$

which gave straight lines when plotted against degradation time. The rate constants, expressed as $\Delta(1/[\eta])$ per hour, are given in Fig. 1 as a function of the pH for two different salt concentrations.

Above pH 10 the rate of degradation increased rapidly with increasing pH; it became approximately proportional to the hydroxyl ion concentration above pH 11. The rate was higher in the solutions containing 1.0 N sodium chloride when pH was above 9. Below this pH the rate was approximately independent of the pH and also was lower in the solution with the higher sodium chloride concentrations. The degradation below pH 10 was supposed to be due, to a large extent, to the oxidative-reductive degradation investigated previously.[8] In the following, only the degradation taking place at pH 10 or above, has been investigated.

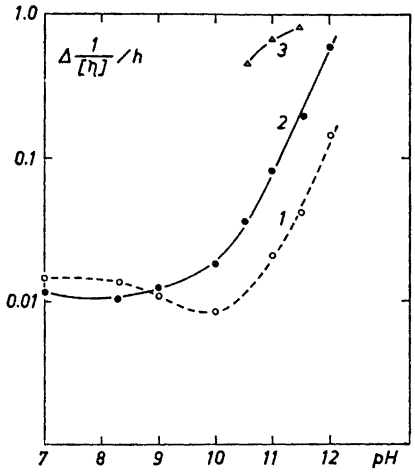


*Fig. 1.* Correlation between pH and rate of degradation of alginate
1. Glycine buffer, 0.1 N sodium chloride.
2. » » 1.0 N » »
3. 1 N carbonate buffer.

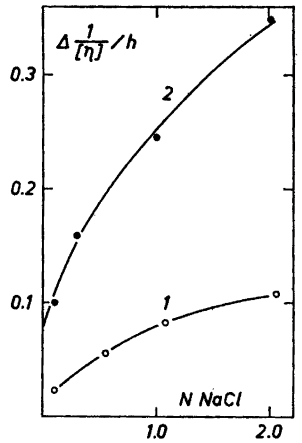


*Fig. 2.* Rate of degradation at pH 11 in the presence of varying amounts of sodium chloride
1. Glycine buffer. 2. 0.05 N sodium carbonate.

The influence of ionic strength on the degradation rate was further examined by using glycine buffer and 0.05 N sodium carbonate, both adjusted to pH 11.0. In Fig. 2 the degradation rates are given as a function of the sodium chloride concentration. For both buffers an increasing ionic strength leads to an increasing rate of degradation. The degradation rate is, however, always much higher in the sodium carbonate solution than in the glycine buffer.

The influence of the concentration of the carbonate buffer on the degradation rate was determined; the results are given in Fig. 3. The pH of the solution was in all cases adjusted to 11.0. The degradation rate varied approximately linearly with the sodium carbonate concentration, the rate being much higher than when sodium chloride was used to increase the ionic strength. The degradation rates in 1 N sodium carbonate buffers adjusted to different pH values were also determined; the rates are given in Fig. 1. The dependence of the pH of the solution was much smaller than when glycine buffer was used.

The results indicate that the carbonate anion may have a catalytic effect on the degradation reaction. If we assume that the degradation in glycine buffer at pH 12 in 1 N sodium chloride is catalysed by only the $OH^-$ anion, we have

$$r = k_{OH^-}\,[OH^-]$$

where $r$ is the rate of the degradation, $[OH^-]$ is the activity of the hydroxyl ions, and $k_{OH^-}$ is a constant which includes the constant alginate concentration. In the same way, we can express the rate of degradation in sodium carbonate solution at pH 11:

$$r = k_{OH^-}\,[OH^-] + k_{CO_3^{2-}}\,[CO_3^{2-}]$$

where $[CO_3^{2-}]$ is the concentration of the carbonate anion. Expressing the rates as $\Delta(1/[\eta])$ per hour and by using the results given in Fig. 1 for calculation of $k_{OH^-}$ and in Fig. 3 for calculation of $k_{CO_3^{2-}}$, we find the numerical values of 60 and 1.6 for the two constants, respectively. Using these constants, the rates of degradation which should be expected in 1 M sodium carbonate solution at pH 10.55, 11 and 11.45 can be calculated and compared to the results given in Fig. 1. The result is given in Table 1. It should be noted that a pos-

*Table 1.* Rate of degradation in carbonate buffers.

| pH | $k_{OH^-}[OH^-]$ | $k_{CO_3^{2-}}[CO_3^{2-}]$ | $r_{calc}$ | $r_{found}$ |
|---|---|---|---|---|
| 10.55 | 0.02 | 0.47 | 0.49 | 0.46 |
| 11.0 | 0.06 | 0.615 | 0.675 | 0.68 |
| 11.45 | 0.17 | 0.695 | 0.865 | 0.83 |

sible catalytic effect of the $HCO_3^-$ anion and of glycine is neglected; the difference in ionic strength of glycine buffer containing 1 N sodium chloride and a 1 N sodium carbonate buffer is not taken into account. The reasonably good agreement between the observed and calculated values indicates that the carbonate anion is a catalyst for the reaction.

The effect of a number of other substances on the degradation rate was investigated and the results are given in Table 2. In all cases the pH was

*Table 2.* Rate of degradation of alginate at pH 11 in the presence of 1 M NaCl and 0.2 M of various salts and organic bases.

| | $\Delta(1/[\eta])$/h |
|---|---|
| 0.2 M $K_3PO_4/K_2HPO_4$ | 1.07 |
| 0.2 » $Na_2CO_3/NaHCO_3$ | 0.65 |
| 0.2 » Glycine | 0.128 |
| 0.2 » Sodium acetate + 0.025 M glycine | 0.089 |
| 0.2 » Sodium citrate + 0.025 M glycine | 0.095 |
| 0.2 » Sodium thiosulfate + 0.025 M glycine | 0.081 |
| 0.2 » Potassium thiocyanate + 0.025 M glycine | 0.077 |
| 0.2 » Triethylamine | 0.021 |
| 0.2 » Methylamine | 0.035 |
| 0.025 M Glycine | 0.08 |

adjusted to 11. In the case where the substances tested had no buffer capacity at this pH, a glycine buffer was used in addition. Sodium chloride was added at a concentration of 1.0 N.

The only two substances showing a catalytic effect were sodium carbonate and potassium phosphate. The results also indicate an effect of glycine, as a higher glycine concentration led to an increase in the degradation rate.

The effect of different cations was also investigated. The different cations are known to form ion pairs with the alginate anion to different extent.[9] As discussed later, the formation of an ion pair might facilitate an elimination

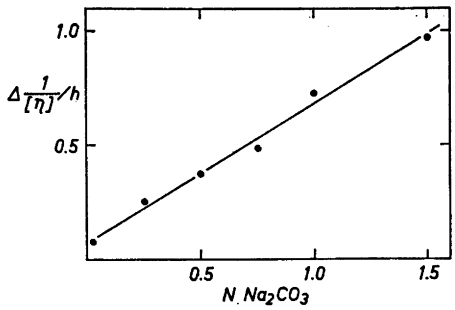


*Fig. 3.* Rate of degradation in sodium carbonate of varying strengths, adjusted to pH 11.

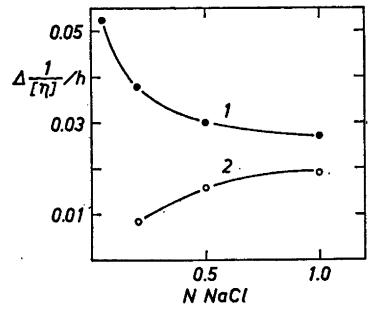


*Fig. 4.* Rate of degradation in glycine buffer at pH 10 in the presence of varying amounts of sodium chloride.
1. In 0.1 N magnesium chloride. 2. Without magnesium chloride.

reaction. The rates of degradation in glycine buffer, at pH 10, and containing 0.1 N magnesium chloride and sodium chloride in varying amounts, were determined; the results are given in Fig. 4. For comparison, the rates of degradation without magnesium chloride are also given in the figure. The rate of degradation is markedly increased in the presence of magnesium chloride. The addition of sodium chloride leads to a decrease of the rate, contrary to the case where no magnesium is present. Magnesium is the only divalent metal giving soluble salts with alginate, hence degradation in the presence of calcium ions had to be carried out as a heterogeneous reaction. Two experiments were performed, both with glycine buffer, pH 10, in the presence of 0.1 N calcium chloride, one without sodium chloride, the other in the presence of 1.0 N sodium chloride. The rates were 0.145 and 0.075, respectively, thus showing a marked increase in degradation rate compared to the magnesium chloride solution. As was the case in the presence of magnesium, the addition of sodium chloride decreased the rate.

2. *Correlation between viscosity decrease and colour with thiobarbituric acid.* The enzymic degradation of alginate is assumed to proceed completely as an elimination process. By correlating the colour formed in the TBA assay and the increase in reducing power, it is possible to calibrate the TBA assay. Fig. 5 gives the optical density in the TBA assay as a function of the reducing power of the solution. The slope of the curve corresponds to an optical density of 0.24 for 0.01 μmole endgroups formed. This is in fair agreement with Preiss and Ashwell,[7] who report an optical density of 0.29 for 0.01 μmole unsaturated derivative.

Fig. 6 gives the correlation between the number of bonds broken, as calculated from the TBA assay by using the results given above and the decrease of viscosity by enzymic degradation. The viscosity decrease is expressed as the increase in the inverse value of the intrinsic viscosity ($\Delta(1/[\eta])$).

Writing the Staudinger equation as $P_n = 1/\lambda \times K[\eta]$, where $P_n$ is the number average degree of polymerization, $\lambda$ the ratio between the weight

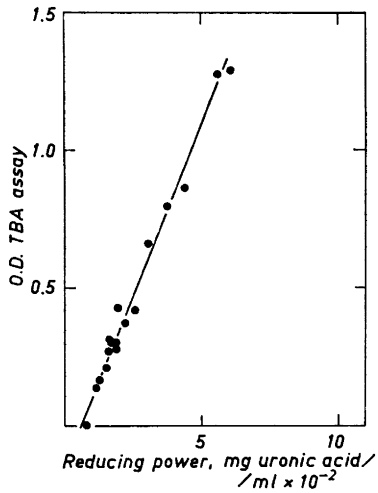


*Fig. 5.* Correlation between reducing power and TBA assay by enzymic degradation.

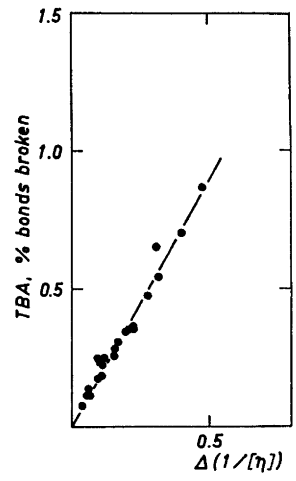


*Fig. 6.* Correlation between viscosity decrease and bonds broken (TBA) by enzymic degradation.

average and the number average molecular weight, and $K$ a constant, and, assuming the exponent in the Staudinger equation to be unity,[10] we can write:

$$1/P_t - 1/P_0 = \lambda/K[\Delta(1/[\eta])]$$

The expression $1/P_t - 1/P_0$ corresponds to the number of bonds broken after the time $t$, as part of the total number of bonds between the monomeric units. This formula allows the calculation of $K/\lambda$ from results of the type given in Fig. 6. From the enzymic degradation the value $K/\lambda = 56$ was calculated.

Due to the rapid destruction of the reducing end-groups in alkaline medium, the correlation between reducing power and TBA assay during alkaline degradation is not easily established. The correlation between the TBA assay and the viscosity decrease was, however, determined for alkaline degradation under different conditions. Fig. 7a gives the results for degradation experiments in glycine buffers at pH 11 and 12 and also for sodium carbonate at pH 11. The rate of degradation in these experiments varied by a factor of 30, but the correlation was the same for all experiments. In order to establish that the TBA reaction was not caused by products formed by degradation from the reducing end of the polymer, the correlation between viscosity decrease and TBA assay was determined for alkaline degradation in the presence of sodium borohydride. The results are given in Fig. 7b and show that the correlation is not influenced by the presence of borohydride. Finally, the correlation was also determined for the degradation in the presence of magnesium and calcium ions. Again, the correlation was the same as in the previous experiments (Fig. 7c).

The correlation between the TBA assay and the viscosity decrease by alkaline degradation, however, is significantly different from that obtained

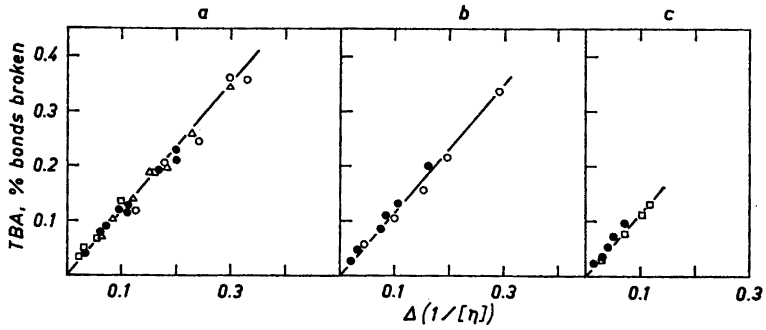


*Fig. 7.* Correlation between viscosity decrease and bonds broken (TBA) by alkaline degradation.

a. □ Glycine buffer, 0.1 N sodium chloride, pH 11, *k*: 0.02.
● Glycine buffer, 1.0 N sodium chloride, pH 11, *k*: 0.08.
○ Glycine buffer, 1.0 N sodium chloride, pH 12, *k*: 0.6.
△ 1.0 N sodium carbonate, pH 11, k:0.66.
b. ● 1 N sodium carbonate, pH 11, 1 % borohydride.
○ Glycine buffer, pH 11, 1 M sodium chloride, 0.2 % borohydride.
c. ● Glycine buffer, 0.1 N magnesium chloride, pH 10.
○ Glycine buffer, 0.1 N calcium chloride, pH 10.

by enzymic degradation, the value of $K/\lambda$ calculated from the alkaline degradation experiments being 85. The same figure was found in experiments where the degradation was carried out at pH 4.0, the increase in reducing power and the decrease in viscosity being correlated.[10]

3. *Rate of degradation of alginic acid methyl ester.* The determination of the rate of degradation of an alginic acid ester is complicated by the simultaneous de-esterification taking place in alkaline medium. The methyl ester preparation used in this work had an intrinsic viscosity too low to allow determination of the degradation rate by viscosity measurements; the rate was, therefore,

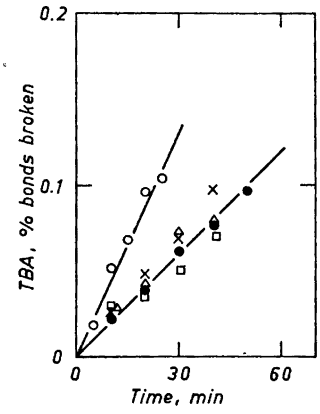


*Fig. 8.* Degradation of alginic acid methyl ester at 20°.
● Glycine buffer, 0.1 N sodium chloride, pH 10.
× Glycine buffer, 1.0 N sodium chloride, pH 10.
□ Glycine buffer, 0.1 N magnesium chloride, pH 10.
○ 0.5 M sodium carbonate buffer, pH 10.

determined only by means of the TBA assay. The degradation was carried out in glycine buffer at pH 10. The rates of both degradation and de-esterification were very high at this pH and the experiments were, therefore, carried out at 20°. An approximately linear rate of degradation was observed for the first 30 to 60 min, as shown in Fig. 8. The effect of addition of sodium chloride, magnesium chloride, and calcium chloride on the degradation rate was determined and found to be small compared with the effect on the rate of degradation of alginate. An experiment in carbonate buffer adjusted to pH 10 was also included, in this case a higher rate being observed.

The degradation rates of alginate and alginic acid methyl ester were compared by using the activation energy of alginate degradation in a carbonate buffer at pH 9.8, which was previously determined [11] to .27 kcal/mole. The ratio between the degradation rate of the methyl ester and the free alginate in glycine buffer, pH 10, was found to vary between $2 \times 10^5$, in the presence of 0.1 N sodium chloride, and $1.5 \times 10^4$, in the presence of 0.1 N calcium chloride.

4. *Comparison between the UV spectra of the products formed by enzymic and alkaline degradation.* The unsaturated uronic acid derivative formed by the enzymic degradation of alginate has an ultraviolet absorption with a sharp maximum at 235 mμ.[7] This was observed by determining at intervals the ultraviolet spectrum of an alginate solution being degraded by extracellular alginase. The spectra are given in Fig. 9.

For comparison, an alginate solution was degraded at 100° in 1 M potassium phosphate, adjusted to pH 11, and the ultraviolet spectrum of the solution determined at intervals. In order to avoid interference from small amounts of carbonate, strong hydrochloric acid was added to the solutions until a pH of approximately 2 was obtained. It has previously been shown [7] that the spectrum of the unsaturated derivative formed by enzymic degradation is not appreciably changed by decreasing the pH to 2.

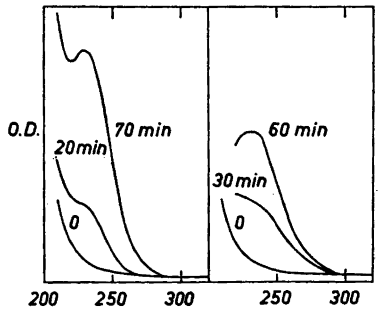


*Fig. 9.* Change in UV spectrum during degradation of alginate.
a) Enzymic degradation. b) Alkaline degradation.

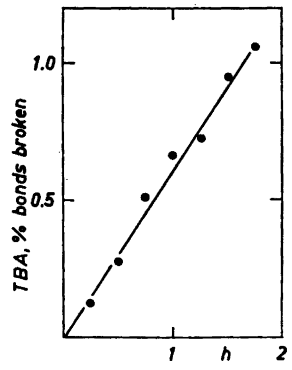


*Fig. 10.* Number of bonds broken as a function of degradation time. 0.5 M phosphate buffer, pH 11.

The spectrum of the compound formed in the TBA reaction was also determined both for enzymic and alkaline degradation of alginate. In both cases the absorption maximum was at 549 mμ and the two spectra were indistinguishable.

## DISCUSSION

In our previous publication [5] we reported that, for a given viscosity decrease, the colour in the TBA assay depended on the pH during the degradation. This was attributed to the fact that the rate of the alkaline degradation decreased with decreasing pH, while other degradation reactions, less dependent on the pH, became responsible for a relatively larger proportion of the degradation. Most important among these reactions is probably the oxidative-reductive degradation caused by the presence of small amounts of phenolic substances which are usually present in alginate preparations.[8,12] We have previously shown [12] that this reaction does not lead to the formation of derivatives which give a colour reaction with TBA.

A significant fact emerging from the present investigation is that the correlation between the colour in the TBA assay and the viscosity decrease is independent of the experimental conditions, provided the pH is 11 or above, even if the absolute rates of the degradation varied by a factor of 30 (Fig. 7a). If two different reactions were the cause of the viscosity decrease, *i.e.* the random depolymerization and a different reaction giving colour in the TBA assay, the rate of the two reactions should vary proportionally to each other when the conditions were changed. The wellknown degradation of the polysaccharides from the reducing end sometimes gives rise to products giving a colour reaction with TBA.[13] The fact that the correlation between TBA colour and viscosity decrease is not influenced by the presence of sodium borohydride during the degradation (Fig. 7b) shows, however, that reactions at the reducing end of the polymer chain play no significant part in the reactions leading to the formation of products reacting in the TBA assay in the present case. It is thus strongly indicated that the decrease of viscosity and the formation of the derivative reacting with TBA is due to the same chemical reaction. The only known reaction which leads at the same time to a random depolymerisation and the formation of a derivative giving colour in the TBA assay is the β-alkoxy-elimination reaction. That this actually is the reaction responsible for the alkaline degradation of alginate is further supported by the absorption maximum at 230 mμ of alkaline degraded alginate solutions; this indicates the formation of unsaturated derivatives of the type well-known from enzymic degradation (Fig. 9).

We have previously found that the enzymic degradation gave a higher optical density in the TBA assay than alkaline degradation for the same viscosity decrease. This applies to all pH values tested. This was confirmed by the results reported in this investigation. We assumed previously that this indicated that the alkaline degradation only partly proceeded as a β-elimination reaction even at pH 11 and that other types of scission of the polymeric chain also took place. While this is certainly the case at pH values below 10, the results in this work indicate that this is not the case at pH values

of 11 or higher. The fact that the correlation between TBA colour and viscosity decrease was independent of the conditions at pH 11, and above, makes it improbable that two different reactions are responsible for the alkaline degradation at these pH values. This is further supported by finding the same value of the factor $K/\lambda$ in the Staudinger equation by alkaline degradation as found by acid degradation.[10] The value of $K/\lambda$ also agrees reasonably well with the value of $K$ found by light scattering measurements.[10] A possible explanation of the different value of $K/\lambda$ by enzymic degradation is a higher value of $\lambda$ due to a lack of randomness in the enzymic degradation.

Alginate is a highly charged polymer, a property which should be expected to determine the conditions which lead to a high rate of the alkali-catalysed $\beta$-alkoxy-elimination reaction. The negative charge of the ionized alginate molecule will make the concentration of negatively charged hydroxyl ions in the proximity of the polymer lower than in the bulk of the solution. This difference should decrease with increasing ionic strength of the solution according to the Debye-Hückel relationship and the Donnan equilibrium. This is in accordance with the marked increase in the rate of the degradation with increasing sodium chloride concentration (Fig. 2).

On a highly charged polymer the cations are supposed to form ion pairs with the negatively charged groups. Measurements carried out by studying osmotic and Donnan equilibria,[9] and by means of potentiometric measurements,[14] indicated that 60—70 % of monovalent cations (like potassium and sodium) form ion pairs with the carboxyl groups in alginate. The corresponding figures for magnesium were found to be 85 % and for calcium and copper over 95 %. The formation of ion pairs may affect the rate of elimination reaction in two ways, both leading to an increase in the rate: Firstly, ion pair formation leads to a decrease in the effective charge of the polymer and, thus, to an increase in the concentration of negative ions close to the polymer chain. Secondly, the effect of a closely attached cation to the negatively charged carboxyl group might be to counteract the electron-donating character of the carboxyl group. Whether the magnitude of this effect is of an order that may have a significant influence on the availability of the C-5 hydrogen atom to nucleophilic attack is not known.

The results reported above show that the rate of degradation increases when cations with increasing tendency for ion pair formation are present (Fig. 4). The fact that addition of sodium chloride decreases the degradation rate when calcium and magnesium ions are present indicates that these ions only are active in increasing the degradation rate when bound to the alginate molecules. Due to the ion exchange equilibrium, the amount of divalent metals bound to the alginate decreases with increasing sodium chloride concentration. Thus, the results clearly indicate that ion pair formation increases the rate of the $\beta$-alkoxy-elimination reaction, but does not allow us to distinguish between the two possible causes of this effect.

Hydroxyl ions are not the only catalyst for the $\beta$-elimination reaction in alginate. Other anions, like the carbonate ions and the phosphate ions, also have a catalytic effect, and the reaction may be described as a general base catalysed reaction. Also the glycine anion seems to have a catalytic effect, while weekly basic anions, like acetate and citrate, are not very active. The

same applies to the two amines tested and to other nucleophilic reagents like thiosulfate and thiocyanate.

As discussed in the introduction, the effect of a carbonyl group and a dissociated carboxyl group at C-6 on the availability of the C-5 hydrogen to nucleophilic attack should be very different. In agreement with this, the rate of β-elimination reaction for alginic acid methyl ester is $10^4$—$10^5$ higher than that of unesterified alginate.

A similar difference was observed by Gadamer [17] for the alkali-catalysed racemisation of tropic acid. Rotation measurements demonstrated a rapid racemisation of the ethyl ester at room temperature, while no reaction could be detected for the tropic acid itself. However, it is well known that this reaction proceeds under more forcing conditions.

As should be expected, the rate of the depolymerization of the neutral alginic acid methyl ester is affected very little by changes in the ionic strength or by the presence of magnesium or calcium ions. The presence of carbonate ions, however, also increases the rate of degradation in this case (Fig. 8).

The possibility that the β-alkoxy-elimination reaction in the alginate is due to small amounts of ester groups remains to be discussed. The very great difference in the depolymerization rate of the esterified and the unesterified polyuronide shows that, if this was the case, the amount of ester groups must be very low — of the order of 0.01 % of the monomeric units. Further degradation of the reaction products in the alkaline solution makes it difficult to follow the alkaline depolymerization to a point where more than about 1 % of the bonds are broken. Within this range, however, a complete linearity between the number of bonds broken and the time of degradation is observed (Fig. 10). This seems to exclude the possibility of the observed β-elimination in alginate being caused by small amounts of ester groups.

Even if the rate of the β-alkoxy-elimination reaction is very much lower for alginate than for the methyl ester, the rate is sufficiently high to be of considerably practical interest. In a solution containing 1 M potassium phosphate adjusted to pH 12 the rate of degradation at 100° corresponds to a breaking of about 5 % of the bonds in one hour; this again corresponds to 40 % of the rate of depolymerization in 0.1 N hydrochloric acid at the same temperature.

## EXPERIMENTAL

For all the experiments in this work alginate prepared from *Laminaria digitata*, Tarva 29/8, was used. The method of preparation has been described elsewhere.[11] The uronic acid composition of the alginic acid was 61 % mannuronic and 49 % guluronic acid. The intrinsic viscosity was 12 dl/g. Alginic acid methyl ester was prepared by treating a suspension of alginic acid in dry ether with diazomethan for 70 h at 0°. The degree of esterification was found by titration to be > 95 %.

The degradation experiments were carried out by mixing a 1 % alginate solution with a buffer solution of the desired strength and composition. The pH was adjusted, when necessary, by adding 2 N HCl or NaOH using a Radiometer pH meter 4, equipped with a glass electrode with a small salt error (G 200 B). The pH was adjusted within ± 0.02 unit. The mixture was then boiled under reflux and a stream of nitrogen passed through the solution. Samples were removed at intervals for analyses.

In the presence of calcium ions the following procedure was followed: Alginate solution (1 %) was mixed in a test-tube with an equal volume of a glycine buffer adjusted to

pH 10, and containing 0.1 M calcium chloride. A gel was formed and the test-tube, with a glass marble on top, was placed in a boiling water bath. One test-tube was used for each time of degradation. After being treated for the required time, the test-tube was cooled, the gel dissolved by addition of ethylenediaminetetraacetic acid and sodium carbonate, and the sample analysed.

The degradation rate of alginic acid methyl ester was determined by mixing a 1 % solution of the ester and buffer solution. After adjustment of the pH, the solutions were kept at 20° in a water bath, occasionally samples being removed for analysis.

The viscosity was determined by means of an Ubbelohde viscometer or a pipette viscometer. The intrinsic viscosity was found by empirical curves of the type described previously.[15] The thiobarbituric acid (TBA) assay was carried out according to Weissbach and Hurwitz,[6] the reducing power being determined by means of the Nelson method.[16]

The spectra were determined by using a Beckman DB spectrophotometer.

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