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The Action of Atomic Hydrogen on Aqueous Solutions

I. Effect on Silver, Cysteine, and Glutathione Solutions[1]

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INTRODUCTION

Radiation preservation of foods frequently results in the formation of undesirable odors and flavors. The action of ionizing radiation on biological products—such as food—is largely indirect. The primary action consists mainly in the radiolysis of water, which results in the formation of various free radicals, such as hydroxyl-(OH), peroxy-(HO_2), and atomic hydrogen (H). These free radicals then combine with the solute as well as with one another, forming a great variety of products. Even though free radicals have some reactions in common—mainly hydrogen abstractions, which result in new free radicals—they also show certain typical reactions which distinguish them from each other. It seemed of considerable interest, therefore, to determine which of the various species of free radicals present in aqueous solutions was responsible for the formation of the odors and flavors.

A point of departure was provided by Batzer and Doty (*1*), who showed that at least some of the odors appearing on irradiation of meat were due to hydrogen sulfide or mercaptans, formed on decomposition of a water-soluble fraction of meat. The decomposing compound turned out to be glutathione, a tripeptide containing glycine, glutamic acid, and cysteine.

Since cysteine ($HS \cdot CH_2 \cdot CH \cdot (NH_2) \cdot COOH$) is the sulfur-containing portion of the glutathione molecule, initial work was carried out with it. Atomic hydrogen was chosen as the reagent for the first series of experiments, since of the products of radiolysis of water it seemed the likeliest to cause the formation of hydrogen sulfide or mercaptans; the strongly oxidizing properties of the other free radicals—OH and HO_2—made them less likely choices.

[1] This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned number 694 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense. This paper was presented at the American Chemical Society Meeting at Atlantic City, New Jersey, September, 1956.

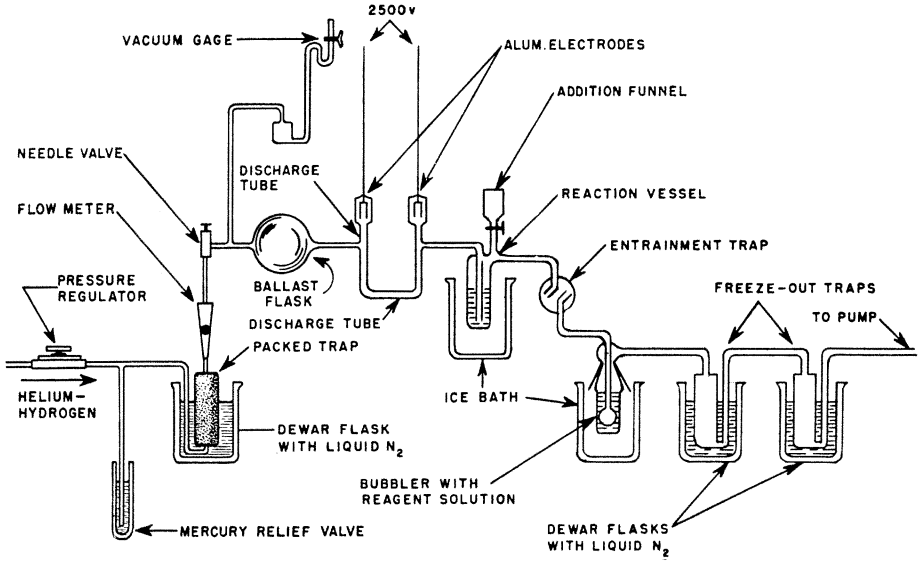

FIG. 1. Equipment for reactions with atomic hydrogen.

THE PRODUCTION OF HYDROGEN ATOMS

Since the radiolysis of water produces OH radicals as well as the desired hydrogen atoms, a different approach was sought. The production of hydrogen atoms by electrical discharge in a hydrogen atmosphere is well established and appeared suitable for our purpose. The problem was to produce an arc at pressures above about 5 mm Hg (the vapor pressure of water at 0° C) and to transport the resulting H atoms into the aqueous solutions.

Equipment

Hydrogen atoms were produced at moderately high pressure (13 to 20 mm Hg) by electrical discharge in a stream of hydrogen diluted with helium. This gas stream was then bubbled through various solutions. The apparatus was a modification of the system described by Harteck and Roeder (*2*), but where these authors used a closed system in which the gases were recirculated, we used a single-pass system. The system is shown schematically in Fig. 1.

The first section of the system consisted of components for purification and flow regulation of the 5% hydrogen–95% helium stream. The gas passed first through a Deoxo catalyst under 800 mm Hg pressure; traces of water and carbon dioxide were removed in the liquid-nitrogen-cooled trap filled with stainless steel packing. The gas then passed through a flowmeter (Fischer-Porter Flowrator 06-150) and a Hoke vacuum needle valve into the reaction section.

The reaction section consisted of the discharge tube and the reaction vessel.

The discharge tube (Fig. 2) had a path length (from electrode to electrode) of about 20 inches. Current to the tube was supplied by a 2500-volt filament transformer capable of delivering 500 ma. The current was limited by a resistor (a 1200-watt hot plate) and measured with a milliameter. The reaction vessle, a simple bubbler, was

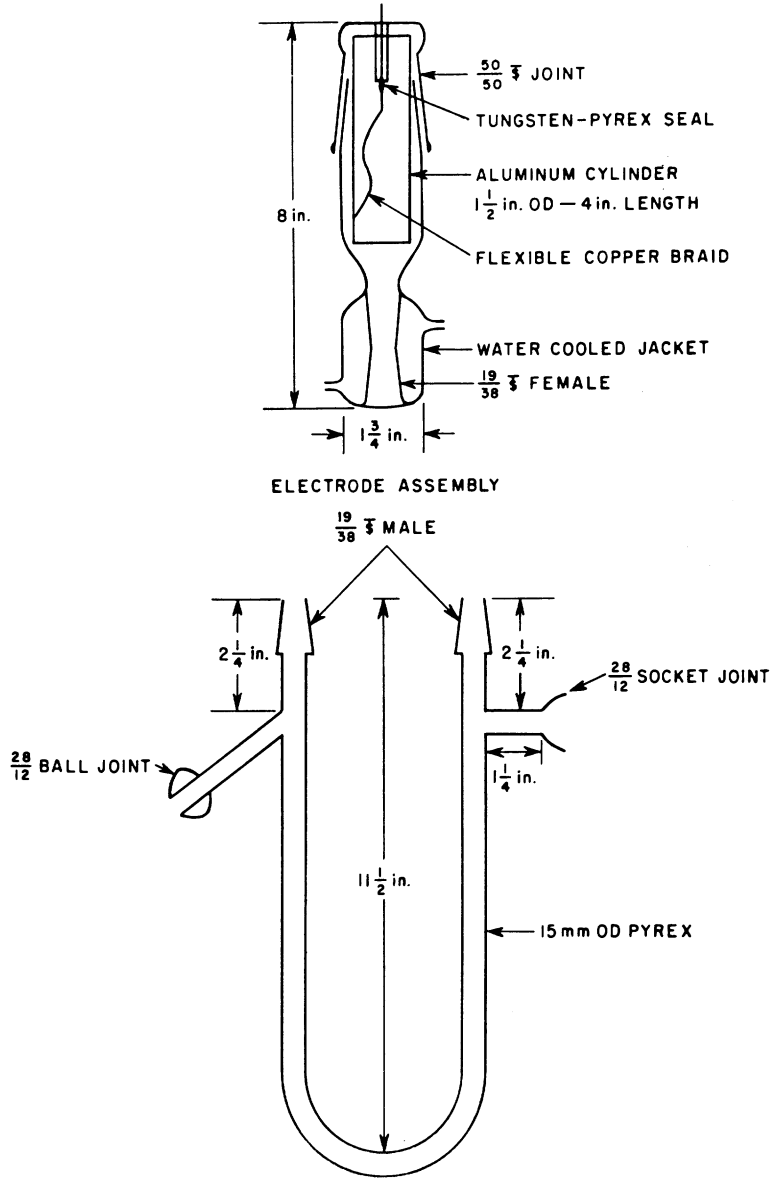

FIG. 2. Diagram of discharge tube.

connected to the discharge tube with 12-mm tubing, which resulted in a stream velocity of the order of 2.5 meters/sec. The interval between the time the gas left the discharge tube and the time it reached the solution was about 0.1 second, which minimized the effect of recombination of the hydrogen atoms. The use of a rare gas as a diluent also retarded the recombination rate, since the recombination requires a third body to remove excess energy and rare gases are not capable of absorbing this energy effectively.

The third section of the line consisted of various ice-cooled and liquid-nitrogen-cooled traps for trapping the reaction products and entrained water vapor. The gas stream was finally removed from the line with a Welch Duo-Seal vacuum pump vented to the outdoors.

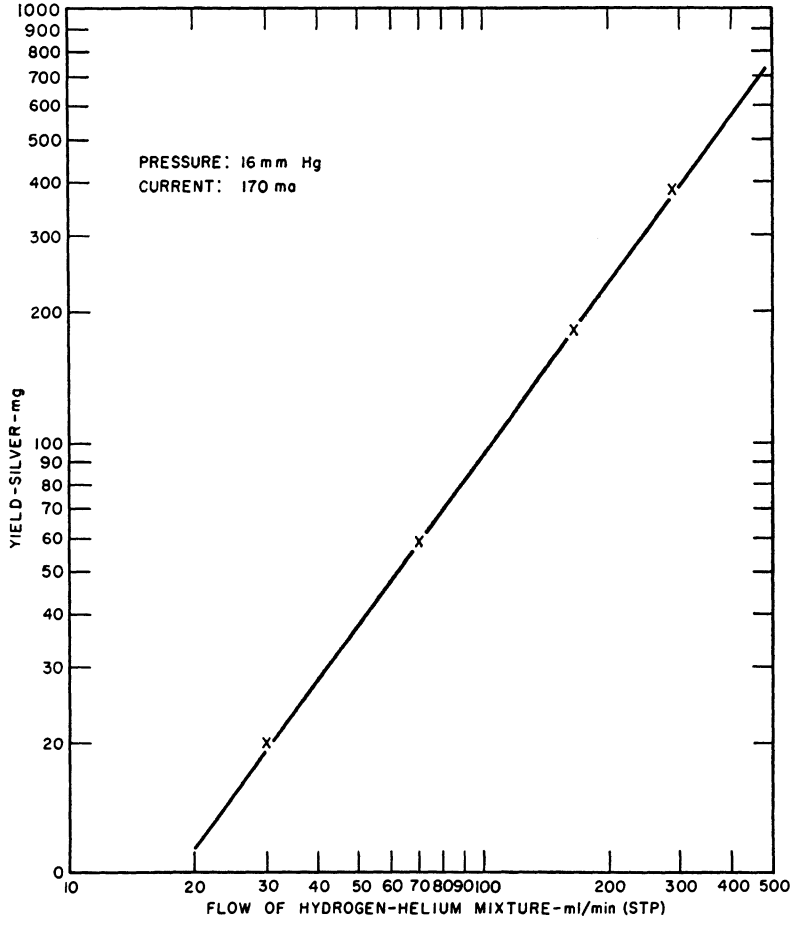

FIG. 3. Effect of flow rate on production of hydrogen atoms.

Procedure

1. *Calibration of equipment—silver runs.* The equipment was calibrated with a 5% silver nitrate solution. This solution is not affected by molecular hydrogen, but atomic hydrogen precipitates metallic silver.

A typical silver run was carried out in the following manner. The pressure in the system on the pump side of the needle valve was reduced to 15 to 20 mm, and the valve opened to allow a hydrogen-helium flow of 275 cc/min (STP). The packed purification column had previously been cooled with liquid nitrogen. The electrical circuit to the discharge tube was then closed. A few minutes was required for sweeping out the residual air in the system with the hydrogen-helium stream. As soon as the red hydrogen arc appeared in the tube, the current was shut off; then 25 ml of the 5% silver nitrate solution was introduced into the reaction vessel through the addition funnel, and the freeze-out traps were immersed in liquid nitrogen. The pressure was adjusted to 15 to 16 mm and the arc started. As soon as the arc had attained

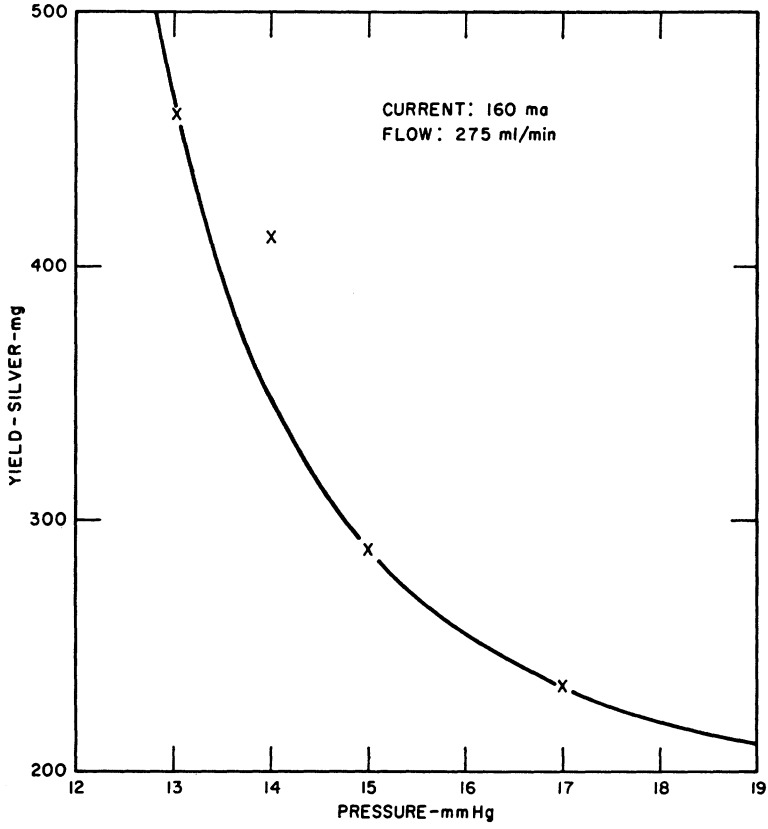

FIG. 4. Effect of pressure on production of hydrogen atoms.

maximum brightness, the current was adjusted to the desired value (normally 170 to 180 ma) by changing the primary voltage of the transformer.

A "normal" silver run lasted 1 hour with the arc in operation. It was necessary to add small volumes of distilled water to the reaction vessel during this time to compensate for evaporation. At the end of the run the arc was shut off, the system brought to atmospheric pressure, and the reaction vessel removed.

The precipitate was filtered off, carefully washed, and redissolved in hot 15% nitric acid. The silver content was determined by a Volhard titration with $N/10$ ammonium thiocyanate in the presence of ferric alum as an indicator.

Reproducible results were obtained only when the equipment was thoroughly cleaned before each run. The discharge tube and the reaction vessels were cleaned by boiling for 15 minutes in 10% alcoholic caustic (100 gm of sodium hydroxide in in 1 liter of 50% aqueous isopropanol), rinsed, and dried. Immediately before use, the glassware was rinsed with 10% phosphoric acid and drip-dried. This resulted in a deactivation of the walls.

2. *Cysteine and glutathione runs.* When reproducible conditions for the production of hydrogen atoms were established, the action of hydrogen atoms on cysteine

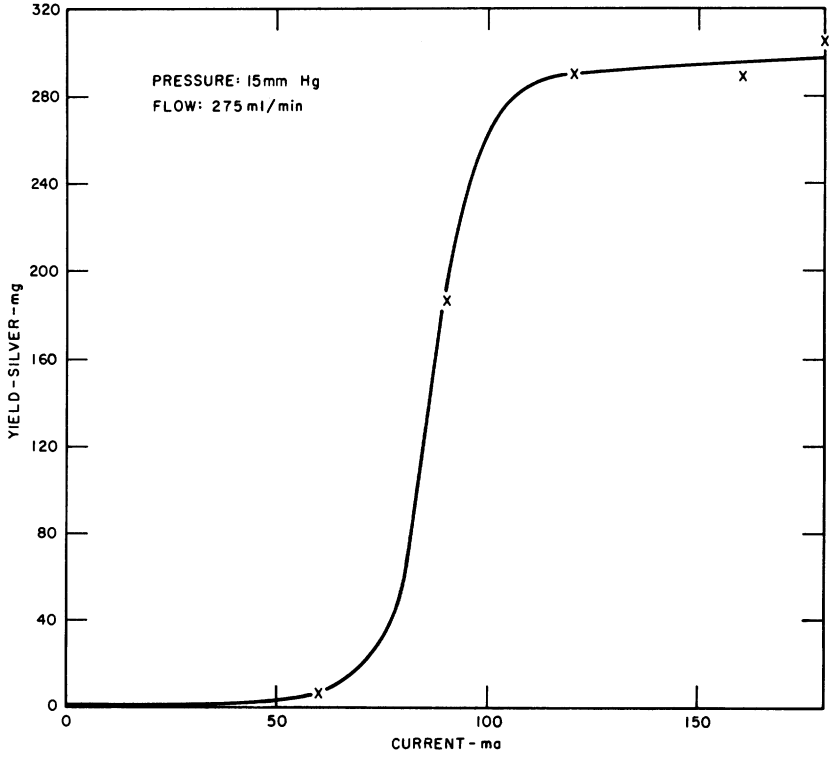

FIG. 5. Effect of current on production of hydrogen atoms.

and glutathione solutions was investigated. Preliminary runs had established that hydrogen sulfide was formed during these reactions. The experimental conditions were unfavorable for efficient removal of H_2S from the gas stream: total pressure was low (13 to 16 mm Hg), the concentration of H_2S in the gas stream was minute ($\sim$0.01%), and pressure drop considerations ruled out efficient gas dispersers. A satisfactory solution to the problem was found in a scrubbing solution of the following composition: 5 ml of 50% zinc sulfate, 10 ml of triethanolamine, and 40 ml of 50% ethylene glycol in water. The glycol lowered the vapor pressure of the solution and minimized the plugging of the freeze-out traps with ice. The triethanolamine scrubber was cooled in an ice bath.

The experimental procedure was as follows. The system was assembled, with a freshly prepared discharge tube and reaction vessel. The scrubber was charged with the $ZnSO_4$-triethanolamine solution, the freeze-out traps cooled with liquid nitrogen, and the pressure lowered to $\sim$15 mm while a stream of hydrogen-helium was passed through the system. As soon as the system had been thoroughly flushed with this gas mixture, a hydrogen arc was produced in the discharge tube. The appropriate solution was then introduced into the reaction vessel through the addition funnel.

The pH of the cysteine solution was adjusted to the desired value with 10 N sodium hydroxide or 6 N hydrochloric acid and measured on a Beckman pH meter, with a "blue" (high pH) glass electrode. Ordinarily a 1-hour run was made with the arc on. The electric current was then turned off, and the pH of the solution adjusted to 1.5 by addition of 6 N HCl, with thymol blue as an internal indicator. The system was swept for 1 hour (with the same gas mixture) to transfer H_2S quantitatively into the triethanolamine scrubber.

The scrubber was then removed and its contents transferred to an Erlenmeyer flask. An excess of $N/100$ (or $N/10$) iodine solution was added, followed immediately by 25 ml of 6 N HCl. The excess iodine was back-titrated, with thiosulfate of the same normality as the iodine solution. Failure to acidify the solution immediately resulted in high values due to the formation of iodoform.

In the case of cysteine, the reaction vessel was removed from the line, its contents transferred to an iodine determination flask, and the pH adjusted to the yellow methyl red end point (pH 6.5 to 7). The air in the flask was swept out with nitrogen, and the stoppered flask permitted to stand overnight. The precipitated cystine was filtered off, washed with water and acetone, air-dried, and weighed.

A blank run was made in the same manner, with fresh solutions adjusted to the same pH, but without turning the arc on. After 1 hour, the pH was adjusted to 1.5 and the sweeping continued for another hour. Hydrogen sulfide was determined as above.

With glutathione the runs were carried out and H_2S determined in the same manner as with cysteine. In addition, the reaction solution was quantitatively transferred to a 100-ml volumetric flask and diluted to volume with distilled water. Aliquots were taken for iodimetric determination of the reduced form of glutathione

(GSH) and the polarographic determination of both the reduced and the oxidized (GSSG) forms.

RESULTS AND DISCUSSION

Silver Runs

The performance of this equipment was determined by measuring the amount of metallic silver produced under a variety of conditions, by the procedure described above.

1. *Reproducibility.* The reproducibility of early runs was very poor. By adapting the rigorous cleaning procedure described in a preceding paragraph, a reproducibility of $\pm 10\%$ was obtained, which was considered adequate for this work.

2. *Effect of flow rate.* Although the spectrum of the hydrogen arc indicates virtually complete dissociation into hydrogen atoms, their extremely rapid rate of recombination results in a rapid decrease of hydrogen atoms downstream from the discharge tube. The system is, therefore, very dependent on the linear flow rate, which determines the residence time of the hydrogen atoms in the lines. The effect of changes in flow rate is shown in Fig. 3. On log-log paper this relationship forms a straight line.

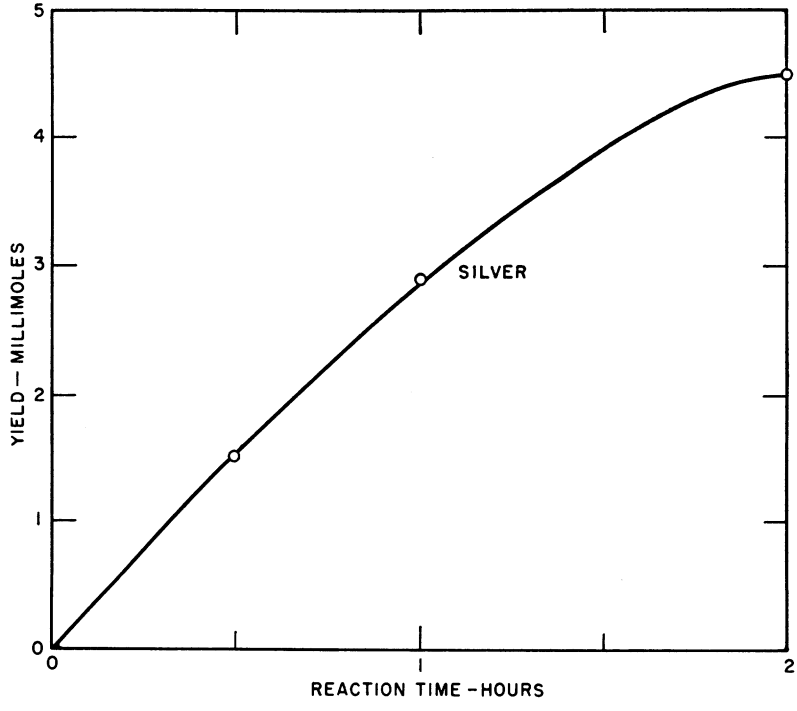

FIG. 6. Effect of "dose" (reaction time) on silver nitrate solution.

3. *Effect of pressure.* Pressure changes have two effects on the production of hydrogen atoms: lower pressures result in an increase of the linear flow rate (at constant feed) and in an increased efficiency of the hydrogen arc. Consequently, the changes found on decreasing the pressure in the system exceed those that would result from the apparent increase in flow rate, as shown in Fig. 4.

4. *Effect of current.* As a first approximation, the production of hydrogen atoms (and metallic silver) should be proportional to the flow of current. As is shown in Fig. 5, this is not so. Rather, there seems to be a threshold of about 100 ma, with only minor effects for an increase from 100 to 180 ma.

5. *Effect of time* ("*dose*"). Variations of the reaction time ("dose") result in a relationship which is shown in Fig. 6 The amount of precipitated silver appears to be a function of the depletion of silver ions in solutions. If we assume that

$$-\frac{d[Ag^+]}{dt} = K[Ag^+],$$

then

$$\ln \frac{[Ag^+]}{[Ag^+]_0} = -Kt$$

where $[Ag^+]_0$ is the concentration of silver at the beginning of the run. Since

$$[Ag^+] = [Ag]_0 - \left[\frac{Ag}{V}\right]$$

$$\ln \frac{[Ag^+]_0 - \left[\frac{Ag}{V}\right]}{[Ag^+]_0} = \ln\left(1 - \frac{Ag}{V[Ag^+]_0}\right) = -Kt$$

where $V\,[Ag^+]_0 = 795$ mg (25 ml of 5 % $AgNO_3$) and Ag the weight of precipitated silver. From this value, the results shown in Table I are obtained.

The Action of Atomic Hydrogen on Cysteine Solutions

1. *The effect of pH changes.* By the technique described in the preceding section, the action of hydrogen atoms on aqueous solutions of cysteine at various pH was determined. The results obtained are shown in Fig. 7.

TABLE I

REACTION CONSTANT OF SILVER REDUCTION

Time (*hr*)	*Yield* (*mg Ag*)	$\frac{Ag}{V\,[Ag^+]_0}$	*K*
½	161	0.203	0.45
1	310	0.390	0.50
2	484	0.610	0.47

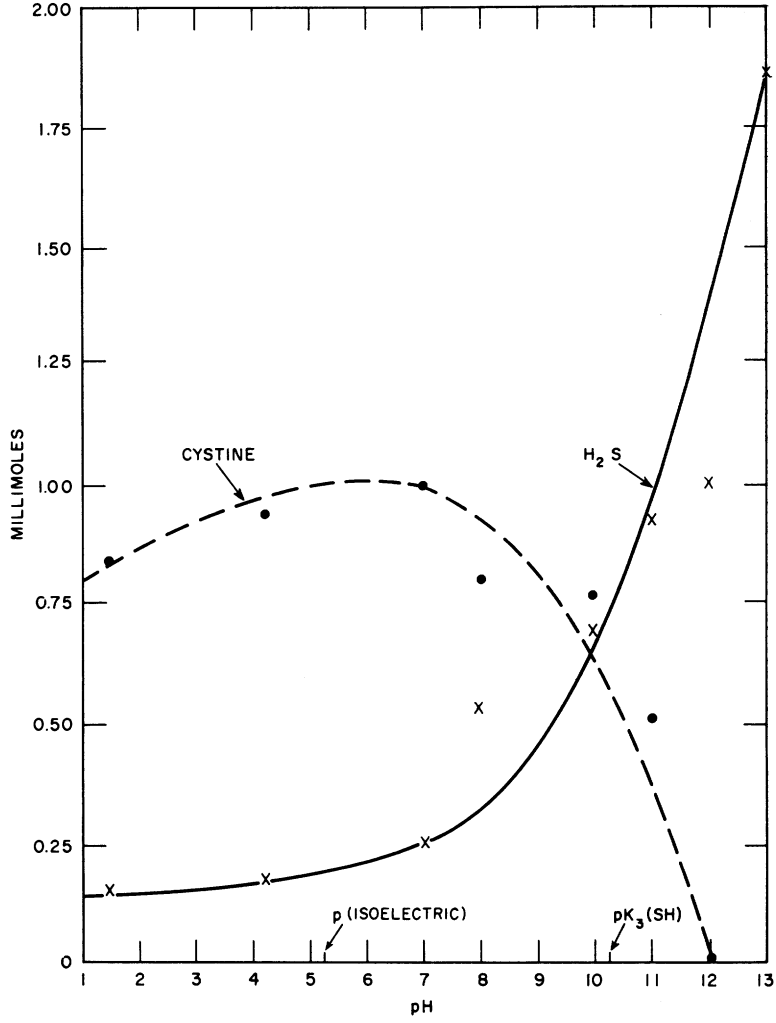

FIG. 7. Action of atomic hydrogen on 5% cysteine solutions. Effect of pH.

As is shown in this figure, the effect of pH changes was pronounced. In acid or neutral solutions (pH 1.5 to 7) not much hydrogen sulfide was formed, but a relatively large amount of the disulfide—cystine—was found. In alkaline solutions (pH 7 to 13) the amount of H_2S increased rapidly; that of cystine decreased to zero at pH 12 or higher.

The results obtained so far can be discussed best in terms of free radical reactions. Possible initial reactions are, in an acid solution,[2]

[2] The figures given for the energy release in these reactions are intended to serve only as a guide, since they were computed from generalized bond energies (*3*).

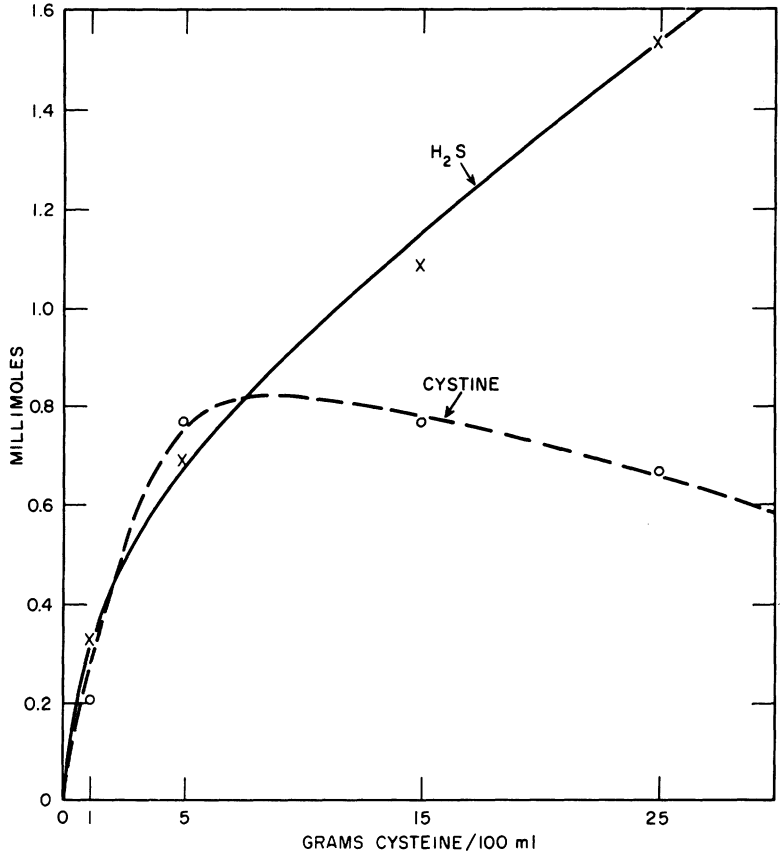

FIG. 8. Action of atomic hydrogen on cysteine solutions. Effect of cysteine concentration.

$$H\cdot + R{-}S{-}H \rightarrow SH\cdot + RH + 31 \text{ kcal} \quad (1)$$

$$H\cdot + R{-}S{-}H \rightarrow RS\cdot + H_2 + 23 \text{ kcal} \quad (2)$$

$$H\cdot + R{-}S{-}H \rightarrow R\cdot + H_2S + 24 \text{ kcal} \quad (3)$$

whereas in highly alkaline solutions only the following initiating reactions may take place, both of which eventually yield H_2S:

$$H\cdot + R{-}S' \rightarrow R\cdot + SH^- + 26 \text{ kcal} \quad (4)$$

$$H\cdot + R{-}S' \rightarrow S^- + RH + 40 \text{ kcal} \quad (5)$$

since at high pH the sulfhydryl group is ionized. There appears to be only one chain-continuing step:

$$R\cdot + R{-}S{-}H \rightarrow RS\cdot + RH + 14 \text{ kcal} \quad (6)$$

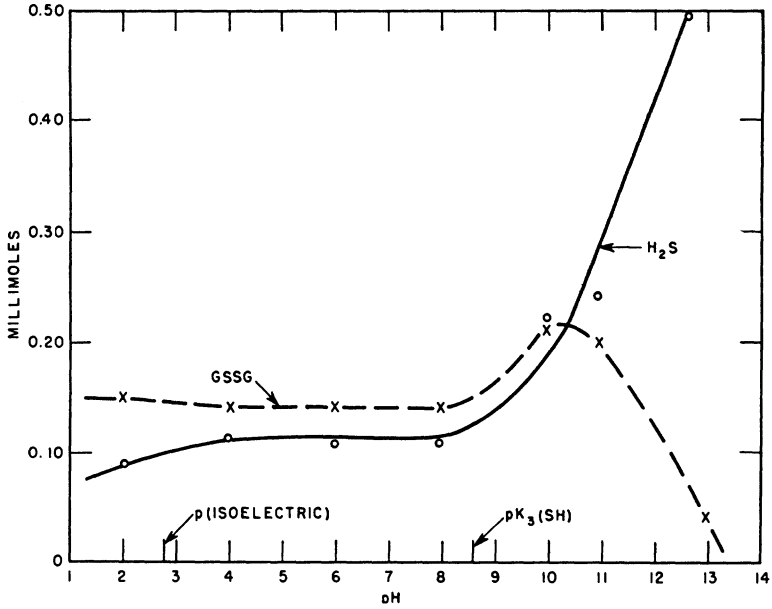

FIG. 9. Action of atomic hydrogen on 1% glutathione solutions. Effect of pH.

since all other reactions result in a chain termination:

$$H\cdot + H\cdot \rightarrow H_2 + 103 \text{ kcal} \quad (7)$$

$$H\cdot + R\cdot \rightarrow RH + 94 \text{ kcal} \quad (8)$$

$$H\cdot + RS. \rightarrow RSH + 30 \text{ kcal} \quad (9)$$

$$H\cdot + SH\cdot \rightarrow H_2S + 80 \text{ kcal} \quad (10)$$

$$R\cdot + R\cdot \rightarrow R{-}R + 69 \text{ kcal} \quad (11)$$

$$R\cdot + RS\cdot \rightarrow R{-}S{-}R + 54 \text{ kcal} \quad (12)$$

$$RS\cdot + RS\cdot \rightarrow RSSR + 38 \text{ kcal} \quad (13)$$

It should be noted that the species resulting from the action of H atoms on very alkaline solutions of cysteine cannot result in the production of cystine according to reaction 13. This may explain the rapid drop in cystine yield in the vicinity of the ionization constant of the sulfhydryl group (pK_3(SH) = 10.2). It appears that the favored reaction is reaction 2, and that only when this reaction is inhibited, as discussed above, is an appreciable amount of H_2S formed.

The thermal desulfuration of cysteine in alkaline solution according to

$$RSH + OH' \rightarrow ROH + RS' \quad (14)$$

is slow even at a pH of 13, provided the temperature is kept low (0 to 5° C). On a few occasions high blanks (and correspondingly higher yields of "live" runs) occurred. These are believed to be due to traces of heavy metal contamination.

2. *The effect of concentration.* The concentration of cysteine was varied from 1 to 25% at pH 10. As can be seen from the results shown in Fig. 8, the amount of H_2S and cystine increase at about the same rate up to a concentration of about 5% cysteine. The H_2S concentration continues to increase; the cystine yields drop off at higher concentrations, indicating other reactions at higher concentrations, or secondary reactions of the cystine originally produced.

The Effect of Atomic Hydrogen on Glutathione

The same equipment and techniques were used to determine the action of hydrogen atoms on glutathione solutions. A somewhat lower concentration was used (1% instead of 5%). The pH was varied from 1.5 to 13. The results are shown in Fig. 9. Compared with cysteine, there is a marked similarity of the shapes of the resulting curves. Allowing for the difference in concentration, the sensitivity of the two compounds toward atomic hydrogen appears to be of the same order of magnitude.

SUMMARY

The equipment described in this paper appears to be capable of producing hydrogen atoms at a controllable and reproducible rate. The experimental arrangement permits reactions of the atomic hydrogen with liquids or solutions boiling as high as or higher than water.

The action of atomic hydrogen on cysteine results in a variety of reactions, of which the most important appear to be the formation of hydrogen sulfide and of cystine, the disulfide (dimer) of cysteine. The reactions are markedly dependent on the pH of the solution. There is no indication of any attack on the amino group, which is in accord with results obtained by other investigators (who used γ-rays). Very similar results were obtained with glutathione.

It is hoped that this approach will be useful in elucidating the role of atomic hydrogen in other reactions and on other types of compounds, as well as the function of additives designed to minimize such reactions.

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