Table I. Heme Marker Band Frequencies

	cm^{-1}		
	III (dp)[a]	IV (ap)[a]	V (dp)[a]
COHb[b]		1584	1631
deoxy-Hb[c]	1546	1552	1607
(Im)$_2$Fe^{II}(PP)[d]	1534	1583	1617
Fe^{II}(MP)[e]	1570	1589	1642
COHb CARS[f]	1549	1559	1609

[a] Band numbering from ref 15; dp = depolarized, ap = anomalously polarized. [b] From ref 11. [c] From ref 8. [d] Bis(imidazole) ferrous protoporphyrin IX (planar, low spin); from ref 15. [e] Ferrous mesoporphyrin IX dimethyl ester (four coordinate, intermediate spin); from ref 15. [f] This work; error in frequencies estimated as ±3 cm^{-1}.

using the known absorptivities and dissociation quantum yield.[12] It is not possible to lower the laser energies sufficiently to reduce photolysis appreciably without losing the CARS signal (which varies linearly with the ω_2 power and as the square of the ω_1 power). The photolyzed COHb molecules have ample time to recombine in the 100 ms between the laser pulses as calculated using the second-order rate constant of 4.5 × 10^6 M^{-1} s^{-1}.[13] In order to ensure that our spectra were not artifacts due to changes which accumulate with repeated laser pulses, we redetermined them with the COHb solution circulating in a flow cell, with the estimated beam transit time being 20 ms. Although the signal/noise ratio deteriorated slightly, the spectral features were unaltered.

It is of interest that the CARS spectra show no evidence of peaks at the positions expected if the iron atom had stayed in the heme plane for a time comparable with 6 ns following the CO dissociation. The basis for this assertion is as follows. It is known that iron(II) tetraphenylporphyrin, without any axial ligands, exists in an intermediate spin, $S = 1$, ground state.[14] The iron atom is in the porphyrin plane and the iron–nitrogen bonds are short,[14] consistent with an empty d$_{x^2-y^2}$ orbital; the six iron(II) valence electrons occupy the four remaining d orbitals, with two electrons remaining unpaired. The resonance Raman spectrum of iron(II) mesoporphyrin,[15] a close analogue of the protoporphyrin contained in Hb, is consistent with this picture. The three bands in the iron(II) mesoporphyrin of interest to this study are found at 1589 (ap) and at 1642 and 1570 cm^{-1} (dp). When two imidazole ligands are bound to iron(II) protoporphyrin, the complex is low spin ($S = 0$) and the bands are at 1583 (ap) and 1617 and 1534 (dp). If CO dissociation from COHb leaves the iron atom transiently in the heme plane, with one axial imidazole ligand, the exact spin state is uncertain. However, the Raman frequencies can confidently be expected to be somewhere between those given above for four- and six-coordinate iron(II) heme (Table I summarizes the marker band frequencies expected for the various geometries along with the CARS data we have obtained). In particular, the ap band is expected at 1583–1589 cm^{-1} and the high frequency dp band is expected at 1617–1642 cm^{-1} for in-plane iron. As these bands are absent in our CARS spectra (see Figure 1), we conclude that the iron atom does not remain in the heme plane for an appreciable fraction of 6 ns.

Alpert et al.[16] found an optical transient with a half-life of ~50 ns following laser photolysis of COHb which they suggested may be associated with a tilt of the porphyrin rings in the globin pocket. They rejected the possibility that the transient was associated with the iron out-of-plane displacement on the grounds that protein-free CO heme did not give the optical transient. This inference is open to the objection that the presence of protein restraints might slow down the iron displacement, but the present result confirms that the iron atom is out of the heme plane long before 50 ns. The photodissociation itself is very fast for COHb. Shank et al.[17] reported at a time constant of <0.5 ps for this process, while Rentzepis

and co-workers measured a value of 11 ps. Conceivably both CO dissociation and iron displacement could occur in a concerted process. Picosecond resonance Raman experiments will be needed to test this possibility.

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R. F. Dallinger, J. R. Nestor, T. G. Spiro*
Department of Chemistry, Princeton University
Princeton, New Jersey 08540

On the "Uncatalyzed" Oxidation of Sulfur(IV) in Aqueous Solutions

Sir:

Despite numerous reports on the rates of the uncatalyzed oxidations of aqueous sulfur(IV) solutions, we now report that the reactions are in fact trace-metal catalyzed. Using small amounts of chelating agents, EDTA and 1,10-phenanthroline, to complex trace metal ions, we have reduced the "uncatalyzed" oxidation rate up to four orders of magnitude at high pH and by a factor of 20 at low pH. We suggest that an analogous technique be used to test the degree to which other autoxidations, previously accepted as uncatalyzed, may be trace-metal catalyzed.

The oxidation of aqueous sulfur(IV) solutions to sulfate by molecular oxygen has attracted widespread attention since the early work of Bigelow,[1] with many studies[2-8] concentrating largely on the kinetics of the previously assumed uncatalyzed reactions. There have been, however, major discrepancies in the reported kinetic parameters despite concerted efforts to eliminate contamination of the reactants from trace impurities. Owing to the extreme susceptibility of the oxidation reaction to catalysis by certain transition metal ions (Cu^{II} concentrations as low as 10^{-8} M have been reported as significantly affecting the sulfite reaction[2]), it appears that the reported anomalies may merely have been due to the varying levels of trace-metal impurities in the water and reactants used in the different investigations.

Rather than trying to achieve lower impurity levels than those in the previous studies, we masked the contribution to the reaction from trace-metal impurities by complexing with an effective chelating agent. EDTA was chosen in studying the

Table I. Effect of EDTA Chelation of the Apparent Uncatalyzed Oxidation of Aqueous Sulfite Solutions

solution A[a]		solution B[c]	
EDTA, M	rate, 10^{-5} M/min	EDTA, M	rate, 10^{-5} M/min
0.0	149	0.0	$(14\,700)^d$
1.0×10^{-8}	65	5.0×10^{-7}	23
5.0×10^{-8}	35	1.0×10^{-6}	3.3
1.0×10^{-7}	26	5.0×10^{-6}	1.5
1.0×10^{-6}	0.2	1.0×10^{-4}	0.2

[a] 0.003 M Na_2SO_3 (6×10^{-8} M Cu impurity *[b]* by atomic absorption analysis. *[b]* Reference 9. *[c]* 0.02 M Na_2SO_3 ($2.3 \times$ Cu impurity*[b]* by atomic absorption analysis. *[d]* Calculated from the Cu analysis plus the data of Mishra and Srivastava.[9]

Table II. Effect of 1,10-Phenanthroline on the Apparent Uncatalyzed Oxidation of Aqueous Sulfur Dioxide Solutions

1,10-phenanthroline, 10^{-5} M	reaction rate, 10^{-7} M/min	
	$[S(IV)]_0$, 0.050 M^a	$[S(IV)]_0$, 0.015 M^a
0.0	68.1	10.5
0.05		10.4
1.0	35.4	
5.0		4.98
10.0	10.7	
100.0	3.45	1.98

[a] 5×10^{-7} M Fe impurity*[b]* by atomic absorption analysis. *[b]* Reference 10.

sulfite oxidation since it is most effective in the high pH range, where it acts as a sexadentate ligand, occupying all the coordination sites of the complexed metal ion. 1,10-Phenanthroline was chosen in the low pH sulfur dioxide reaction since, unlike EDTA, its chelating effectiveness is not significantly reduced by acidity. It is also important to note that both chelating agents used are not capable of acting as an oxidation inhibitor in the classic sense of terminating free-radical chains. The effects of EDTA and 1,10-phenanthroline were investigated at 25 °C in a quartz Morton reactor modified to facilitate oxygen mass transfer and allow for rapid sample withdrawal. The kinetics were established by monitoring the sulfur(IV) concentration iodometrically as a function of time. The high pH (9.2–9.4) sulfite solutions were prepared using sodium sulfite salt while the low pH (1.65–1.95) sulfur dioxide solutions were prepared by sparging SO_2 gas into solution.

By using two separate sources of highly purified water, denoted as A and B, high pH kinetic studies were performed on two sets of sulfite solutions of significantly differing levels of trace-metal impurities. Atomic absorption analysis (Jarrell Ash Model 810 spectrophotometer) detected Cu and Fe impurities of 6×10^{-8} and 8×10^{-9} M, respectively, in water source A with corresponding impurity levels of 2.3×10^{-7} and 5×10^{-7} M in source B. Co and Mn, the other potentially active catalysts, could not be detected within the limits of analysis ($\sim 1 \times 10^{-8}$ M) in either water supply. Kinetic studies of the low pH reaction were all performed using deionized water from source B.

The effect of EDTA concentration on the rate of the apparent uncatalyzed sulfite oxidation is shown in Table I. Note that for both solutions studied very marked reductions in the rate were observed at relatively low EDTA concentrations, with the rate reduced over four orders of magnitude in solution B at an EDTA concentration of 1.0×10^{-4} M. Also note that the reaction in solution B, in the absence of EDTA, was mass-transfer limited. The rate quoted in Table I is quite approximate and has been estimated from the known Cu impurity level and extrapolation of the kinetic data of Mishra and Srivastava.[9]

The effect of 1,10-phenanthroline on the apparent uncatalyzed oxidation rate of low pH sulfur dioxide solutions is shown in Table II. Note that, although the effects are much less marked than those observed in the high pH reaction, the apparent uncatalyzed oxidation rate is substantially reduced by the addition of 1,10-phenanthroline which complexes the iron impurities in solution.[10]

These results clearly suggest that the oxidations of both high pH sulfite and low pH sulfur dioxide solutions, previously accepted as uncatalyzed, are in fact due primarily to trace-metal impurities acting as catalysts. Note, however, that measurable reaction rates were still obtained in the presence of excess chelating agent in both the high and low pH solutions, suggesting that the reaction may also proceed by some parallel

thermal or photoinitiated reaction, but at a greatly reduced rate. It should also be noted that, although this is the first such evidence reported on the oxidation of sulfur(IV) solutions, other studies[11,12] have revealed trace-metal catalysis in certain previously accepted uncatalyzed hydrocarbon autoxidations.

The conclusions drawn from the high pH studies were further supported by a study of the comparative efficiencies of various aromatic free-radical inhibitors (phenol, hydroquinone, resorcinol, phloroglucinol, pyrogallol, and pyrocatechol) on both the Cu-catalyzed and apparent uncatalyzed reactions. Pyrocatechol, which is capable of chelating transition metal ions, was found to have a markedly greater inhibiting influence than the other inhibitors which have little or no complexing abilities.

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Albin Huss, Jr., Phooi Kong Lim, C. A. Eckert*
Department of Chemical Engineering
University of Illinois, Urbana, Illinois 61801

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Role of Anhydrovinblastine in the Biosynthesis of the Antitumor Dimeric Indole Alkaloids

Sir:

For the past decade numerous attempts have been made in this laboratory[1] to establish the identity of those monomeric indole alkaloids which serve as the building blocks for the antitumor alkaloids of *Catharanthus roseus,* exemplified by vinblastine (VLB, **1**), vincristine (VCR, **2**), leurosine (**3**), and leurosidine (**4**).[2] Thus, while vindoline (**5**) appears to serve as a satisfactory, intact precursor for the *Aspidosperma* segment of **1** (0.05% incorporation[1]), the derivation of the "iboga-like"