The Role of the Superoxide Radical in the OsO_4-catalysed Decomposition of Hydrogen Peroxide

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It is shown by direct e.s.r. spectrometry that the superoxide radical is formed in the OsO_4-catalysed decomposition of hydrogen peroxide. The initial rate of superoxide formation was found to be proportional to the 0.6 power of the concentration of hydrogen peroxide and the first power of the catalyst concentration. This is interpreted in terms of the formation of a peroxo-osmic acid, the anion of which decomposes into superoxide and osmium(VII) radicals. The latter is oxidized by hydrogen peroxide at pH < 9, but reduced by the peroxide anion at higher pH. Since the reoxidation of osmium(VI) by hydrogen peroxide slows down when the pH is increased, the decomposition of hydrogen peroxide goes through a maximum. As both superoxide and osmium(VII) were found to be oxidized by tetranitromethane, the decomposition of hydrogen peroxide is inhibited at pH < 9, but accelerated at pH > 9, for at higher pH the catalyst is transformed by this reagent into its more active [osmium(VIII)] form.

In a previous paper [1] it was shown that the OH radical is involved in the catalysed decomposition of hydrogen peroxide. In the presence of radical scavengers, such as different dyes, propan-2-ol, *etc.*, the rate of dioxygen evolution decreases to various extents, depending on the pH, but the decomposition cannot be stopped entirely (especially above pH 9). This indicates that other routes are also involved in the catalysed decomposition. It was also shown that the O_2^- radical can be detected in the catalysed process.[1] The role of the latter entity is examined in this paper.

EXPERIMENTAL

Chemically pure preparations were usually used without further purification. Tetranitromethane was recrystallized eight times from 0.8 mol dm^{-3} sulphuric acid immediately before use. Triply distilled water was used throughout the experiments. *N*-Chloro-2,2,6,6-tetramethylpiperidin-4-ol (ctpo) and its *N*-bromo-analogue (btpo) were prepared from 2,2,6,6-tetramethylpiperidin-4-ol according to the method of Rigo *et al.*[2,3] A Beckman DB-G spectrophotometer was used for kinetic measurements, while spectra were recorded with a Unicam SP 800 spectrophotometer. A Radiometer pH 26 pH-meter was used for pH measurements.

Detection of the O_2^- *Radical.*—In a reaction mixture containing hydrogen peroxide (0.1 mol dm^{-3}), phosphate buffer (0.1 mol dm^{-3}) of appropriate pH, and osmium tetraoxide (10^{-6} mol dm^{-3}), formation of the O_2^- radical was detected by e.s.r. spectroscopy. The reaction was started by adding osmium tetraoxide solution to the reaction mixture. After 2 min, samples (0.5 cm^3) were introduced into the measuring capillaries and then immersed immediately into liquid nitrogen. The e.s.r. spectra were recorded near to liquid-nitrogen temperature with a JEOL-JES-PE spectrometer.

Kinetic Measurements.—The catalysed decomposition of hydrogen peroxide was followed by two methods. (*i*) The volume of dioxygen evolved was measured with the simple apparatus described elsewhere.[1] Osmium tetraoxide was mixed with phosphate buffer (0.1 mol dm^{-3}) of appropriate pH in the reaction vessel and thermostatted for 20 min. The vessel was then closed tightly and the reaction started by injection of hydrogen peroxide through the septum with the aid of an all-glass syringe. The solution was stirred during the entire run. (*ii*) Changes in the concentration of hydrogen peroxide were estimated titrimetrically. Samples (1—5 cm^3) withdrawn from the reaction mixture were added to a known excess of acidified arsenious acid solution and back-titrated with standard 0.05 N cerium(IV) sulphate using ferroin as indicator. This indirect method of analysis was chosen to avoid the induced reaction occurring in the H_2O_2–OsO_4–Ce^{IV} system.[4]

The rate of consumption of the inhibitor dyes was determined spectrophotometrically as described elsewhere.[1]

Evaluation of Kinetic Results.—Initial rates were obtained both numerically and graphically, using concentration *vs.* time curves. In some cases when the gas volume *vs.* time curves showed short incubation periods (*ca.* 0.15—0.20 cm^3), due to the fact that the reaction mixture was not saturated with dioxygen, only the sufficiently linear parts of the curves were used to determine the initial rates.

RESULTS

Formation and pH-*Dependence of the* O_2^- *Radical.*—The e.s.r. spectra of frozen reaction mixtures show a radical of axial symmetry. From a comparison of the g values with the literature data the radical was assigned to the superoxide ion. The $g_{\parallel}$ tensor was found to depend on the pH (2.076, 2.087, and 2.115 at pH 10.1, 11, and 13.5 respectively) whilst $g_{\perp}$ was 2.001 at each pH. These values agree well with data obtained by Knowles *et al.*[5] and Symonyan and Nalbandyan.[6] As may be seen in Figure 1, curve (2), the concentration of the superoxide radical increases with increasing pH (the reaction is quenched after 2 min by cooling to liquid-nitrogen temperature).

Superoxide radicals can also be detected by $C(NO_2)_4$, which is known to react very rapidly to give nitroform, $CH(NO_2)_3$. The formation of nitroform was followed spectrophotometrically at 350 nm (a region where the other components of the reaction mixture do not absorb). In contrast with the results obtained by direct e.s.r. measurement of the O_2^- radical concentration, on using $C(NO_2)_4$ it was found that the initial rate of formation of nitroform varies with the pH according to a curve, with a maximum at pH 10.6 [Figure 1, curve (1)]. In the pH range 6.5—9.0 the plot of the logarithm of the rate of formation of nitroform *vs.* pH is linear, with a slope of one.

Effect of $C(NO_2)_4$ *on the Bleaching of* NN-*Dimethyl*-p-*nitrosoaniline* (RNO).—When $C(NO_2)_4$ and RNO were added simultaneously to the reaction mixture in comparable quantities at pH < 8.5, the bleaching of RNO was considerably suppressed by $C(NO_2)_4$, whilst RNO did not exert any influence on the function of $C(NO_2)_4$, *i.e.* the rate of formation of nitroform was not influenced by the presence of RNO.

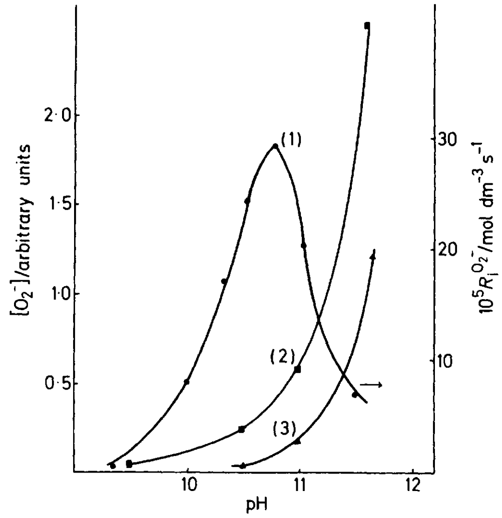


FIGURE 1 Rates of formation of the superoxide radical and nitroform as functions of pH: (1) rate of formation of nitroform at 2.15×10^{-8} mol dm^{-3} OsO_4, 5.34×10^{-3} mol dm^{-3} H_2O_2, 1.73×10^{-4} mol dm^{-3} $C(NO_2)_4$, 0.1 mol dm^{-3} phosphate buffer, and 293 K; (2) concentration of O_2^- (in arbitrary units) after 2 min conversion time, determined by e.s.r. spectroscopy. Spectra of frozen samples taken near liquid-nitrogen temperature; 1×10^{-6} mol dm^{-3} OsO_4, 0.1 mol dm^{-3} H_2O_2, and 0.1 mol dm^{-3} phosphate buffer. Curve (3) as in (2) but in the absence of catalyst

Effect of $C(NO_2)_4$ *on the Rate of Dioxygen Evolution.*—At pH < 9.0, well defined incubation periods were obtained in plots of evolved dioxygen *vs.* time when $C(NO_2)_4$ was applied in the reaction mixtures. When the induction period was over, *i.e.* all the $C(NO_2)_4$ had been reduced, dioxygen evolution started at a higher rate. However, this rate was always lower than the rate in the absence of $C(NO_2)_4$, owing to the reaction between nitroform and OH. It was checked that the rate of dioxygen evolution was equal to the rate for a reaction mixture containing OH scavenger in a quantity kinetically equivalent to the nitroform formed during the incubation period. Due to the $CH(NO_2)_3$ + OH reaction, the concentration of nitroform passes through a maximum during the run. At pH > 9 the catalysed decomposition reaction is accelerated by the introduction of $C(NO_2)_4$ (see Table 1). The enhancement of the rate of dioxygen evolution is proportional to the concentration of $C(NO_2)_4$. After all the $C(NO_2)_4$ has been transformed to nitroform, the rate of decomposition falls back near to the value obtained in the absence of $C(NO_2)_4$. When the concentration of $C(NO_2)_4$ is equal to or exceeds that of hydrogen peroxide, the stoicheiometry $\Delta[O_2]/\Delta[H_2O_2]$ has a value of 1 : 1 instead of the 1 : 2 obtained in the absence or presence of only a small amount of $C(NO_2)_4$. In this case the mole ratio of the reaction product $\Delta[CH(NO_2)_3]/\Delta[O_2]$ is 2 : 1 (Table 1).

TABLE 1

Influence of $C(NO_2)_4$ on the initial rate and stoicheiometry of the OsO_4-catalysed decomposition of hydrogen peroxide *

	Initial rate/10^{-5} mol dm^{-3} s^{-1}			
pH	$[C(NO_2)_4] = 0$	$[C(NO_2)_4] > 0$	$\frac{\Delta[O_2]}{\Delta[H_2O_2]}$	$\frac{\Delta[CH(NO_2)_3]}{\Delta[O_2]}$
9.6	1.5	8.0	0.98	1.67
9.7	2.2	12.0	0.98	1.75
10.03	4.6	45.0	0.96	1.87
10.13	5.8	57.0	1.04	1.88
10.55	9.7	96.0	0.99	1.90
11.05	8.3	93.0	1.00	1.95
11.18	6.0	70.0	1.00	2.08
11.54	3.6	40.0	0.94	1.99
11.97	2.0	32.0	0.98	2.00

* Conditions: 3.67×10^{-6} mol dm^{-3} OsO_4; 4.6×10^{-3} mol dm^{-3} H_2O_2; 1.3×10^{-3} mol dm^{-3} $C(NO_2)_4$; 0.1 mol dm^{-3} phosphate buffer; 293 K.

Determination of the Rate of Initiation involving the Superoxide Radical.—As mentioned above, at pH < 9 the addition of $C(NO_2)_4$ to the reaction mixture results in an induction period of dioxygen evolution, the length of which could be determined with satisfactory accuracy. The average rate of superoxide formation is given by equation (i), where

$$R_i^{O_2^-} = \{[C(NO_2)_4]_0/\tau\} - R_{bgr} \qquad (i)$$

τ is the length of the induction period in s, $[C(NO_2)_4]_0$ is the initial concentration of $C(NO_2)_4$, and R_{bgr} is the rate of the background reaction.

Another method was also used to determine $R_i^{O_2^-}$. Under various experimental conditions $C(NO_2)_4$ was applied in increasing quantities and the rate of formation of nitroform (determined graphically) as a function of the initial concentration of $C(NO_2)_4$ gave curves having limiting values. Under conditions where all radicals were captured by $C(NO_2)_4$, half of the limiting rate was taken as the rate of initiation. The factor 0.5 comes from the stoicheiometry of the reaction (see below).

The $R_i^{O_2^-}$ values obtained by spectrophotometry agree reasonably well with those calculated from the length of the induction period up to pH 8.5 (Table 2). Near the upper end of this pH range the values obtained by the latter method proved to be somewhat higher than those determined by spectrophotometry. At pH > 9, $R_i^{O_2^-}$ determined by the latter method was found to be considerably

TABLE 2

Rate of initiation involving the superoxide radical [a]

	$10^7 R_i^{O_2^-}$/mol dm^{-3} s^{-1}			
pH	*b*	*c*	$\frac{-(d[H_2O_2]/dt)_0}{10^{-7}\ \text{mol dm}^{-3}\ \text{s}^{-1}}$	Chain length
6.5	0.20	0.20	2.3	11.5
7.0	0.58	0.56	17.9	31.0
7.5	1.62	1.80	66.9	41.2
7.7	3.25	6.20	107.0	32.9
8.0	5.73	10.20	166.0	29.0
8.25	18.70	16.80	226.0	12.0
8.5	31.10	33.60	312.3	10.0
9.0	75.00	—	383.5	5.1

[a] Conditions: 1.0×10^{-6} mol dm^{-3} OsO_4; 3.1×10^{-2} mol dm^{-3} H_2O_2; 2.26×10^{-4} mol dm^{-3} $C(NO_2)_4$; 0.1 mol dm^{-3} phosphate buffer; 293 K. [b] Obtained by spectrophotometry. [c] Obtained from induction period.

greater than the overall rate of dioxygen evolution in the absence of $C(NO_2)_4$.

Dependence of the Rate of Initiation on the Analytical Concentration of Hydrogen Peroxide and Osmium Tetraoxide.— At pH < 9 the dependence of $R_i^{O_2^-}$ on the total concentrations of hydrogen peroxide and osmium tetraoxide can be described by the empirical equation (ii)

$$R_i^{O_2^-} = \text{constant}\cdot[H_2O_2]^n[OsO_4]^m \qquad \text{(ii)}$$

where $n = 0.6$ and $m = 1$ in the range of 0.016—0.30 mol dm^{-3} H_2O_2 and 5×10^{-8} —2.5×10^{-7} mol dm^{-3} OsO_4 (see Figure 2).

Investigations in the Presence of ctpo *or* btpo.—It was found that neither ctpo nor btpo reacts with either osmium(VIII), osmium(VI), or hydrogen peroxide. When ctpo was used the formation of *N*-oxyl radicals could not be observed by e.s.r. spectroscopy, either at pH 8.5 or at 10.6; btpo furnished the same result. On the other hand, in the neighbourhood of pH 8.5 or below, the rate of dioxygen evolution decreased when ctpo was added to the reaction mixture in increasing quantities, and reached a limiting value which was just half of that obtained in the absence of ctpo.

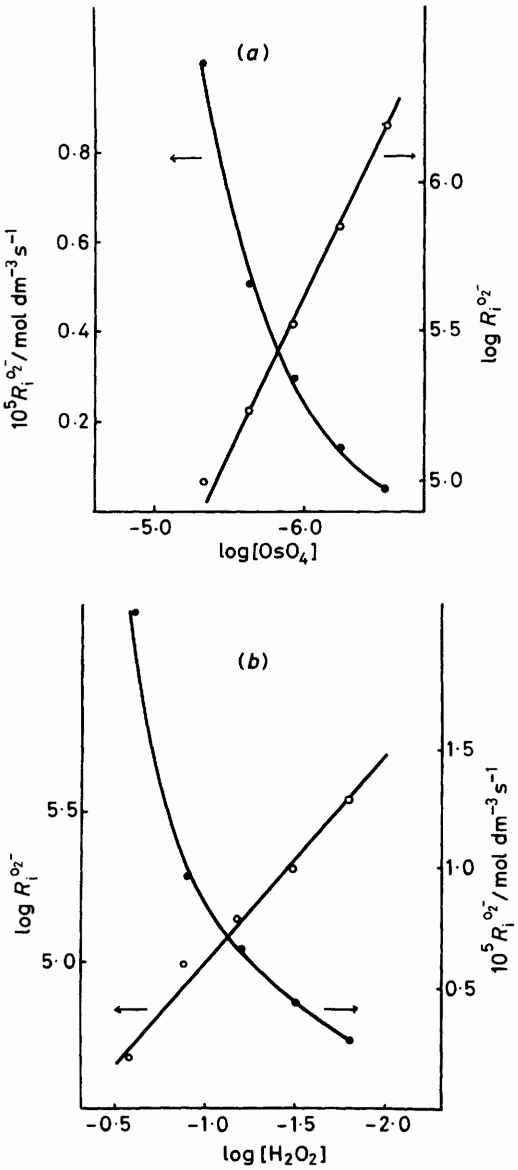


FIGURE 2 Dependence of the rate of initiation involving the O_2^- radical on the total concentrations of OsO_4 (*a*) and H_2O_2 (*b*). Conditions: pH 8.40; 0.1 mol dm^{-3} phosphate buffer; 293 K; 3.47×10^{-2} mol dm^{-3} H_2O_2; and 3.04×10^{-6} mol dm^{-3} OsO_4

The bleaching of RNO can also be diminished by adding ctpo. At pH < 8.5 the rate of bleaching reached a limiting value of one half the initial value when ctpo was applied in increasing quantities. At pH > 8.5 the rate-decreasing effect of ctpo on dioxygen evolution gradually increased; at pH 9 and 11 only 8—10 and 1—2% dioxygen was evolved respectively, in comparison to the value obtained in the absence of ctpo. It is worth noting that at higher pH progressively lower concentrations of ctpo were sufficient to obtain the suppressed limiting rate of dioxygen evolution. It should be added that under such experimental conditions a strong optical interaction was observed between ctpo and osmium tetraoxide at 230 nm. The absorbance of the mixture was considerably greater than would be expected based on the additivity of the absorbances of the components, and increased in time up to 1 h. A somewhat smaller optical interaction was found by using the corresponding piperidine derivative.

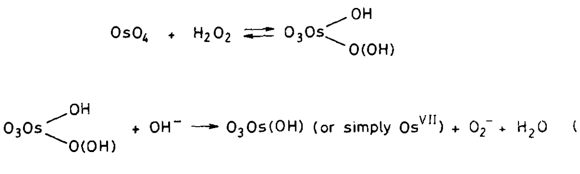
DISCUSSION

With e.s.r. spectroscopy it was proved directly that the superoxide radical is formed during the osmium tetraoxide-catalysed decomposition of hydrogen peroxide. The present investigations show that the radical concentration increases with increasing pH (Figure 1). The use of ctpo and btpo for detection of the superoxide radical was not successful in this system. The ctpo reacts with the superoxide radical by two paths; the main path yields piperidine whilst the second path produces the stable *N*-oxyl radical in a yield of 5%. Further, the *N*-oxyl radical can easily be attacked by the OH radical (and by the superoxide radical to a lesser extent), which is why we failed to observe the formation of the *N*-oxyl radical in spite of the relatively high concentration of the superoxide radical.

The reaction between $C(NO_2)_4$ and the superoxide radical is quite fast ($k_{O_2^- + C(NO_2)_4} = 1.9 \times 10^9$ dm^3 mol^{-1} s^{-1}); [7] it produces nitroform, which can be measured sensitively ($\varepsilon = 1.5 \times 10^4$ dm^3 mol^{-1} cm^{-1} [8] at 350 nm). The use of $C(NO_2)_4$, however, is not entirely convenient as it reacts with the peroxide anion in the absence of any catalyst [see equation (iii)]. Fortunately,

$$C(NO_2)_4 + HO_2^- + OH^- \longrightarrow C(NO_2)_3^- + NO_2^- + O_2 + H_2O \qquad \text{(iii)}$$

this background reaction ($k_{C(NO_2)_4 + HO_2^-} = 7.0 \times 10^2$ dm^3 mol^{-1} s^{-1}) [9] is slower than the catalysed reaction and can be taken into consideration. In connection with the use of $C(NO_2)_4$, it must also be borne in mind that nitroform is an effective scavenger for the OH radical ($k_{OH + CH(NO_2)_3} = 10^9$ dm^3 mol^{-1} s^{-1}).[7] This reaction gives a colourless product, and consequently the simultaneous occurrence of the OH radical may lead to underestimation of the concentration of the superoxide radical. The $C(NO_2)_4$ results in an induction period during the catalysed decomposition of hydrogen peroxide. To explain this it is plausible to assume that peroxo-osmic acid formed in the reaction between hydrogen peroxide and osmium tetraoxide decomposes into osmium(VII) and the superoxide radical in alkaline medium [see equation (1)].



$$OsO_4 + H_2O_2 \rightleftharpoons O_3Os(OH)(O(OH))$$

$$O_3Os(OH)(O(OH)) + OH^- \longrightarrow O_3Os(OH)\ (\text{or simply } Os^{VII}) + O_2^- + H_2O \quad (1)$$

During the induction period $C(NO_2)_4$ undergoes reaction with the superoxide radical with stoicheiometry shown in equation (2). The osmium(VII) formed in reaction (1) being a reactive species attacks hydrogen peroxide, and the OH radical is produced [see equation (3)]. This can be scavenged very effectively by RNO.[10,11]

$$C(NO_2)_4 + O_2^- \longrightarrow C(NO_2)_3^- + NO_2 + O_2 \quad (2)$$

$$Os^{VII} + H_2O_2 \longrightarrow Os^{VIII} + OH + OH^- \quad (3)$$

Since the bleaching of RNO can be inhibited in the presence of $C(NO_2)_4$, this indicates that the osmium(VII) species are more effectively removed by the $C(NO_2)_4$ [equation (4)] than by reaction (3), and therefore in the presence of $C(NO_2)_4$ the OH radical is hardly formed.

$$C(NO_2)_4 + Os^{VII} \longrightarrow C(NO_2)_3^- + NO_2 + Os^{VIII} \quad (4)$$

At pH > 9, $C(NO_2)_4$ first elevates then diminishes the rate of dioxygen evolution in the catalysed decomposition of hydrogen peroxide. The rate increase is probably connected with the fact that at the higher pH the rate of reaction (2) exceeds the rate of dismutation of the superoxide radical [$k_{HO_2 + O_2^-} = (1.02 \pm 0.49) \times 10^8$ dm^3 mol^{-1} s^{-1}] [12] [equation (5)] because the concentration of

$$HO_2 + O_2^- \longrightarrow O_2 + HO_2^- \quad (5)$$

HO_2 decreases by an order of magnitude per pH unit increase, and $k_{O_2^- + O_2^-}$ is less than 0.35 dm^3 mol^{-1} s^{-1}.[12] Furthermore, in the presence of $C(NO_2)_4$, osmium(VIII) is restored by reaction (4) more quickly than by reaction (3), therefore the initiation [equation (1)] will also be enhanced. The sum of steps (1), (2), and (4) gives the following stoicheiometry; *i.e.* in the presence of $C(NO_2)_4$

$$H_2O_2 + 2\,C(NO_2)_4 + 2\,OH^- \longrightarrow O_2 + 2C(NO_2)_3^- + 2\,NO_2 + 2\,H_2O$$

in sufficient quantity $\Delta[O_2]/\Delta[H_2O_2]$ is 1 : 1 instead of 1 : 2 found in the OsO_4-catalysed decomposition of hydrogen peroxide. Another stoicheiometric relationship can also be deduced: $\Delta[CH(NO_2)_3]/\Delta[O_2] = 2 : 1$, as found experimentally.

However, at higher pH the reaction of osmium(VII) with a peroxide anion [equation (6)] starts to compete effectively with reaction (3) and the osmium will be increasingly transformed into its less-efficient form, and from then on the rate of dioxygen evolution will be governed by the rate of regeneration of the active form of the catalyst [equation (7)].

$$Os^{VII} + HO_2^- + OH^- \longrightarrow Os^{VI} + O_2 + H_2O \quad (6)$$

$$Os^{VI} + H_2O_2 + 2\,H^+ \longrightarrow Os^{VIII} + 2\,H_2O \quad (7)$$

The decrease in the rate of nitroform formation at pH > 10.6 can be explained by the effective competition of reaction (6) with reaction (7).

At pH < 9 the addition of ctpo to the reaction mixture results in a drop in the rate of the catalysed reaction. When ctpo is applied in sufficient quantity a limiting rate of dioxygen evolution can be reached which is exactly half the rate observed in the absence of ctpo. Halving of the rate of RNO bleaching was similarly found when sufficient ctpo was added at lower pH. These findings can be explained in that, in contrast with $C(NO_2)_4$, ctpo removes the superoxide radical quantitatively but does not react with osmium(VII). On increasing the pH the dramatic increase of the effectiveness of ctpo in the catalysed decomposition of hydrogen peroxide is much more connected with the complexation of osmium than with the more effective removal of the superoxide radical, although it was stated by Rigo *et al.*[2] that the scavenging of superoxide radical (and the formation of *N*-oxyl radical) is enhanced on increasing the pH from 7 to 12.

The rate of initiation [reaction (1)] is found to be inversely proportional to the hydrogen-ion concentration. This can be seen in Table 2, where data obtained by using different methods are collected. If the initial rate of the catalysed decomposition of hydrogen peroxide is divided by the appropriate $R_i^{O_2^-}$ value determined under similar experimental conditions, the length of the chain is obtained.

The crucial point of this mechanism is that the stationary concentration ratio of $[Os^{VIII}]/[Os^{VI}]$ decreases with increasing pH. Since both the reduction of osmium(VIII) and the oxidation of osmium(VI) consume hydrogen peroxide, it is reasonable to assume that the highest efficiency of the decomposition lies at that pH where the rates of both reactions become equal. The valence change of osmium during the decomposition of hydrogen peroxide at different pH is now under investigation.

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