# Rate Studies on the Hydrolysis of Niacinamide

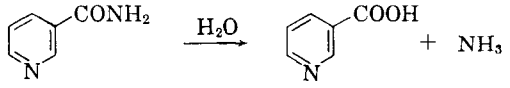
By PER FINHOLT† and TAKERU HIGUCHI

**The apparent first-order rate constant corresponding to the hydrolysis of niacinamide has been determined over the pH range 0.4–11.3 in purely aqueous solutions at 89.4°. Although the pH-rate profile shows a minimum between pH 4 and 6, the reaction velocity is relatively slow over the usual pharmaceutical range. Surprisingly, the second-order proton catalyzed rate constants for the free vitamin and its protonated form appear to be roughly the same. The hydrolytic reaction appears to be subject to both general acid and general base catalysis.**

NIACINAMIDE is one of the most stable of the vitamins in common use. It has been reported (1) that a 10% niacinamide solution in water may be autoclaved at 120° for 20 minutes without any degradation occurring. The great stability of niacinamide is probably the reason why there has been so little work done on the kinetics of the niacinamide hydrolysis. The only kinetic study reported in the literature was made by Jellinek and Gordon (2), who studied the hydrolysis in hydrochloric acid solutions. They found that it differed from the hydrolysis of other amides such as benzamide in that the first-order rate constants for the hydrolysis did not pass through a maximum between 3 and 4 *N* hydrochloric acid. There was a steady increase in the rate constant with increasing hydrochloric acid concentration from 0.05 *N* up to 8.4 *N*. Jellinek and Gordon did not study the hydrolysis in less acidic solution nor in alkaline medium.

The scope of the present work was to study the hydrolysis of niacinamide both in acidic and in alkaline solution, and to determine to what extent the hydrolysis is catalyzed by hydrogen and hydroxyl ions and by general acids and bases. It is known from the literature that the hydrolysis of amides may be subject to general acid base catalysis. As an example may be mentioned the hydrolysis of chloramphenicol, which is catalyzed by ammonia and ammonium ions, mono- and dihydrogen phosphate ions (3), undissociated acetic acid, mono- and dihydrogen citrate ions, and monohydrogen oxalate, succinate, tartrate, fumarate, phthalate, malonate, glutarate, and adipate ions (4–6).

It is assumed that the breakdown of niacinamide can be attributed solely to the hydrolytic cleavage of the amide linkage according to the equation

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## EXPERIMENTAL

**Materials.**—The niacinamide used was niacinamide U.S.P. from Mallinckrodt, recrystallized from benzene. All reagents used were of analytical grade.

**Assay.**—The study of the hydrolysis of niacinamide required an assay method which permitted an accurate determination of niacinamide in the presence of nicotinic acid. For the separation of the two compounds an ion exchange procedure recommended by Sweeney and Hall (7) was used According to this, nicotinic acid at pH 4.5–5 was quantitatively adsorbed by the anion exchange resin IRA-400, while niacinamide was not adsorbed and was determined in the eluate.

For the determination of niacinamide in the eluate, ultraviolet spectrophotometry was used. The absorption spectrum of niacinamide has a maximum at 262 mμ. The molar absorptivity is dependent on pH, as seen from Fig. 1. The protonated form of the amide has a higher molar absorptivity than the nonprotonated form. In order to convert the amide completely to its protonated form, 10% concentrated hydrochloric acid was added to the eluate before the reading in the spectrophotometer, a Cary recording model 11MS,

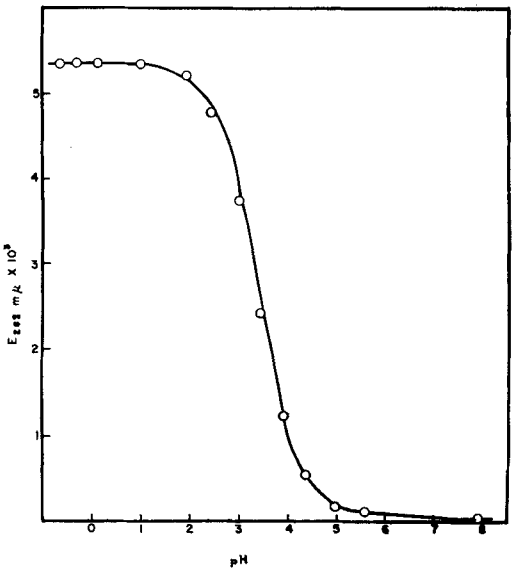



Fig. 1.—Plot showing the pH dependency of the molecular extinction coefficient of niacinamide at the absorption maximum at 262 mμ.

was made. A linear relationship between $A_{262m\mu}$ and concentrations of niacinamide ranging from 1 to 5 mg. in 100 ml. was found.

The complete assay method was as follows: An aliquot part of the sample, usually 2.00 ml., is added to the ion exchange column (Fig. 2) and adjusted to a pH of about 5. The column is washed with water. To the washings are added 10 ml. of the concentrated hydrochloric acid and water to 100 ml., and the absorbance measured in U.V. The content of niacinamide in the solution is read from a standard curve.

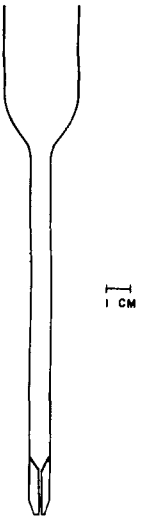



Fig. 2.—Ion exchange tube used for the separation of niacinamide and nicotinic acid.

By analyzing 30 samples with known content of niacinamide and niacin, 99–100.3% (average 99.6%) of the niacinamide was recovered. Sweeney and Hall found that about 0.8% of the niacinamide was hydrolyzed during the ionic exchange procedure. According to our results the amount hydrolyzed was even less. In no case will the hydrolysis on the column have any influence on the results of our kinetic studies.

High amounts of electrolytes in the solution on the column may, to some extent, displace the nicotinate ion from the column. With normal sodium chloride, for example, it was possible to elute nicotinic acid quantitatively. To analyze samples containing relatively high amounts of electrolytes it was, therefore, first necessary to determine the amount of the sample which may be collected through the column without getting any nicotinic acid in the eluate.

The procedure described proved to be satisfactory even in the presence of catechol, since this compound was firmly adsorbed by the resin.

**Hydrolysis Experiments.**—In most cases the following procedure was used: 122 mg. (0.001 *M*) of niacinamide was dissolved in 100 ml. of the appropriate buffer solution. The solution was placed in 5-ml. ampuls and heated to 89.4° in a constant temperature bath. Ampuls were taken out of the bath at appropriate intervals, cooled in ice, and the solution was analyzed. When a mixture of catechol and catecholate was used as buffer, the air in the ampuls was replaced by nitrogen.

In cases where the half-life was shorter than 2–3 hours, e.g., in strongly alkaline solution, the following technique was used: the alkaline buffer solution was heated to 89.4° in a 100-ml. volumetric flask in a constant temperature bath, and 122 mg. of niacinamide dissolved in 1 ml. of the same solution were added. Five-milliliter samples were withdrawn at appropriate intervals, mixed with a calculated amount of 50% sulfuric acid, and cooled in ice.

As far as those experiments are concerned where the main purpose was determination of the catalytic effect of a certain buffer, a series of solutions of different buffer concentrations but the same pH and ionic strength were required. To be able to calculate the composition of these solutions the pKa of the acid component in the buffer mixture was determined at 90° and at the actual ionic strength. The following equations were used for the actual calculations

At pH values less than 4

$$pH = pKa + \log \frac{[base] + [H^+]}{[acid] - [H^+]}$$

At pH values 4–8

$$pH = pKa + \log \frac{[base]}{[acid]}$$

At pH values greater than 8

$$pH = pKa + \log \frac{[base] - [OH^-]}{[acid] + [OH^-]}$$

[acid] and [base] are the measured amounts of the acid and the base component of the buffer mixture.

pH was determined at 90° with a Beckman expanded scale pH meter.

**Determination of pKa of the Prontonated Form of Niacinamide.**—The pKa of the protonated form of niacinamide was determined spectrophotometrically at room temperature, using the equation

$$pKa = pH - \log \frac{a_{NH^+} - a}{a - a_N}$$

where $a_{NH^+}$ = molecular extinction coefficient of the protonated form of niacinamide, $a_N$ = molecular extinction coefficient of the nonprotonated form of niacinamide, $a$ = the observed molecular extinction coefficient, and $a_{NH^+}$ and $a_N$ were determined in solutions of pure $NH^+$ and N. A pKa of 3.40 was found.

pKa was determined electrometrically at 25° and at 90° ($\mu = 0.5$). The following values were found: pKa at 25° = 3.42 and pKa at 90° = 3.12.

## RESULTS AND DISCUSSION

**Order of Reaction with Respect to Niacinamide.**—The rate of disappearance of niacinamide from the solutions was found to be strictly first order with respect to niacinamide at pH values from 0.4 up to 11.3. There was a linear relationship between time and logarithm of residual niacinamide concentration, as shown in Fig. 3.

**Primary Salt Effect.**—In strongly acidic solution the first step in the hydrolytic process was presumed

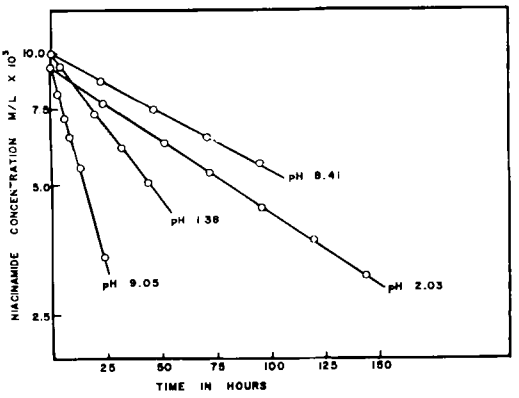
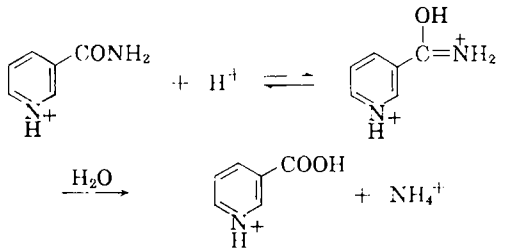



Fig. 3. Plots showing the overall first-order character of the hydrolysis of niacinamide at different pH values and 89.4°. For the actual runs 0.05*N* perchloric acid (pH 1.38), 0.5*M* phosphate (pH 2.03), 0.05*M* borate (pH 8.41), and 0.15*M* catecholate (pH 9.05) were used.

to be a reaction between hydrogen ions and the protonated form of the amide




Since a reaction between two charged species was involved in the hydrolytic process, a primary salt effect was expected. The existence of such an effect is obvious from Fig. 4 where the log of the rate constant $k$ has been plotted against the square root of the ionic strength $\mu$. The $k$ values were found by making runs using 0.1 *N* hydrochloric acid or 0.02 *N* perchloric acid or 0.2 *N* perchloric acid to which different amounts of sodium chloride had been added. According to the theory of Brönsted and Bjerrum, there is a linear relationship between log $k$ and the square root of the ionic strength when two charged species react in dilute solution ($\sqrt{\mu} < 0.2$). At higher concentrations the situation is more complex. It is therefore rather surprising that we find a nearly linear relationship between log $K$ and $\sqrt{\mu}$ at $\sqrt{\mu}$ values from 0.2 up to 1, as shown in Fig. 4. Because of the primary salt effect in acid solutions all experiments in the acid region were carried out at the same ionic strength, namely $\mu = 0.5$.

In alkaline solution (pH 7–12) niacinamide will exist in the noncharged form. Experiments carried out in strongly alkaline solution showed, as expected, that there was no primary salt effect under these conditions. The same half-lives were found with 0.1 *N* sodium hydroxide as with 0.1 *N* sodium hydroxide containing 0.5 *M* sodium chloride (Fig. 5); and the same values were obtained with 0.025 *N* barium hydroxide as with 0.025 *N* barium hydroxide containing 0.5 *M* sodium chloride.

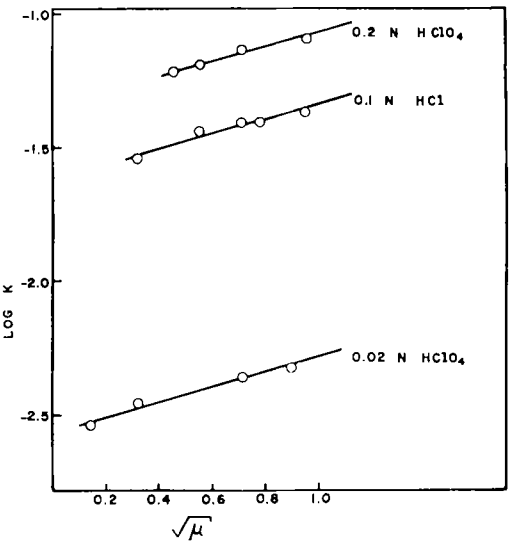



Fig. 4. Plots showing the effect of the ionic strength ($\mu$) on the rate of niacinamide hydrolysis in strong acids at 89.4°. The ionic strength of the acids was varied by addition of different amounts of sodium chloride.

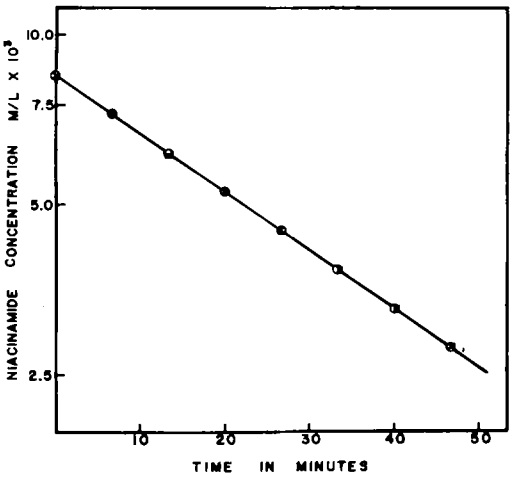



Fig. 5.—Plot showing the rate of niacinamide hydrolysis in 0.1*N* sodium hydroxide (o) and in 0.1*N* sodium hydroxide containing 0.5*M* sodium chloride (x). The coincidence of the experimentally determined points shows that the rate of niacinamide hydrolysis is independent of ionic strength.

**Catalytic Effect of Hydrogen Ions and Hydroxyl Ions on the Hydrolysis of Niacinamide.** The catalytic effect of hydrogen ions on the hydrolysis of the protonated form of niacinamide was determined by using hydrochloric acid or perchloric acid ($[H^+] \gtrsim 0.1$) as solvents. See Table I. At these high hydrogen ion concentrations niacinamide will exist only in the protonated form.

The catalytic effect of hydroxyl ions was determined by using solutions of sodium hydroxide or barium hydroxide as solvents. See Table II.

**Catalytic Effect of General Acids and Bases on the Hydrolysis of Niacinamide.** The catalytic

TABLE I.—CATALYTIC EFFECT OF HYDROGEN IONS ON THE HYDROLYSIS OF THE PROTONATED FORM OF NIACINAMIDE

| Acid | [H+] | $k$, hr.$^{-1}$ × $10^2$ | $k_{H+}$,[a] hr.$^{-1}$ mol.$^{-1}$ L. × 10 |
|---|---|---|---|
| HCl | 0.10 | 3.41 | 3.41 |
| HCl | 0.50 | 19.4 | 3.88 |
| $HClO_4$ | 0.20 | 6.79 | 3.40 |

[a] Average: $k_{H+}$ = 3.6 × $10^{-1}$ hr.$^{-1}$ mol.$^{-1}$ L.

TABLE II.—CATALYTIC EFFECT OF HYDROXYL IONS ON THE HYDROLYSIS OF NIACINAMIDE

| Base | [OH−] | $k$, hr.$^{-1}$ × 10 | $k_{OH-}$,[a] hr.$^{-1}$ mol.$^{-1}$ L. |
|---|---|---|---|
| NaOH | 0.05 | 6.87 | 13.7 |
| NaOH | 0.10 | 14.0 | 14 |
| NaOH | 0.20 | 32.8 | 16.4 |
| $Ba(OH)_2$ | 0.02 | 2.75 | 13.8 |
| $Ba(OH)_2$ | 0.025 | 3.16 | 12.6 |
| $Ba(OH)_2$ | 0.05 | 6.87 | 13.7 |
| $Ba(OH)_2$ | 0.10 | 17.7 | 17.7 |

[a] Average: $k_{OH-}$ = 14.6 hr.$^{-1}$ mol.$^{-1}$ L.

effect of certain buffers was determined by making runs with each buffer, varying the total buffer concentration but keeping the pH and the ionic strength constant in each series. By plotting the observed $k$ values against buffer concentration, straight lines were obtained, the slopes of which were a measure of the catalytic activity of the buffers.

Figure 6 shows that phosphate buffers with pH 10.19 and pH 10.33 have a catalytic effect on the hydrolysis of niacinamide. At pH 8.64 the effect is very small. The phosphate buffers with pH 8.64 were solutions of $Na_2HPO_4$. The catalytic constant of $HPO_4^-$ is obviously very small compared to the catalytic constant of $PO_4^{\equiv}$. The small slope of the line corresponding to pH 8.64 may be attributed partly to the amount of $PO_4^{\equiv}$ ions present in the $Na_2HPO_4$ solutions.

For the hydrolysis of niacinamide in the actual phosphate buffers the following equation is valid

$$k = k_0 + k_{HPO_4^-}[HPO_4^-] + k_{PO_4^{\equiv}}[PO_4^{\equiv}] \qquad \text{(Eq. 1)}$$

At pH 10.33 and pH 10.19 $k_{HPO_4^-}[HPO_4^-]$ is negligible compared to $k_{PO_4^{\equiv}}[PO_4^{\equiv}]$ and may be omitted, thus giving

$$k = k_0 + k_{PO_4^{\equiv}}[PO_4^{\equiv}] \qquad \text{(Eq. 2)}$$

$k_0$ is equal to $k$ at zero buffer concentration, i.e., equal to the intercept of the lines with the $y$-axis.

$[PO_4^{\equiv}]$ may be calculated from the equation

$$\frac{[PO_4^{\equiv}]}{C - [PO_4^{\equiv}]} = \frac{ka_3}{[H^+]} \qquad \text{(Eq. 3)}$$

where $C$ = total buffer concentration and $ka_3$ = $10^{-10.72}$ (89.4° and $\mu$ = 0.75).[1]

Using Eqs. 2 and 3, the run at pH 10.33 gives $k_{PO_4^{\equiv}}$ = 6.8, the run at pH 10.19 gives $k_{PO_4^{\equiv}}$ = 6.5. Average $k_{PO_4^{\equiv}}$ = 6.65 hr.$^{-1}$ mol.$^{-1}$ L.

Figure 7 shows the catalytic effect of phosphate buffers pH 6.40–7.70. There is an increase in $k$ with increasing buffer concentration especially at

[1] A 0.2 $M$ phosphate buffer pH 10.19 has $\mu$ = 0.74.

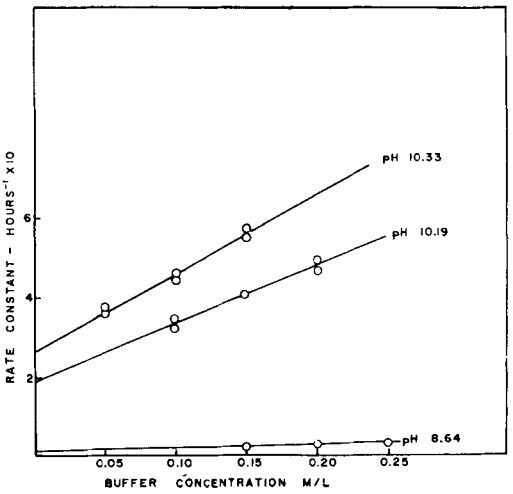



Fig. 6.—Plots showing the effect of phosphate concentration on the pseudo first-order rate constant of niacinamide hydrolysis at fixed pH values. All the runs were made at 89.4° and at ionic strength of 0.75.

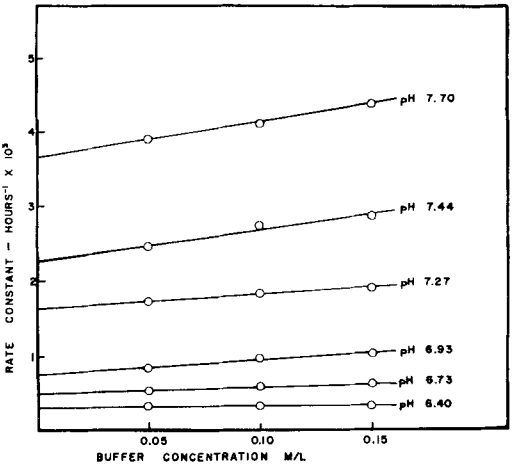



Fig. 7.—Plots showing the effect of phosphate concentration on the pseudo first-order rate constant of niacinamide hydrolysis at fixed pH values. All the runs were made at 89.4° and at ionic strength of 0.75.

the highest pH values. The catalytic effect of these buffers seems, however, to be mainly due to the catalytic effect of the $PO_4^{\equiv}$ ions present. The following calculation indicates this.

At pH 7.7 the increase in $k$ going from zero buffer concentration up to 0.15 $M$ is 0.72 × $10^{-3}$. At pH 7.7 $[PO_4^{\equiv}]$ = 1.26 × $10^{-4}$ calculated according to the equations

$$[PO_4^{\equiv}] = \frac{ka_2 \times ka_3}{[H^+]^2}[H_2PO_4^-]$$

$$\frac{0.15 - [H_2PO_4^-]}{[H_2PO_4^-]} = \frac{ka_2}{[H^+]}$$

where $ka_2$ = $10^{-6.45}$ (90° and $\mu$ = 0.75). $k_{PO_4^{\equiv}}$ × $[PO_4^{\equiv}]$ = 6.65 × 1.26 × $10^{-4}$ = 0.84 × $10^{-3}$, i.e., nearly equal to the increase in $k$.

A similar calculation using the results from the experiments at the lower pH values show that in most cases the increase in $k$ is a little higher than what would have been expected if $PO_4^{\equiv}$ was the only catalytic species.

The catalytic effect of $H_2PO_4^-$ is shown in Fig. 8. If we assume that the increase in $k$ may be attributed solely to a catalytic effect of $H_2PO_4^-$ on the nonprotonated form of niacinamide, calculation gives as result

$$k_{H_2PO_4^-} = 4.4 \times 10^{-4} \text{ hr.}^{-1} \text{ mol.}^{-1} \text{ L.}$$

Figure 9 seems to indicate that $H_2PO_4^-$ has a more pronounced catalytic effect on the hydrolysis of the protonated form of niacinamide than on the hydrolysis of the nonprotonated form. There also seems to be a catalytic effect of the undissociated phosphoric acid on the protonated form of the amide.

Higuchi and collaborators (6) have shown that the "half salt" of dibasic carboxylic acids, such as oxalic, succinic, tartaric, fumaric, phthalic, malonic, glutaric, and adipic acid has a strong catalytic

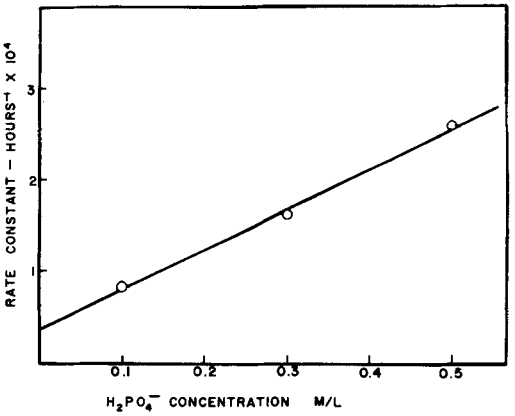



Fig. 8.—Plot showing the effect of dihydrogen phosphate concentration on the pseudo first-order rate constant of niacinamide hydrolysis at 89.4° and at ionic strength of 0.5 and pH-4.50.

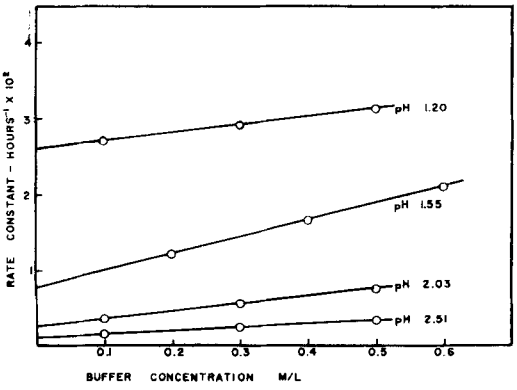



Fig. 9.—Plots showing the effect of phosphate concentration on the pseudo first-order rate constant of niacinamide hydrolysis at fixed pH values. All the runs were made at 89.4° and at ionic strength of 0.5.

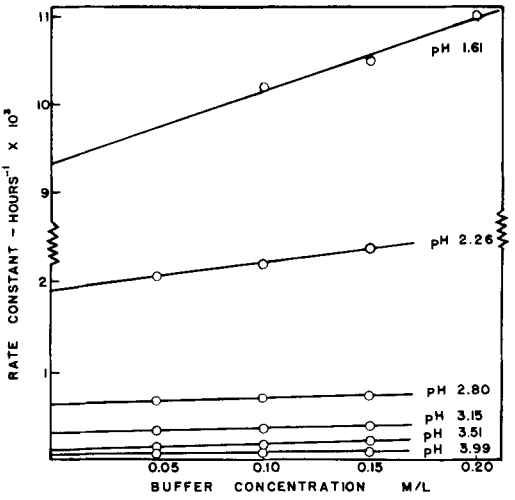



Fig. 10.—Plots showing the effect of oxalate concentration on the pseudo first-order rate constant of niacinamide hydrolysis at fixed pH values. All the runs were made at 89.4° and at ionic strength of 0.5.

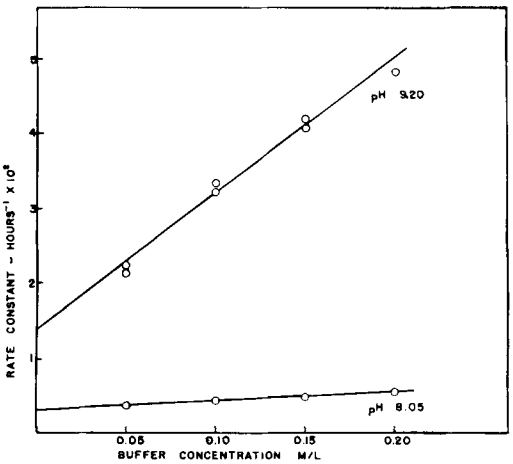



Fig. 11.—Plots showing the effect of catecholate concentration on the pseudo first-order rate constant of niacinamide hydrolysis at fixed pH values. All the runs were made at 89.4° and at ionic strength of 0.2.

effect on the hydrolysis of chloramphenicol. A similar effect could not be demonstrated for the "half salt" of oxalic acid on the hydrolysis of niacinamide (Fig. 10, curve marked pH 2.80). With decreasing pH, i.e., with an increase in the concentration of undissociated oxalic acid and of protonated niacinamide, there is a slight increase in the catalytic effect of the oxalate buffers.

Catechol catalyzes, according to Higuchi and collaborators (6), the hydrolysis of phosphonate esters. In the present work the influence of catechol on niacinamide hydrolysis has been studied (Fig. 11). The great difference in slope between the two lines on Fig. 11 seems to indicate that the catalytic effect of the catecholate buffers must be attributed mainly to the double charged catechol

anion. pKa of catechol was determined to be 8.65 at 89.4° and $\mu = 0.2$, which was the ionic strength used in the hydrolysis experiments.

The evaluation of the catalytic effect of borate buffers gives rise to certain problems, because these buffers do not contain only undissociated $H_3BO_3$ and $H_2BO_3^-$ ions, but also the ions $BO_2^-$ and $B_4O_7^=$. The actual concentration of each of these ions cannot be determined and it is therefore impossible to make an accurate calculation of the ionic strength of the buffer solutions and to calculate the catalytic constant of each species. The first of the difficulties mentioned may be handled by using the buffer in a relatively low concentration and adding a relatively great amount of a neutral salt. The ionic strength will then be determined mainly by the neutral salt. In our experiments the total buffer concentration never exceeded 0.15 *M* and sodium chloride was added to $\mu = 0.75$.

pKa of boric acid was determined to be 8.42 at 90° and $\mu = 0.75$. It is obvious from Fig. 12 that the catalytic effect of borate buffers is due mainly to the "borate" ions, not to undissociated boric acid. A calculation of the catalytic constant of the borate ions, assuming they all exist as $H_2BO_3^-$, gave results varying from $0.7 \times 10^{-1}$ (at pH 7.65) up to $1.2 \times 10^{-1}$ (at pH 8.77).

**pH-Rate Profile of Niacinamide Hydrolysis.**—The pH-rate profile (Fig. 13) shows the relation between pH and log $k$ at zero buffer concentration. pH of the strongly acid solutions at 89.4° and $\mu = 0.5$ were calculated from the equation

$$\text{pH} = -\log [H^+] + 0.08$$

The above relationship between pH and $[H^+]$ was obtained as the result of pH measurements in acids of known hydrogen ion concentration at 89.4° and $\mu = 0.5$. In a similar manner we found the following relationship between pH and $[OH^-]$ at 89.4° and $\mu = 0.75$

$$\text{pH} = 12.0 + \log [OH^-]$$

This equation was used for calculating the pH of the strongly basic solutions.

In the alkaline region the phosphate and borate buffers were adjusted to an ionic strength of 0.75. The ionic strength of the catecholate buffers was 0.20. The pH of these buffers at $\mu = 0.75$ were found to be equal to the pH at $\mu = 0.20$ less 0.15.

At zero buffer concentration the following reactions will contribute to the overall velocity of the hydrolysis

$N^+ + H^+ \rightarrow$ products, rate constant: $k_{H^+}'$
$N + H^+ \rightarrow$ products, rate constant: $k_{H^+}''$
$N^+ + H_2O \rightarrow$ products, rate constant: $k_{H_2O}'$
$N + H_2O \rightarrow$ products, rate constant: $k_{H_2O}''$
$N^+ + OH^- \rightarrow$ products, rate constant: $k_{OH^-}'$
$N + OH^- \rightarrow$ products, rate constant: $k_{OH^-}''$
$N^+$ = protonated form of niacinamide
$N$ = nonprotonated form of niacinamide
$[N_T] = [N] + [N^+]$

The overall velocity is equal to the sum of the rates of all these reactions

$$-\frac{d[N_T]}{dt} = k_{H^+}'[N^+][H^+] + k_{H^+}''[N][H^+] + k_{H_2O}'[N^+][H_2O] + [k_{H_2O}''][N][H_2O] + [k_{OH^-}'][N^+][OH^-] + [k_{OH^-}''][N][OH^-] \quad \text{(Eq. 4)}$$

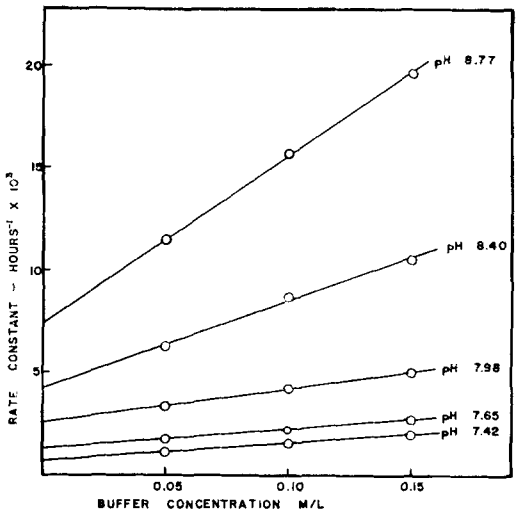



Fig. 12.—Plots showing the effect of borate concentration on the pseudo first-order rate constant of niacinamide hydrolysis at fixed pH values. All the runs were made at 89.4° and at ionic strength of 0.75.

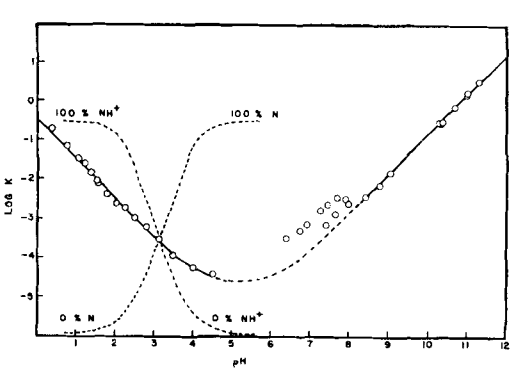



Fig. 13.—Plot showing the relation between pH and the log of the pseudo first-order rate constant of niacinamide hydrolysis at 89.4° and zero buffer concentration. The dotted S-shaped curves show the amount of the protonated ($NH^+$) and nonprotonated (N) form of niacinamide in relation to pH.

In fairly strongly acidic solution the last four terms of Eq. 4 will be negligible compared to the first two terms and may be omitted, thus giving

$$-\frac{d[N_T]}{dt} = k_{H^+}'[N^+][H^+] + k_{H^+}''[N][H^+]$$

Because of the overall first-order character of the hydrolysis the following equation is valid

$$-\frac{d[N_T]}{dt} = K[N_T] \quad \text{(Eq. 5)}$$

Combining the last two equations and the equation

$$k_a = \frac{[N][H^+]}{[N^+]}$$

gives

$$k = [H^+]\frac{k_{H^+}'[H^+] + k_{H^+}''(K_a)}{[H^+] + k_a}$$

The fact that the pH-rate profile is a nearly straight line from pH 0.4 to pH 3.5 shows that $k_{H^+}'$ and $k_{H^+}''$ must be of the same order of magnitude, thus giving

$$k = [H^+][k_{H^+}'] = [H^+]\, k_{H^+}''$$

$$\log k = -pH + 0.08 + \log k_{H^+}'$$

If $k_{H^+}''$ had been different from $k_{H^+}'$ there would have been a break in the curve around pH = pKa = 3.1.

In fairly strongly basic solution the five first terms in Eq. 4 may be omitted. Doing this and combining with Eq. 5 gives

$$k = k_{OH^-}''\,[OH^-]$$

$$\log k = pH - 12 + \log k_{OH^-}''$$

In accordance with this last equation the pH-rate profile is a straight line between pH 8.4 and 11.3. Between pH 6.4 and 8 however, the experimentally determined $k$ values deviate from the straight line. The deviations were greatest when phosphate was used as buffer [the 6 points lying farthest away from the dotted line (Fig. 13) correspond to $k$ values obtained from experiments with phosphate as buffer]. The deviations were relatively small when borate was used as buffer (corresponding to the 3 points closest to the dotted line). With catechol catecholate as the buffer, the deviation was intermediate (the point with the coordinates 7.9–2.5).

In the pH region 6.4–8 the data suggest that reactions other than the hydrolysis catalyzed by hydroxyl ions and the buffers may participate significantly. It is difficult to say what these reactions are. One must keep in mind, however, that in the pH region 6.4–8 the half-life of niacinamide is very long, 10–100 days. This means that the niacinamide solutions in our experiments had to be heated at 89.4° from one to several weeks. During this long period of heating the solution will attack the glass surface and extract products such as silicates and metals, which may well have a catalytic effect. Phosphates are known to be especially aggressive against glass. In addition to this, the glass surface itself may act as a catalyst, thus promoting a heterogeneous catalysis.

The pH-rate profile seems to level off earlier than would have been the case if $k_{H_2O}'$ and $k_{H_2O}''$ had been negligible. This means that at pH 4.5–6 the overall velocity is mainly determined by the noncatalyzed hydrolysis.

## SUMMARY AND CONCLUSIONS

1. The hydrolysis of niacinamide was found to be first order with respect to niacinamide over a wide range of hydrogen ion concentration (pH 0.4–11.3).

2. The rate of the hydrolysis has a minimum at pH 4.5–6. In this region, the half-life of niacinamide was about 1000 days at 89.4°.

3. The hydrolysis is catalyzed by hydroxyl ions and hydrogen ions. The catalytic constant of the hydroxyl ions was found to be $k_{OH^-} = 14.6$ L. hr.$^{-1}$ mol.$^{-1}$ at 89.4°. The catalytic effect of the hydrogen ions was determined to be nearly the same for the hydrolysis of the protonated form of niacinamide as for the hydrolysis of the nonprotonated form, $k_{H^+} = 3.6 \times 10^{-1}$ L.hr.$^{-1}$ mol.$^{-1}$ at 89.4°.

4. The hydrolysis is catalyzed by general acids and bases. The highest effect was produced by the triply charged phosphate ion. Considerable effects by the doubly charged catechol ion and the borate anions were also noted. Mono- and dihydrogen phosphate ions were found to have a very small effect on the hydrolysis of the nonprotonated form of niacinamide. Monohydrogen phosphate seemed to have a somewhat stronger effect on the hydrolysis of the protonated form. The hydrolysis of this form also appeared to be slightly catalyzed by undissociated phosphoric acid and oxalic acid.

5. A positive, primary salt effect was noted in strongly acidic solution; but none in strongly alkaline solution was noted.

## REFERENCES

(1) Mikkelsen, V. H., *Arch. Pharm. Chemi*, **46**, 479(1939).
(2) Jellinek, H. H. G., and Gordon, A., *J. Phys. & Colloid Chem.*, **53**, 996(1949).
(3) Trolle-Lassen, C., *Arch. Pharm. Chemi*, **60**, 689(1953).
(4) Higuchi, T., and Marcus, A. D., THIS JOURNAL, **43**, 530(1954).
(5) Higuchi, T., Marcus, A. D., and Bias, C. D., *ibid.* **43**, 129(1954).
(6) Higuchi, T., Progress report 1, May 20, 1959, and Progress report 2 and 3, March 3, 1960, to Army Chemical Warfare Laboratories, Contract number DA 18-108-405-CML-265.
(7) Sweeney, J. P., and Hall, W. L., *J. Assoc. Offic. Agr. Chemists*, **36**, 1018 (1953).