A Kinetic Study of the Chromium(VI)−Hydrogen Peroxide Reaction. Role of the Diperoxochromate(VI) Intermediates

Joaquin F. Perez-Benito* and Conchita Arias

Departamento de Quimica Fisica, Facultad de Quimica, Universidad de Barcelona, Marti i Franques, 1, 08028 Barcelona, Spain

Received: November 20, 1996; In Final Form: April 4, 1997[⊗]

The kinetics of the Cr(VI)−H_2O_2 reaction has been studied in aqueous solutions (pH = 4.6−7.3) in the presence of either phosphate or acetate buffers. In those media, Cr(VI) behaves both as a catalyst (being recovered unchanged) for the dismutation of H_2O_2 and as an oxidant (being reduced to Cr(III)). High concentrations of H_2O_2, buffer, or H^+ favor the reduction Cr(VI) → Cr(III). A combination of spectrophotometric and titrimetric measurements has allowed us to obtain the initial rates of disappearance of both Cr(VI) ($v_{c,o}$) and H_2O_2 ($v_{p,o}$) as well as an apparent value of the overall initial rate of formation ($v'_{i,o}$) of two relatively stable, violet intermediates, identified in this work as two diperoxochromate(VI) complexes differing in the absence (C_1) or presence (C_2) of a phosphate (or acetate) ligand. Both $v_{c,o}$ and $v'_{i,o}$ are of first order in both Cr(VI) and H_2O_2, whereas $v_{p,o}$ follows the law $v_{p,o} = ((k_p[H_2O_2]_o + k'_p[H_2O_2]_o^2)[Cr(VI)]_o)/(1 + k''_p[H_2O_2]_o^2)$, k_p, k'_p, and k''_p being pseudo rate constants dependent on both the total buffer concentration and pH. The three initial rates increase with rising total buffer concentration, the effect caused by phosphate ions being greater than that caused by acetate ions; $v_{c,o}$ and $v'_{i,o}$ show acid catalysis, whereas the $v_{p,o}$ vs pH plots are bell shaped. Anomalous Arrhenius plots have been found in the three cases. A mechanism is proposed where the intermediates C_1 and C_2 both have an active role in the conversion $H_2O_2 \rightarrow O_2$ and where C_2 is involved in the generation of most of the Cr(III) formed under the experimental conditions of our work. Of some biological relevance might be the finding that adenine seems to stabilize some peroxochromium intermediate complex, the adenine molecule being destroyed during the course of the Cr(VI)−H_2O_2 reaction.

Introduction

Chromium(VI) is a potent, well-established carcinogen,[1] present in the environment as an air and soil pollutant[2,3] and capable of easily crossing the cell membrane as chromate ion with the aid of nonspecific anion carriers,[4] whose interaction with biological molecules has gained some attention lately.[5,6] On its hand, hydrogen peroxide, formed in aerobic living organisms as a byproduct of the oxygen cellular metabolism,[7] is a molecule of considerable biological importance, mainly because of its facility to produce potentially damaging free radicals: with one-electron oxidants it yields superoxide radicals[8] (widely considered as toxic,[9] although their toxicity remains somehow controversial[10,11]), whereas with one-electron reductants it yields hydroxyl radicals[8,12] (very dangerous for living organisms due to their ability to react with almost every biomolecule,[13] including DNA[14]). In recent years, it has been proposed that hydrogen peroxide might collaborate with other species present in the cellular medium (mainly with thiols such as glutathione[15−17]) in the activation of chromium(VI) required to produce its toxic and carcinogenic effects.[18−20]

The chromium(VI)−hydrogen peroxide reaction presents an unusually complicated kinetic behavior that makes the elucidation of its mechanism (in spite of numerous studies extended over a period of almost a century[21−22]) very complex.[23] In particular, it involves some intermediates stable enough to be directly detected, but the nature of the intermediates (that seems to depend markedly on the pH of the medium) is better known when the reaction takes place in very acidic solutions.[24−28] We have studied this reaction under near-physiological pH conditions and found that a relatively stable, violet intermediate, whose rate of formation is notably influenced by the nature and concentration of the buffer present in the solution, is involved in the mechanism. An interesting feature of this kinetic system is that a reversible step of the mechanism can be considered in rapid pre-equilibrium as far as the rate of disappearance of hydrogen peroxide is concerned but not with regard to the disappearance of chromium(VI), due to the different time scale for the disappearance of the two species.

Experimental Section

Deionized water was purified by distillation followed by treatment with a Millipore system before being used as solvent. Hydrogen peroxide (30% solution, without stabilizing additives) was purchased from Fluka. Potassium dichromate and all the other chemicals used were purchased from Merck (analytical grade). The pH of the solutions was kept constant during the kinetic runs with either phosphate (KH_2PO_4−K_2HPO_4) or acetate (CH_3CO_2H−$NaCH_3CO_2$) buffers, and it was measured with a Metrohm 605 pH meter provided with a glass-calomel combination electrode. When necessary, the ionic strength was controlled with potassium nitrate. The temperature was kept constant during each kinetic run with a conventional thermostatic bath provided with both heating and cooling devices. The decay of the reactant chromium(VI) and the formation of the violet intermediate were followed at 372 and 497 nm, respectively, in 1 cm thermostated cells with a Varian Cary 219 UV−vis spectrophotometer. The decay of hydrogen peroxide was followed by titration with potassium permanganate in the presence of sulfuric acid. The spectra of the chromium(III) formed as a reaction product and of the violet intermediate were recorded with a Hitachi U 2000 UV−vis spectrophotometer.

[⊗] Abstract published in *Advance ACS Abstracts,* June 1, 1997.

S1089-5639(96)03868-6 CCC: $14.00

TABLE 1: Spectral Properties of Several Chromium(III) Complexes

species	λ_{max} (nm)	λ_{max} (nm)	source
Cr(III)−GSSG	395	545	ref 28[a]
$Cr(NO_3)_3$	408	572	this work[b]
Cr(III)−phosphate	430	603	this work[c]
$Cr_3(O_2)_2^{5+}$	433	625	ref 29[d]
$Cr_2O_2^{4+}$	439	634	ref 29[d]

[a] Spectrum of the chromium(III) complex with glutathionyl disulfide formed from the reduction of chromium(VI) with glutathione. [b] Spectrum of chromium(III) nitrate in neutral aqueous solution. [c] Spectrum of the chromium(III)−phosphate complex postulated as a reaction product from the reduction of chromium(VI) (2.56×10^{-4} M) by hydrogen peroxide (0.196 M), at $[KH_2PO_4] = 0.216$ M, $[K_2HPO_4] = 0.024$ M, pH 5.63, and 25.0 °C. [d] Spectra of two peroxochromium(III) species formed from the reduction of chromium(VI) by hydrogen peroxide in perchloric acid aqueous solutions.

Results

Stoichiometry. Our results indicate that two stoichiometric equations are required for the Cr(VI)−H_2O_2 system. In one of them Cr(VI) behaves as an oxidant for hydrogen peroxide

$$2HCrO_4^- + 3H_2O_2 + 8H^+ = 2Cr^{3+} + 3O_2 + 8H_2O \quad (1)$$

whereas in the other Cr(VI) behaves as a catalyst for its dismutation

$$2H_2O_2 = O_2 + 2H_2O \quad (2)$$

Molecular oxygen was easily detected as one of the reaction products by means of its characteristic bubbles. Chromium was present in the product solution as a mixture of Cr(VI) (detected by its absorption in the 350−370 nm region, depending on the pH of the solution) and Cr(III). The visible spectrum of the latter, obtained in the presence of phosphate buffer, showed the two weak absorption bands characteristic of this oxidation state of chromium (Figure 1S in the Supporting Information), but they were localized at 430 ($\epsilon = 86$ M^{-1} cm^{-1}) and 603 ($\epsilon = 63$ M^{-1} cm^{-1}) nm, which represents a certain shift with respect to the bands corresponding to other Cr(III) species[29,30] (Table 1). This shift may be attributed to the existence in this product of a phosphate ligand coming from the buffer, given the ability of Cr(III) to form complexes with a wide variety of ligands.[31]

From the spectrophotometric measurements, the percentages of Cr(VI) and Cr(III) at the end of the reaction could be deduced. The proportion of Cr(III) increased as the $[H_2O_2]_o/[Cr(VI)]_o$ ratio (Figure 1), the total phosphate concentration (Figure 1 inset), or the acidity of the medium (either in the presence of phosphate (Figure 2) or acetate (Figure 2 inset) buffers) was raised.

Detection of a Relatively Stable Intermediate. A violet intermediate could be observed in many of the experiments. Its spectrum presented a wide absorption band with a maximum at 497 nm (Figure 2S in the Supporting Information), and its appearance was more easily visible at high concentrations of both hydrogen peroxide and total buffer and at low pH.

Kinetic Data. The progress of the reaction was followed by monitoring the decay of hydrogen peroxide by permanganate titration. The plots corresponding to various initial concentrations of hydrogen peroxide are shown in Figure 3. In addition, both the decay of the reactant Cr(VI) (spectrophotometric measurements at 372 nm) and the formation of the violet intermediate (spectrophotometric measurements at 497 nm) were also monitored. In none of the cases could the experimental data be fitted to a simple rate law, probably because of the nonstoichiometric nature of the reaction. Moreover, in the experiments with a high initial concentration of hydrogen peroxide, the spectrophotometric measurements at both 372 and 497 nm were increasingly perturbed as the reaction advanced

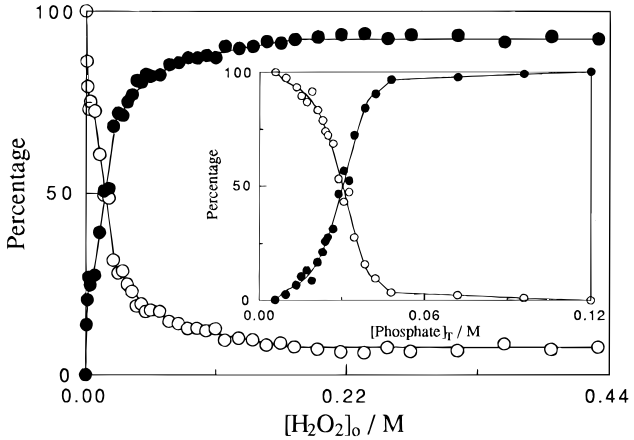


Figure 1. Effect of the initial concentration of H_2O_2 on the percentages of Cr(VI) (empty circles) and Cr(III) (filled circles) in the final product mixture at $[Cr(VI)]_o = 2.56 \times 10^{-4}$ M, $[KH_2PO_4] = [K_2HPO_4] = 0.060$ M, pH 6.71, and 25.2 °C. Inset: Effect of the total buffer concentration ($[KH_2PO_4] + [K_2HPO_4]$) on the percentages of Cr(VI) (empty circles) and Cr(III) (filled circles) in the final product mixture at $[Cr(VI)]_o = 2.56 \times 10^{-4}$ M, $[H_2O_2]_o = 0.196$ M, pH 6.31, ionic strength 2.22 M (KNO_3), and 25.2 °C.

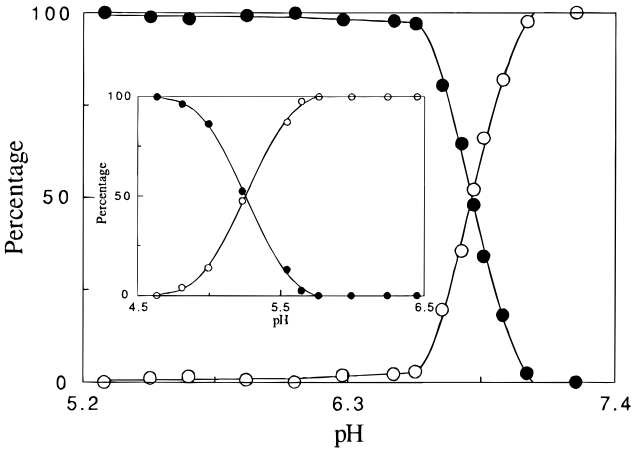


Figure 2. Effect of the pH on the percentages of Cr(VI) (empty circles) and Cr(III) (filled circles) in the final product mixture at $[Cr(VI)]_o = 2.56 \times 10^{-4}$ M, $[H_2O_2]_o = 0.196$ M, $[KH_2PO_4] + [K_2HPO_4] = 0.120$ M, ionic strength 2.22 M (KNO_3), and 25.2 °C. Inset: The same plots for acetate buffer ($[CH_3CO_2H] + [NaCH_3CO_2] = 0.120$ M, the other experimental conditions as in the case of phosphate buffer).

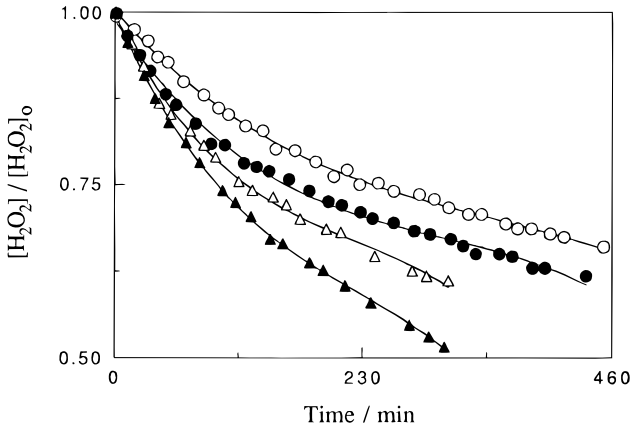


Figure 3. Dependence of the $[H_2O_2]/[H_2O_2]_o$ ratio on time for the reaction between Cr(VI) (2.56×10^{-4} M) and H_2O_2 in the presence of a KH_2PO_4 (0.060 M)−K_2HPO_4 (0.060 M) buffer, at pH 6.71 and 25.2 °C. $[H_2O_2]_o = 0.106$ (empty circles), 0.195 (filled circles), 0.392 (empty triangles), and 0.586 (filled triangles) M.

TABLE 2: Effect of the Initial Concentration of Chromium(VI) on the Initial Rates[a]

$[Cr(VI)]_o$ (10^{-4} M)	$v_{c,o}$ (10^{-7} M s^{-1})	$v_{p,o}$ (10^{-5} M s^{-1})	$v'_{i,o}$ (10^{-4} s^{-1})
0.67	1.02 ± 0.02	0.27 ± 0.04	0.40 ± 0.01
1.33	2.04 ± 0.08	0.49 ± 0.01	0.94 ± 0.13
2.00	3.02 ± 0.01	0.81 ± 0.02	1.32 ± 0.06
2.66	3.87 ± 0.03	1.02 ± 0.04	1.72 ± 0.13
3.33	4.90 ± 0.03	1.44 ± 0.03	2.57 ± 0.06
4.00	5.54 ± 0.09	1.66 ± 0.06	2.47 ± 0.06
5.33	7.84 ± 0.21	2.10 ± 0.01	4.12 ± 0.33
6.66	8.86 ± 0.61	2.83 ± 0.10	4.59 ± 0.31

[a] $[H_2O_2]_o = 0.196$ M, $[KH_2PO_4] = [K_2HPO_4] = 0.060$ M, pH 6.71, and 25.2 °C.

because of the accumulation of oxygen bubbles on the cell walls. Hence, we have used the initial-rates method by determining the initial rates of disappearance of both chromium(VI) ($v_{c,o} = -(d[Cr(VI)]/dt)_{t=0}$) and hydrogen peroxide ($v_{p,o} = -(d[H_2O_2]/dt)_{t=0}$), as well as an apparent value of the initial rate of formation of the violet intermediate ($v'_{i,o} = (dA_{497}/dt)_{t=0}$). The real value of the latter initial rate ($v_{i,o}$) could not be obtained because the molar absorption coefficient of the violet intermediate was unknown.

The three initial rates presented a first-order dependence on the initial Cr(VI) concentration (Table 2). Both $v_{c,o}$ and $v'_{i,o}$ presented a first-order dependence also on the initial hydrogen peroxide concentration (Figure 4 inset). A certain deviation with respect to that behavior was observed in $v_{c,o}$ at high $[H_2O_2]_o$, but it might be due to a systematic error caused by the difficulty of extrapolating accurate values of the initial rate when the reaction was too fast. The $v_{p,o}$ data could be fitted to a much more complicated function, approaching a saturation-like situation at high values of $[H_2O_2]_o$ (Figure 4). The dependencies found for the initial rates on the initial concentrations of both chromium(VI) and hydrogen peroxide were given by the rate laws

$$v_{c,o} = k_c[Cr(VI)]_o\,[H_2O_2]_o \qquad (3)$$

$$v_{p,o} = \frac{(k_p[H_2O_2]_o + k'_p[H_2O_2]_o^2)[Cr(VI)]_o}{1 + k''_p[H_2O_2]_o^2} \qquad (4)$$

the law found for $v'_{i,o}$ being similar to that found for $v_{c,o}$ (eq 3). It should be noticed that eq 4 agrees well with the rate law found by Kobozev and Galbreich[32] for the same reaction,

The values of $v_{c,o}$ increased with decreasing pH (Figure 5 top), approaching a saturation-like situation at low pH (provided that the phosphate concentration was high enough), whereas at high pH its values were slightly negative (denoting that, at high pH, formation of free Cr(VI) was predominant over its destruction when the spectrophotometric measurements were taken). The $v_{p,o}$ vs pH plots showed a bell-shaped profile that was more pronounced as the total buffer concentration increased (Figure 5 bottom). Very similar bell-shaped plots were reported for the reduction of Cr(VI) by thiols such as penicillamine[33] and glutathione[34] in the same pH range. The $v_{p,o}/v_{c,o}$ ratio decreased as the acidity of the medium was raised (Table 3), approaching at low pH the value expected for the noncatalytic oxidation of hydrogen peroxide by Cr(VI) (eq 1). Thus, both the stoichiometric (Figure 2) and kinetic (Table 3) determinations indicate that at high pH Cr(VI) behaves as a catalyst of the dismutation of hydrogen peroxide, whereas at low pH its role is rather that of a reactant (oxidizing agent).

An acceleratory effect caused by the buffer on the disappearance of both reactant Cr(VI) and hydrogen peroxide and on the formation of the violet intermediate was found. At the same total buffer concentration, the acceleratory effect caused by phosphate ions was greater than that caused by acetate ions, and this could be observed for the rates of disappearance of both reactant Cr(VI) (Figure 6 top) and hydrogen peroxide (Figure 6 bottom) and also for the apparent rate of formation of the violet intermediate (Figure 6 top, inset). This agrees with the results reported for the Cr(VI)−thiol reactions.[33,34]

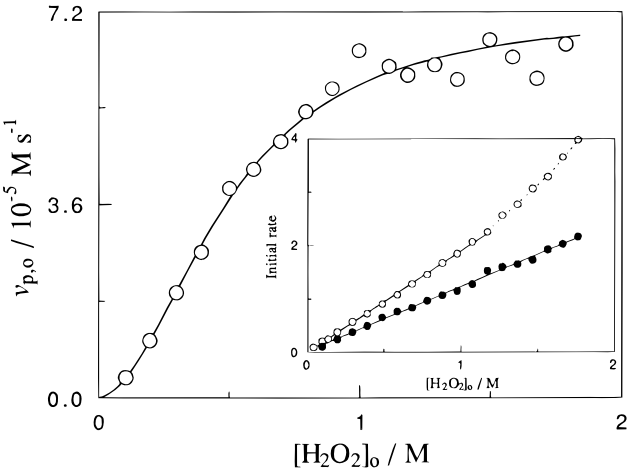


Figure 4. Effect of the initial concentration of H_2O_2 on its own initial rate of disappearance for its reaction with Cr(VI) (2.56×10^{-4} M), in the presence of a KH_2PO_4 (0.060 M)−K_2HPO_4 (0.060 M) buffer at pH 6.71 and 25.2 °C. The solid line is the prediction based on the rate law (eq 4) with $k_p = 3.25 \times 10^{-2}$ M^{-1} s^{-1}, $k'_p = 1.05$ M^{-2} s^{-1}, and $k''_p = 3.77$ M^{-2}. Inset: Effect of the initial concentration of H_2O_2 on the initial values of both the rate of disappearance of Cr(VI) (empty circles, in 10^{-6} M s^{-1}) and the apparent rate of formation of the violet intermediate (filled circles, in 10^{-3} s^{-1}), under the same experimental conditions as the former plot.

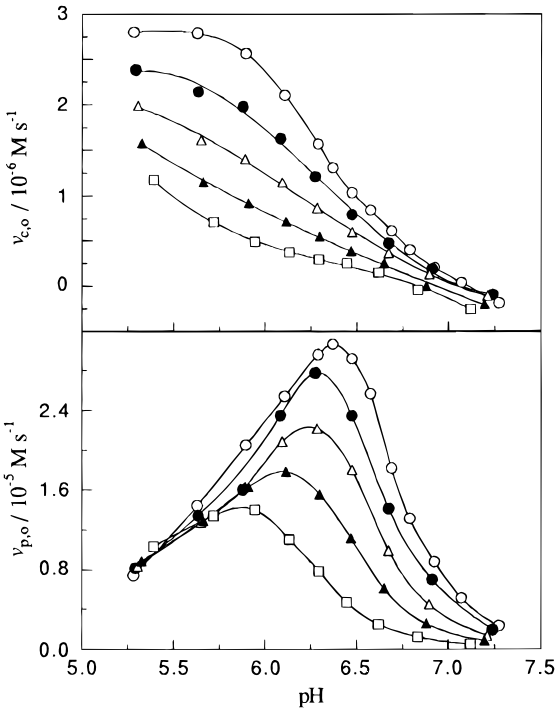


Figure 5. Effect of the pH on the initial rates of disappearance of both Cr(VI) (top) and H_2O_2 (bottom) for the reaction between Cr(VI) (2.56×10^{-4} M) and H_2O_2 (0.196 M) in the presence of a KH_2PO_4−K_2HPO_4 buffer at ionic strength 2.22 M (KNO_3) and 25.2 °C. Total phosphate concentrations: 0.024 M (squares), 0.048 M (filled triangles), 0.072 M (empty triangles), 0.096 M (filled circles), and 0.120 M (empty circles).

TABLE 3: Effect of the pH on the Ratio between the Initial Rates of Disappearance of Hydrogen Peroxide and Chromium(VI)[a]

pH	$v_{p,o}/v_{c,o}$
6.93	42.8 ± 2.0
6.79	32.6 ± 1.7
6.69	29.7 ± 1.5
6.58	30.5 ± 0.5
6.48	28.2 ± 1.4
6.37	23.4 ± 1.2
6.29	18.9 ± 1.0
6.11	12.1 ± 0.5
5.90	8.0 ± 0.2
5.63	5.2 ± 0.4
5.28	2.6 ± 0.3

[a] $[Cr(VI)]_o = 2.56 \times 10^{-4}$ M, $[H_2O_2]_o = 0.196$ M, $[KH_2PO_4] + [K_2HPO_4] = 0.120$ M, ionic strength 2.22M (KNO_3), and 25.2 °C.

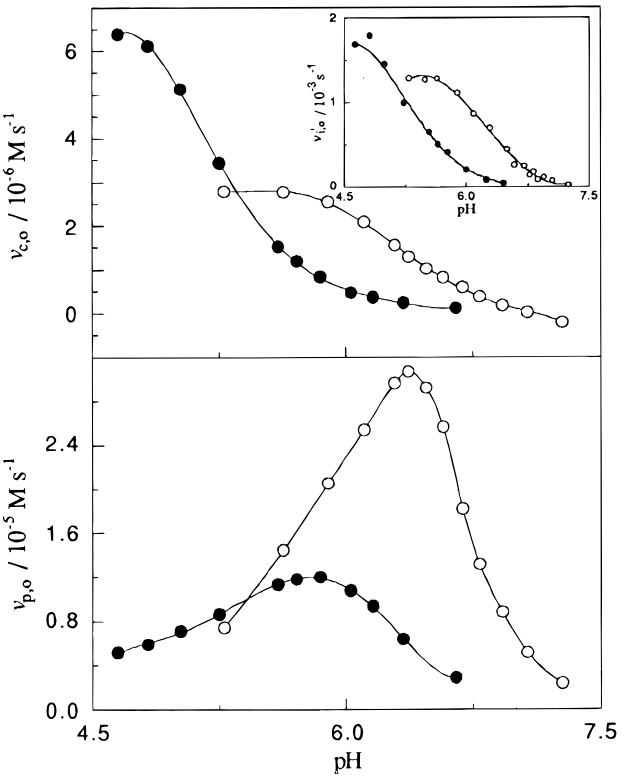


Figure 6. Effect of the pH on the initial values of the rates of disappearance of both Cr(VI) (top) and H_2O_2 (bottom) and of the apparent rate of formation of the violet intermediate (top, inset) for the reaction between Cr(VI) (2.56×10^{-4} M) and H_2O_2 (0.196 M) in the presence of a buffer of total concentration (acid + base) 0.120 M, at ionic strength 2.22 M (KNO_3), and 25.2 °C. Buffers include $KH_2PO_4-K_2HPO_4$ (empty circles) and $CH_3CO_2H-NaCH_3CO_2$ (filled circles).

We have also studied the effect of the total phosphate concentration on the apparent initial rate of formation of the violet intermediate. The $v'_{i,o}$ vs $[\text{phosphate}]_T$ plot showed an upward-concave curvature (Figure 7), and the data could be fitted to a function of the type (Figure 7 inset):

$$v'_{i,o} = a + b[\text{phosphate}]_T^2 \quad (5)$$

When potassium nitrate was added to the solutions in the presence of phosphate buffer, an increase of both $v_{c,o}$ and $v_{p,o}$ was observed (Tables 1S and 2S in the Supporting Information). However, the rate values had to be corrected to discount the effect due to the decrease of pH caused by the change of the equilibrium constant of the buffer. Once corrected, the rate values indicated that an increase of the ionic strength resulted

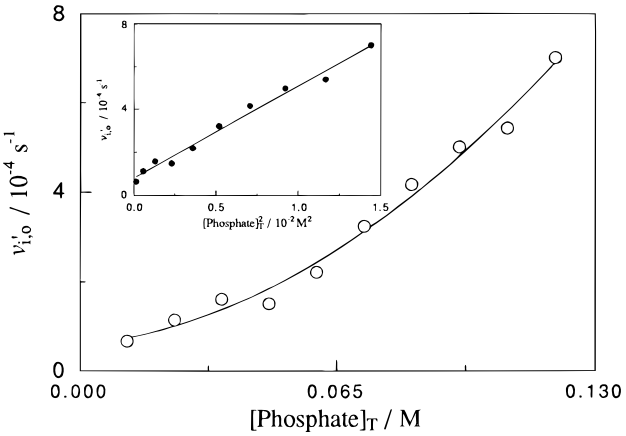


Figure 7. Effect of the total buffer concentration ($[KH_2PO_4] + [K_2HPO_4]$) on the initial value of the apparent rate of formation of the violet intermediate, for the reaction between Cr(VI) (2.56×10^{-4} M) and H_2O_2 (0.196 M) at pH 6.30, ionic strength 2.22 M (KNO_3), and 25.2 °C. Inset: Linearization of the former curve ($v'_{i,o}$ vs $[\text{phosphate}]_T^2$ plot).

in an increase of $v_{c,o}$ and a slight decrease of $v_{p,o}$. An increase of the temperature from 15 to 35 °C had an unusual effect on the reaction, since none of the three initial rates studied ($v_{c,o}$, $v_{p,o}$, and $v'_{i,o}$) fulfilled the Arrhenius law (Figure 3S in the Supporting Information). These results are coherent with the kinetic anomalies reported by Kobozev and Galbreich[35] for the dependence of the Cr(VI)−H_2O_2 reaction rate on temperature.

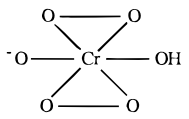
Acidity Equilibrium Constants. We have determined the pK_a values of three different acidic species that, according to our results, have a certain role in the mechanism of the Cr(VI)−H_2O_2 reaction. At ionic strength 2.22 M (KNO_3) and 25.0 °C, the pK_a values found by us for $H_2PO_4^-$, CH_3CO_2H, and $HCrO_4^-$ were 6.29, 4.64, and 5.70, respectively. The first two values were determined by pH measurements of solutions with equal concentrations of the acidic and basic forms of the buffer. The third value was determined from combined spectrophotometric and pH measurements of solutions in the pH range 5.2−7.3, and it is coherent with the pK_a value 5.66 reported for $HCrO_4^-$ at ionic strength 2.10 M (KCl).[34]

Discussion

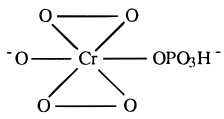
Formation of the Violet Intermediates. It is widely accepted that the Cr(VI)−H_2O_2 reaction in strongly acidic aqueous solutions involves a relatively stable intermediate of well-known structure: the oxodiperoxochromium(VI), $CrO(O_2)_2$.[25] However, this blue species shows an absorption band at 580 nm,[36] and the value reported for its formation equilibrium constant[37] indicates that in neutral or slightly acidic aqueous solutions it is present in a concentration too minute to be directly observed. Thus, the violet intermediate detected in this work and absorbing light at 497 nm must necessarily have a different nature. In fact, our results seem to indicate that two different relatively stable intermediates are involved in the Cr(VI)−H_2O_2 reaction in near-neutrality aqueous solutions. One of them (complex C_1) is formed irrespectively of whether the reaction takes place in the absence or presence of buffer, whereas the other (complex C_2) is formed only when phosphate ions are present in the medium. These complexes might have the structures proposed in Scheme 1.

We can see that both involve a chromium atom in the oxidation state VI and two peroxo groups. Hence, their structures are related to that of $CrO(O_2)_2$. The empirical formula for C_1, $[CrO(O_2)_2OH]^-$, is in accordance with the one proposed by Griffith[38] for the violet intermediate observed by him. On

SCHEME 1



C_1



C_2

the other hand, although we have not found any report on the existence of a complex such as C_2, several complexes of diperoxomolibdenum(VI) with phosphate ligands have been isolated.[39] Actually, according to our findings, provided that phosphate ions are present in the medium, the formation of complex C_2 from the reactants seems to be much more favored than that of C_1. Thus, under the experimental conditions of our work, the greater contribution to the absorption peak observed at 497 nm should be attributed to C_2. In the presence of acetate ions, a third complex similar to C_2 but with an $OCOCH_3$ ligand (instead of OPO_3H^-) is probably involved in the reaction.

For the formation of C_1 we propose the following steps:

$$HCrO_4^- \overset{K_1}{\rightleftharpoons} CrO_4^{2-} + H^+ \quad (6)$$

$$HCrO_4^- + H_2O_2 \xrightarrow[\text{slow}]{k_{II}} Cr(O_2)O_3H^- + H_2O \quad (7)$$

$$Cr(O_2)O_3H^- + H_2O_2 \rightarrow C_1 + H_2O \quad (8)$$

These steps are proposed for the formation of C_2:

$$H_2PO_4^- \overset{K_{III}}{\rightleftharpoons} HPO_4^{2-} + H^+ \quad (9)$$

$$HCrO_4^- + H_2PO_4^- \overset{K_{IV}}{\rightleftharpoons} [HO_3P{-}O{-}CrO_3]^{2-} + H_2O \quad (10)$$

$$H_2O_2 + H_2PO_4^- \overset{K_V}{\rightleftharpoons} H_2O_2{\cdot}H_2PO_4^- \quad (11)$$

$$[HO_3P{-}O{-}CrO_3]^{2-} + H_2O_2{\cdot}H_2PO_4^- \xrightarrow[\text{slow}]{k_{VI}} [CrO_2(O_2)(OPO_3H)]^{2-} + H_2PO_4^- + H_2O \quad (12)$$

$$[CrO_2(O_2)(OPO_3H)]^{2-} + H_2O_2 \rightarrow C_2 + H_2O \quad (13)$$

In both reaction pathways the entrance of the first peroxo group into the Cr(VI) moiety takes place in the rate-determining step, whereas the entrance of the second peroxo group takes place in a subsequent, fast reaction. This is in agreement with the results reported by Funahashi, Uchida, and Tanaka[36] for the formation of $CrO(O_2)_2$ in acidic medium. The existence of the complex $[HO_3P{-}O{-}CrO_3]^{2-}$ (formed in a reversible, fast step) is known.[40,41] The role of the phosphate group in the proposed adduct $H_2O_2{\cdot}H_2PO_4^-$ might be that of increasing the electron density of the peroxo group to facilitate its nucleophilic attack on the chromium atom in the rate-determining step.

According to the mechanism proposed, the overall initial rate of formation of the relatively stable intermediates C_1 and C_2 would be:

$$v_{i,o} = \left\{ k_{II} + \frac{K_{IV}K_V k_{VI}[\text{phosphate}]_T^2[H^+]^2}{(K_{III} + [H^+])^2} \right\} \times \frac{[Cr(VI)]_o[H_2O_2]_o[H^+]}{K_I + [H^+]} \quad (14)$$

where $[Cr(VI)]_o = [HCrO_4^-]_o + [CrO_4^{2-}]_o$, and $[\text{phosphate}]_T = [H_2PO_4^-] + [HPO_4^{2-}]$.

Disappearance of Hydrogen Peroxide. For the conversion of hydrogen peroxide into oxygen in the absence of any buffer, the experimental results found in this work lead us to propose the following mechanism involving two reaction pathways. One of them does not require the formation of any diperoxochromate(VI) intermediate

$$HCrO_4^- + H_2O_2 \xrightarrow[\text{slow}]{k_{VII}} H_2Cr^{V}O_4^- + HO_2^{\bullet} \quad (15)$$

$$H_2Cr^{V}O_4^- + H_2O_2 \rightarrow HCrO_4^- + {}^{\bullet}OH + H_2O \quad (16)$$

whereas the other requires the formation of that intermediate

$$HCrO_4^- + 2H_2O_2 \overset{K_{VIII}}{\rightleftharpoons} C_1 + 2H_2O \quad (17)$$

$$C_1 \xrightarrow[\text{slow}]{k_{IX}} HCrO_4^- + O_2 \quad (18)$$

For the reaction in the presence of phosphate buffer we propose a single pathway:

$$HCrO_4^- + 2H_2O_2 + H_2PO_4^- \overset{K_X}{\rightleftharpoons} C_2 + 3H_2O \quad (19)$$

$$C_2 + OH^- \xrightarrow[\text{slow}]{k_{XI}} [Cr^{V}O(O_2)(OH)(OPO_3H)]^{2-} + O_2^{\bullet-} \quad (20)$$

$$[Cr^{V}O(O_2)(OH)(OPO_3H)]^{2-} + OH^- \rightarrow CrO_4^{2-} + H_2PO_4^- + {}^{\bullet}OH \quad (21)$$

$$[Cr^{V}O(O_2)(OH)(OPO_3H)]^{2-} + H^+ \rightarrow [Cr^{III}(OPO_3H)]^+ + O_2 + 2OH^- \quad (22)$$

Equations 15 and 16 constitute a cycle of a type that is often invoked to explain the catalytic effect that is caused on the decomposition of hydrogen peroxide by substances containing elements (usually metals of the transition series) with two adjacent, easily accessible oxidation states.[8,42] The formation of hydroxyl free radicals from the oxidation of Cr(V) by H_2O_2 is supported by many experimental results.[43−45]

With respect to the two pathways involving the formation of diperoxochromate(VI) complexes, it should be noticed that complexes C_1 and C_2 are now assumed to be in rapid pre-equilibria with the reactants. Effectively, eqs 3 and 4 suggest that, from the point of view of the rates of both the disappearance of Cr(VI) and formation of the violet intermediate, C_1 and C_2 are not in rapid pre-equilibrium with the reagents, whereas, from the point of view of the rate of disappearance of hydrogen peroxide, the rapid pre-equilibrium approximation may be applied. The reason for this peculiar situation may be found in the different time scale for the acquisition of the kinetic data. Whereas for the determination of both $v_{c,o}$ and $v'_{i,o}$ the absorbances of the reacting mixture (at 372 and 497 nm, respectively) were measured only during the first minute of the reaction, $v_{p,o}$ for each kinetic experiment was extrapolated from permanganate titrations performed during a period of at least 40 minutes. Therefore, the spectrophotometric readings allowed us to obtain kinetic data on the rapid formation of the relatively stable intermediates C_1 and C_2, but they were already in quasi-equilibrium with the reactants when the titrations took place.

In our mechanism the diperoxochromate(VI) intermediates are active with respect to the conversion of hydrogen peroxide into oxygen, whereas for other authors[46,47] a monoperoxo

complex was active and the diperoxo complex inactive. In fact, it is known that, under alkaline conditions, the decomposition of complex C_1 yields chromate and oxygen.[48] For the decomposition of the peroxochromate(V) complex formed in eq 20 we have proposed two routes, predominating one in alkaline medium (with the formation of Cr(VI) and hydroxyl free radicals (eq 21)) and the other in acidic medium (with the formation of Cr(III) and oxygen (eq 22)). Actually, a peroxochromate(V) complex is known to yield Cr(VI) by alkaline decomposition and Cr(III) by acidic decomposition.[49,50] Moreover, there are some proofs indicating that under neutral conditions the decomposition of a Cr(V) peroxocomplex yields hydroxyl free radicals,[51] but at pH 1.1 a mixture of Cr(VI) and H_2O_2 is not able to oxidize uracil in conditions under which other transition-metal ions in combination with H_2O_2 can oxidize it,[13] thus suggesting that in very acidic solutions the interaction of Cr(VI) and H_2O_2 does not result in the formation of hydroxyl free radicals. This can easily be accounted for if we consider that, under conditions much more acidic than those used in our work, eq 22 will predominate over eq 21, whereas the Cr(V) formed in eq 15 will presumably behave toward H_2O_2 as an oxidant rather than as a reductant (given that protonation of an oxyanion is known to result in an enhancement of its oxidizing capability), thus leading to the formation of Cr(III) and O_2 rather than to that of Cr(VI) and hydroxyl free radicals.

The rate law for the initial disappearance of hydrogen peroxide that can be deduced from the mechanism proposed matches that found experimentally (eq 4) with

$$k_p = \frac{2k_{VII}[H^+]}{K_I + [H^+]} \tag{23}$$

$$k'_p = 2\left(K_{VIII}k_{IX} + \frac{K_W K_X k_{XI}[\text{phosphate}]_T}{K_{III} + [H^+]}\right)\frac{[H^+]}{K_I + [H^+]} \tag{24}$$

$$k''_p = \left(K_{VIII} + \frac{K_X[\text{phosphate}]_T\,[H^+]}{K_{III} + [H^+]}\right)\frac{[H^+]}{K_I + [H^+]} \tag{25}$$

where K_W stands for the water ionic product.

The mechanism proposed can adequately explain the results found in this work in both the stoichiometric and kinetic experiments. Moreover, the effect attributed by us to phosphate ions is coherent with a recent report by Beck, Nagy, and Szekely,[23] who found that those ions greatly increase the extent and rate of the Cr(VI) → Cr(III) reduction caused by H_2O_2 in weakly acidic medium. It is also interesting to notice that, in all the cases studied by us, the plots obtained for acetate buffer showed a certain displacement toward the lower-pH region with respect to those obtained for phosphate buffer. This is probably due to the difference between the pK_a values for the acidic forms of the two buffers (see above).

Disappearance of Reactant Chromium(VI). Since, according to the mechanism proposed, the only chromium species with a concentration high enough to be taken into account in a matter balance are the reactant Cr(VI), the two relatively stable intermediates C_1 and C_2, and the product Cr(III), we can write the approximate equation:

$$v_{c,o} = v_{i,o} + \left(\frac{d[Cr(III)]}{dt}\right)_{t=0} \tag{26}$$

But, since we have assumed that most of the Cr(III) is formed from the destruction of a relatively stable intermediate (C_2), the initial rate of formation of Cr(III) is expected to be negligible (given that $[C_2]_o = 0$). This is probably true at all experimental conditions except at very low pH, since in very acidic solutions the reduction by H_2O_2 of the Cr(V) formed in eq 15 would represent a likely alternative to its oxidation by H_2O_2 (eq 16), so that Cr(III) might be formed without passing through C_2. Therefore, the initial rate of disappearance of reactant Cr(VI) ($v_{c,o}$) is expected to change with the experimental variables (at all conditions except at very low pH) very much like the apparent initial rate of formation of the relatively stable intermediates ($v'_{i,o}$) does, what is indeed confirmed by the results found in the present work.

Under conditions of pH high enough, we obtained slightly negative values of $v_{c,o}$ (Figure 5 top). This might mean that, under those conditions, the intermediates C_1 and C_2 were in quasi-equilibrium with the reactants (thus having already reached their maximum concentrations) not only when the titrations were done but also when the spectrophotometric readings started to be made. Hence, what we followed under those conditions was the recuperation of free Cr(VI) from C_1 and C_2 caused by the shift of the quasi-equilibria given in eqs 17 and 19 toward the reactant side as H_2O_2 was consumed.

Biological Implications. It is generally accepted that Cr(VI) cannot develop its mutagenic effects unless it is previously activated by its intracellular reaction with appropriate biological compounds.[52] Given that the peptidic thiol glutathione (GSH) is thought to be present in most cells in a concentration (around 10^{-3} M in typical mammalian cells[53]) that is several orders of magnitude higher than that of H_2O_2 ($\leq 10^{-5}$ M at best[54,55]), it seems at first view that the former is more likely the activating agent for Cr(VI) than the latter.

However, given the conditions usually existing in cells (concentrations of both H_2O_2 and $H_2PO_4^-$ lower than those used in our study and physiological pH $\approx$ 7), the results found in this work (Figures 1 and 2) predict that Cr(VI) *behaves toward H_2O_2 inside the cell as a catalyst* rather than as an oxidizing agent, since, under those conditions, the predominant reaction pathway is expected to be the one constituted by eqs 15 and 16. This means that, provided that Cr(VI) and H_2O_2 can encounter each other in the cellular medium, each Cr(VI) ion would be capable of reacting with a very high number of H_2O_2 molecules, yielding a considerable amount of hydroxyl free radicals without being reduced in the process. This might presumably overcome the initial advantage of GSH as the predominant activating agent for Cr(VI) because of its higher concentration, given that GSH reduces Cr(VI) to Cr(III) in an stoichiometric manner.[34] Those $^{\bullet}$OH radicals (formed in eq 16) might either oxidize some DNA residues (such as guanosine) or produce extensive DNA strand breakage.[52]

Actually, some in vivo experiments showed that the toxicity of Cr(VI) in bacterial cells increased when the concentration of catalase (enzyme involved in the elimination of H_2O_2) decreased,[54] and also that a decrease of the cellular concentration of H_2O_2 in mammalian cells led to a decrease of DNA single-strand breaks, in spite of the fact that the concentration of GSH increased simultaneously.[56] In addition, some very recent in vitro experiments with isolated DNA indicated that Cr(V) (an intermediate known to be formed in the cellular metabolism of Cr(VI) and often invoked as a likely mutagenic agent[57]) is not directly involved in DNA damage unless previously activated by oxygen, and that the damage could be suppressed by the addition of catalase.[58] All these results suggest that hydrogen peroxide might have a significant role in the expression of the toxic and mutagenic effects of chromium(VI).

We have monitored the decay of absorbance at 372 nm for the Cr(VI)−H_2O_2 system both in the absence and presence of

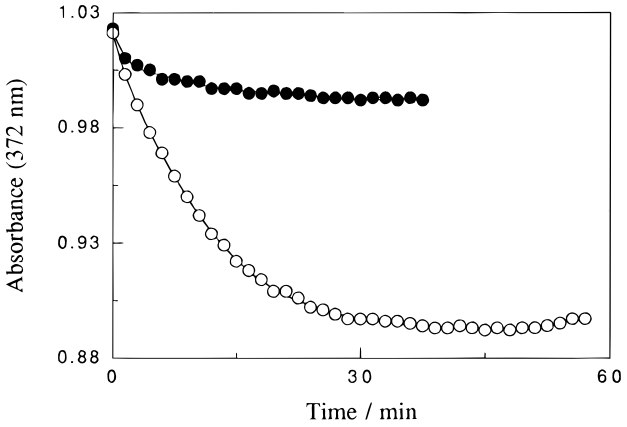


Figure 8. Absorbance at 372 nm vs time plots for the reaction between Cr(VI) (2.56×10^{-4} M) and H_2O_2 (0.395 M) in the presence of a KH_2PO_4 (0.012 M)−K_2HPO_4 (0.012 M) buffer, at pH 6.88 and 25.2 °C. [Adenine] = 0 (filled circles) and 3.60×10^{-4} M (empty circles).

adenine, all the other experimental conditions remaining unaltered (Figure 8). Under those conditions ($[KH_2PO_4] = [K_2HPO_4] = 0.012$ M), and in both cases, the absorbance first decreased, passed through a minimum, and then started to increase slowly till finally (several days later) reaching a value close to the initial one. However, the decrease of absorbance was much more pronounced when adenine was present in the solution (even at a concentration so low as 3.60×10^{-4} M), and the UV−vis spectra indicated that at the end of the reaction both H_2O_2 and adenine had been totally consumed whereas all the initial Cr(VI) was recuperated unchanged. On the contrary, when the concentration of the phosphate buffer was high enough ($[KH_2PO_4] = [K_2HPO_4] = 0.060$ M) for Cr(VI) being completely reduced to Cr(III) instead of behaving as a catalyst, no noticeable change in the absorbance decay was provoked by the presence of adenine in the solution.

These results seem to suggest that adenine is capable of stabilizing temporarily some peroxochromium intermediate complex, and that the adenine molecule is eventually destroyed in the process. Actually, various nitrogen-containing donors such as diethylentriamine are known to be capable of acting as stabilizing ligands for the diperoxochromium(IV) complex, $Cr(O_2)_2$.[59] On the other hand, Bianchi and co-workers have reported that after the entrance of Cr(VI) into the cell (hamster fibroblasts) an enhancement of the conversion of adenine into hypoxanthine can be observed.[60] A possible explanation is that the Cr(VI)−H_2O_2 system might catalyze the well-known hydrolysis of adenine into hypoxanthine and ammonia, probably through a peroxochromium−adenine intermediate complex, thus provoking the appearance of some DNA lesions.[61] If those lesions are not repaired by the action of the enzyme hypoxanthine-DNA glycosylase, during the DNA replication the abnormal base hypoxanthine (HX) would behave as a fake guanine (G), coupling with cytosine (C) instead of with thymine (T) as the original base adenine (A) would do, thus resulting (after two consecutive DNA replications) in an A·T → G·C transition mutation.[62] If confirmed by future work, this might certainly represent one of the possible routes for the development of chromium-induced cancer.

Supporting Information Available: Tables of the effect of the ionic strength and figures of the visible spectra of both the chromium(III)−phosphate complex and the violet intermediate and the effect of the temperature (5 pages). Ordering information is given on any current masthead page.

References and Notes

(1) O'Brien, P.; Wang, G. *Polyhedron* **1993**, *12*, 1409.
(2) Arslan, P.; Beltrame, M.; Tomasi, A. *Biochim. Biophys. Acta* **1987**, *931*, 10.
(3) Goodgame, D. M. L.; Joy, A. M. *Inorg. Chim. Acta* **1987**, *135*, L5.
(4) O'Brien, P.; Wang, G. *J. Chem. Soc., Chem. Commun.* **1992**, 690.
(5) Connett, P. H.; Wetterhahn, K. E. *J. Am. Chem. Soc.* **1985**, *107*, 4282.
(6) Moghaddas, S.; Gelerinter, E.; Bose, R. N. *J. Inorg. Biochem.* **1995**, *57*, 135.
(7) Sagripanti, J. L.; Kraemer, K. H. *J. Biol. Chem.* **1989**, *264*, 1729.
(8) Barb, W. G.; Baxendale, J. H.; George, P.; Hargrave, K. R. *Trans. Faraday Soc.* **1951**, *47*, 462, 591.
(9) Fridovich, I. *Acc. Chem. Res.* **1972**, *5*, 321.
(10) Sawyer, D. T.; Valentine, J. S. *Acc. Chem. Res.* **1981**, *14*, 393.
(11) Ghosh, S. K.; Saha, S. K.; Ghosh, M. C.; Bose, R. N.; Reed, J. W.; Gould, E. S. *Inorg. Chem.* **1992**, *31*, 3358.
(12) Masarwa, M.; Cohen, H.; Meyerstein, D.; Hickman, D. L.; Bakac, A.; Espenson, J. H. *J. Am. Chem. Soc.* **1988**, *110*, 4293.
(13) Conte, V.; Di Furia, F.; Moro, S. *Gazz. Chim. Ital.* **1995**, *125*, 563.
(14) Vieira, A. J. S. C.; Steenken, S. *J. Am. Chem. Soc.* **1990**, *112*, 6986.
(15) Brauer, S. L.; Wetterhahn, K. E. *J. Am. Chem. Soc.* **1991**, *113*, 3001.
(16) O'Brien, P.; Wang, G.; Wyatt, P. B. *Polyhedron* **1992**, *11*, 3211.
(17) Perez-Benito, J. F.; Arias, C.; Lamrhari, D. *New J. Chem.* **1994**, *18*, 663.
(18) Wolf, T.; Bolt, H. M.; Ottenwaelder, H. *Toxicol. Lett.* **1989**, *47*, 295.
(19) Aiyar, J.; Berkovits, H. J.; Floyd, R. A.; Wetterhahn, K. E. *Environ. Health Perspect.* **1991**, *92*, 53.
(20) Luo, H.; Lu, Y.; Shi, X.; Mao, Y.; Dalal, N. S. *Ann. Clin. Lab. Sci.* **1996**, *26*, 185.
(21) Baxendale, J. H. *Adv. Catal.* **1952**, *4*, 31.
(22) Brown, S. B.; Jones, P.; Suggett, A. *Prog. Inorg. Chem.* **1970**, *13*, 159.
(23) Beck, M. T.; Nagy, I. P.; Szekely, G. *Int. J. Chem. Kinet.* **1991**, *23*, 881.
(24) Bogdanov, G. A.; Petrova, G. L. *Zh. Fiz. Khim.* **1955**, *29*, 84.
(25) Evans, D. F. *J. Chem. Soc.* **1957**, 4013.
(26) Morrow, J. I.; Pinkowitz, R. A.; Laufer, J. *Inorg. Chem.* **1966**, *5*, 934.
(27) Orhanovic, M.; Wilkins, R. G. *J. Am. Chem. Soc.* **1967**, *89*, 278.
(28) Ranganathan, C. K.; Ramasami, T.; Ramaswamy, D.; Santappa, M. *Inorg. Chem.* **1989**, *28*, 1306.
(29) O'Brien, P.; Ozolins, Z.; Wang, G. *Inorg. Chim. Acta* **1989**, *166*, 301.
(30) Adams, A. C.; Crook, J. R.; Bockhoff, F.; King, E. L. *J. Am. Chem. Soc.* **1968**, *90*, 5761.
(31) Cotton, F. A.; Wilkinson, G.; Gaus, P. L. *Basic Inorganic Chemistry*; Wiley: New York, 1995; p 558.
(32) Kobozev, N. I.; Galbreich, A. *Acta Physicochim. URSS* **1945**, *20*, 479.
(33) Perez-Benito, J. F.; Lamrhari, D.; Arias, C. *Can. J. Chem.* **1994**, *72*, 1637.
(34) Perez-Benito, J. F.; Lamrhari, D.; Arias, C. *J. Phys. Chem.* **1994**, *98*, 12621.
(35) Kobozev, N. I.; Galbreich, A. *J. Phys. Chem. (USSR)* **1940**, *14*, 1550.
(36) Funahashi, S.; Uchida, F.; Tanaka, M. *Inorg. Chem.* **1978**, *17*, 2784.
(37) Al-Ajlouni, A. M.; Espenson, J. H.; Bakac, A. *Inorg. Chem.* **1993**, *32*, 3162.
(38) Griffith, W. P. *J. Chem. Soc.* **1962**, 3948.
(39) Fields, E. K. *J. Am. Chem. Soc.* **1958**, *80*, 2358.
(40) Holloway, F. *J. Am. Chem. Soc.* **1952**, *74*, 224.
(41) Frennesson, S. A.; Beattie, J. K.; Haight, G. P. *J. Am. Chem. Soc.* **1968**, *90*, 6018.
(42) Yamamoto, K.; Kawanishi, S. *J. Biol. Chem.* **1989**, *264*, 15435.
(43) Shi, X.; Dalal, N. S. *Biochem. Biophys. Res. Commun.* **1989**, *163*, 627; *Free Radical Res. Commun.* **1990**, *10*, 17; *Arch. Biochem. Biophys.* **1990**, *277*, 342, *281*, 90.
(44) Shi, X.; Dalal, N. S.; Vallyathan, V. *Arch. Biochem. Biophys.* **1991**, *290*, 381.
(45) Shi, X.; Sun, X.; Gannett, P. M.; Dalal, N. S. *Arch. Biochem. Biophys.* **1992**, *293*, 281.
(46) Haggett, M. L.; Jones, P.; Wynne-Jones, W. F. K. *Discuss. Faraday Soc.* **1960**, 153.
(47) Flood, P.; Lewis, T. J.; Richards, D. H. *J. Chem. Soc.* **1963**, 2446.
(48) Connor, J. A.; Ebsworth, E. A. V. *Adv. Inorg. Chem. Radiochem.* **1964**, *6*, 279.
(49) Quane, D.; Earley, J. E. *J. Am. Chem. Soc.* **1965**, *87*, 3823.
(50) Brown, S. B.; Edwards, J. O.; Herman, I. J.; Jones, P.; Mills, J. R.; Earley, J. E. *Inorg. Chim. Acta* **1969**, *3*, 351.

(51) Aiyar, J.; Berkovits, H. J.; Floyd, R. A.; Wetterhahn, K. E. *Chem. Res. Toxicol.* **1990**, *3*, 595.
(52) Aiyar, J.; Borges, K. M.; Floyd, R. A.; Wetterhahn, K. E. *Toxicol. Environ. Chem.* **1989**, *22*, 135.
(53) O'Brien, P.; Barrett, J.; Swanson, F. *Inorg. Chim. Acta* **1985**, *108*, L19.
(54) Itoh, M.; Nakamura, M.; Suzuki, T.; Kawai, K.; Horitsu, H.; Takamizawa, K. *J. Biochem.* **1995**, *117*, 780.
(55) Prütz, W. A. *J. Biochem. Biophys. Methods* **1996**, *32*, 125.
(56) Sugiyama, M.; Tsuzuki, K.; Haramaki, N. *Mutat. Res.* **1993**, *299*, 95.
(57) Kortenkamp, A.; Ozolins, Z.; Beyersmann, D.; O'Brien, P. *Mutat. Res.* **1989**, *216*, 19.
(58) Kortenkamp, A.; Casadevali, M.; Cruz, P. *Ann. Clin. Lab. Sci.* **1996**, *26*, 160.
(59) Ghosh, S. K.; Gould, E. S. *Inorg. Chem.* **1989**, *28*, 3651.
(60) Bianchi, V.; Debetto, P.; Zantedeschi, A.; Levis, A. G. *Toxicology* **1982**, *25*, 19.
(61) Singer, B.; Kusmierek, J. T. *Ann. Rev. Biochem.* **1982**, *52*, 655.
(62) Karran, P.; Lindahl, T. *Biochemistry* **1980**, *19*, 6005.