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THE EFFECT OF CUPRIC ION ON THE RADIATION
CHEMISTRY OF AQUEOUS GLYCINE

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Rodney L. S. Willix and Warren M. Garrison

October 1964

THE EFFECT OF CUPRIC ION ON THE RADIATION CHEMISTRY OF AQUEOUS GLYCINE¹

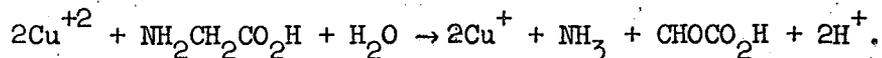
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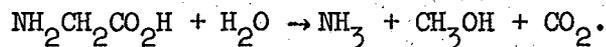
October 1964

Abstract

A study has been made of the effects of cupric ion on the radiation chemistry of oxygen-free glycine solutions over the pH range 3.0 to 9.0. At pH < 6, the hydrated electron e_{aq}^- , derived from water, is removed by Cu^{2+} through simple capture; the hydroxyl radical is scavenged by glycine to give the $NH_2\dot{C}HCO_2H$ radical. The overall reaction stoichiometry is given by:



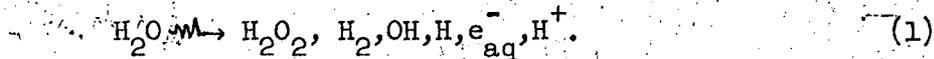
At pH > 6 an additional reaction occurs with the stoichiometry:



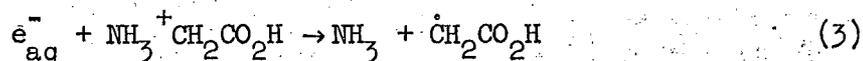
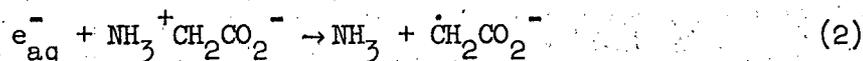
The latter process is associated with capture of e_{aq}^- by the bis-glycinato-Cu(II) chelate. A detailed reaction scheme for the formation of the observed products over the pH range 3.0 to 9.0 is described.

Introduction

The hydrated electron, e_{aq}^- , is the principal reducing species in the radiation decomposition of water^{2,3}



and has been shown to react with both neutral and acid forms of the simpler amino acids via the deamination steps⁴

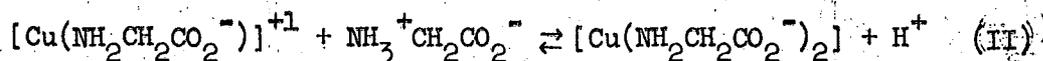
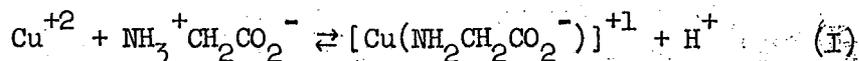


where the rate constants for reaction of e_{aq}^- with the zwitterion and acid forms of glycine via reactions (2) and (3) are $\leq 10^7 M^{-1}sec^{-1}$,^{4,5} and $\sim 10^{10} M^{-1}sec^{-1}$,⁴ respectively. Capture of e_{aq}^- by the acid form of the amino acid occurs in competition with the conversion reaction:

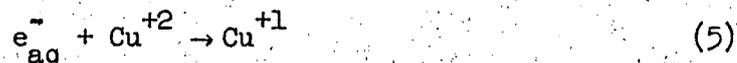


where $k_4 \approx 2 \times 10^{10} M^{-1}sec^{-1}$.⁶

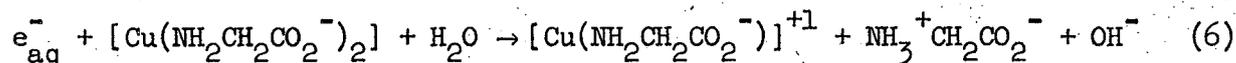
Now, the effects of transition metal ions on the reactions of e_{aq}^- in aqueous solutions of the amino acids are of particular interest because such ions are effectively chelated by the amino acid according to the pH dependent equilibria:



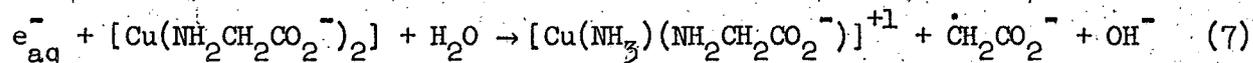
with $K_I = 10^{8.5}$, $K_{II} = 10^7$ at 20°C .⁷ And, since the free Cu^{2+} ion has been shown to react rapidly with e_{aq}^-



with $k_5 = 3 \times 10^{10} \text{ M}^{-1} \text{ sec}^{-1}$,⁸ there arises the interesting chemical and biological question of whether the glycine-Cu(II) chelates react with e_{aq}^- by analogous simple capture



or by a path that leads to chemical degradation of the ligand by reaction akin to step (3), i.e.,



where eqs. 6 and 7 represent overall stoichiometries.

This study provides substantiating evidence for the reductive deamination of amino acids by e_{aq}^- and affirms that e_{aq}^- reacts with bis-glycinato Cu(II) with chemical degradation of the ligand.

Experimental

Materials. Commercially available glycine (Nutritional Biochem. Corp.) recrystallized three times from water, was used throughout. The ^{14}C - (carboxyl carbon) labelled glycine was purified chromatographically on a Dowex 50 cation-exchange column (hydrogen form), HCl of progressively increasing concentration (0 to 4 N) serving as the eluting solvent.⁹ The hydrochloride salt was then passed through an anion exchange column (Dowex 1, acetate form) to remove chloride ion, acetic acid was removed by distillation, and the glycine was then recrystallized from water. Reagent-grade copper sulphate-pentahydrate was used directly as the source of cupric ion. Similarly reagent-grade sodium formate was employed without further purification in the competition experiments. pH adjustments were performed with dilute H_2SO_4 and NaOH solutions. Bis-glycinato-Cu(II) was prepared by the method of Sen, et al.¹⁰

Analyses. Ammonia was determined by the absorption at 410 m μ on the addition of Nessler reagent to the 0.08 N H_2SO_4 solution from a Conway cell. The other compartment of the Conway cell contained saturated K_2CO_3 solution to which an aliquot of the target was added. The allowed time for diffusion was twelve hours.

Glyoxylic acid and formaldehyde were identified by paper chromatography of their 2:4 dinitrophenylhydrazone derivatives against authentic material with butanol/ammonia and methanol/heptane respectively as the ascending solvents. Their amounts were determined by the method of Johnson and Scholes¹¹ with the modification that the target solution was first passed through a Dowex 50 column to remove copper and glycine. Glyoxylic acid was determined with 10^{-3} M DNP reagent (little formaldehyde reacting at this concentration) and the absorption of the alkaline solution measured at 450 m μ . Formaldehyde was determined by the extraction technique¹¹ and the absorption of the DNP derivative in alkaline solution measured over a range of wavelengths between 420 and 480 m μ .

Where necessary, spectrophotometric calibration curves allowed for the time-dependent decay of the absorbing species.

Acetic and succinic acids were assessed by chromatographing the ^{14}C -labelled target effluent (containing inactive carrier acids) of a Dowex 50 column, on silicic acid.¹² Chloroform containing increasing amounts of butanol was the eluting solvent, and the positions of the acetic and succinic acid peaks were located by titration with 0.02 NaOH. The activity was then determined on a "Nuclear Chicago" counter and scaler (utilizing Matheson "Q" gas - 98% helium, 2% butane).

In a probe for nitrogen containing products basic enough to be retained on a Dowex 50 column from a target through-put, the technique described for purifying the labelled glycine was utilized.

Gas analyses were made by adjusting the pH to < 1 with H_2SO_4 , removing the material volatile at -79°C and analyzing the contents in a mass spectrometer, after a total pressure measurement.

All targets were degassed on a vacuum line with a rotary oil pump and a series of three liquid nitrogen traps. The amount of residual O_2 in such a sample was shown to be < 0.2 micromoles i.e., $< 2 \times 10^{-5}$ M.

Stock solutions of glycine, copper sulphate and sodium formate were standardized gravimetrically. The concentration of cupric ion was further checked by the absorption at $750 \text{ m}\mu$ ($\epsilon_{\text{Cu}^{2+}} \sim 10$).

Radiation Source. All irradiations were carried out with the 200 C ^{60}Co - γ facility (dose rate $5.9 \rightarrow 5.3 \times 10^{16} \text{ eV cc}^{-1} \text{ min}^{-1}$), at room temperature. The dose rate was determined with the Fricke dosimeter (10^{-3} M Fe(II), 0.8 N H_2SO_4) using the values $G(\text{Fe(III)}) = 15.6$ and $\epsilon_{\text{Fe(III)}} = 2130$ at 22°C .

Target solutions were 10 milliliters in volume and were irradiated in Pyrex cells. Analyses were commenced immediately after irradiation. Control experiments on unirradiated targets established that no NH_3 was formed in the

absence of irradiation and that the CO_2 level was less than 0.05 micromoles (1% of CO_2 found in experiments).

Results

Over the pH range 3-6, the ammonia yields from 0.3 M glycine decrease with increasing concentrations of divalent copper and approach a limiting steady value of $G \approx 2.2$ at Cu(II) concentrations above ~ 0.03 M. (See Fig. 1.) The effect of Cu(II) becomes less pronounced as the pH is increased above 7 and at pH 8.5, $G(\text{NH}_3)$ is essentially unaffected by Cu(II). (At pH 8.5 a small increase in $G(\text{NH}_3)$ is observed for $[\text{Cu(II)}] > 0.02$ M.) Ammonia yields from 0.3 M glycine in the presence and absence of copper over the pH range ~ 3 to ~ 9 are summarized in Fig. 2.

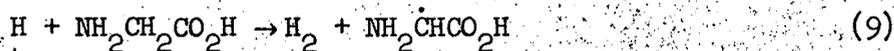
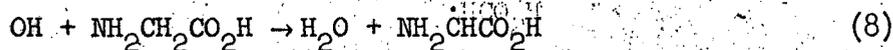
In the pH range below 6, the principal organic products are glyoxylic acid and formaldehyde with $G(\text{NH}_3) \approx G(\text{CHOCO}_2\text{H}) + G(\text{CH}_2\text{O})$ and $G(\text{CH}_2\text{O}) \approx G(\text{CO}_2)$. (See Table I.) The yield of each of these carbonyl compounds remains essentially constant with increasing pH, but $G(\text{CO}_2)$ increases from 0.5 at pH 3.2 to a value of 3.7 at pH 8.5. For alkaline solutions there is the approximation $G(\text{NH}_3) \approx G(\text{CHOCO}_2\text{H}) + G(\text{CH}_2\text{O}) + G'(\text{CO}_2)$ where $G'(\text{CO}_2) = G(\text{CO}_2) - G(\text{CH}_2\text{O})$. Acetic and succinic acids are minor products over the entire pH range studied.

Also, as shown in Table I, product yields from 0.02 M (saturated) solution of the preformed chelate, bis-glycinato-Cu(II), are nearly identical with those obtained with 0.03 M Cu(II) in 0.3 M glycine, at pH 8. However, solutions of preformed copper glycinate behave somewhat differently under radiolysis in that reduction of Cu(II) results in precipitation of Cu_2O . Precipitation is not observed if excess glycine is present.

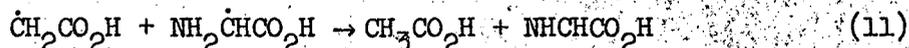
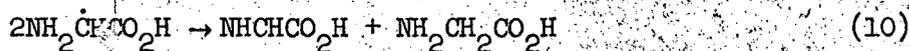
The addition of formate ion, which is relatively unreactive towards e_{aq}^- but an effective scavenger of OH radicals¹³ results in a decrease in $G(NH_3)$ both in acid and alkaline solutions (Fig. 3). At pH 4.0 the ammonia yield falls to a limiting value of $G(NH_3) \approx 0.8$ with increasing formate concentration, whereas at pH 8.6 it levels off at the higher value $G(NH_3) \approx 3.3$. The carbonyl yield is also lower in the presence of formate and the residual yield appears to be pH independent with $G(CHOCO_2H) + G(CH_2O) \approx 0.6 + 0.2 \approx 0.8$.

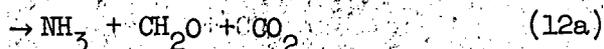
Discussion

The radiation chemistry of the simpler amino acids in aqueous solution at pH values above ~ 3 is consistent with a reaction scheme⁴ in which e_{aq}^- is captured by the amino acid via reactions (2), (3) and OH and H are removed in steps (8), (9) given below in terms of the neutral molecules for simplicity. The inference is that the net charge of the amino acid influences the relative rates but not the form of these reactions



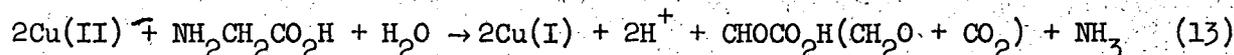
The subsequent reactions include





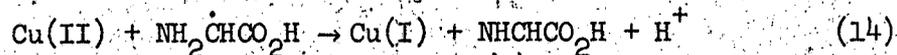
In addition there is some radical combination to give diaminosuccinic, amino-succinic and succinic acids in relatively low yield. Reactions of both OH and e_{aq}^- with glycine zwitterion have been shown to be relatively slow^{14,4} and this is reflected in the rapid fall-off of product yields in neutral glycine solutions with decreasing glycine concentrations below ~ 0.1 M. The saddle at pH 6 to 7 in the pH-yield curve of ammonia from 0.3 M glycine (Fig. 2) is attributed to the relative ineffectiveness of the zwitterion as compared with the acid and basic forms of glycine in scavenging oxidizing and reducing species from within the spur. The drop in $G(\text{NH}_3)$ with decreasing pH below ~ 3 results from the competition of H_3O^+ with $\text{NH}_3^+\text{CH}_2\text{CO}_2\text{H}$ for e_{aq}^- via reactions (4) and (3) respectively. The effect of pH on the yields of other major products including hydrogen, glyoxylic acid and acetic acid are in accord with this formulation.⁹

Now, the presence of > 0.02 M Cu(II) leads to a considerable simplification in the radiation chemistry of aqueous glycine below pH 6. Under these conditions cupric ion is present either as Cu^{+2} or $[\text{Cu}(\text{NH}_2\text{CH}_2\text{CO}_2^-)]^{+1}$,⁷ with the appropriate number of water molecules of hydration. The stoichiometry of radiation-chemical change is given by

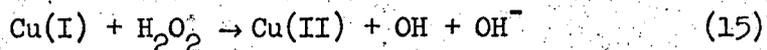


with $G(\text{NH}_3) \approx G(\text{RCHO}) \approx 2.2$. A consistent explanation is that the reducing species in the form of e_{aq}^- or H is preferentially scavenged by Cu^{+2} or $[\text{Cu}(\text{NH}_2\text{CH}_2\text{CO}_2^-)]^{+1}$ to give cuprous ion without net chemical effect on glycine. Glycine degradation

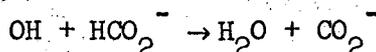
is ascribed wholly to OH attack via reaction (8) followed by the stoichiometry



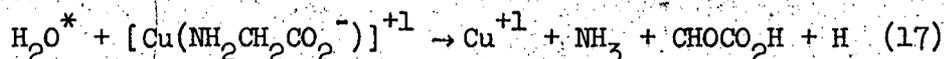
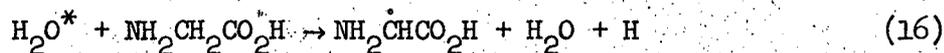
and by reaction (12) or (12a) along with some contribution from the back reaction



We anticipated that $G(\text{NH}_3)$ would drop to zero with the addition of formate ion in increasing concentration because of the competing reaction

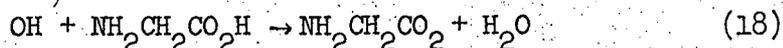


The fact that $G(\text{NH}_3)$ and $G(\text{RCHO})$ level off at $G \approx 0.8$ at formate concentrations above 0.1 M suggests a) that the Cu^{+1} in reaction (15) is present in the chelate form $[\text{Cu}(\text{NH}_2\text{CH}_2\text{CO}_2^-)]^{+1}$ and as a result the OH radical is liberated in close proximity to a glycine molecule and is not then available for scavenging by moderate concentrations of formate in the bulk. Or b) that a relatively long-lived excited water molecule¹⁵ is formed with $G \approx 0.8$ and reacts preferentially with glycine— for example

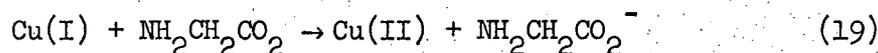


We note also that the difference in $G(\text{NH}_3)$ in the absence and in the presence of formate (i.e., $2.3 - 0.8 = 1.5$) should correspond to a minimum of at least $G_{\text{OH}} \approx 2.1$,

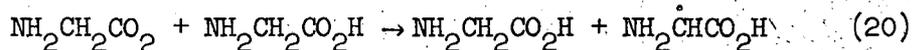
the accepted value for the yield of OH radicals with γ rays.¹⁶ We suggest that OH reacts not only at the α -carbon position as indicated in eq. 8 but also at the carboxyl group:



Unlike $\text{NH}_2\text{CHCO}_2\text{H}$, the $\text{NH}_2\text{CH}_2\text{CO}_2$ species is not oxidized by Cu(II) but instead is reduced via the back reaction



In the absence of Cu(II), the production of $\text{NH}_2\text{CH}_2\text{CO}_2$ is obscured by

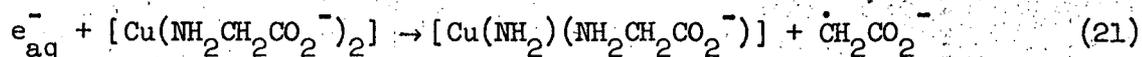


As the pH of the glycine-Cu(II) system is increased above pH 6, the concentration of the bis-glycinato-Cu(II) chelate increases sharply⁷ and at pH 8.5 essentially all of the Cu^{+2} is so bound. The carbonyl yield, $G(\text{CHOCO}_2\text{H}) + G(\text{CH}_2\text{O})$, remains essentially constant with increasing alkalinity indicating that oxidation by OH via the steps (8) and (14) retains the stoichiometry given by eq. 13. The abrupt increase in $G(\text{NH}_3)$ and $G(\text{CO}_2)$ over the pH range 6 to 9 is associated with the onset of a competing reaction of e_{aq}^- that leads to glycine deamination. One possibility, of course, is that the uncharged bis-glycinato-Cu(II) chelate is relatively unreactive towards e_{aq}^- compared with Cu^{+2} and $[\text{Cu}(\text{NH}_2\text{CH}_2\text{CO}_2^-)]^{+1}$ so that reaction of e_{aq}^- with the zwitterion becomes the dominant process at high pH values. We note, however, that solutions of preformed bis-glycinato-Cu(II) at pH 8 also give $G(\text{NH}_3) \approx 5.0$ (and maximal yields of the other products) even though

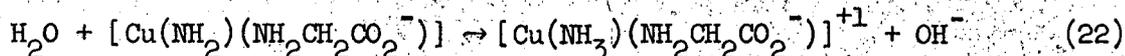
the concentration of free glycine in such solutions is less than 10^{-4} M. We conclude therefore that bis-glycinato-Cu(II) scavenges e_{aq}^- and propose reaction (7) as satisfying the overall experimental requirements.

The addition of formate to the glycine-Cu(II) system at pH 8.6 reduces $G(NH_3)$ from ~ 5.0 to a limiting value of about 3.3 (see Fig. 3). Assuming reaction (15) or (16), (17) contributes $G(NH_3) \approx G(RCHO) \approx 0.8$, we then have $G_{e_{aq}^-} = 3.3 - 0.8 \approx 2.5$ in satisfactory agreement with current findings.¹⁶

Although some perturbation of the carbon-oxygen stretching frequencies in the carboxyl group does occur on complex formation¹⁷ the spectroscopic evidence suggests that the bonding between copper and the carboxyl group in copper glycinate is essentially electrostatic whereas the Cu--N bond is covalent.¹⁰ It is reasonable then to infer that the energy released on capture of e_{aq}^- by $[Cu(NH_2CH_2CO_2^-)_2]$ is available to rupture the N-C bond

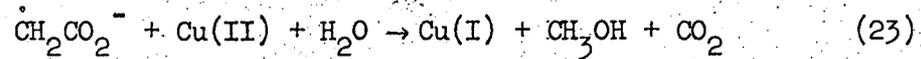


and that the hydrolysis



follows, to give the stoichiometry of eq. 7.

The carboxymethylene radical so formed is a strong oxidizing agent and one might anticipate that it would abstract hydrogen as proposed in step (11) for the Cu-free system. However in the presence of Cu(II) the product stoichiometries require $\dot{C}H_2CO_2^-$ to be removed by the equivalent of



Reaction (23) is analogous to the recently reported oxidation of methyl radicals (from t-butyl hydroperoxides) to methanol by cupric ion in aqueous solution.¹⁸

Acknowledgment

The authors wish to acknowledge the assistance and advice freely given by Drs. M. Kland-English, M. Jayko, and B. M. Weeks during various aspects of this work.

Footnotes and References

- (1) Work performed under the auspices of the U.S. Atomic Energy Commission.
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Table I

Product yields from 0.30 M glycine, 0.03-0.04 M Cu(II) solutions[†]
(Dose = 10^{19} eV cc⁻¹)

pH	G(NH ₃)	G(CHOCO ₂ H)	G(CH ₂ O)	G(CO ₂)	G(H ₂)	G(CH ₃ CO ₂ H)	G(<u>succinic</u> acid)
3.0	2.2±0.2	1.8	0.53	0.5		0.04	0.07
8.5	5.0±0.2	1.8	0.77	3.7	0.45	0.12	0.24
		2.1	0.85				

[†]Preformed 0.02 M bis-glycinato Cu(II)

8.4	5.0±0.2	2.2	0.54	3.1			
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Fig. 1. Yield of ammonia as a function of cupric ion concentration in 0.3 M glycine solutions.

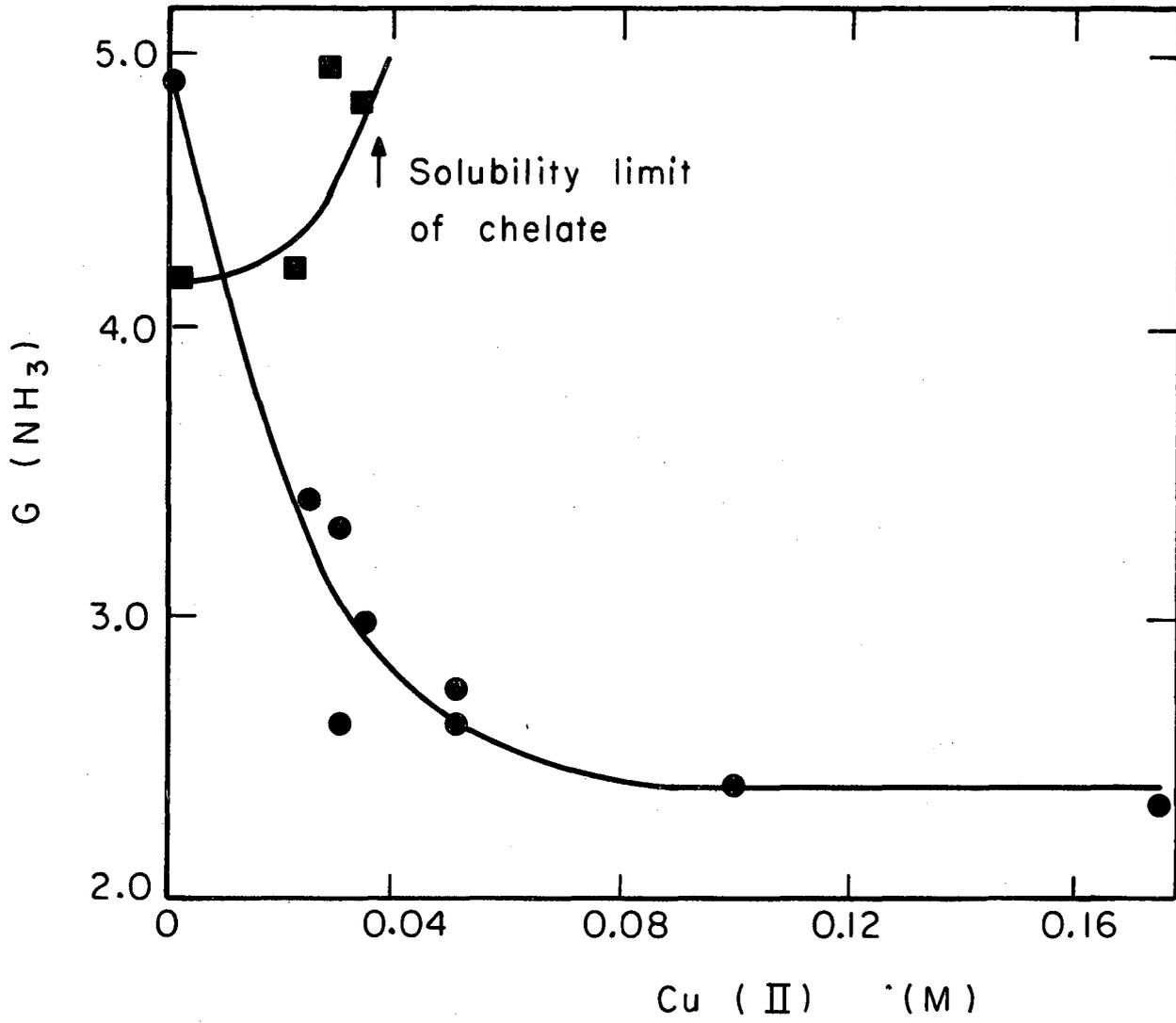
● pH 3.0 ■ pH 8.5

Fig. 2. Yield of ammonia as a function of pH in 0.3 M glycine solutions.

---● glycine
— glycine plus ■ 0.025, X 0.030,
I 0.035, ▲ 0.040, M Cu(II)

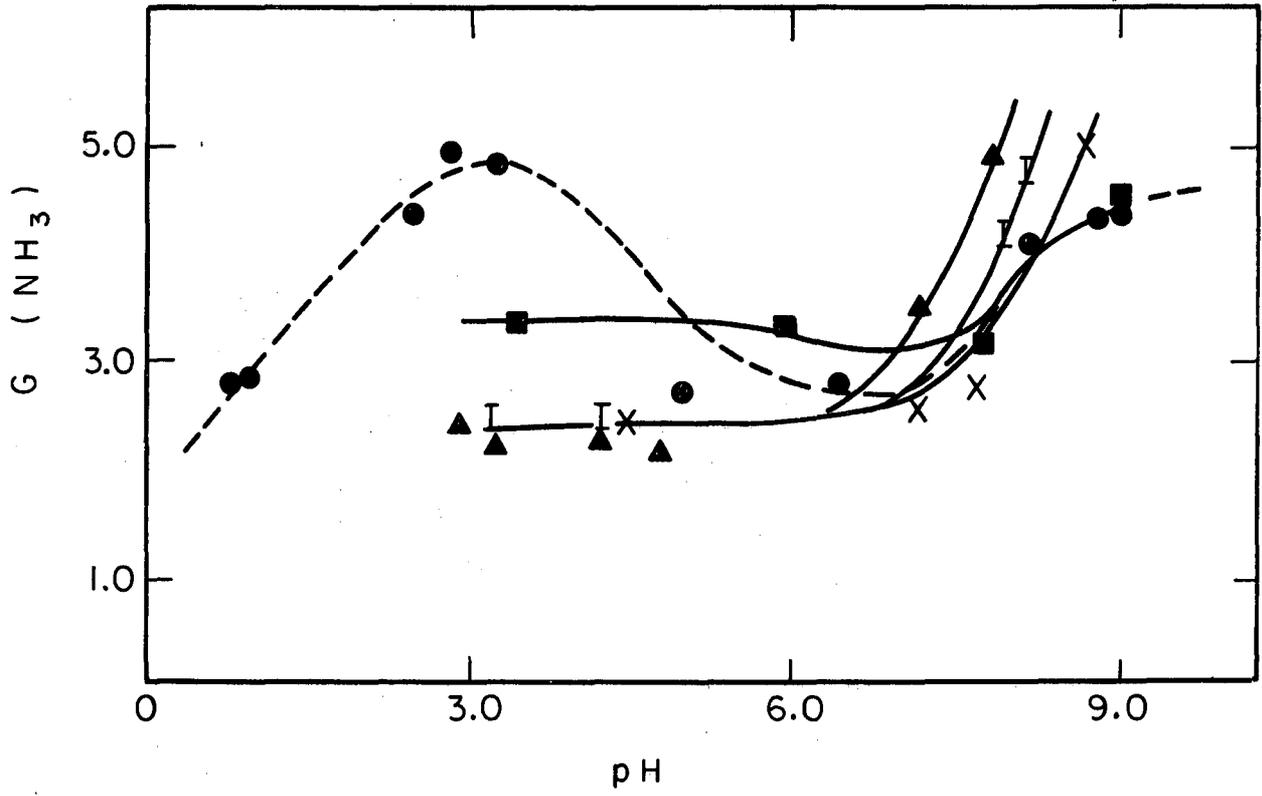
Fig. 3. Effect of formate ion on ammonia yield in 0.3 M glycine solutions.

⊥ pH 8.6, 0.035 M Cu(II)
† pH 4.0, 0.040 M Cu(II)



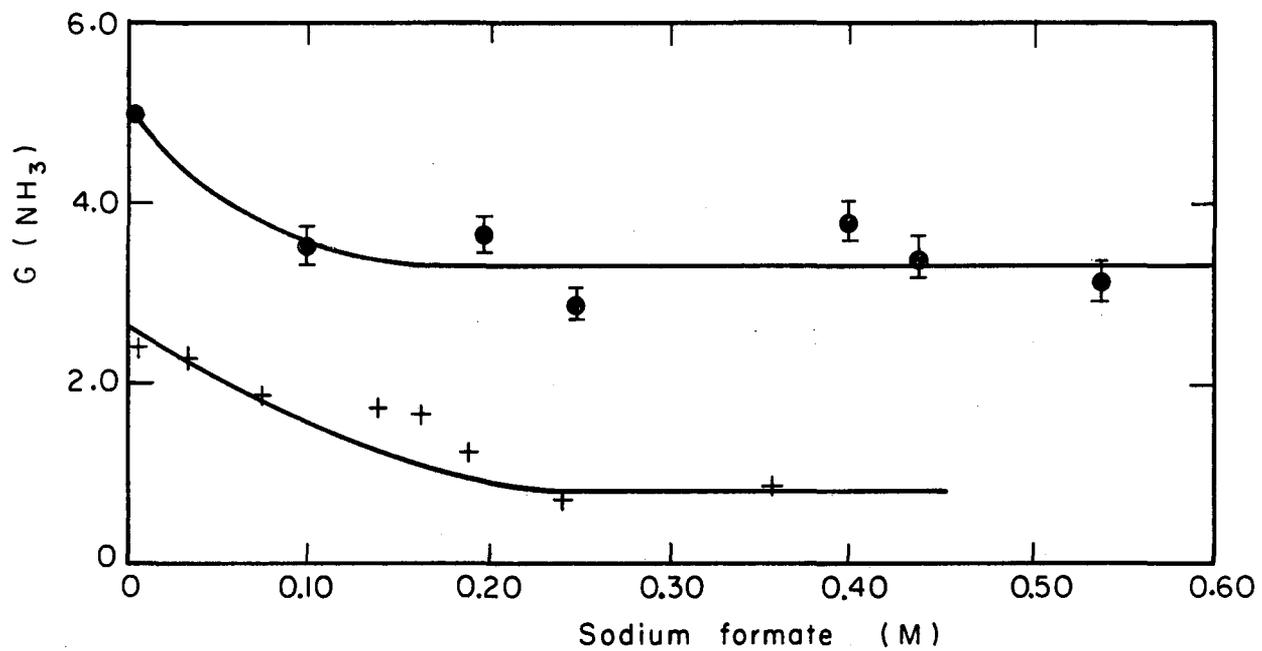
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Fig. 1



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Fig. 2



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Fig. 3

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