

# Formic Acid: A Significant but Ignored Product in the Miller–Urey Experiment

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## Abstract

In 1953 Stanley Miller tested a hypothesis put forward by A.I. Oparin and, later, J.B.S. Haldane concerning the origin of life. The classic experiment is frequently cited in the popular literature and programing as evidence supporting the formation of primitive cellular organisms from polypeptides that self-organized over millions of years. Yet many of the reaction products from Miller's experiment are more problematic for the model than advocates readily admit. Among these is formic acid, reportedly present at about double the combined concentrations of identified amino acids including glycine, alanine, aspartic acid, and glutamic acid. Formic acid can produce a significant negative impact on the formation and stability of components such as a hypothetical polypeptide. Consequently, advocates of the Oparin-Haldane-Miller theory are faced with an acute problem. The present paper explores the reaction chemistry of aqueous formic acid and an imaginary polypeptide assumed to be present in the primordial ocean.

## Introduction

Stanley Miller's spark discharge treatment of methane, water, hydrogen, and ammonia continues to be cited as a plausible model that simulates endogenous production of amino acids in the prebiotic world (Fox and Strasdeit, 2009). In subsequent steps, it is believed that this diverse suite of amino acids combined to form high molecular-weight polypeptides, which in turn supplied critical

raw materials to form the first universal common ancestor (UCA) (Fitz et al., 2007). This imagined scenario, known as the "peptide world," draws from Miller's original findings, but precariously so, as our examination will show.

While a graduate student of Harold C. Urey at the University of Chicago, Miller reported a curious experiment that would initiate empirical origin-of-life studies (Miller, 1953). In the short

*Science* article, Miller discusses the results of exposing ammonia, methane, water, and hydrogen to electrical discharge, which he concludes "may have played a significant role in the formation of compounds in the primitive earth" (Miller, 1953, p. 528).

The apparatus consists of a round-bottom flask attached to a trap simulating the oceanic environment and another flask fixed with electrodes simulating the atmosphere bristling with lightning. Gaseous hydrogen, methane, and ammonia (10, 20, and 20 cm Hg respectively) were charged into the apparatus, and water in the small flask was boiled. This highly reducing composition was

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exposed to a spark discharge produced by a Tesla coil peaking at 60,000 volts.

The mixture was exposed to continuous sparking over a one-week period, resulting in significant observable changes. Miller (1953) reported the presence of "glycine, alpha-alanine and beta-alanine" and tentatively reported finding "aspartic acid and a-amino-n-butyric acid." He further reported that other amino acids were "undoubtedly present but in smaller amounts" (Miller, 1953, p. 529). He estimated the total yield was in the "milligram range," and reported "yellow" compounds that could not be extracted with ether (Miller, 1953).

Miller followed this short paper with a detailed, quantitative and qualitative

report of the products obtained through spark discharge (Miller, 1955). Here Miller discloses an array of organic compounds (Table I). One of the most significant reaction products is formic acid. Formic acid (also called methanoic acid, formula HCOOH) is the simplest carboxylic acid. It is toxic and used as a preservative and antibacterial agent. It also occurs naturally, in the venom of bee and ant stings. Miller reported a concentration of 4% formic acid, based upon the amount of methane initially added. In contrast, the total yield of the most abundant amino acids (d,l-glycine and d,l-alanine) were all less than 4%. Miller emphasized the formation of the few amino acids and apparently

considered the additional compound suite (including formic acid) largely a less important detail.

Although not considered in great depth here, it is fair to note that most geologists no longer accept a highly reducing atmosphere, as Miller originally proposed (Johnson et al., 2008). Recent investigators point out that the ancient earth lacked an insulating or protective ozone layer. The absence of the ozone layer would allow high energy UV radiation from the sun to produce oxygen (O<sub>2</sub>) and hydrogen (H<sub>2</sub>) from water on the earth. Free oxygen would rapidly decompose organic compounds. Proponents of chemical evolution, including Miller, Urey, and Melvin Calvin, admit

**Table I (from Boudreaux and DeMassa, 2010)**  
**MAJOR CHEMICAL PRODUCTS IN MILLER'S EXPERIMENT**

Chemical Name	Chemical Formula	Symbol	Moles (x 10 <sup>-4</sup> )	Molarity <sup>a</sup> (x 10 <sup>-4</sup> )	pKa <sup>b</sup>	[H <sub>3</sub> O <sup>+</sup> ] <sup>c</sup> (x 10 <sup>-4</sup> )
Formic acid	HCO <sub>2</sub> H	FA	23.3	47	3.75	29
Glycine	NH <sub>2</sub> CH <sub>2</sub> CO <sub>2</sub> H	Gly	6.3	13	2.34	77
Glycolic acid	HOCH <sub>2</sub> CO <sub>2</sub> H	GIA	5.6	10	3.83	12
d,l Alanine	NH <sub>2</sub> (CH <sub>3</sub> )CHCO <sub>2</sub> H	d,l,Ala	3.4	6.8	2.33	18
Lactic acid	H <sub>3</sub> CCH(OH)CO <sub>2</sub> H	LcA	3.1	6.2	3.86	9.3
B. Alanine	NH <sub>2</sub> (CH <sub>3</sub> )CHCO <sub>2</sub> H	β,Ala	1.5	3.0	3.51	9.6
Acetic acid	H <sub>3</sub> CCO <sub>2</sub> H	AcA	1.5	3.0	4.36	3.6
Propionic acid	H <sub>3</sub> CCH <sub>2</sub> CO <sub>2</sub> H	PrA	1.3	2.6	4.87	1.9
(Iminodiacetic acid) <sup>d</sup>	-	-	(0.9)	-	-	-
Total pH						1.8 <sup>e</sup>

a. Reported by Miller (1953; 1955). For example, formic acid is at 0.0023 moles.

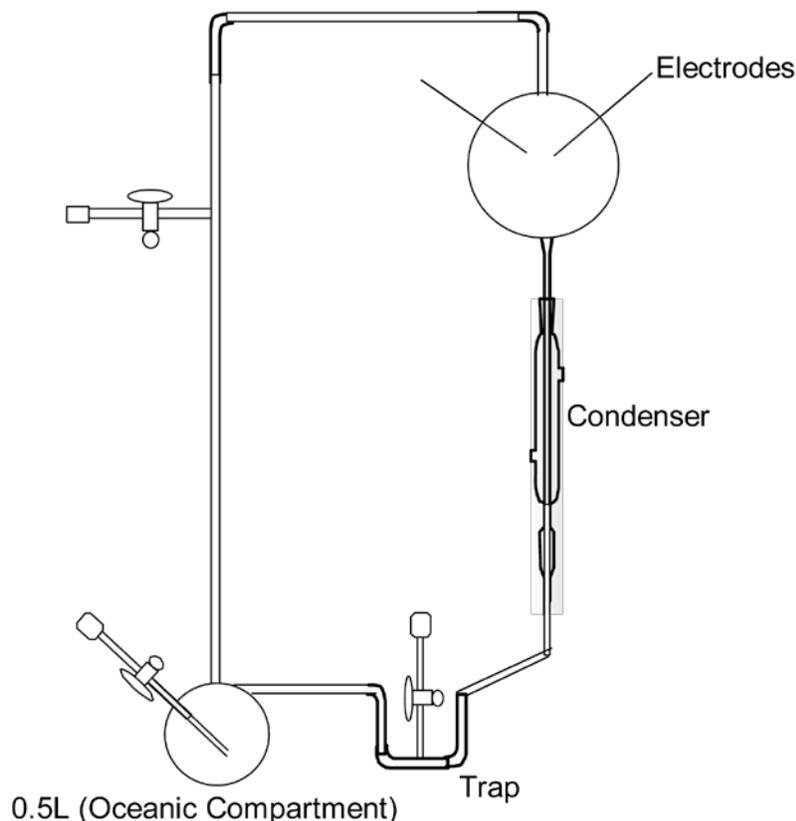
b. Based on 0.5L volume, as reported.

c. Calculated at equilibrium. No reactions between components accounted for.

d. This and all subsequent products in amounts too small to be significant.

e. Net pH of all H<sub>3</sub>O<sup>+</sup> in solution.

f. Other reported compounds (< 10<sup>-4</sup> moles each) a-hydroxybutyric acid, sarcosine, formaldehyde, (d,l)-a-amino-n-butyric acid, acetaldehyde, succinic acid, iminoaceticpropionic acid, n-methyl urea, (d,l)-n-methyl alanine, (d,l)-glutamic acid urea, (d,l)-aspartic acid, homoserine2-Me-Ser, B-hydroxy-Asp, ornithine2-Me-Glu, phenyl alanine.



**Figure 1.** A schematic representation of the Miller apparatus, emulating the synthesis of organic compounds in the supposed prebiotic earth. The circulating mixture passed over tungsten electrodes sparking for one week, consuming  $1.5 \times 10^3$  kcal of energy. Quantitative and qualitative results were reported for both the gaseous and dissolved components.

that an oxygen atmosphere like ours today would make Miller-type reactions impossible.

Although Miller's experimental conditions are generally considered obsolete, the study's popularity persists. Discussions of his work are still offered as evidence for biomolecule origins in many textbooks (e.g., Chiras, 2012, p. 449–450, Mader, 2011, p. 317, Starr et al., 2009, p. 310), popular media sources (e.g., PBS Report, 1998), and commonly taught in many public schools. Clearly, young students are still being given the impression that this experiment demonstrates natural processes for the origin of life. For example, the Escambia County

School District school district posted sample questions for students taking exams concerning the origin of life. Among these questions was the following (Escambia County School District, 2011, emphasis added):

The Miller-Urey experiment of 1953 was designed to test the hypothesis that lightning supplied the energy needed to turn atmospheric gases into organic molecules such as amino acids. *Which of the following describes why the Miller-Urey theory is widely accepted today?*

A. Amino acids spontaneously form from molecules in the atmosphere today.

B. Organic molecules are present today in extremely high concentrations.

C. The process of synthesizing organic molecules from a mixture of gases has been successfully modeled in the laboratory.

D. No other alternative hypotheses have been introduced.

While the specific approach of the Miller-Urey experiment is not widely accepted today as a valid mechanism, many researchers still offer its results as supportive evidence for the materialistic origin of organic molecules, which subsequently formed the first life forms.

In this light, we need to address another misunderstanding. The experiment's production of formic acid as a major reaction product is frequently ignored. The presence of this formic acid has a potential significant chemical impact on the hypothetical polypeptide that is predicted by the peptide-world hypothesis.

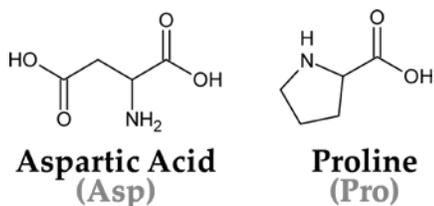
### Consequences of Formic Acid Formation

A recent report reexamined Miller's 1953 original samples obtained from a derivative experiment involving injection of steam into the spark (Johnson et al., 2008). In this new analysis, six additional amino acids were discovered. Aliphatic amines were discovered, including methylamine, ethylamine, isopropylamine, and normal propylamine ( $< 10^{-5}$  moles relative to glycine = 1). It is uncertain as to whether these products may have formed over the 56 years since the experiments were initiated or if these compounds were formed initially but were below the original detection limits of the equipment used during Miller's time. It is also uncertain whether the original samples in Miller's other experiments, including apparatus 1, may have included these additional compounds. However, for completeness we will list these new findings. Formic acid and

other simple acids were not evaluated in the reexamination, so their concentrations are unknown (Jeffery Bada, 2009, personal communication).

If Miller's reaction mixture is representative of the primordial soup, formic acid not only would have inhibited polypeptide formation (amino acid condensation), as thermodynamic treatments have shown (Boudreaux and De Massa, 2010), but also would have actually inhibited peptide formation even in the presence of selected condensing agents thought present on the early earth (Chang et al., 1969).

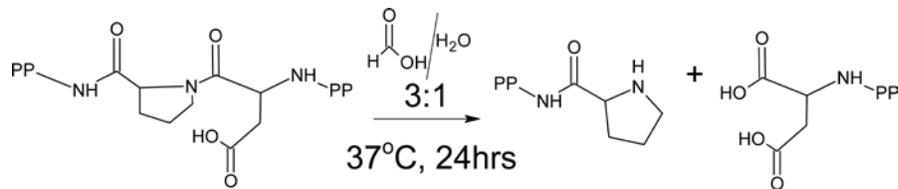
Assuming polypeptides were present at some time in a Miller-type soup, the formic acid is such an effective protein denaturant that the polypeptides would have depolymerized in the presence of formic acid. Workers have shown that treatment of common fodder, such as maize with formic acid, leads to a significant increase in free amino acid (Fairbairn and Phillip, 1992). One study discusses fragmentation between a prolyl (proline) and aspartyl (aspartate) linkage of a large protein (Anders and Consigli, 1983). Ring et al. (1972) identified both amino acids in spark discharge reaction mixtures. This is an important point regarding the stability of a hypothetical polypeptide, discussed below.



The protein was heated at 37°C for 24 hours using 75% formic acid (25% water) (Figure 2).

Akiya and Savage (2004) believe formic acid promotes spontaneous hydrolysis (water cleavage of the peptide bond) at the site of the proline nitrogen atom (Figure 3).

Less aggressive acids catalyze hydrolysis reactions as well. For example, us-

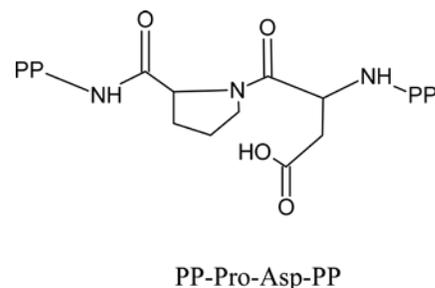


**Figure 2. Hydrolysis of Pro-Asp linkage in aqueous formic acid mixture.**

ing fairly mild conditions, proteins have been digested with formic acid (Hua et al., 2006). In another study, hydrolysis of peptides occurred in 5% formic acid solution at 37°C for 6 hours (Funatsu and Taguchi, 1988). This work suggests that at the very least, formic acid would have been highly disruptive to polypeptide chain stability in a primordial soup (or lagoon) containing adjacent amino acids like aspartate and proline.

In yet another study, formic acid/water mixtures (pH 2, near the calculated pH of Miller's mixture, see Appendix) were routinely used to digest proteins containing aspartic acid (Rosenburg, 2005). Smith reports cleavage at Asp-X peptide bonds facilitated by the adjacent carboxylic acid side group. Smith also calls "formic acid a good protein denaturant ... and that low pH facilitates a number of side reactions at the glutamic acid side carboxylic acid residue (Walker, 1994). Arai et al. (1999) found that less acidic pH's (6.0) have also been found to favor peptide hydrolysis.

Kinetic studies explain why glutamic acid (or aspartic acid) facilitates chain cleavage (Joshi et al., 2005). The study suggests that Asp-mediated cleavage involved nucleophilic attack of the side-chain carboxylic acid group on the protonated carbonyl carbon of the peptide bond. Fragmentation of the polypeptide follows with hydrolysis of the terminal anhydride moiety. These findings suggest that a hypothetical polypeptide (or oligopeptide) in a Miller-type broth enriched with formic acid



**Figure 3. A prolyl-aspartyl linkage in a polypeptide chain (PP).**

would similarly fragment (Figure 4). It should also be noted that Miller did not report any peptides such as di-peptides or polypeptides.

Other research has invoked this neighboring group catalysis effect (carboxyl group displacement of the nitrogen of the peptide bond), reducing a tetrapeptide (klerval) to a tripeptide (Won et al., 1999). The findings of Joshi et al. (2005) also suggest how Asp-Pro linkages might rupture in a formic acid enriched environment (Figure 5). In Miller's subsequent work, Asp and Pro amino acids were identified in spark discharge experiments by employing a gaseous mixture of methane, nitrogen, water, and ammonia (Ring et al., 1972).

At least one suggestion drawn from these observations is that a hypothetical prebiotic polypeptide containing amino acid species, such as aspartic acid and glutamic acid (Glu-X, Asp-X, Pro-Asp,

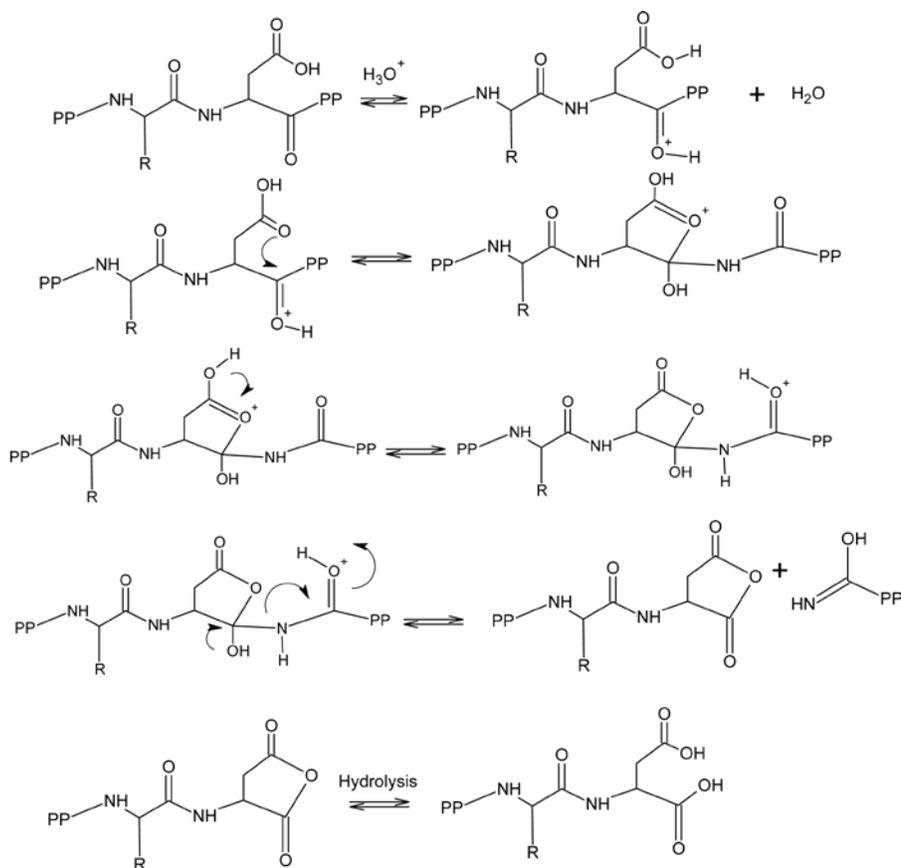


Figure 4. Proposed hydrolysis mechanism of polypeptide chain via Asp neighboring group nucleophilic attack (several steps omitted in anhydride ring opening).

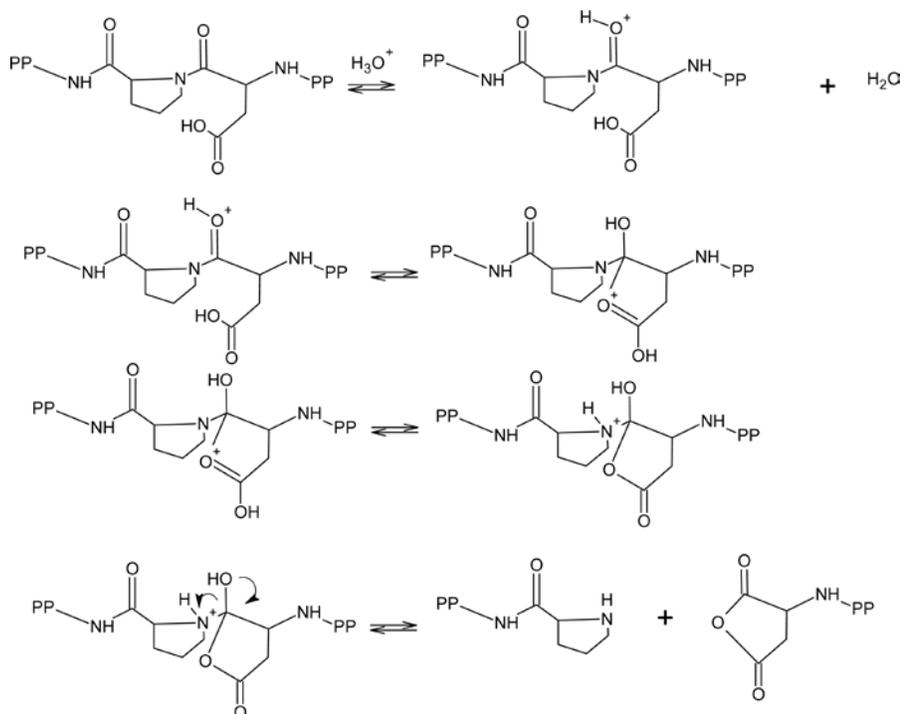


Figure 5. Proposed mechanism of hydrolysis for Pro-Asp linkage.

where X is an adjacent amino acid), both of which were formed in Miller's spark discharge experiments, would hydrolyze in the presence of formic acid. The neighboring group effect would enhance chain fragmentation. It is likely that formic acid would promote cleavage reactions elsewhere on a hypothetical polypeptide chain. This would have been further assisted by other Miller diacid compounds incorporated into the chain (e.g., iminodiacetic acid, Table I).

## Conclusion

Although the reducing atmosphere on earth required for Miller's scenario is no longer accepted by geoscientists, Miller's discovery of amino acids continues to be offered as support for the Oparin-Haldane origin-of-life hypothesis (Miller et al., 1997). A PBS Report (1998) states, Miller's work showed that compounds necessary for life could have been formed in an environment without free oxygen, confirming Haldane's theory. The creation of amino acids from Earth's raw materials may well have been the first step of evolution. It also opens the possibility (since the proposed atmosphere was based on proportions of elements in the universe) that similar atoms and amino acids could have formed elsewhere, on planets formed in the same manner as Earth.

Ignored in these considerations is the impact of various reaction products, such as formic acid, on subsequent hypothesized chemical events leading to the hypothetical proto-cell (i.e., the universal common ancestor).

As we have shown, the highly acidic environment in Miller's "soup" (neglecting potential degradation due to other energy sources; e.g., temperature or light) makes a hostile nursery for either developing polypeptides or promoting polypeptide stability. The problem is further frustrated by the presence of neighboring functional groups that as-

sist with chain cleavage. It is therefore altogether unclear how polypeptides could form, or even survive, in this highly acidic milieu.

To produce peptides and maintain them for further chemical evolution, the peptides formed must be isolated from all the cumulated degrading chemicals present in the reaction mixture. This point is also true for the required chiral purity of amino acids and coagents needed to produce a hypothetical biologically active polypeptide. How this isolation could naturally occur can only be the product of speculation and presents a monumental impasse for the hypothesis of chemical evolution.

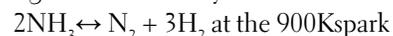
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## Appendix

According to Miller (1955) the initial gas pressures were:  $\text{NH}_3$ -20 cm,  $\text{CH}_4$ -20 cm and  $\text{H}_2$ -10 cm in 5.5 L vessel. As the 5L of water were introduced into the vessel and heated to boiling, the total pressure varied from 60–80 cm. Thus water vapor pressure between 10–30 cm was also present in the gas mixture.

From the general gas equation:  $PV = nRT$ , the number of moles of  $\text{NH}_3 = 0.057$  in  $V=5.5\text{L}$  and  $T = 35^\circ\text{C} = 308\text{K}$ . This amounts to 0.011 moles/L. But the  $\text{NH}_3$  is subjected to sparking prior to its dissolution in the water, which was subsequently introduced. Thus the following reaction initially occurs:



discharge temperature the approximate value of the equilibrium constant for this reaction is  $K_{eq} = 750$ , showing that the dissociation of ammonia is very favorable (Miller (1955, p. 2354, Table 1, etc.).

$NH_3$  provided 10% of the  $NH_3 + N_2 + H_2$  mix; hence, when cooled to  $35\text{ C}^\circ$  the  $NH_3$  partial pressure is 50 mm, i.e.  $0.1 \times 500\text{mm} = 50\text{ mm}$  or  $0.066\text{ atm}$ . From the general gas equation, the number of moles is  $n = 0.0144$  moles in

$5.5\text{L} = 0.0026$  moles/L. At temperatures approaching the boiling point of water ( $90\text{--}100\text{ C}^\circ$ ) the maximum solubility of ammonia is about 7%; thus,  $0.07 \times 0.0026$  moles/L =  $1.8 \times 10^{-4}$  moles/L remain after the reaction.

Considering the aqueous solution dissociation equilibrium:  $NH_3(aq) \leftrightarrow NH_4^+ + OH^-$ , the molar concentrations are:  $[NH_3] = (1.8 \times 10^{-4} - x)$ ,  $[NH_4^+] =$

$[OH^-] = x$  and  $K_b = 1.8 \times 10^{-5}$ . Solving the equilibrium for  $x = 4.9 \times 10^{-5}\text{M} = [OH^-]$ . The total sum of all acids in Miller's mix of products yields  $1.6 \times 10^{-2}\text{ M} = [H^+]$  (see Table 1 from a previous paper). Hence,  $1.6 \times 10^{-3}\text{M } [H^+] + 4.9 \times 10^{-5}\text{ M } [OH^-] = 4.9 \times 10^{-5}\text{M } \{HOH\}$  and the remaining  $[H^+] = 1.6 \times 10^{-2} - 4.9 \times 10^{-5} = 1.595 \times 10^{-2}\text{M}$  (actually  $1.6 \times 10^{-2}\text{ M}$ ), for which the  $\text{pH} = 1.80$ .