ACTA CHEMICA SCANDINAVICA 19 (1965) 143–152

Kinetic Studies on the Degradation of Alginic Acid by Hydrogen Peroxide in the Presence of Iron Salts

OLAV SMIDSRØD, ARNE HAUG and BJØRN LARSEN

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

The degradation of alginate by aqueous hydrogen peroxide in the presence of iron salts has been followed by viscosity measurements, and the concomitant decomposition of the hydrogen peroxide was followed titrimetrically. The observed correlation between the rate of degradation of the alginate and the concentrations of hydrogen peroxide and iron salts suggests that the degradation is brought about by hydroxyl radicals, formed from the hydrogen peroxide according to the reactions postulated by Weiss. The rate of decomposition of the hydrogen peroxide in the presence of alginate or other carbohydrates is different from that expected on the basis of Weiss' reactions, possibly due to the formation of a complex between the carbohydrate, the ferrous or ferric ions, and the hydrogen peroxide molecules.

A previous publication [1] discussed the degradation of alginate in the presence of autoxidizable, reducing compounds. The observations were explained by assuming the following reactions to take place:

(1) Autoxidation of the reducing compound leads to the formation of peroxide.
(2) Reaction of the peroxide with the reducing compound, leads to formation of radicals.
(3) Destruction of free radicals due to reaction with the reducing compound.
(4) Reaction between the radicals and alginate, leading to degradation of the alginate.

The purpose of the present paper is to study further the last three of the reactions described above. Reaction (1) was eliminated by adding hydrogen peroxide to the solution. Iron salts, with ferrous ions as the active reducing agent, were used because this system has been found to be particularly active in the degradation of polysaccharides, and because the reaction between hydrogen peroxide and iron salts has been the subject of numerous investigations.

Weiss [2] and Baxendale [3] have reviewed the literature on the decomposition of hydrogen peroxide in the presence of ferrous ions, and both have given a

set of reactions that explains the experimental observations. We here consider the set of reactions given by Weiss:

1. $Fe^{2+} + H_2O_2 \xrightarrow{k_1} Fe^{3+} + OH^- + OH\cdot$
2. $OH\cdot + H_2O_2 \xrightarrow{k_2} HO_2\cdot + H_2O$
3. $O_2^-\cdot + H_2O_2 \xrightarrow{k_3} O_2 + OH\cdot + OH^-$
4. $Fe^{2+} + OH\cdot \xrightarrow{k_4} Fe^{3+} + OH^-$
5. $Fe^{3+} + O_2^-\cdot \xrightarrow{k_5} Fe^{2+} + O_2$
6. $Fe^{2+} + HO_2\cdot \xrightarrow{k_6} Fe^{3+} + HO_2^-$
7. $Fe^{3+} + HO_2^- \xrightarrow{k_7} Fe^{2+} + HO_2\cdot$
8. $HO_2\cdot \overset{K_{HO_2}}{\rightleftharpoons} H^+ + O_2^-\cdot$
9. $H_2O_2 \overset{K_{H_2O_2}}{\rightleftharpoons} H^+ + HO_2^-$

In addition we shall have to use the following two reactions for the degradation of alginate and buffer ions respectively (the term "buffer ions" here includes all substances which may be attacked by radicals with exception of those given in reactions 1—10):

10. $Alg + OH\cdot \xrightarrow{k_a}$ Products.
11. $xB + OH\cdot \xrightarrow{k_B}$ Products.

Using this set of reactions and the principle that the concentrations of radicals in the stationary state must be constant, it is possible to obtain simple relations which are well suited for experimental tests. When the amount of hydrogen peroxide is high compared with that of the other reactants present, the following simplified relation for the decomposition of hydrogen peroxide should apply:[2]

$$-d[H_2O_2]/dt = 2k_1[Fe^{2+}]\,[H_2O_2] \tag{1}$$

i.e., the decomposition is a first order reaction with respect to the concentration of hydrogen peroxide. For the degradation of alginate we get the following relation:

$$-\frac{d[Alg]}{dt} = \frac{k_a k_1[Fe^{2+}]\,[H_2O_2]\,[Alg]}{k_2[H_2O_2] + k_4[Fe^{2+}] + k_a[Alg] + k_B[B]^x} \tag{2a}$$

which, when hydrogen peroxide is in excess, simplifies to

$$-\frac{d[Alg]}{dt} = \frac{k_a\, k_1}{k_2}\,[Fe^{2+}][Alg] \tag{2}$$

By combining eqn. (2a) with the complete expression for the decomposition of hydrogen peroxide, we find the following correlation between the decomposition of hydrogen peroxide and the degradation of alginate:

$$\frac{d[Alg]}{dt} = \frac{k_a\,[Alg](d[H_2O_2]/dt)}{2k_2[H_2O_2] + k_4[Fe^{2+}] + k_a[Alg] + k_B[B]^x} \tag{3a}$$

which again in the presence of an excess of hydrogen peroxide may be simplified to:

$$\frac{d[Alg]}{dt} = \frac{k_a}{2k_2}\,\frac{[Alg]}{[H_2O_2]}\,\frac{d[H_2O_2]}{dt} \tag{3}$$

In this work the degradation of alginate was followed by viscosity measurements. If P_t is the number average degree of polymerization at the time t, the concentration of bonds available for degradation is proportional to $1-(1/P_t)$.

According to eqn. (2) the degradation of alginate is a first order reaction with respect to the concentration of alginate and may be written:

$$\ln\frac{[Alg]_0}{[Alg]_t} = \ln\frac{[1-(1/P_0)]}{[1-(1/P_t)]} = \frac{k_a\,k_1}{k_2}\,[Fe^{2+}]\,t = kt$$

For high values of P this equation may be simplified to

$$\frac{1}{P_t} - \frac{1}{P_0} = kt \tag{4}$$

Assuming the exponent in the Staudinger equation to be one,[4,5] we have $P = (1/\lambda)[\eta]\cdot K$, where K is a constant and λ is the ratio between the weight-average and the number-average molecular weight. Eqn. (4) may thus be written

$$\frac{\lambda}{K}\left(\frac{1}{[\eta]_t} - \frac{1}{[\eta]_0}\right) = kt$$

The fact that plots of $\Delta(1/[\eta])$ against time were shown to be linear for degradation of alginate in the presence of most reducing agents indicates that λ does not change appreciably during the degradation. This is further supported by the observation that plots of $\Delta(1/[\eta])$ *versus* number of bonds broken by enzymatic degradation are linear.[6] In this work we have used the value 58 for K/λ (Ref. 4).

Eqn. (2) may thus be tested by observing the change in viscosity of the alginate, expressed as $\Delta(1/[\eta])$, while equation (1) may be examined by following the decrease in concentration of hydrogen peroxide by titration.

As shown in the set of equations given above, ferrous ions are formed by reduction of ferric ions in two of the postulated reactions, while ferrous ions are oxidized to ferric ions by three of the reactions. In the stationary state an equilibrium is thus set up between divalent and trivalent iron, and when the concentration of hydrogen peroxide is high the amount of ferrous ions may be shown to be proportional to the total amount of iron added to the solution. Apart from a short induction period before the equilibrium is estab-

lished, the rate of degradation is, therefore, independent of whether the iron is added as trivalent or divalent ions.

EXPERIMENTAL

The preparation of alginate has been described in a previous publication.[7] In all the experiments alginate prepared from *Laminaria digitata*, harvested at Tarva 29/8–61, has been used. A commercial preparation of methylcellulose was used in a few experiments. The hydrogen peroxide, as well as all salts and buffer components used in the experiments, were analytical grade reagents. As a catalyst for the destruction of hydrogen peroxide $FeCl_3 \cdot 6H_2O$ was used. Water purified on ion exchange columns was used in all the experiments.

The degradation experiments were carried out in Ubbelohde viscometers placed in a thermostatic bath. The viscosity was determined at intervals, and the intrinsic viscosity was found by using correlation curves of the type described earlier.[8] When the viscosity became too low to be measured, the test solutions were transferred to flasks, and samples were removed at intervals for the determination of hydrogen peroxide. The concentration of hydrogen peroxide was determined by titration with potassium permanganate.[9]

A 0.3 % solution of alginate and a temperature of 20.0°C was used if no other values are given in the text. McIlvain buffer (0.05 M citric acid + 0.1 M Na_2HPO_4), adjusted to ionic strength 0.2 by means of sodium chloride, was used in most of the experiments. When no buffer ions should be present, the pH of the alginate solution was adjusted to 4 by addition of hydrochloric acid and sodium chloride added to 0.1 N.

RESULTS

The decrease of viscosity of alginate and the degradation of hydrogen peroxide were determined for different amounts of iron present in the solution. In Fig. 1 the rate constants

$$k_{Alg} = A \frac{1}{P} /t \text{ and } k_{H_2O_2} = \ln \frac{[H_2O_2]_0}{[H_2O_2]_t} /t$$

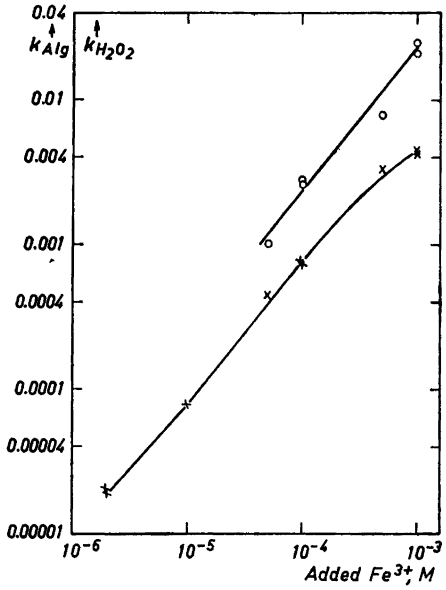

Fig. 1. Rate constants for the degradation of alginate and hydrogen peroxide as a function of the total amount of ferric ions added. The reaction is carried out in McIlvain buffer, pH 6 and with 0.1 M hydrogen peroxide initially present. O = hydrogen peroxide, × = alginate.

are shown as functions of the total amount of iron added to the solution, using hours as the time unit. The experiments were carried out in phosphate-citrate buffer (McIlvain buffer) at pH 6 and 0.1 M hydrogen peroxide was added initially to the solution.

The rate of the decomposition of hydrogen peroxide is proportional to the amount of ferric chloride added to the solution, in agreement with eqn. (1). The rate of alginate degradation is also proportional to the amount of iron salts in the solution at iron contents lower than 10^{-4} M while the increase of the rate is slightly smaller at higher iron salt concentrations. Thus, the ratio between the degradation rates of alginate and hydrogen peroxide decreases slightly with increasing concentration of iron salts. This is in good agreement with eqn. (3a).

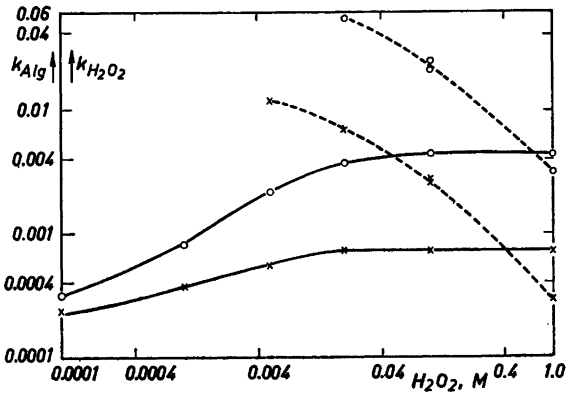

Fig. 2. Rate constants for the degradation of alginate and hydrogen peroxide as a function of the concentration of hydrogen peroxide. The reaction is carried out in McIlvain buffer, pH 6 and at two different concentrations of ferric ions added. O = 10^{-3} M Fe^{3+}, × = 10^{-4} M Fe^{3+}, ——— = alginate, – – – – = hydrogen peroxide.

The dependence of the rate constants on the concentration of hydrogen peroxide is shown in Fig. 2. The rate of degradation of alginate is independent of the amount of hydrogen peroxide present when the concentration of hydrogen peroxide is high, in good agreement with eqn. (2). At lower concentration of hydrogen peroxide, where the approximations leading to the simplified eqn. (2) are not valid, the rate of alginate degradation increases with increasing hydrogen peroxide concentration, as would be expected according to eqn. (2a).

The rate constant of hydrogen peroxide degradation, expressed as described above, should, for a first order reaction like eqn. (1), be independent of the hydrogen peroxide concentration. As shown in Fig. 2, this is, however, not in agreement with our experimental results. The rate constants decrease with increasing hydrogen peroxide concentration, and the results indicate that the degradation of hydrogen peroxide in this experiment is approximately a zero order reaction at high concentrations of hydrogen peroxide.

Table 1. Rate constants of hydrogen peroxide destruction in 0.1 and 1 M H_2O_2.

$$k = \ln \frac{[H_2O_2]_0}{[H_2O_2]_t} / t$$

Medium	pH	Temp. °C	M $FeCl_3$	k 0.1 M	k 1.0 M	$k_{1.0}/k_{0.1}$
0.01 M Nitric acid	2.0	20.0	4×10^{-3}	0.105	0.115	1.09
Alginate, McIlvain buffer	6.0	20.0	10^{-3}	0.025	0.0032	0.128
McIlvain buffer	6.0	20.0	10^{-3}	0.029	0.0032	0.11
0.05 M Acetate buffer	4.0	50.7	10^{-4}	0.004	0.0048	1.2
Alginate, 0.05 M acetate buffer	4.0	50.7	10^{-4}	0.01	0.0016	0.16
Alginate, without buffer	4.0	50.7	10^{-4}	0.012	0.002	0.166

In acid medium Andersen [10] has found that the degradation of hydrogen peroxide is approximately a first order reaction, as would be expected from Weiss' equations. We repeated Andersen's experiments using 0.01 N nitric acid and 4×10^{-3} M ferric chloride and found under these conditions that the rate constant of the hydrogen peroxide degradation was only slightly dependent on the hydrogen peroxide concentration as shown in Table 1. Our results were therefore in good agreement with those of Andersen.

The difference between the results of the experiments shown in Fig. 2 and the experiments described above may be due to different pH or may be caused by the presence of carbohydrate or buffer ions. The rate constants of the hydrogen peroxide destruction in a solution containing only McIlvain buffer and no alginate were determined and the results are given in Table 1. The order of reaction was close to zero. In order to test solutions containing only alginate and no buffer ions, experiments were carried out at pH 4, where the alginate itself has some buffer capacity. The rate constants of hydrogen peroxide degradation were determined in acetate buffer containing no alginate, acetate buffer and alginate, and in an alginate solution containing no buffer ions. The results are given in Table 1, and clearly show that in the presence of alginate the destruction of hydrogen peroxide is approximately a zero order reaction, while in acetate buffer alone, the reaction order is close to one, as was the case at low pH in the absence of carbohydrate.

In order to establish if this effect of alginate is associated with the presence of carboxyl groups in the carbohydrate, or if it is a general feature of all carbohydrates, the rate constants of hydrogen peroxide destruction were determined in the presence of methylcellulose and glucose. The experiments were carried out in 0.01 M nitric acid and the results are shown in Figs. 3 and 4, respectively. The result clearly indicates that the presence of carbohydrate changes the order of reaction of the hydrogen peroxide destruction to approximately zero.

According to the results in Table 1, acetate buffer does not change the order of reaction of hydrogen peroxide destruction. The acetate ions would, however, be expected to be oxidized by radicals as formulated in reaction (11) in the set of postulated reactions given in the introduction. Acetate and

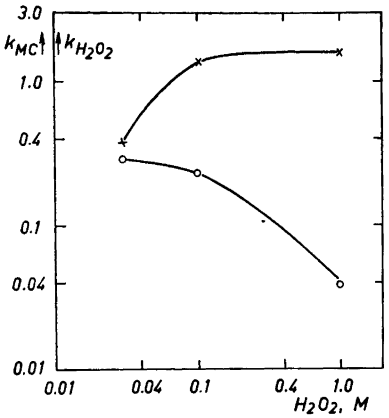

Fig. 3. Rate constants for the degradation of hydrogen peroxide and methylcellulose as a function of the concentration of hydrogen peroxide. The reaction is carried out in 0.01 M HNO_3 with 4×10^{-3} M $FeCl_3$ and 0.75 % methylcellulose. O = hydrogen peroxide, × = methylcellulose, $k_{MC} = \Delta(1/[\eta])/t$.

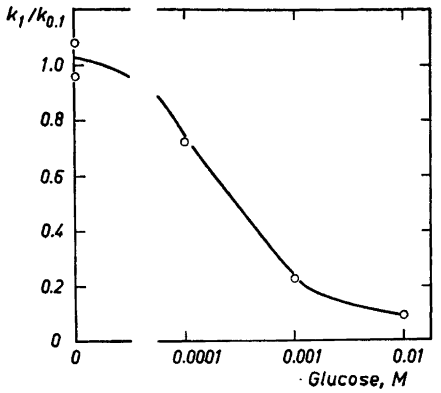

Fig. 4. The ratio between rate constants of hydrogen peroxide destruction in 1 and 0.1 M H_2O_2 as a function of the concentration of glucose. 0.01 M HNO_3, 4×10^{-3} M $FeCl_3$.

related substances were, therefore, chosen to investigate this effect further.

The effect of buffer ions that would be expected according to the theory is shown in eqn. (2a), which, for this purpose, may be simplified to

$$-\frac{d[Alg]}{dt} = \frac{C}{D + k_B[B]^x} \tag{5}$$

where C includes all the terms in the numerator and D all the terms in the denominator which do not involve the buffer ions, *i.e.* D includes all reactions leading to destruction of radicals except those due to the buffer ions. Eqn. (5) shows that the effect of the buffer ions should preferably be examined under conditions where D is low, *e.g.* with relatively small amounts of hydrogen peroxide present. This is illustrated in Table 2, showing the rate constants of alginate degradation at two different concentrations of hydrogen peroxide, with and without acetate buffer.

Table 3 gives the rate constants for alginate degradation in solutions containing 0.1 M hydrogen peroxide and 0.1 M of various carboxylic acids

Table 2. Rate constants for alginate degradation in 0.1 and 1 M H_2O_2 with and without acetate buffer. All the solutions contain 0.1 N NaCl, 10^{-4} M $FeCl_3$ and are adjusted to pH 4.0 with HCl or NaOH.

	0.1 M H_2O_2	1 M H_2O_2
Without buffer	0.0028	0.0028
0.05 M Acetic acid/acetate	0.00062	0.00175

Table 3. Rate constants for alginate degradation in solutions containing 0.1 M of different alcohols and buffer systems. 10^{-4} M $FeCl_3$, 0.1 M H_2O_2, pH = 4.0, 0.1 N NaCl.

Without buffer	0.0028	Without buffer	0.0028
Formic acid/formate	0.00069	Methanol	0.00051
Acetic acid/acetate	0.0004	Ethanol	0.00041
Propionic acid/propionate	0.000088	Propanol	0.000154

and alcohols. The rate constants decrease with increasing molecular weight both for acids and alcohols.

The effect of varying the concentration of added propanol was investigated, and Fig. 5 shows the rate constants of alginate degradation as a function of the propanol concentration. The shape of the curve is in agreement with eqn. (5), as shown by the calculated curve in Fig. 5, where C and D have arbitrarily been chosen as 0.00251 and 1, respectively, and x and k_B have been chosen as 0.7 and 74 to obtain the best possible fit.

When the pH is varied in buffered solutions the proportion between the buffer components is changed, and the effect of the buffer ions on the degradation of hydrogen peroxide and alginate may, therefore, be different at different pH-values. The effect of the proton concentrations may, therefore, be obscured by such effects. It is of considerable practical importance, however, to know the degradation rates at different pH-values, and the experiments given in Fig. 6 show that the rate of the alginate degradation increases more slowly with increasing pH-values than the rate of the hydrogen peroxide splitting reaction.

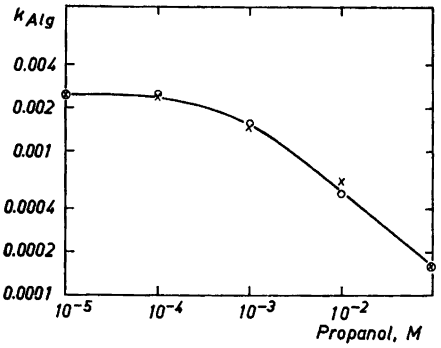

Fig. 5. Rate constants for the degradation of alginate with different amounts of propanol present. 0.1 M H_2O_2, 10^{-4} M $FeCl_3$, 0.1 N NaCl, pH 4.0. O = observed values, × = calculated values.

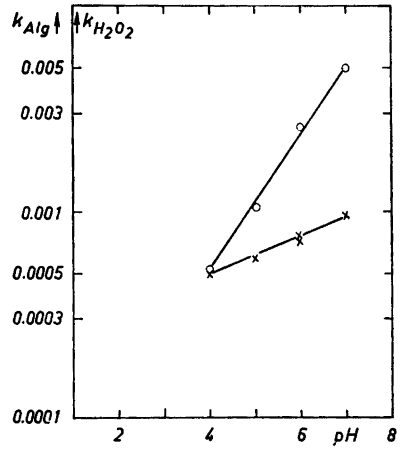

Fig. 6. Rate constants for the degradation of alginate and hydrogen peroxide at different pH-values. McIlvain buffer, 0.1 M H_2O_2, 10^{-4} M Fe^{3+}. O = hydrogen peroxide, × = alginate.

DISCUSSION

The correlation between the concentration of iron salts and observed rate constants, both for hydrogen peroxide destruction and for alginate degradation is in good agreement with the equations based on the series of reactions given by Weiss. The correlation between the rate of alginate degradation and the hydrogen peroxide concentration is also in good agreement with the theory of radical attack on the alginate molecule, and demonstrates clearly that the degradation is not caused by a direct chemical attack of the hydrogen peroxide itself.

The rate of destruction of hydrogen peroxide does not, however, accord with Weiss' series of reactions when substances like carbohydrates, or the constituents of the McIlvain buffer (phosphate-citrate) are present. In solutions containing only nitric acid or acetate buffer, however, the observed rates are in good agreement with Weiss' equations. Zero order reactions are often found in cases where a complex between the catalyst and the reactant is formed, and where the destruction of this complex is the rate determining step in the reaction. The following set of equations may be postulated in addition to those given by Weiss:

$B + Fe \rightleftharpoons \{BFe\}$, equilibrium constant K_1.
$\{BFe\} + H_2O_2 \rightleftharpoons \{BFe\ H_2O_2\}$, equilibrium constant K_2.
$\{BFe\ H_2O_2\} \xrightarrow{k'}$ Products, rate determining step.

In this case, B stands for a compound which may form a complex with ferric or ferrous ions. With the destruction of the complex $\{BFe\ H_2O_2\}$ as the rate determining step we may write:

$$-\frac{d[H_2O_2]}{dt} = k'[\{BFe\ H_2O_2\}] = k' \frac{[Fe]_0\,[H_2O_2]}{1/K_2 + [H_2O_2]} \qquad (6)$$

if we assume B to be in large excess compared to the total amount of iron salt, Fe_0. Eqn. (6) shows that with increasing concentration of hydrogen peroxide the reaction approaches a zero order reaction. It seems reasonable to assume that the observed deviations from the equations based on Weiss' set of reactions are due to such complex formation. It should be pointed out that the low solubility of ferric hydroxide makes it possible to use ferrous ions as catalyst for the hydrogen peroxide degradation only at low values of pH, or when an iron complexing agent is present in the solution.

Buffer ions which do not form complexes with iron salts do not lead to a change of reaction order, but have a pronounced influence on the concentration of free radicals in the solution. The effect of the buffer ions on the rate of degradation of alginate is very pronounced as illustrated in Table 3, and from a practical point of view this "protecting effect" of buffer ions is of considerable importance. We may, therefore, distinguish between two different types of buffer ion effects in the hydrogen peroxide degradation reaction: (1) The change of reaction order of the hydrogen peroxide degradation caused by buffer ions which form a complex with ferric or ferrous ions and (2) the radical destroying effect of buffer ions which may be oxidized by free radicals. In

many cases buffer ions of type (1) may be attacked by radicals and thus also belong to type (2).

In some of the experiments the first order rate constants were found to vary with time, due to changes in hydrogen peroxide concentration or to formation of reaction products which influence the reaction rate. In all cases, therefore, the initial reaction rates have been used. Due to the sensitivity of the viscosity method it is possible to obtain reliable reaction rates for the alginate degradation when only 0.1—1 % of the bonds in the alginate molecule are broken. This advantage and the simplicity of the viscosity measurements, makes determinations of the degradation of alginate or other suitable polysaccharides a very convenient tool for the study of radical producing reactions. Assuming the alginate degradation to occur according to the reaction

$$\text{Alg} + \text{OH}\cdot \xrightarrow{k_a} \text{Products}$$

the degradation rate is proportional to the concentration of hydroxyl radicals, and may conveniently be used for the study of the effect of other substances on the radical concentration, as illustrated in Table 3. The results of this investigation show, however, that the presence of polysaccharides changes the reaction mechanism of the hydrogen peroxide degradation catalyzed by iron salts, and the method is, therefore, limited to the study of the type of reaction which takes place in the presence of iron complexing agents. With organic reducing agents no such limitation should occur, and in such systems the method should be well suited for the study of the radical concentration.

REFERENCES

1. Smidsrød, O., Haug, A. and Larsen, B. *Acta Chem. Scand.* **17** (1963) 2628.
2. Weiss, J. *Advan. Catalysis* **4** (1952) 343.
3. Baxendale, J. H. *Advan. Catalysis* **4** (1952) 31.
4. Donnan, F. G. and Rose, R. S. *Can. J. Research* **28 B** (1950) 105.
5. Cook, W. H. and Smith, D. B. *Can. J. Biochem. Physiol.* **32** (1954) 227.
6. Haug, A., Larsen, B. and Smidsrød, O. *Acta Chem. Scand.* **17** (1963) 1466.
7. Haug, A. *Acta Chem. Scand.* **13** (1959) 601.
8. Haug, A. and Smidsrød, O. *Acta Chem. Scand.* **16** (1962) 1569.
9. Kolthoff, I. M. and Sandell, E. B. *Textbook of Quantitative Inorganic Analysis*, The Macmillan Company, New York 1952, p. 574.
10. Andersen, V. S. *Acta Chem. Scand.* **2** (1948) 1.

Received October 2, 1964.