

## Dissociation of Alginic Acid

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The dissociation of alginic acid has been studied by a number of authors<sup>1-5</sup>. All these investigations were carried out on the assumption that alginic acid is a homogeneous and well-defined compound. Recently, however, it has been shown that alginates are chemically heterogeneous compounds<sup>6,7</sup>, and that alginates from different sources may vary considerably in chemical composition<sup>8,9</sup>. It has also been shown that ion exchange properties of alginates depend on the proportion of D-mannuronic and L-guluronic acid present in the alginate. The present note reports an investigation on the correlation between acid strength and uronic acid composition of alginates.

**Materials and methods.** The sampling of seaweed and preparation of alginate and alginate fractions have been described earlier<sup>7,8</sup>. Alginic acid was prepared by shaking sodium alginate with a large volume of 0.1 N hydrochloric acid over-night. The alginic acid was washed thoroughly several times with distilled water and dried *in vacuo*.

As alginic acid is insoluble in water, part of the titration curve must be determined for a two-phase system. To obtain equilibrium, it was found necessary to shake the alginate suspension for at least one hour after the addition of alkali. The following procedure was therefore adopted: Small samples (25 mg) of alginic acid were suspended in water or sodium chloride solution and the chosen amount of 0.1 N sodium hydroxide solution added. When the measurements were carried out in the presence of sodium chloride, the amounts of sodium was kept constant throughout the titration curve (mequiv. NaCl + mequiv. NaOH = const.). The volume was chosen so that the alginate concentration was 0.025 N. The suspension was shaken over-night and the pH was determined by a Beckman Zeromatic pH-meter. As a standard buffer, a 0.05 M potassium-hydrogenphthalate solution was used. When measurements were carried out in sodium chloride solution, salt was added to the buffer solution to obtain the same strength as in the test solution.

The pH of half neutralized alginic acid was determined in the following way: Alginic acid was suspended in water, and sodium hydroxide

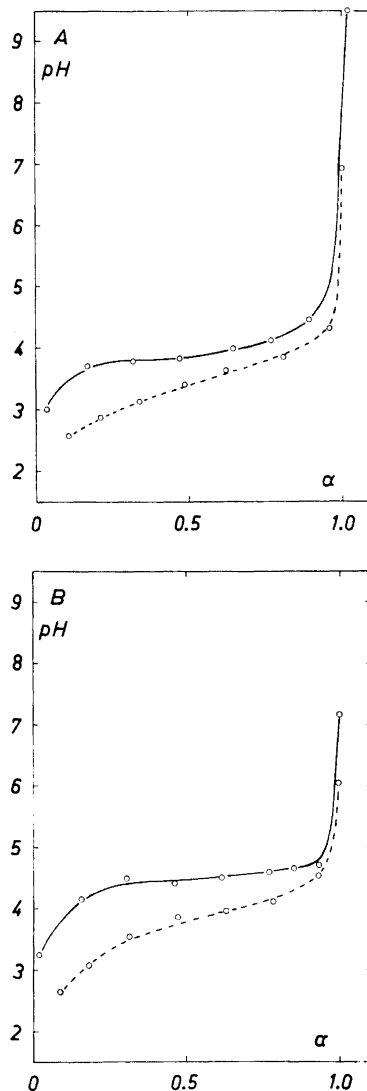


Fig. 1. A) *L. digitata*, Kråkvågøy, 3/7; ---- water and ——— 0.1 N NaCl. B) *L. hyperborea* stipes, Hustad, 12/2; ---- water and ---- 0.1 N NaCl.

added in slight excess. By titrating with 0.1 N hydrochloric acid the amount of alkali consumed by the alginic acid was determined. To another sample of alginic acid half the determined amount of alkali was added, and the pH was measured after equilibrium was obtained.

Table 1.

Source of alginate	Medium	$n$	$pK_s'$
<i>L. hyperborea</i> , stipes, Hustad, 26/2	Water	0.40	4.36
» » » » 12/2	»	0.34	4.41
» » » » 12/2	(NaCl + NaOH) = 0.1 N	0.87	3.74
<i>L. digitata</i> , Kråkvågøy, 3/7	Water	0.44	3.93
» » » » 3/7	(NaCl + NaOH) = 0.1 N	0.92	3.42
» » Espevær, 4/7	Water	0.70	3.87
» » » » 4/7	(NaCl + NaOH) = 0.025 N	0.98	3.70
» » » » 4/7	(NaCl + NaOH) = 0.1 N	0.90	3.42
» » » » 8/1	Water	0.60	3.94
» » » » 8/1, $K_1Mn_8$	»	»	3.75*
» » » » 8/1, $K_8Mn_1$	»	»	4.00*
» » Tarva, 3/7	»	»	3.84*
» » » » 3/7, $K_1Mn_8$	»	»	3.72*
» » » » 3/7, $K_8Mn_1$	»	»	4.10*

Concentration of alginate in all cases was 0.025 N.

\* pH for half neutralized samples.

Fig. 1 shows typical titration curves of alginic acid from *Laminaria digitata* and *Laminaria hyperborea* stipes, in water and in 0.1 N sodium chloride solution. If the results are calculated according to the modified Henderson equation  $pH = pK_s' + n \log \frac{a}{1-a}$ , the values given in Table 1 are obtained for the constants  $pK_s'$  and  $n$ . The degree of neutralization  $a$  is corrected for the presence of free hydrogen ions. The table also shows the pH of half neutralized samples of alginate fractions prepared by precipitation with potassium chloride and manganous sulphate<sup>9</sup>. If necessary, the observed pH has been corrected for the presence of free hydrogen ions to give the  $pK_s'$  value.

Both  $n$  and  $pK_s'$  vary with varying amounts of sodium chloride in the solution. With increasing ionic strength  $n$  increases towards 1, while the normal behaviour for polyacids is that  $n$  is higher than 1 at low ionic strength and decreases towards 1 when the ionic strength is increased. The abnormal behaviour may, at least partly, be due to the fact that part of the titration is carried out in a two-phase system, where the Donnan equilibrium will cause a considerable difference between the pH in the external solution and the pH in the gel, particularly when the ionic strength is low. As normal for polyacids the  $pK_s'$  decreases with increasing ionic strength. The con-

centration of alginic acid markedly influenced the value of the  $pK_s'$  of alginic acid in water. When the alginate concentration was varied from 0.06 to 0.006 N the pH of half neutralized samples increased by approximately 0.4 units, while no significant variation with dilution was observed when the medium was 0.1 N sodium chloride solution.

Alginate from *L. hyperborea* stipes contains more guluronic than mannuronic acid<sup>9</sup>, while the opposite is the case for alginate from *L. digitata*. The fraction which is soluble in potassium chloride solution and precipitated by manganous ions ( $K_1Mn_8$ ) is enriched in guluronic acid residues, while the opposite fraction ( $K_8Mn_1$ ) is enriched in mannuronic acid residues. The results in Table 1 thus clearly indicate that there exists a correlation between the  $pK_s'$  values and the uronic acid composition of alginates, so that alginates rich in guluronic acid residues have a higher  $pK_s'$  value than alginates containing more mannuronic acid.

Samples of mannuronic and guluronic lactones were prepared from hydrolysates of alginic acid according to the method of Fischer and Dörfel<sup>8</sup>. The lactones were transformed to salts by adding alkali, and the acids liberated by running the solutions through a column of Dowex 50 ion exchange resin. The uronic acid solutions were immediately titrated with 0.1 N

sodium hydroxide solution, and the  $pK_s$  values of 3.38 and 3.65 were found for mannuronic and guluronic acid, respectively. The differences observed in the  $pK_s'$  values for polymers with different uronic acid composition are thus in good agreement with the  $pK_s$  values found for the monomeric units.

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## Experimental Determination of Br $f$ Values by Electron Diffraction

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A study of the electron diffraction pattern of the  $\text{Br}_2$  molecule was carried out in conjunction with a Fulbright research grant to Norway. From data taken on the Oslo diffraction unit, it had appeared that there existed an anomaly in the intensity of the electron scattering from bromine compounds. For the compounds ordinarily studied, usually hydrocarbons and chlorine substituted hydrocarbons, it was always possible to bring the observed atomic scattering into almost exact coincidence with that calculated theoretically<sup>1</sup>. This could not be done for bromine containing com-

pounds. Since the  $\text{Br}_2$  molecule is the simplest of bromine compounds, and since it also exhibits the apparent anomaly, the diffraction pattern from this molecule was studied in detail.

Except for constants, the total intensity observed in electron scattering by a diatomic molecule is given by

$$I \propto \frac{1}{s^4} \left[ g^2(s) + ZS + g^2(s)e^{-ds^2} \frac{\sin sr}{sr} \right]$$

where the first two terms in brackets constitute the atomic scattering, and the third term is the molecular effect. The term  $ZS$  is the incoherent atomic scattering which is usually relatively small;  $s$  is equal to  $(4\pi/\lambda) \sin \Theta/2$ ;  $d$  is the damping factor; and  $g(s)$  is the amplitude of the coherently scattered wave. Within the limits of the Born approximation,  $g(s)$  is equal to  $(Z-f)$ , where  $f$  is the ordinary X-ray atomic form factor. The data is recorded photographically, and since a flat plate plus a rotating sector are employed, suitable correction must be made. Since the sector effectively compensates for the well known  $\csc^4 \Theta/2$  effect, the over-all variation in intensity is sufficiently small that recording the intensity photographically introduces little error.

It was established that the difficulty of calculating the atomic scattering lay mainly in the use of the bromine  $f$  values obtained from statistical atomic models. For small values of  $s$ , the intensity is highly sensitive

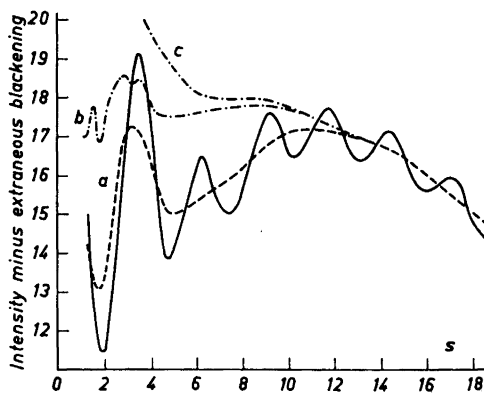


Fig. 1. Intensity in electron diffraction pattern from  $\text{Br}_2$ : (a) Observed atomic scattering; (b) predicted atomic scattering from Thomas-Fermi atom with exchange; (c) predicted atomic scattering for Thomas-Fermi atom without exchange.

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