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# The Polarographic Behavior of the Condensation Product of Glyoxal with *o*-Phenylenediamine, and Its Application to the Determination of Glyoxal

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In buffer solutions containing excess *o*-phenylene-diamine (OPD), glyoxal is condensed with OPD and the condensation product produces two diffusion-controlled reduction waves ($E_{1/2}=-0.211$ and $-0.358$ V. vs. SCE, pH=1.6) at pH values below 2.5 and a single well-defined reduction wave ($E_{1/2}=-0.782$ V., pH=8.5) at pH values above 2.5. In the pH range between 2 and 8, an ill-defined reduction wave, which seems to be a catalytic hydrogen wave brought about by the reduction product, is also observed at a more positive potential than a normal hydrogen wave. A linear relationship between the limiting current and the concentration of glyoxal has been obtained under the following conditions, which are recommended for the practical determination of glyoxal; $(1—2)\times10^{-2}$ M for the concentration of OPD, 7—10 for pH, 25°C for the temperature, and 30—60 min. for the condensation time. The application was made to the determination of the purity of a commercial glyoxal aqueous solution, and also to the determination of the glyoxal which is present in ethylene glycol as an impurity. A mechanism for the electrode reactions of the condensation product (i. e., quinoxaline) of glyoxal with OPD was postulated.

It was first reported by Winkel and Proske[1] that glyoxal shows an unstable reduction wave at $-1.50$ V. in an ammonium chloride solution. The half-wave potential was found by Mackinney and Temmer[2] to be $-1.41$ V. vs. SCE in a biphthalate buffer at pH 3.4. However, no remarks on the current were made in either paper. More detailed results on the polarographic behavior of glyoxal in various buffer solutions and the mechanisms of its electrolytic reduction have been reported by Elving and Bennett[3]; the reduction wave is typically kinetic-controlled in its character, and hence the limiting current varies with the temperature and with the pH of electrolytic solution, and also decreases with time in the alkaline region because of the instability of glyoxal. Therefore, strict experimental conditions seem to be required if this reduction wave is to be used in the polarographic determination of glyoxal.

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1) A. Winkel and G. Proske, *Ber.*, **69**, 1917 (1936).

2) G. Mackinney and O. Temmer, *J. Am. Chem. Soc.*, **70**, 3586 (1948).

3) P. J. Elving and C. E. Bennett, ibid., **76**, 1412 (1954).

One of the present authors has previously reported that the condensation products of dehydro-L-ascorbic acid (DAA) and its related compounds with *o*-phenylenediamine (OPD) show diffusion-controlled reduction waves at a dropping mercury electrode, and that these reduction waves can be applied to the determination of DAA and its related compounds.[4]

An analogous study of glyoxal was made for the present work, and it was found that the condensation product of glyoxal with OPD gives a diffusion controlled reduction wave characteristic of glyoxal.

The present paper will describe the polarographic behavior of the condensation product and a method of analyzing glyoxal by using this reduction wave. Furthermore, some consideration will be given to the mechanism of the electrode reactions of the condensation product (i. e., quinoxaline) of glyoxal with OPD.

## Experimental

**Materials and Reagents.**—The glyoxal was a commercial product (about a 40% aqueous solution) from Wako Pure Chemical Industries, Ltd.; it was used without further purification. Glyoxal-sodium bisulfite monohydrate was prepared according to the method of Ronzio and Waugh.[5] Quinoxaline was prepared by Jones and McLaughlin's method.[6]

Stock solutions of glyoxal and quinoxaline were prepared by using redistilled water or 99% ethanol as the solvent. As the standard solution of glyoxal, $1\times10^{-2}$ M aqueous solution of the pure crystalline monohydrate of glyoxal-sodium bisulfite was used[7]; it was prepared by dissolving the specimen directly into redistilled water.

OPD was a commercial product; it was checked to give no reduction wave in the absence of glyoxal under the present experimental conditions. The stock solution (about 0.1 M) of OPD was prepared fresh daily by dissolving OPD directly in redistilled water. No maximum suppressor was used, since the condensation product showed no maximum under the present polarographic conditions. All the other chemicals were of the reagent grade or better.

**Buffer Solutions.**—Walpole's buffer for pH values from 1 to 2.5, an acetate buffer for pH 4.3, McIlvanie's buffer for pH 2.2 to 8.0, mixtures of disodium hydrogen phosphate with sodium hydroxide for pH above 8, and Britton-Robinson's buffer (BR buffer) for pH 1.8 to 12.0 were used for examining the effect of pH and, at the same time, for checking the effects of the components of the buffer solution.

**Apparatus and Procedure.**—The polarographic measurements were carried out with a Yanagimoto Automatic Recording Polarograph (Type PA 101). The characteristics of the capillary used were $m=1.14$ mg./sec. and $t=4.00$ sec./drop in the buffer solution (pH=4.0) when the height of the mercury reservoir was 71.0 cm. and when the applied potential was 0 V. vs. SCE at 25°C.

From the necessity of employing a thermostat for temperature control, a modified H-type electrolytic cell with a potasium nitrate-saturated agar bridge and a sintered glass disk was used. The accuracy of the temperature control was ±0.2°C. A saturated calomel electrode was used as the reference electrode. The cell resistance was less than 240 Ω. To check the potential at the dropping mercury electrode, a Shimadzu Potentiometer (Type 3P) was used, while the final pH of the polarographic solutions was measured with a Yanagimoto pH-meter (Type 42) after the polarographic measurements had been made, when necessary. For the experiments of controlled-potential electrolysis, a Yanagimoto Controlled-Potential Electrolyser (MFG. No. 258) was used.

The experimental procedure was as follows: a given volume (usually 1 to 4 ml.) of the glyoxal stock solution was placed in a 20-ml. volumetric flask; a given volume (usually 1 to 4 ml.) of a 0.1 M aqueous solution of OPD and 10 ml. of an appropriate buffer solution were added, and then the flask was filled up to the mark with redistilled water. A part of the mixture was transferred to an electrolytic cell, and the cell was kept in the thermostat for a given time to allow the condensation reaction to take place. At the same time the dissolved oxygen was removed by bubbling nitrogen gas. After this period of time, the polarograms are recorded.

**Controlled-potential Electrolysis.**—In order to get information concerning the reversibility of the electrode reaction and to ascertain the number of electrons

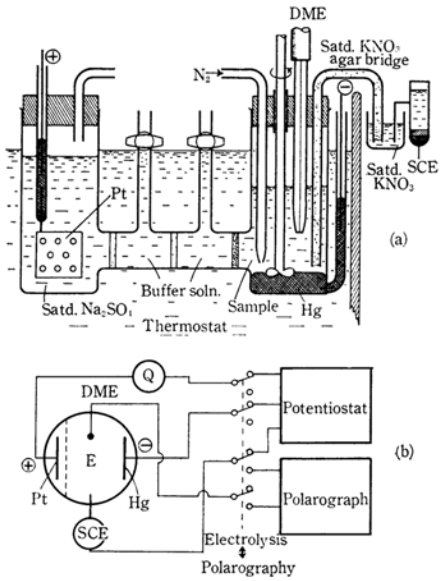



Fig. 1. Controlled-potential electrolysis.
(a) Electrolysis cell, (b) Schematical diagram of the circuit for the electrolysis and polarography.
E: Electrolysis cell (a), Q: gas coulometer.

4) T. Wasa, M. Takagi and S. Ono, This Bulletin, **34**, 518 (1961).
5) R. Ronzio and T. D. Waugh, "Organic Syntheses," **24**, 61 (1944).
6) R. G. Jones and K. C. McLaughlin, ibid., **30**, 86 (1950).
7) C. S. Wise, C. L. Mehltretter and J. W. Van Cleve, *Anal. Chem.*, **31**, 1241 (1959).

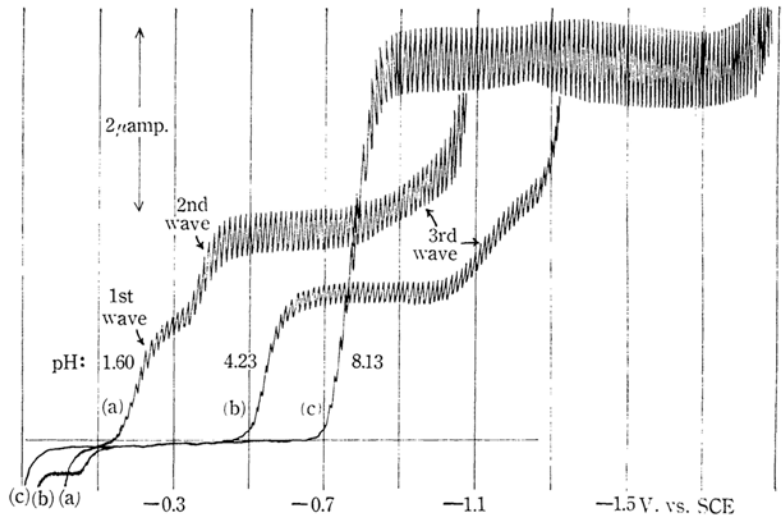



Fig. 2. Polarograms of condensation product in BR buffer at 25°C.
Glyoxal: $8\times10^{-4}$ M, OPD: $1\times10^{-2}$ M, Condensation: 60 min.

participating in the electrode reaction, controlled-potential electrolysis[8-10] was carried out by using the cell shown in Fig. 1.

The experimental procedure was as follows: about 45 ml. of a buffer solution (pH 8.9) was placed in the cathode compartment of the electrolytic cell, and the cell was placed in the thermostat. The solution and the mercury pool cathode (area, about 15 $cm^2$) were stirred vigorously with a glass propellor stirrer immersed in the mercury-solution interface, and the dissolving oxygen was removed by bubbling nitrogen gas. After the dissolved oxygen had been removed, electrolysis was carried on at a fixed cathode potential (e. g., −1.20 V. vs. SCE) until the current became negligibly small. The circuit was then connected to the polarograph, and 5 ml. of a quinoxaline solution of known concentration, which had been deaerated, was pipetted into the cell. After mixing, the inlet tube of nitrogen gas was pulled up and nitrogen gas was passed over the solution. The stirrer was stopped, and a polarogram was taken. The inlet tube of nitrogen gas was then pushed down again into the solution and the stirring was re-commenced; then the electrolysis was carried out at −1.20 V. vs. SCE, with automatic control, while nitrogen gas was being bubbled through the solution. After a given period, electrolysis was stopped, the electricity required for the electrolysis was measured by a gas coulometer,[10] and a polarogram was recorded according to the procedures described above. These procedures were repeated several times during the course of the electrolysis.

## Results and Discussion

**Reduction Waves of the Condensation Product of Glyoxal with OPD.**—A well-defined reduction wave was obtained when glyoxal was treated with OPD in appropriate buffer solutions by the procedure described above, as may be seen in Fig. 2 (a, b and c). The reduction wave showed all the typical characteristics of a diffusion-controlled current: proportionality between the wave height and the square root of the height of the mercury reservoir (Fig. 3), and a small temperature

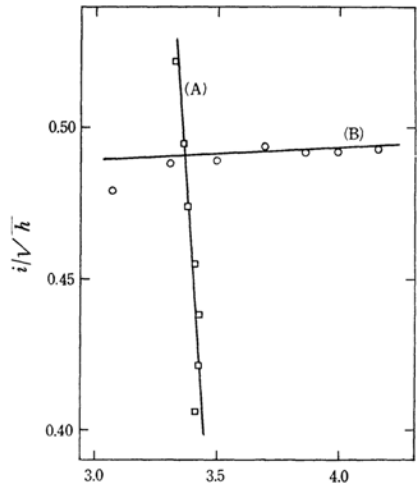



Fig. 3. Relationships between the limiting current ($i$) and the height ($h$) of mercury reservoir.
Glyoxal: (A) $3.2\times10^{-2}$ M without OPD at pH 7.0, (B) $8\times10^{-4}$ M with $1\times10^{-2}$ M OPD at pH 8.0. Condensation: 60 min. at 25°C.

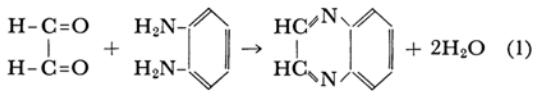
8) S. Ono, M. Takagi and T. Wasa, This Bulletin, **31**, 356 (1958).

9) N. Tanaka, T. Nozoe, T. Takamura and S. Kitahara, ibid., **31**, 327 (1958).

10) J. A. Page and J. J. Lingane, *Anal. Chim. Acta*, **16**, 175 (1957).

coefficient of 1.9%/deg. Figure 3 also includes the results obtained with glyoxal only.

The shape and the half-wave potential of this reduction wave were practically identical with those of the quinoxaline prepared by Jones and McLaughlin's method.[6] Therefore, the condensation product of glyoxal with OPD under the present experimental conditions is probably quinoxaline, as has been reported by Hinsberg[11] and Liebigs[12], and the reaction may be written as:




glyoxal OPD quinoxaline

*The Stability of the Stock Solution of Glyoxal.*— Figure 4 shows the change in the limiting currents of the condensation products with the time elapsed (at a room temperature of about 15°C) after the preparation of the stock solutions of glyoxal using 0.1 N sulfuric acid, 0.01 N sulfuric acid, water, or 99% ethanol. Figure 4 indicates that glyoxal in the ethanolic stock solution is the most stable; therefore, the ethanolic stock solution was used for the fundamental experiment. However, for analytical purposes the aqueous stock solution was also used, because the limiting current remained almost constant for a month or more when the condensation was carried out in an alkaline buffered solution.

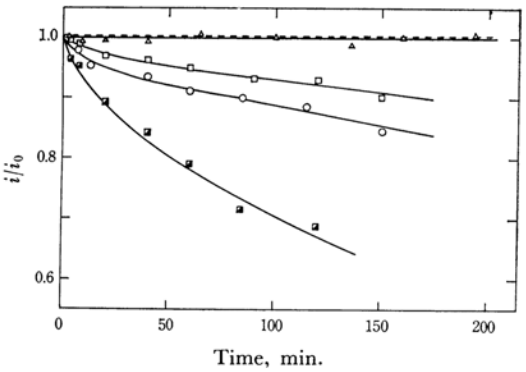



Fig. 4. Stability of the stock solution of glyoxal. Solvent of stock solution: △ 99% ethanol, ○ water, □ 0.01 N $H_2SO_4$, ◪ 0.1 N $H_2SO_4$. Condensation: $1\times10^{-2}$ M OPD, 30 min. at 25°C in BR buffer. —— Condensation was carried out at pH 3.80, ---- at pH 8.90 with aqueous stock solution. $i_0$: initial wave height.

*The Effect of pH.*—In order to examine the effect of pH on the polarographic behavior of the condensation product of glyoxal with OPD, the concentrations of glyoxal and OPD were kept constant and only the pH of the mixture was changed (Fig. 5). The limiting currents of the condensation products were measured 60 min. after the addition of OPD at 25°C, since they increase with time during the first 40 min., attaining maximum values which remain almost constant for a further 24 hr. over all the pH range studied. Figure 5 shows that the limiting current of the condensation product shows its

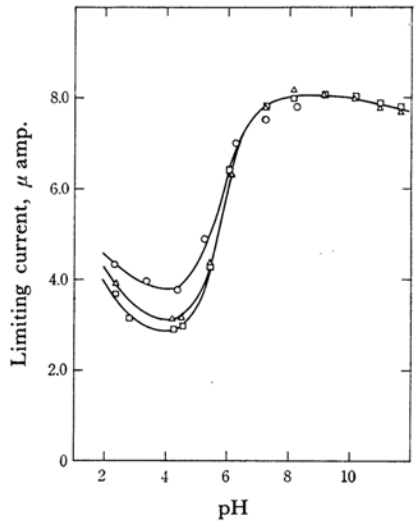
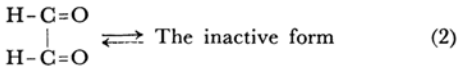



Fig. 5. pH-dependence of the limiting current of the condensation product at 25°C.
Condensation: $1.6\times10^{-3}$ M glyoxal, $1\times10^{-2}$ M OPD, 60 min. ○ one day after the preparation of ethanolic stock solution of glyoxal, △ 16 days, □ 21 days.

minimum value at pH about 4, and that it increases remarkably at pH above 5, reaching a maximum value in the pH of 7 to 10. The same behavior was not observed in the polarographic investigation of quinoxaline.[13] Also, Fig. 5 shows that the limiting current decreases slowly, at the rate of about 2%/day, in the pH range between 2 and 6 with the time elapsed after the preparation of the stock solution of glyoxal. These results seem to indicate that glyoxal in the stock solution changes into a form inactive (dimer or hydrate form) in the reaction with OPD and that this change is almost irreversible at a pH value of about 4, but that it becomes reversible and the rate of producing glyoxal from the inactive form increases upon either an increase or a decrease in pH from about 4.




At pH values lower than 2.5 the splitting of the reduction wave into two waves which have almost equal heights was observed (Fig. 2). At the pH

11) O. Hinsberg, *Ber.*, **17**, 320 (1884).
12) H. Liebigs, *Ann. der Chem.*, **237**, 334 (1887).
13) M. Takagi, private communication to the authors.

between 2 and 8, an ill-defined and irreversible reduction wave was also observed at a more positive potential than the reduction wave of the hydrogen ion; it showed its maximum value at a pH value of about 4 and disappeared at pH values above 8. Further, when the condensation took place at a pH value of about 4, a small oxidation wave ($E_{1/2}=-0.07$ V. vs. SCE), which seems to be due to the condensation product of an aldehyde group of glyoxal with OPD, was observed (Fig. 2, b).

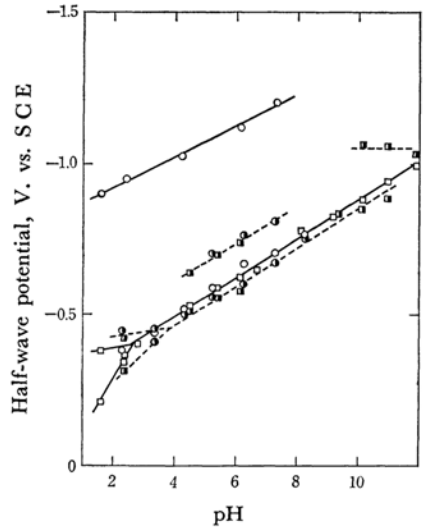



Fig. 6. pH-dependence of the half-wave (summit) potential.
○, ◑: McIlvaine buffer, □, ◧: BR buffer, -○-□-: half-wave potential, --◑--◧--: summit potential, 25°C.

The changes in the half-wave potentials of the condensation product with the pH value are presented in Fig. 6, in which the summit potentials ascertained by a. c. polarography are also shown. As may be seen in Fig. 6, a linear relationship has been obtained; its inclination was about 60 mV./pH at pH values above 2.5. Though no further experiments on the condensation product have been made by a. c. polarography, the changes in the summit potentials, the peak height and the shape of the peak with the pH value are obviously somewhat complicated. A typical a. c. polarogram was observed in the 8—10 pH range.

The influence of the components of buffer solutions on the half-wave potential, the wave height and the shape of the wave was examined, but no appreciable change has been observed.

*The Effect of the Concentration of OPD.*—Figure 7 shows the relationship at 25°C between the limiting current and the time elapsed after the addition of OPD to an electrolytic solution containing glyoxal and BR buffer with a pH value of 8.90, when the concentration of glyoxal was kept constant approximately at $8\times10^{-4}$ M and the concentration of OPD was changed. With the increase in the concentration of OPD, the growing velocity of the wave increased, so the time required for the condensation was shortened.

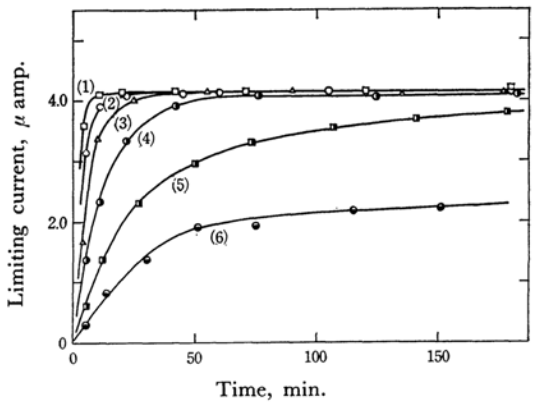



Fig. 7. Effect of the concentration of OPD.
OPD: (1) $2\times10^{-2}$, (2) $1\times10^{-2}$, (3) $5\times10^{-3}$, (4) $2\times10^{-3}$, (5) $1\times10^{-3}$, (6) $5\times10^{-4}$ M.
Glyoxal: $8\times10^{-4}$ M.
Condensation was carried out at pH 8.90 at 25°C.

*The Effect of the Temperature.*—Figure 8 shows the effect of the temperature on the reaction time. When the concentrations of glyoxal and OPD were kept constant and the experimental temperature was raised, the time required for condensation was shortened over all the pH range studied, while it was observed that the limiting current of the condensation product, after its maximum value was attained, decreased slightly with time at temperatures above 45°C.

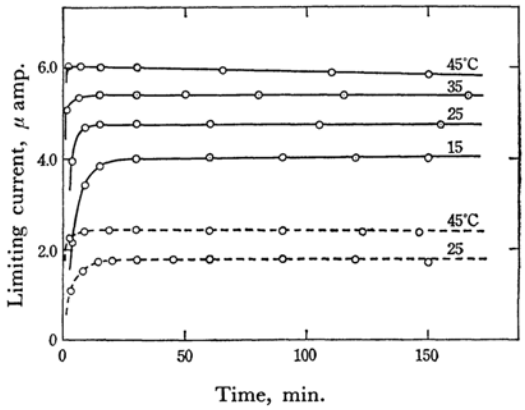



Fig. 8. Effect of the temperature.
Glyoxal: $9.3\times10^{-4}$ M, OPD: $1\times10^{-2}$ M
—— at pH 8.90, ---- at pH 3.80

**Electrolysis and Coulometry at a Constant Potential.**—Figure 9 shows the change in the polarograms of quinoxaline, the condensation product of glyoxal with OPD, during the course of electrolysis. In the early stages of electrolysis,

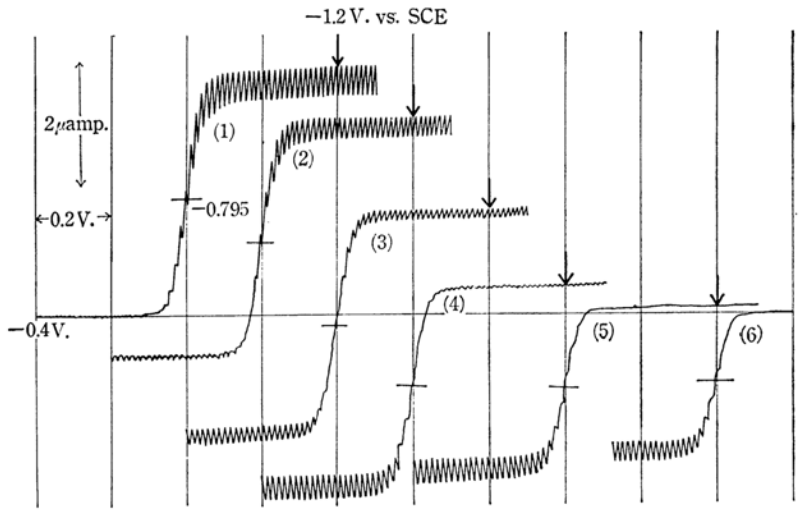
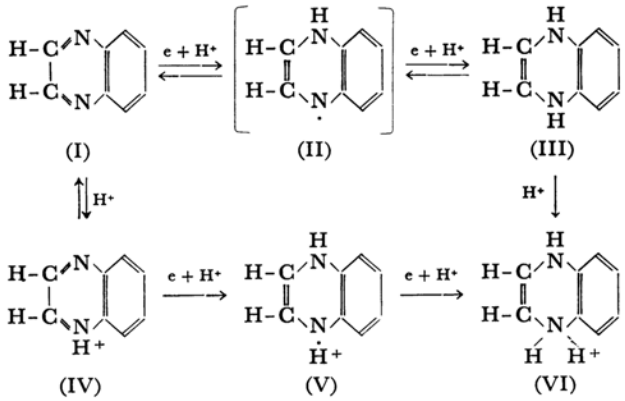



Fig. 9. Controlled-potential electrolysis of quinoxaline at −1.2 V. in BR buffer (pH 8.70) containing 10% ethanol.
Quinoxaline: $7.2\times10^{-4}$ M. (1) before electrolysis, (2) after 5, (3) 15, (4) 25, (5) 35, and (6) 55 min. at 20°C.




the anodic wave which is due to the oxidation of the electrolytic reduction product grows at the expense of the reduction wave of quinoxaline. On further electrolysis, the height of the anodic wave decreases slowly with time, and at the same time it is observed that the electrolytic solution becomes muddy; this is probably caused by the low solubility of the reduction product in an aqueous solution. However, the electrode reaction is reversible because the half-wave potential of the anodic wave is identical with that of the reduction wave of the condensation product (quinoxaline).

The results of the coulometric study are summarized in Table I. From this study one can conclude that the electrode reaction requires two electrons at pH 9.15.

The mechanism of the electrolytic reduction of quinoxaline is analogous to that of phenazine.[14,15] In strongly acidic solutions, the reduction of quinoxaline occurs in two well-separated steps

Table I. Estimation of the number of electrons required for the reduction of quinoxaline

Initial amount of quinoxaline: $4.985\times10^{-5}$ mol. in 50 ml., pH 9.15, containing 10% alcohol, 20°C

| Electrolysis time min. | Quantity of electricity consumed coulomb | Limiting current of quinoxaline μamp. | Amount of quinoxaline reduced $\times10^{-5}$ mol. | *n* |
|---|---|---|---|---|
| 0 | 0 | 3.59 | | |
| 5 | 1.144 | 3.14 | 0.623 | 1.903 |
| 10 | 2.178 | 2.78 | 1.127 | 2.003 |
| 20 | 3.425 | 2.29 | 1.805 | 1.966 |
| 25 | 3.991 | 2.10 | 2.069 | 1.999 |
| 35 | 4.862 | 1.74 | 2.567 | 1.963 |
| 55 | 6.132 | 1.33 | 3.141 | 2.023 |

14) R. C. Kaye and H. I. Stonehill, *J. Chem. Soc.*, **1952**, 3240.
15) Y. Asahi, *J. Pharm. Soc. Japan* (*Yakugaku Zasshi*), **80**, 679 (1960).

(see Fig. 2, a), each corresponding to the taking-up of one electron, since the two wave heights almost coincide with each other and the sum of the two wave heights is equal to that of the single wave in the neutral and alkaline ranges. As the pH increases, these waves move closer together, unitl at pH values higher than 2.5 they coalesce into a single two-electron step (see Fig. 2, b, c).

From the observation described above, it may be assumed that in acidic solutions of pH values lower than 2.5 a stable semiquinone (V) is formed in the first reduction step, and that its stability rapidly decreases as the pH value increases. The mechanisms of the electrolytic reductions may be expressed as follows:

$$\begin{array}{ccccc} O & \rightleftarrows & [O] & \rightleftarrows & O \\ \downarrow\uparrow & & & & \downarrow \\ O & \rightarrow & O & - & O \end{array}$$

The third wave (see Fig. 2), though no particular experiments have been made with this reduction wave, is considered to be a catalytic hydrogen wave of the protonated reduction product as in the case of the nitrogen-containing heterocyclic compounds.[16]

**Application to the Determination of Glyoxal in Commercial Products.**—On the basis of the above results, the following conditions may be recommended as the most practical conditions for the determination of glyoxal by utilizing the wave of the condensation product of glyoxal with OPD: $(1-2)\times10^{-2}$ M for the concentration of OPD, 7—10 for the pH range, 25°C for the temperature, and 30—60 min. for the condensation time. Under these conditions a linear relation was obtained between the concentration of glyoxal and the limiting current of the condensation product in the range of $(0.2—20)\times10^{-4}$ M, as is shown in Fig. 10.

For applying the present polarographic method to the determination of glyoxal in commercial products, the following procedure was employed: 1—5 g. of a commercial reagent (about 40% aqueous solution) was transferred to the 1 l. volumetric flask and diluted to the mark with distilled water. Then, in a run, 2 ml. of the diluted sample was placed in a 20-ml. volumetric flask, 10 ml. of a BR buffer with a pH value of 9.0 and 2 ml. of the aqueous solution of OPD (0.1 M) were added, and then the volume was made up to the mark with distilled water. A part of the mixture was taken in an electrolytic cell and immersed in a thermostat at 25°C for 45 min., during which time dissolved oxygen was removed by the bubbling nitrogen gas. After that, a ploarogram was taken in the usual way (A). In another run,

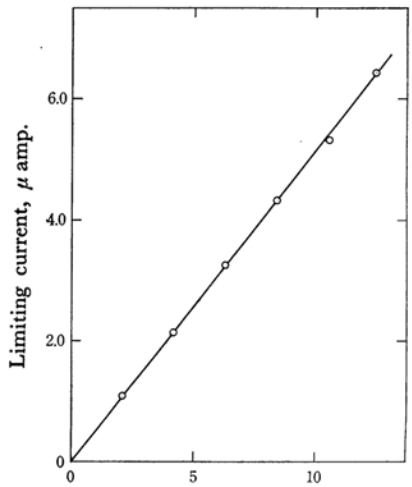



Fig. 10. Linear relationship between the limiting current of the condensation product and the concentration of glyoxal.

2 ml. of the diluted sample was placed in a 20 ml. volumetric flask, and 1 ml. of a standard solution containing glyoxal sodium bisulfite of $1\times10^{-3}$ M, 10 ml. of a BR buffer of pH 9.0, and 2 ml. of the solution of OPD were added. After that, the same procedure as in the former run was followed, and a polarogram was recorded (B). By comparing the two polarograms, (A) and (B), the glyoxal content in the original sample was calculated. Some of the analytical results obtained are listed in Table II, together with those obtained by the titrimetric method[17] based on Cannizaro's reaction. The results obtained by the two methods are in good agreement with each other.

As another application, the determination of glyoxal in commercial products of ethylene glycol containing glyoxal and glycolaldehyde as impurities

TABLE II. THE RESULTS OF THE DETERMINATION OF GLYOXAL IN COMMERCIAL PRODUCT

Concn. of OPD: $1\times10^{-2}$ M, pH 9.0, 25°C

| Sample taken* g. | Polarographic | | Titrimetric** content % |
|---|---|---|---|
| | Found g. | Content % | |
| 1.267 | 0.539 | 42.5 | 42.8 |
| 1.259 | 0.530 | 42.1 | |
| 1.271 | 0.548 | 43.1 | 43.2 |
| 2.476 | 1.067 | 43.1 | |
| 2.481 | 1.052 | 42.4 | |

* Commercial product of glyoxal (about 40%).
** Determination by titration method based on Cannizaro's reaction.

16) P. Zuman, "Organic Polarographic Analysis," Pergamon Press, Oxford (1964), p. 100; see also M. Brezina and P. Zuman, "Polarography in Medicine, Biochemistry and Pharmacy," Interscience Publishers, New York (1958), p. 374.

17) J. G. Baldinus and I. Rothberg, *Anal. Chem.*, **32**, 1176 (1960).

was carried out. In this determination, the polarographic procedure described above was followed after the following pretreatment of the ethylene glycol sample: the sample was diluted to 2 to 5 times with distilled water, and the resulting diluted solution was allowed to stand overnight to effect hydrolysis, because glyoxal in concentrated ethylene glycol is considered to exist as acetal, which is inactive for OPD. The results obtained by the present polarographic method are given in Table III, together with those obtained by the titration method[18] using hydroxylamine hydrochloride. Though glycolaldehyde shows an anodic wave which is characteristic of monoaldehyde in the buffer solution containing OPD,* the presence of glycolaldehyde does not seriously interfere with the determination of glyoxal by the polarographic OPD method, because it does not give the waves in the potential range used for the determination of glyoxal. On the other hand, when the determination of glyoxal was carried out by the titration method, the presence of glycolaldehyde interfered seriously with the determination of glyoxal.

When such substances as methanol, ethanol, ethylene glycol, glycolic acid and acetone exist in the sample, the limiting current of the condensation product decreases slightly with an increase in the concentration of these substances. However, as long as the determination of the glyoxal is carried out by the standard addition method described above, these substances do not cause any interference, since the substances are not polarographically reducible under the present conditions.

Table III. The results of the determination of glyoxal in commercial ethylene glycol

Concn. of OPD: $1\times10^{-2}$ M, pH 9.0, 25°C

| Exp. No. | Content of glyoxal, % | |
|---|---|---|
| | Polarographic | Titrimetric* |
| 1 | 0.0122 | 0.1979 |
| 2 | 0.0120 | 0.1975 |
| 3 | 0.0121 | 0.1983 |

* Hydroxylamine titration method (glyoxal + glycolaldehyde).

Aldehydes such as formaldehyde, acetaldehyde, propylaldehyde, butylaldehyde and glycolaldehyde give the anodic waves which have been described in the case of glycolaldehyde under the present experimental conditions, but they do not give reduction waves, whereas glyoxal does. Therefore, the presence of the aldehydes does not cause any interference with the determination of glyoxal. Furthermore, the presence of sulfite ion does not interfere, because electrolytic solutions are alkaline when they have pH values of 8—10.

Methylglyoxal, biacetyl and glyoxylic acid easily react with OPD under the present conditions and give reduction waves the half-wave potentials of which are −0.802, −0.851 and −0.830 V. respectively at pH 8.5. Therefore, the presence of these substances interferes with the determination of glyoxal.

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18) Sidney Siggia, "Quantitative Organic Analysis Via Functional Groups," Wiley, New York (1963). p. 73.

* The details of this kind of observations will be given elsewhere.