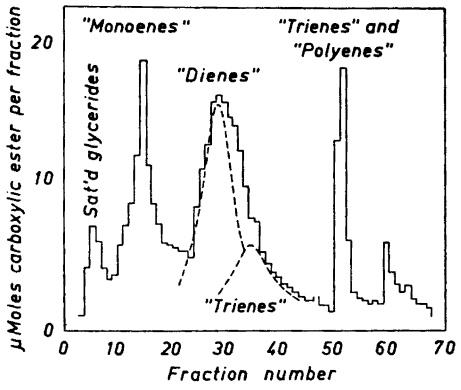

Fig. 1. Chromatography of 117 µmoles of serum triglycerides on a 6.0 g column (13 × 70 mm) of silicic acid-silver nitrate. Fraction volume: 5 ml. Recovery: 106 %. Fractions 1—40 were eluted with petroleum ether containing 0—100 % toluene. The 'polyenoic' fractions were recovered with chloroform and chloroform-methanol (20:1) (v/v).

bonds in each triglyceride molecule is constant within the 'monoenoic', 'dienoic', and 'trienoic' peaks in Fig. 1. This means that the serum triglycerides contained about 6 % saturated, 21 % 'monoenoic', 35 % 'dienoic', and 38 % 'trienoic' and 'polyenoic' glycerides.

Table 1 suggests among other things that the 'dienoic' triglycerides were mainly of 'monopalmito-diolein' type, whereas the amount of the 'dipalmito-monolinolein' type seemed to be surprisingly small. Similar analysis of the 'polyenoic' glycerides is not yet possible because of their incomplete separation. However, it is apparent that they contained rather large amounts both of fully saturated and of tetra-, penta-, and hexaenoic acids.

The monochain lipids are, of course, easier to separate than the polychain lipids. — We have thus obtained very satisfactory separations of, *e.g.* methyl-esters on the silicic acid-silver nitrate adsorbent *. — But nevertheless we think that the real value of de Vries' method lies in its application for the analysis of chain combinations in the polychain lipids.

* 'Monoenoic fatty acids of serum sphingomyelins'; Under preparation.

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1. De Vries, B. *Chem. & Ind. London* **1962** 1049.
2. Haahti, E., Nikkari, T. and Juva, K. *Acta Chem. Scand.* **17** (1963) 538.
3. Salminen, A., Renkonen, O.-V. and Renkonen, O. *Ann. Med. Exptl. Biol. Fenniae (Helsinki)* **38** (1960) 447.
4. Hallgren, B., Stenhagen, S., Svanborg, A. and Svennerholm, L. *J. Clin. Invest.* **39** (1960) 1424.
5. De Vries, B. *A lecture at the Course of Chromatographic Methods in Lipid Research*, Milan, Italy 1962.
6. Renkonen, O. *Biochim. Biophys. Acta* **54** (1961) 361.
7. Renkonen, O. *Biochim. Biophys. Acta* **56** (1962) 367.

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The Degradation of Alginates at Different pH Values

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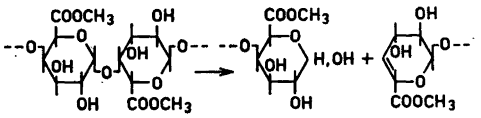
Recent investigations have shown [1-3] that pectin is rapidly degraded both in alkali and in neutral solutions and that this degradation leads to the formation of unsaturated uronic acid derivatives. The carbonyl group of the methyl ester is essential for this reaction and the degradation is regarded as a β-elimination reaction:



Consequently, the free acids are known to be more stable in alkaline solutions, and whether the same reaction mechanism occurs with a free carboxyl group in the 6-position does not seem to be definitely established [4].

Enzymic degradation of pectin [5], chondroitin sulphate [6] and alginic acid [7,8] has been shown to give rise to unsaturated compounds and the reaction is supposed to be an elimination reaction similar [9] to the reaction described above.

The α,β unsaturated uronic acid derivatives formed in the elimination reaction described above give formyl-pyruvic acid by oxidation with periodate. Formyl-pyruvic acid has been shown to give a colour reaction with thiobarbituric acid [10].

In a study of the stability of alginate solutions, the decrease of viscosity at different values of pH was determined. By using the thiobarbituric acid reaction and correlating the optical density with the decrease of viscosity, it seemed possible to determine to what extent the β-elimination mechanism was responsible for the degradation. Enzymic degradation was used for comparison. Some of the results obtained are given here.

Material and methods. Alginate prepared from *Laminaria digitata* was used throughout this investigation. The method of preparation of the alginate has been described elsewhere[11]. The thiobarbituric acid reaction was carried out according to Weissbach and Hurwitz[10]. The alginate concentration was 0.5 %, and the results of the colour reaction are given in terms of the optical density, using 0.2 ml sample of the 0.5 % alginate solution. Viscosity measurements were made using Ubbelohde viscometers, and the intrinsic viscosity calculated from empirical curves as described earlier[12]. The thermal degradation was carried out by heating buffered alginate solutions in stoppered flasks at suitable temperatures. Samples were taken at intervals for determination of viscosity and for the colour reaction. The following buffer systems were used:

pH 4—8: Mc Ilvaine buffer
pH 8—11: Carbonate buffer
pH 12.7—13: Sodium hydroxide.

The ionic strength was adjusted to 0.2 by addition of sodium chloride. Enzymic degradation was carried out as described elsewhere[14].

When the degree of polymerization is above 10, it has been shown [13] that for a first order reaction

$$kt = \frac{1}{DP_t} - \frac{1}{DP_0}$$

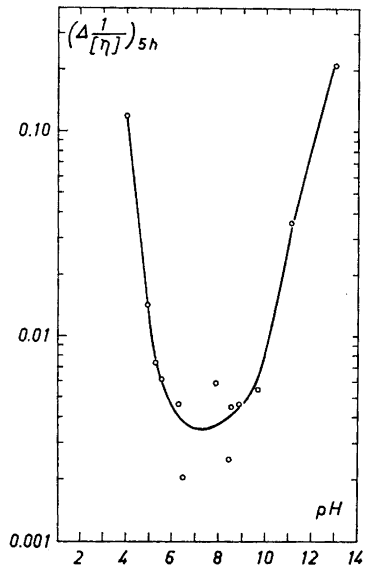


Fig. 1. Rate of degradation at different values of pH. Alginate prepared from *L. digitata*, Tarva, 29/8. $[\eta]_0 = 30.0$. Heated 5 h at 68°C.

If a in the modified Staudinger equation is 1, then 1/DP is proportional to $1/[\eta]$ and we have

$$k't = \frac{1}{[\eta]_t} - \frac{1}{[\eta]_0} = \Delta\frac{1}{[\eta]}$$

Thus, for a first order reaction $\Delta(1/[\eta])$ should be proportional to the number of bonds broken in the chain molecule.

Plots of $\Delta(1/[\eta])$ *versus* time of degradation was found to be linear in the region investigated ($[\eta] > 3$ dl/g). The degradation by heating for 5 h at 68° at different values of pH is shown in Fig. 1. As expected, the rate of degradation is strongly dependent on the pH. Degradation is very slow in the region between pH 5 and 10. This renders reliable estimate of the degradation rate difficult, particularly as traces of impurities have been shown to influence the degradation rate (see subsequent note, p. 1473).

The correlation between the decrease in viscosity and the formation of colour with thiobarbituric acid is given in Fig. 2. In order to obtain a sufficient degradation for a reliable estimate of colour in the

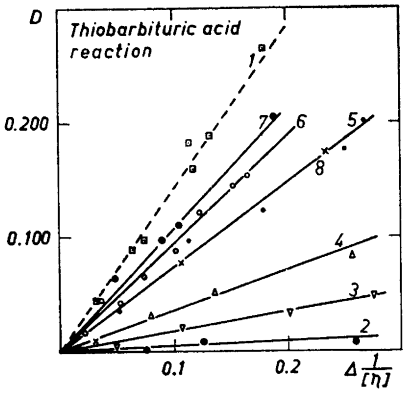


Fig. 2. The correlation between thiobarbituric acid reaction and decrease of viscosity. Alginate prepared from *L. digitata*, Espevær, 10/6. $[\eta]_0 = 24$.

1.	Enzymic degradation		
2.	Thermal	degradation	pH 4.5
3.	»	»	pH 5.2
4.	»	»	pH 6.8
5.	»	»	pH 8.8
6.	»	»	pH 9.8
7.	»	»	pH 10.8
8.	»	»	pH 12.7

thiobarbituric acid reaction, the temperature was increased to 80°. Experiments at pH 12.7 and 4.5 were, however, conducted at 62°.

The slope of the curves in Fig. 2 indicates to what extent the β-elimination reaction is responsible for the degradation of the alginate. Assuming that the degradation in all cases proceeds at random and that the enzymic degradation occurs solely according to the elimination mechanism, we can calculate the percentage of β-elimination in the degradation reaction. The results are given in Fig. 3. The apparent decrease of β-elimination at pH 12.7 is most probably due to degradation of the unsaturated uronic acid derivative in the alkaline medium. The assumption of random degradation is supported by the fact that no dialysable material giving colour reaction with thiobarbituric acid was detected by dialysis of the alginate solutions after degradation ($[\eta]>3$).

As expected, the part played by β-elimination in the degradation of alginate depends very markedly on the pH, but even in neutral and slightly acid medium

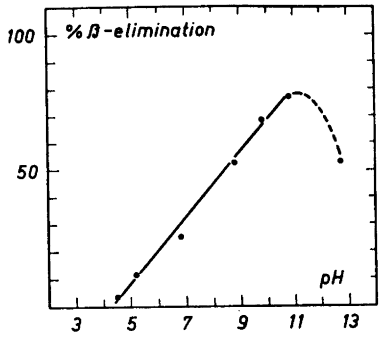


Fig. 3. β-Elimination reaction as a function of the pH, calculated as per cent of total degradation.

a significant part of the degradation occurs as β-elimination. From Fig. 1 it is evident that the rate of degradation increases very markedly when the pH increases above 10 or decreases below 5. From Fig. 3 it follows that above 10 the increased rate is due to increasing rate of the β-elimination reaction, while at pH values less than 5 the well known proton catalysed hydrolysis is responsible for the rapid degradation.

1. Vollmert, B. *Makromol. Chem.* **5** (1950) 110.
2. Albersheim, P. *Biochem. Biophys. Res. Commun.* **1** (1959) 253.
3. Albersheim, P., Neukom, H. and Deuel, H. *Arch. Biochem. Biophys.* **90** (1960) 46.
4. Whistler, R. L. and BeMiller, J. N. *J. Am. Chem. Soc.* **82** (1960) 457.
5. Nagel, C. W. and Vaughn, R. H. *Arch. Biochem. Biophys.* **94** (1961) 328.
6. Suzuki, S. *J. Biol. Chem.* **235** (1960) 3580.
7. Tsujino, I. and Saito, T. *Nature* **192** (1961) 970.
8. Preiss, J. and Ashwell, G. *J. Biol. Chem.* **237** (1961) 309.
9. Ludowieg, J., Vennesland, B. and Dorfman, A. *J. Biol. Chem.* **234** (1961) 333.
10. Weissbach, A. and Hurwitz, J. *J. Biol. Chem.* **234** (1959) 705.
11. Haug, A. *Acta Chem. Scand* **13** (1959) 601.
12. Haug, A. and Smidsrød, O. *Acta Chem. Scand.* **16** (1962) 1569.
13. Marchessault, H. and Rånby, B. G. *Svensk Papperstid.* **62** (1959) 230.
14. Haug, A. and Larsen, B. *Acta Chem. Scand.* **17** (1963). *In press.*

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