Volume 28
Number 2
January 25, 1989

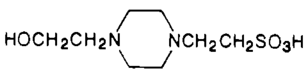
Inorganic Chemistry

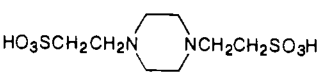
Communications

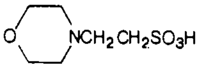
Oxidation of Tertiary Amine Buffers by Copper(II)

Sir:

The problem of metal ion complexation and precipitation inherent in the use of the common inorganic buffers carbonate and phosphate has led to the widespread use of noncoordinating organic buffers such as HEPES, PIPES, MES, and related analogues.[1,2]


HEPES


PIPES


MES

A study appeared recently[3] which cautioned against the danger that HEPES could be oxidized by Cu(II), especially when the latter bore a high oxidation potential such as in (batho)$_2$Cu$^{\text{II}}$ ($E°$ = 0.62 V vs NHE compared to 0.167 V for the aquo ion,[4] batho = 2,9-dimethyl-4,7-diphenyl-1,10-phenanthrolinedisulfonate). This study interpreted the oxidation in terms of oxidation of the primary alcohol group of HEPES and reported a similar oxidation of the structurally related alcohol *N*,*N*-dimethylethanolamine (DMEA). The fact that simple alcohols (methanol, ethanol, 2-propanol, glycerol) were not oxidized was rationalized in terms of a prerequisite complex formation with Cu(II) that occurred for HEPES and DMEA via coordination with the amino nitrogen.[5] Curiously, Tris, tris(hydroxymethyl)aminomethane, which was acknowledged to be capable of complexing metal ions, failed to be oxidized.

We have been studying the mechanism of copper-mediated oxidation of nitrogen-containing compounds, and the reported reaction of HEPES and DMEA with (batho)$_2$Cu$^{\text{II}}$ appeared to us to represent tertiary amine oxidation rather than alcohol oxidation. In the present study, we have clarified and reinterpreted the characteristics of the earlier reported oxidations. Most importantly, we demonstrate that *the oxidations of HEPES and DMEA are examples of general amine oxidation chemistry and that other non-alcoholic tertiary amine buffers such as PIPES and MES are similarly subject to oxidation* by (batho)$_2$Cu$^{\text{II}}$.

The earlier study on (batho)$_2$Cu$^{\text{II}}$ oxidations[3] made the following claims. (1) The rate is first order in HEPES and second order in Cu(II). (2) The pH rate dependence displays a linear increase of log (rate) with increasing pH at low pH (pH < pK_a), which plateaus at higher pH ($\geq$pK_a). (3) The reaction is inhibited by the strong chelating agents NTA (nitrilotriacetic acid) and EDTA (ethylenediaminetetraacetic acid). (4) (phen)$_3$Fe$^{\text{III}}$ (phen = 1,10-phenanthroline) does not oxidize HEPES even though it is a stronger oxidizing agent ($E°$ = 1.1 V vs NHE[6]) than (batho)$_2$Cu$^{\text{II}}$.

In interpreting the observed reactions of HEPES and DMEA as alcohol oxidations, the previous workers rationalized the second-order dependence in Cu(II) and observed pH rate profile in terms of rapid preassociation of the substrate with one Cu(II)–batho,[5] which required an unprotonated amino nitrogen, and subsequent rate-limiting oxidation by a second Cu(II). The claimed unreactivity of (phen)$_3$Fe$^{\text{III}}$ was then attributed to the recognized low affinity of Fe(III) for aliphatic amines.

Our results[7] run contrary to many of the conclusions reached in the previous study. First, we find that the rate is strictly *first order in Cu(II)*. This can be seen from the observed invariance of $t_{1/2}$ with [Cu(II)] in Table I (second order in Cu(II) requires that $t_{1/2}$ vary inversely with [Cu(II)]). The previously claimed second-order dependence in Cu(II) was based only on the apparent linearity of the pseudo-second-order kinetic plot (carried out to 50% reaction). Actually, when we plotted our individual kinetic runs according to first- and second-order behavior, both plots were fairly linear up to 50% reaction (though the former still exhibited a better linear fit, especially beyond 50% reaction).[8] Notwithstanding, it is the dependence of $t_{1/2}$ on [Cu(II)] that provides an unambiguous assessment of the true kinetic order.

Second, the reaction is an *amine oxidation*, not an alcohol oxidation, as would be expected on the basis of the lower ionization potential of nitrogen vs that of oxygen. Although product identity does not permit such distinction,[10] our designation of the reaction as an amine oxidation is clearly demonstrated (Table II) by (i) the nearly identical oxidation rates of DMEA and the corre-

---

(1) Good, N. E.; Winget, G. D.; Winter, W.; Connolly, T. N.; Izawa, S.; Singh, R. M. M. *Biochemistry* **1966**, *5*, 467.

(2) Ferguson, W. J.; Braunschweiger, K. I.; Braunschweiger, W. R.; Smith, J. R.; McCormick, J. J.; Wasmann, C. C.; Jarvis, N. P.; Bell, D. H.; Good, N. E. *Anal. Biochem.* **1980**, *104*, 300.

(3) Hegetschweiler, K.; Saltman, P. *Inorg. Chem.* **1986**, *25*, 107.

(4) Lappin, A. G.; Youngblood, M. P.; Margerum, D. W. *Inorg. Chem.* **1980**, *19* 407.

(5) (batho)$_2$Cu$^{\text{II}}$ is known to form pentacoordinate species in solution: Al-Shatti, N.; Lappin, A. G.; Sykes, A. G. *Inorg. Chem.* **1981**, *20*, 1466.

(6) Moore, G. R.; Williams, R. J. P. *Coord. Chem. Rev.* **1976**, *18*, 125.

(7) Reactions were carried out at 25.0 °C as done previously,[3] with use of a 10-fold excess of [batho] over [CuSO$_4$], both at low concentration compared to that of the organic substrate, and monitored through measurement of ΔA_{483}. Pseudo-first-order plots were linear to >75% reaction. The numbers listed in Tables I–III represent the average of two runs in most cases. The buffers used were of the highest purity available commercially (Aldrich), and all amines were fractionally distilled under N$_2$ shortly before use.

(8) In order to avoid the possible occurrence of "special" salt effects such as those observed in ferricyanide oxidation of amines,[9] most of our data were obtained in the absence of ancillary salt used by the previous workers to maintain constant ionic strength (1 M NaCl). However, the kinetic order in Cu(II) was not changed upon switching to their identical conditions (Table I, entry 4).

(9) Audeh, C. A.; Lindsay Smith, J. R. *J. Chem. Soc. B* **1970**, 1280.

(10) For example, DMEA undergoes an overall four-electron oxidation to glyoxal and dimethylamine. Consistent mechanisms can be written involving either oxidative N-dealkylation or alcohol oxidation as the initial step, followed by the expected rapid oxidation of the resulting glycolaldehyde or dimethylaminoacetaldehyde, respectively. In the case of HEPES, which transfers a total of six electrons,[3] a similar product-based mechanistic ambiguity is expected.

0020-1669/89/1328-0169$01.50/0 © 1989 American Chemical Society

Table I. Effect of [Cu(II)] on the Rate of Amine Oxidations by $(batho)_2Cu^{II}$

substrate	pH	$t_{1/2}$, min			10^4k_{obsd},[c] s^{-1}
		$[Cu^{II}]_0$ = 0.025 mM	$[Cu^{II}]_0$ = 0.10 mM	$[Cu^{II}]_0$ = 0.25 mM	
$(CH_3)_2NCH_2CH_2OH$	8.1[a]	19.4	19.5	20.0	5.88
HEPES	8.1[a]	4.6	4.6	4.4	25.3
HEPES	6.8[a]	20.0	19.8	19.7	5.82
HEPES	6.8[b]	22.0	22.0	24.0	5.10

[a] $[substrate]_0$ = 0.05 M, [batho] = 2.5 mM, $[KH_2PO_4]$ = 0.10 M adjusted to the required pH with KOH, 25 °C. [b] Same conditions except no phosphate buffer and [NaCl] = 1.0 M. [c] Average for three $[Cu(II)]_0$ concentrations.

Table II. Effect of Structure on the Rate of $(batho)_2Cu^{II}$ Oxidations[a]

compd	10^4k_{obsd}, s^{-1}	compd	10^4k_{obsd}, s^{-1}
$(CH_3)_2NCH_2CH_2OH$	6.0	$H_2NCH_2CH_2OH$	0.027
$(CH_3)_2NCH_2CH_2OCH_3$	4.8	$(C_5H_4N)CH_2CH_2OH$[b]	0.014
$CH_3NHCH_2CH_2OH$	1.8		

[a] $[substrate]_0$ = 0.05 M, $[Cu(II)]_0$ = 0.25 mM, [batho] = 2.5 mM, $[KH_2PO_4]$ = 0.10 M adjusted to pH 8.1 with KOH, 25.0 °C. [b] 2-(2-Hydroxyethyl)pyridine.

Table III. Effect of pH on the Rate of Oxidation of Buffers by $(batho)_2Cu^{II}$

	pK_a[b]	10^4k_{obsd},[a] s^{-1}			
		pH = 5.5	pH = 6.8	pH = 8.1	pH = 10.7
MES	6.15	0.029	0.13	0.27	0.020
PIPES	6.80	0.22	2.7	5.5	0.11
HEPES	7.55	0.29	5.9	25.0	0.91
HEPES[c]	7.55		5.1		0.28
HEPES[d]	7.55		5.9		1.9

[a] $[substrate]_0$ = 0.05 M, $[Cu(II)]_0$ = 0.25 mM, [batho] = 2.5 mM, $[KH_2PO_4]$ = 0.10 M adjusted to the required pH with KOH, 25.0 °C. [b] Sigma Chemical Co. 1988 catalog, p 313. [c] Same as above except [batho] = 1.0 mM. [d] Same as above except [batho] = 5.0 mM.

sponding methyl ether, (ii) the successively lower oxidation rates of the secondary and primary amine analogues of the tertiary amine DMEA,[11] (iii) the minimal reactivity of 2-(2-hydroxyethyl)pyridine, which should possess at least some of the coordinating potential of DMEA, and (iv) the observed oxidations of the two common non-alcoholic tertiary amine buffers PIPES and MES. The slower oxidation of the morpholine-based buffer MES compared to that of the piperazine-based buffers PIPES and HEPES undoubtedly arises from the electron-withdrawing effect of the β-oxygen. Thus, the previously reported unreactivity of simple alcohols is not a consequence of their poor coordinating capacity but merely of the unfavorable potential for their oxidation by $(batho)_2Cu^{II}$. Moreover, the inertness of Tris, which, in view of its ability to complex metal ions[12] was unexpected, is now easily understood in terms of it being a primary rather than tertiary amine.[11]

The data listed in Table III are consistent with the previous report[3] that the rates of the $(batho)_2Cu^{II}$ oxidations increase with increasing pH up to the pK_a of the amine. Clearly, the free-base form of the amine is required if the reaction involves oxidation at nitrogen. At even higher pH, however, the rate again declines. This decrease is believed to be a consequence of the conversion of $(batho)_2Cu^{II}$ to a hydroxide complex, which is an ineffective oxidant. The fact that increasing [batho] at high pH (but not low pH) increases the reaction rate (Table III, entries 4 and 5) suggests that the unreactive complex formed is the dihydroxy-bridged dimer $[(batho)Cu(OH)_2Cu(batho)]^{2+}$, with displacement of one batho ligand per copper, rather than the pentacoordinate $(batho)_2CuOH^+$ species.[13] We have confirmed the general occurrence of a bell-shaped pH rate profile for the oxidation of a large series of amines by the aqueous Cu(II)–batho reagent.[14]

Our demonstration of first-order dependence in Cu(II) is consistent with a rate-limiting electron transfer from amine to $(batho)_2Cu^{II}$, analogous to the mechanism proposed by Lindsay Smith[15,16] for ferricyanide oxidation of amines (tertiary amines are ultimately dehydrogenated to iminium species, which hydrolyze to secondary amine and aldehyde). In fact, the ferricyanide studies made us suspect the reported[3] inability of $(phen)_3Fe^{III}$ to oxidize HEPES, especially considering that $E°$ = 1.1 V vs NHE for this complex[6] compared to 0.75–1.05 V for tertiary amines.[11,16] Using the authentic $(phen)_3Fe^{III}$ perchlorate complex,[17] we found that HEPES was rapidly oxidized, even at low pH, where the amino nitrogen is protonated.[18] Perhaps the failure of the previous workers to observe reactions is that they had used $[(phen)FeCl_3]_2$, a doubly chloride-bridged dimer that forms in the attempted preparation of $(phen)_3Fe^{III}$ from $FeCl_3$ and phen in acetic acid.[19] This dimeric complex, containing only a single phen ligand per Fe(III), is a much weaker oxidant, and we have found it incapable of oxidizing even very reactive tertiary amines such as *N*-methylpyrrolidine.[16] We do not dispute the claimed lower affinity of Fe(III) than of Cu(II) for aliphatic amines. However, the ferricyanide oxidations reported by Lindsay Smith, as well as those observed by us for $(batho)_2Cu^{II}$ and $(phen)_3Fe^{III}$ in the current study, are probably best described in terms of outer-sphere electron transfer, for which a coordinative interaction between oxidant and reductant is not required.

In conclusion, the previous observation that cautioned against the use of HEPES buffer in studies involving the Cu(II)–batho complex[3] is well served. However, since the cause of this reactivity is the presence of the tertiary amine function, all tertiary amine buffers should be avoided in such cases. Moreover, it is expected that any metal ion complex with a redox potential in excess of 0.6 V vs NHE will be capable of oxidizing such tertiary amine buffers, whether by inner- or outer-sphere mechanisms. An extensive study on the kinetics and mechanism of aqueous $(batho)_2Cu^{II}$ oxidations of amines in general will be reported in a full paper.[14]

Acknowledgment. We thank M. P. Singh for preliminary work on this project, Prof. Fred Urbach for helpful discussions, and the NSF (Grant No. CHE 87-06263) for financial support.

Registry No. HEPES, 7365-45-9; PIPES, 4432-31-9; MES, 5625-37-6; $(batho)_2Cu^{II}$, 72244-80-5; $(CH_3)_2NCH_2CH_2OH$, 108-01-0; $(CH_3)_2NCH_2CH_2OCH_3$, 3030-44-2; $CH_3NHCH_2CH_2OH$, 109-83-1; $H_2NCH_2CH_2OH$, 141-43-5; $(C_5H_4N)CH_2CH_2OH$, 103-74-2.

Department of Chemistry
Case Western Reserve University
Cleveland, Ohio 44106

Fengjiang Wang
Lawrence M. Sayre*

Received July 8, 1988

---

(11) One-electron oxidation of amines follows the trend 3° > 2° > 1°: Mann, C. K. *Anal. Chem.* **1964**, *36*, 2424. Masui, M.; Sayo, H.; Tsuda, Y. *J. Chem. Soc. B* **1968**, 973.

(12) Masi, D.; Mealli, L.; Sabat, M.; Sabatini, A.; Vacca, A.; Zanobini, F. *Helv. Chim. Acta* **1984**, *67*, 1818.

(13) The analogous phenanthroline complex $[(phen)Cu^{II}(OH)Cu^{II}(phen)]^{2+}$ is known: Harris, C. M.; Sinn, E.; Walker, W. R.; Woolliams, P. R. *Aust. J. Chem.* **1968**, *21*, 631.

(14) Sayre, L. M.; Wang, F. Manuscript in preparation.

(15) Lindsay Smith, J. R.; Mead, L. A. V. *J. Chem. Soc., Perkin Trans. 2* **1973**, 206.

(16) Lindsay Smith, J. R.; Masheder, D. *J. Chem. Soc., Perkin Trans. 2* **1976**, 47.

(17) Schilt, A. A.; Taylor, R. C. *J. Inorg. Nucl. Chem.* **1959**, *9*, 211.

(18) Kinetics of oxidation by $(phen)_3Fe^{III}$ were followed by monitoring the appearance of $(phen)_3Fe^{II}$ at 510 nm: Sutin, N.; Gordon, B. M. *J. Am. Chem. Soc.* **1961**, *83*, 70. The measured k_{obsd} values are 5.8×10^{-3} and 1.1×10^{-2} s^{-1} at pH 3.4 and 4.5, respectively. This reaction may even represent an alcohol rather than an amine oxidation at these pH values, but simple tertiary amines are also oxidized.[14]

(19) Brandt, W. W.; Howsmon, W. B., Jr. *J. Am. Chem. Soc.* **1954**, *76*, 6319.