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The Polarographic Behavior of the Condensation Products of *o*-Phenylenediamine with Glycolaldehyde and with Glyceraldehyde, and Its Application to the Determination of Glycolaldehyde and Glyceraldehyde

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Glycolaldehyde and glyceraldehyde condense with *o*-phenylenediamine (OPD) in a buffered solution (pH 2—10) containing an excess of the latter to produce the corresponding condensation products. Both condensation products give a single diffusion-controlled oxidation wave ($E_{1/2}$: −0.08 V *vs.* SCE for both aldehydes at pH 6.0) at the dropping mercury electrode, and their electrode reactions are irreversible over all the pH range studied (pH 2—10). The limiting currents of both waves show their maximum values at pH values of about 6 and decrease either with an increase or a decrease in the pH from about 6. From the results of the present polarographic investigations, it has been deduced that the condensation products of OPD with glycolaldehyde and with glyceraldehyde are probably benzimidazoline derivatives. The equilibrium constants for the condensation reaction of glycolaldehyde and glyceraldehyde with OPD were calculated to be 551 and 294 *l*/mol respectively at 25°C. A linear relationship between the limiting current of the condensation product and the concentration [$(0.5—30)\times10^{-4}$ M] of glycolaldehyde or glyceraldehyde was obtained under the following conditions, which are recommended for the practical determination: 5.5—6.5 for pH, 2×10^{-2} M for the concentration of OPD, 25°C for the temperature, and 5—60 min for the condensation time. The present method was applied to the determination of the glycolaldehyde present as an impurity in a commercially-available ethylene glycol.

It has been reported by several authors that glycolaldehyde[1] and glyceraldehyde[2-4] show a single reduction wave at the dropping mercury electrode in neutral and alkaline solutions, and that this wave can be applied to the detection and determination of glycolaldehyde[5] and glyceraldehyde.[4,6] However, their polarographic behavior is very similar to that of formaldehyde[7,8]: the limiting current varies with the pH of the electrolytic solution and with the temperature, and also decreases with the time in the alkaline region because of the instability of the two substances. Experimental conditions need, therefore, to be strictly controlled if this reduction wave is to be used for the polarographic determination of glycolaldehyde and glyceraldehyde.

During the course of an investigation of the polarographic behavior of α-dicarbonyl compounds[9,10] in various buffer solutions containing excess *o*-phenylenediamine (OPD), it has been found that glycolaldehyde is easily condensed with OPD and that the condensation product shows an anodic wave which is characteristic of the monoaldehyde.

The present paper will describe the polarographic behavior of the condensation products of OPD with glycolaldehyde and with glyceraldehyde, and a method for determining glycolaldehyde and glyceraldehyde by utilizing these oxidation waves.

Experimental

Glycolaldehyde was prepared according to the procedure of Fischer and his co-workers.[11,12] Its melting point of 95.0—96.0°C agreed with that given in the

1) R. Bieber and G. Trümpler, *Helv. Chim. Acta*, **31**, 5 (1948).
2) A. Winkel and G. Proske, *Ber.*, **69**, 1917 (1936).
3) M. Fedoroňko, J. Königstein and K. Linek, *Collection Czech. Chem. Communs.*, **30**, 4297 (1965).
4) J. Trnka, *Sborník Mezinárod. Polarog. Sjezdu Praze, 1st Congr.*, **1951**, Pt. III, 518; *Chem. Abstr.*, **47**, 11037 (1953).
5) V. G. Brudz, Yu. I. Vainshtein, Yu. A. Davydovskaya, D. A. Drapkina and I. S. Markovich, *Zavodskaya Lab.*, **27**, 1087 (1961); *Chem. Abstr.*, **56**, 10916 (1962).
6) W. Stoll, E. Waldmann, V. Prey and H. Berbalk, *Monatsh.*, **83**, 988 (1952).
7) R. Bieber and G. Trümpler, *Helv. Chim. Acta*, **30**, 706 (1947).
8) K. Veselý and R. Brdička, *Collection Czech. Chem. Communs.*, **12**, 313 (1947).
9) S. Musha, T. Wasa and T. Naito, This Bulletin, **39**, 1902 (1966).
10) T. Wasa and S. Musha, *ibid.*, **40**, 1616 (1967).
11) H. O. L. Fischer and C. Taube, *Ber.*, **60**, 1704 (1927).
12) H. O. L. Fischer and L. Feldmann, *ibid.*, **62**, 854 (1929).

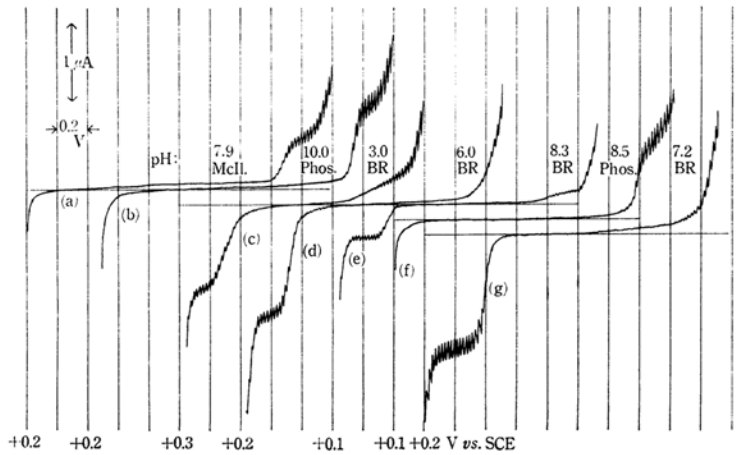


Fig. 1. Polarograms of glycolaldehyde and glyceraldehyde with or without OPD at 25°C.
Glycolaldehyde: (a), (b), 7.4×10^{-4}; (c)—(e), 3.7×10^{-4} M
Glyceraldehyde: (f), 1×10^{-3}; (g), 5×10^{-4} M
OPD: (c)—(e), (g), 2×10^{-2} M Condensation: 60 min

literature.[13] DL-glyceraldehyde was a commercial product from the Tokyo Kasei Kogyo Co., Ltd.; it was used without further purification. All the other chemicals were the same as were used in previous works.[9,10] As for the buffer solutions, Britton-Robinson (BR), McIlvaine (McII), and phosphate (Phos) buffers were employed. No maximum suppressor was added, since no maximum was observed under the present polarographic conditions.

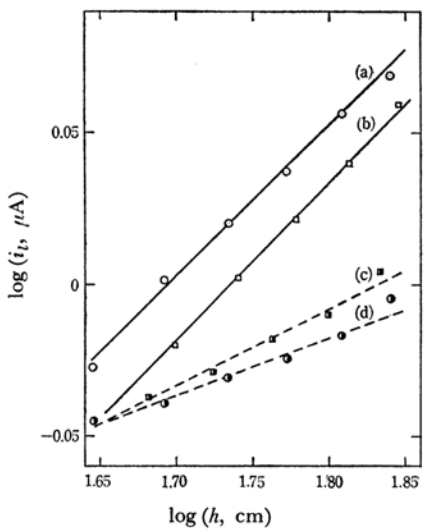


Fig. 2. Plots of log (i_l) against log (h) at 25°C.
Glycolaldehyde: (a) 3×10^{-4}; (d) 1.3×10^{-3} M
Glyceraldehyde: (b) 4×10^{-4}; (c) 2×10^{-3} M
OPD: (a), (b) 2×10^{-2} M Condensation, 30 min
pH: (a) 6.1 (BR); (b) 7.0 (BR), (c), (d) 7.9 (McII) —— Anodic wave with OPD, - - - Cathodic wave without OPD

13) H. J. H. Fenton and H. Jackson, *J. Chem. Soc.*, **75**, 575 (1899).

The apparatus and the experimental procedures were the same as have been reported in previous papers.[9,10] Characteristics of capillary: $m=1.143$ mg/sec, $t=4.00$ sec/drop in the buffer solution at 0 V *vs.* SCE.

Results and Discussion

Reduction Waves of Glycolaldehyde and Glyceraldehyde. Representative polarograms for the reduction of glycolaldehyde and glyceraldehyde

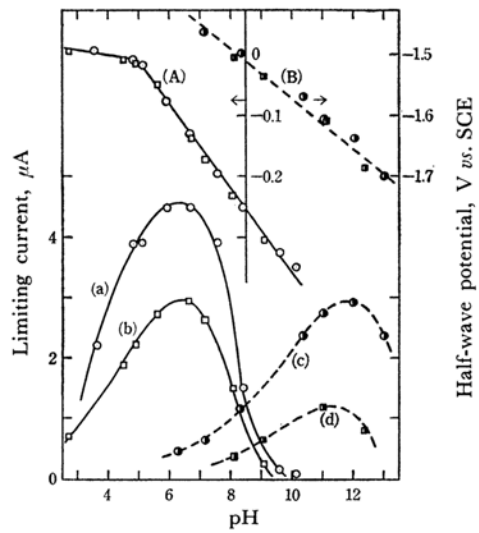
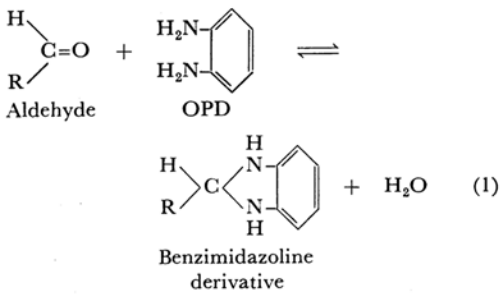


Fig. 3. pH-dependence of the limiting currents and the half-wave potentials at 25°C.
○, ◑: Glycolaldehyde (1×10^{-3} M);
□, ■: Glyceraldehyde (1×10^{-3} M)
—— Anodic wave in BR buffer with 2×10^{-2} M OPD, 60 min, - - - Cathodic wave in McIlvaine's and phosphate buffer without OPD

in various buffer solutions at 25°C are shown in Fig. 1 (a, b, and f). The reduction waves can be said to be partially reaction-controlled, since the slopes of the plot[14] of the logarithm of the wave height (i_l) at a pH value of 7.9 at 25°C against the logarithm of the height of the mercury reservoir (h) are 0.19 for glycolaldehyde and 0.27 for glyceraldehyde (Fig. 2, c and d). The limiting currents at pH values above 6 increase with an increase in the pH value and reach a maximum value at pH 12 (Fig. 3, c and d); it was also observed that they decrease with the time at pH values of about 12 because of their instability. The half-wave potentials of these reduction waves shift towards a more negative potential with an increase in the pH value (Fig. 3, B). These results are almost entirely in accordance with those obtained by Bieber and Trümpler,[1] and by Fedoroňko and his co-workers.[3]

Oxidation Waves of the Condensation Products of Glycolaldehyde and Glyceraldehyde with OPD. A well-defined oxidation wave was obtained, as may be seen in Fig. 1 (c, d, e, and g), when either glycolaldehyde or glyceraldehyde was treated with OPD in appropriate buffer solutions by the procedure described above. The slopes of the lines in Fig. 2 (a and b) are 0.50 for glycolaldehyde and 0.51 for glyceraldehyde; therefore, it can be said that these oxidation waves are diffusion-controlled. The condensation products responsible for this anodic wave are probably benzimidazoline derivatives, and the reaction may be written as:



Glycolaldehyde: $R=-CH_2OH$;
Glyceraldehyde: $R=-CHOHCH_2OH$

Change in the Concentrations of Glycolaldehyde and Glyceraldehyde in Stock Solutions. Figure 4 shows the relationship between the limiting currents of the condensation products and the time elapsed (at a room temperature of about 20°C) after the preparation of the stock solution. In an aqueous solution, the limiting currents increase with the time for the first 3 hr for glycolaldehyde and for the first 20 hr for glyceraldehyde; after attaining the maximum value, they remain almost constant for a week or more. These results agree with those given in the literature;[12,13,15] glycolaldehyde is present as

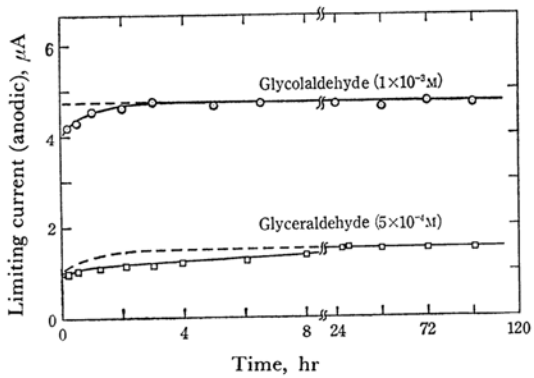


Fig. 4. Change of the stock solution with time elapsed after its preparation.
Concentration of stock solution: 1×10^{-2} M in water (——) and 0.01 N sulfuric acid (- - -).
Condensation: 2×10^{-2} M OPD, 25°C, pH 6.0, 20 min

a dimer in a crystalline state, and the dimer is completely converted into a monomer when the aqueous solution is allowed to stand overnight. In 0.01 N sulfuric acid, the change from dimer to monomer seems to be accelerated by acid-catalysis.

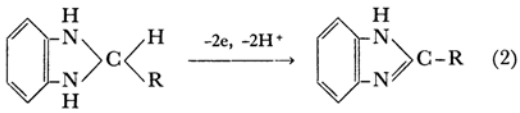
The Effect of pH. When the concentrations of aldehydes and OPD were kept constant and only the pH of the mixture was changed, the relationships between the limiting currents of the condensation products measured 60 min after the addition of OPD at 25°C and the pH are as shown in Fig. 3 (a and b). The condensation products of both aldehydes behave the same at the dropping mercury electrode: the limiting currents reach their maximum values at a pH value of about 6, and decrease with either an increase or a decrease in pH from about 6. It seems that the decrease in the limiting current with an increase in the pH is due to the decrease in the rate of the condensation reaction, while the decrease with a decrease in the pH is due to the inactivation of OPD by protonation.[16]

The changes in the half-wave potentials of the condensation products of both aldehydes with the pH value are also presented in Fig. 3 (A). A linear relationship has been obtained; its inclination was about −70 mV/pH at pH values above 5.

A normal a.c. polarogram was not observed except for a small peak which seems to be due to the adsorption of the condensation product. Moreover, no reduction wave was obtained with the oxidation product by controlled-potential electrolysis.*1 Therefore, the electrode reaction of the condensation product may be concluded to be an irreversible reaction. By comparing the height of the anodic wave with that of the reduction wave of quinoxalines at the same concentration, a two-

14) W. B. Swann, W. M. McNabb and J. F. Hazel, *Anal. Chim. Acta*, **28**, 441 (1963).
15) N. P. McCleland, *J. Chem. Soc.*, **99**, 1825 (1911).
16) H. B. Mark, Jr., *J. Electroanal. Chem.*, **7**, 276 (1964).
*1 The details will be given elsewhere.

electron oxidation was deduced for the anodic wave of the condensation product; therefore, the electrode reaction may be written as:



(2)

Benzimidazoline derivative — Benzimidazole derivative

As it may be considered that glycolaldehyde and glyceraldehyde form complexes with the borate ion, as in the case of fructose,[14] the effects of the components of buffer solutions (BR, McIlvaine and phosphate buffers) on the half-wave potential, the wave height, and the shape of the wave were examined, but no appreciable change was observed. However, the anodic wave of the condensation product was steepened with an increase in the ionic strength of the electrolytic solution, making the measurement of wave height easy.

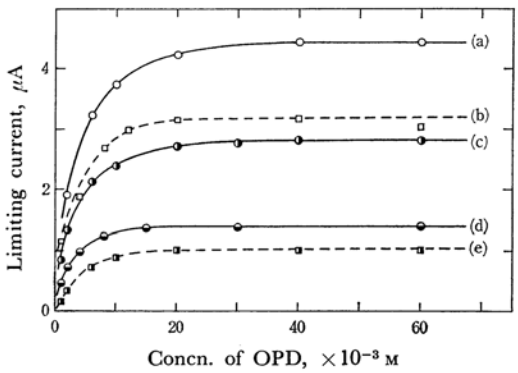


Fig. 5. Relationship between the limiting current (anodic) and the concentration of OPD at 25°C.
—— glycolaldehyde (a) 11.35×10^{-4}; (c) 7.17×10^{-4}; (d) 3.70×10^{-4} M
- - - glyceraldehyde (b) 10.61×10^{-4}, (e) 3.58×10^{-4} M
Condensation: 60 min, pH 6.0

The Effect of the Concentration of OPD. Figure 5 shows the relationship between the limiting currents measured 60 min after the addition of OPD at 25°C and the concentration of OPD in a BR buffer with a pH of 6.0. As is evident in Fig. 5, the condensation reaction attains an equilibrium state which may be expressed in the following general form:

$$\text{Aldehyde}+n\ \text{OPD} \rightleftarrows \text{Condensation product}+m\ H_2O \qquad (3)$$

If it is assumed that n is equal to unity and that the concentration of water is constant, the equilibrium constant,[17] K', is given by:

$$K' = x/(a-x)(b-x) \qquad (4)$$

where a and b are the initial concentrations of aldehydes and OPD respectively. x, the concentration of the condensation product produced, is calculated from the polarographic data in Fig. 5 by the following equation:

$$x = ai_l/i_{l\,\max.} \qquad (5)$$

where i_l is the wave height at various values of a and b. $i_{l\,\max.}$ is the wave height when the condensation reaction has been completed. From Eqs. (4) and (5):

$$\log\,[i_l/(i_{l\,\max.}-i_l)] = \log K' + \log\,(b-x) \qquad (6)$$

Figure 6 shows the relationship between $\log[i_l/(i_{l\,\max.}-i_l)]$ and $\log\,(b-x)$, these values being calculated from the data of Fig. 5. For the reaction of glycolaldehyde with OPD, with the limiting current at $b=6\times10^{-2}$ M in Fig. 5 being used as $i_{l\,\max.}$, it was observed that the plots showed a linear relationship and that the inclination was equal to unity. Therefore, Eq. (1), assuming a one-to-one condensation, adequately describes the predominant reaction at least. For the reaction of glyceraldehyde with OPD, $i_{l\,\max.}$ was determined by a trial-and-error method, so the inclination of the plots in Fig. 6 may become unity. The equilibrium constants obtained from

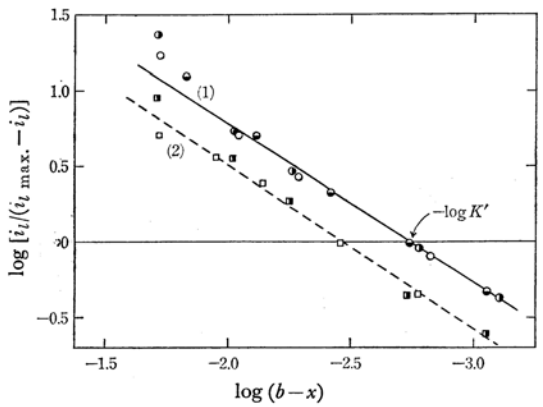


Fig. 6. Plots of $\log\,[i_l/(i_{l\,\max.}-i_l)]$ against $\log\,(b-x)$ at 25°C.
(1) glycolaldehyde (○ 11.35×10^{-4}; ◑ 7.17×10^{-4} and ◒ 3.70×10^{-4} M), (2) glyceraldehyde (□ 10.61×10^{-4} and ◨ 3.58×10^{-4} M).

Table 1. Equilibrium constants for the condensation reaction in BR buffer (pH 6.0), 25°C, 60 min

Aldehydes	Concentration $\times10^{-4}$ M	$i_{l\,\max.}$ μA	Slope	K' l/mol
Glycolaldehyde	11.35	4.468	0.990	543
	7.17	2.820	1.006	559
	3.70	1.456	1.055	552
Glyceraldehyde	10.61	(3.791)	—	296
	3.58	(1.128)	—	292

17) P. Zuman, "Organic Polarographic Analysis," Pergamon, Oxford (1964), p. 232.

Fig. 6 are presented in Table 1. As can be seen from Table 1, the equilibrium constants for both aldehydes are relatively small. For analytical purposes, therefore, the concentration of OPD in the reaction mixture should be kept in a large excess and constant.

The Effect of the Temperature. The condensation reaction immediately attains its equilibrium state at pH values lower than about 6, and the temperature coefficient of the anodic wave at about 20°C is almost equal to that obtained for the diffusion-controlled current, *i.e.*, 1.5—2%/deg. At temperatures about 45°C, it was observed that the anodic limiting current, after attaining its maximum value, decreased with the time and that, at the same time, a new reduction wave ($E_{1/2}$: about −0.7 V *vs.* SCE), which may be due to quinoxaline, was produced. At pH values above 6, the time required for the condensation was shortened when the temperature rose.

The Effect of Aeration. In order to examine the effect of aeration on the wave height of the condensation product, aeration was carried out by bubbling air through the electrolytic solution in the early stages of the condensation reaction; the results obtained are presented in Table 2. In Table 2, no remarkable change in the limiting current is observed as a result of aeration for about 10 min, when a large excess of OPD was present in the reaction mixture. However, for analytical purposes, it is necessary to remove the dissolving oxygen by bubbling nitrogen immediately after the preparation of the electrolytic solution.

TABLE 2. EFFECT OF AERATION ON THE LIMITING CURRENT OF THE CONDENSATION PRODUCT
Glycolaldehyde: *ca.* 1×10^{-3} M, Condensation: 2×10^{-2} M OPD, pH 6.0, 25°C, 30 min

Time, min	0	2.5	5.0	10.0	20.0
i, μA	4.09	4.08	4.06	4.00	3.54

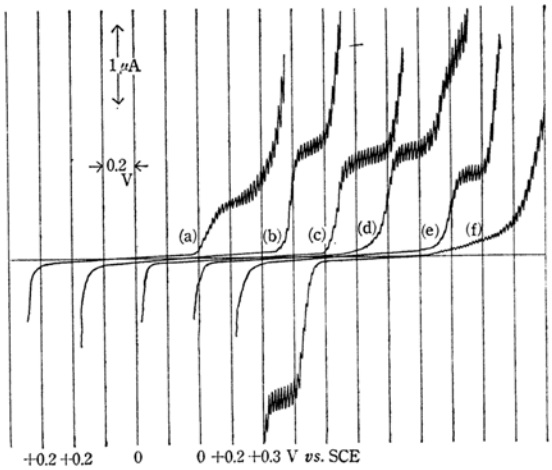


Fig. 7. Polarograms of glycolaldehyde in BR buffer containing various amines at 25°C.
Glycolaldehyde: (a)—(e), 1×10^{-3}; (f), 5×10^{-4} M.
Amine: 2×10^{-2} M, (a), aniline (pH 3.0); (b), methylamine (pH 9.0), (c), ethylenediamine (pH 9.0); (d), *p*-phenylenediamine (pH 9.0); (e), *m*-phenylenediamine (pH 9.0) and (f), 4-chloro-*o*-phenylenediamine (pH 3.0). All polarograms were recorded at 60 min after the addition of amines.

The Reaction with Other Amines. Polarograms of glycolaldehyde in an electrolytic solution containing aniline, methylamine, ethylenediamine, *p*-phenylenediamine, *m*-phenylenediamine, or 4-chloro-*o*-phenylenediamine are shown in Fig. 7. In the solutions containing amines (2×10^{-2} M) except for 4-chloro-*o*-phenylenediamine, a reduction wave ($E_{1/2}$: −0.9——1.3 V *vs.* SCE) which seems to be due to the Schiff base was observed, but no anodic wave was obtained. On the other hand, with 4-chloro-*o*-phenylenediamine, an anodic wave similar to that obtained with OPD was observed.

The Reduction Wave of the Condensation Product of OPD with Glycolaldehyde and with Glyceraldehyde. In a previous paper,[10] the heating of a reaction mixture containing $(1—2)\times10^{-2}$ M OPD and $(1—2)\times10^{-2}$ M sodium sulfite was recommended for the determination of partially-polymerized biacetyl. Under the same conditions, it was observed that glycolaldehyde and glyceraldehyde are easily condensed with OPD to produce a condensation product which shows a single, well-defined reduction wave, but no oxidation wave (*cf.* Fig. 8). The effects of the pH of the reaction mixture on the limiting currents and the half-wave potentials are shown in Fig. 9. A linear relationship between the limiting current of the condensation product and the concentration of glycolaldehyde or glyceraldehyde was obtained at the pH value at which the current exhibits its maximum

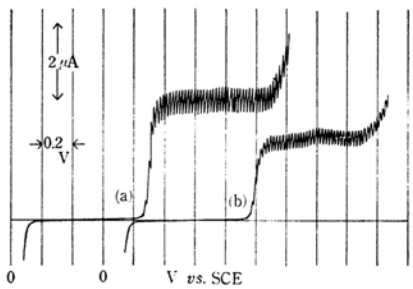


Fig. 8. Reduction waves of the condensation products.
(a) 1×10^{-3} M glyceraldehyde in BR buffer (pH 10.8)
(b) 1×10^{-3} M glycolaldehyde in 0.1 N NaOH (pH 13)
Condensation: 2×10^{-2} M OPD, 2×10^{-2} M Na_2SO_3, 100°C, 5 min
Both polarograms were recorded at 25°C.

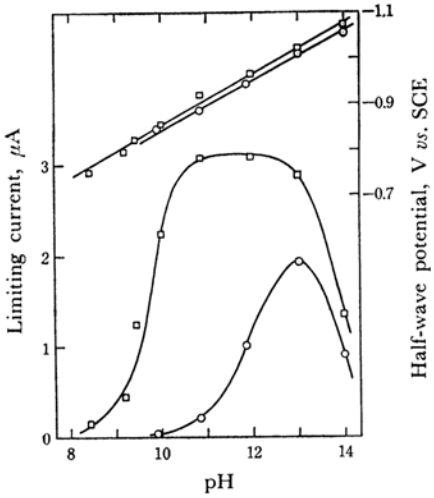


Fig. 9. Effects of pH on the reduction waves of the condensation products.
□ 1×10^{-3} M glyceraldehyde;
○ 1×10^{-3} M glycolaldehyde
Condensation: 2×10^{-2} M OPD, 0.2 M Na_2SO_3, 100°C, 5 min
Polarograms were recorded at 25°C.

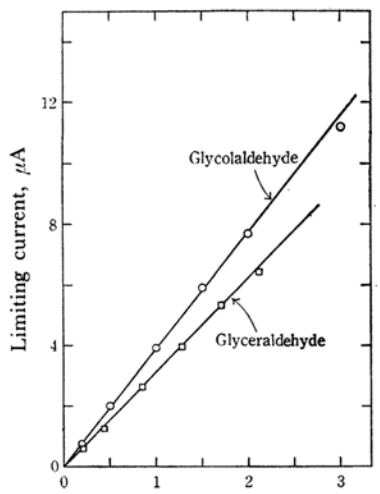


Fig. 10. Linear relationship between the limiting currents (anodic) of the condensation products and the concentrations of glycolaldehyde and glyceraldehyde in BR buffer (pH 6.0) at 25°C.
OPD: 2×10^{-2} M Condensation: 25°C, 20 min

value. Though no further experiments on the reduction waves of the condensation products of glycolaldehyde and glyceraldehyde with OPD have been made, these condensation products are probably quinoxaline derivatives and the reduction wave can probably also be applied to the determination of the aldehydes.

Application to the Determination of Glycolaldehyde and Glyceraldehyde. On the basis of the above results, the following conditions can be recommended for the determination of glycolaldehyde and glyceraldehyde by using the oxidation wave of the condensation product with OPD: 5.5—6.5 for pH range, 2×10^{-2} M for the concentration of OPD, 25°C for the temperature, and 5—60 min for the condensation time. Moreover, it is desirable that the ionic strength of the reaction mixture be kept as high as possible by adding potassium nitrate, and that the dissolving oxygen be removed by bubbling nitrogen as soon as possible after the preparation of the electrolytic solution. Under these conditions, a linear relation was obtained between the concentrations of glycolaldehyde and glyceraldehyde and the limiting currents of their condensation products in the range of $(0.5—30)\times10^{-4}$ M, as is shown in Fig. 10.

For applying the present OPD method to the determination of glycolaldehyde in commercial products of ethylene glycol containing glycolaldehyde and glyoxal as impurities, the following procedure was employed: the sample was diluted up to 2 to 10 times with distilled water, and the resulting diluted solution was allowed to stand overnight.[9] Then, in a run, 2 m*l* of the diluted sample was placed in a 20-m*l* volumetric flask, 10 m*l* of a BR buffer (pH, 6.0) containing 1 M potassium nitrate and 4 m*l* 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 placed in an electrolysis cell and immersed in a thermostat at 25°C for 60 min, during which time the dissolved oxygen was removed by bubbling nitrogen. After that, a polarogram was recorded in the usual way (A). In another run, 2 m*l* of the diluted sample was placed in a 20-m*l* volumetric flask, and 1 m*l* of a standard solution [$(1—2)\times10^{-3}$ M] of glycolaldehyde, 10 m*l* of a BR buffer (pH, 6.0) containing 1 M potassium nitrate, and 4 m*l* of the solution (0.1 M) of OPD were added. Then, the same procedure as in the former run was followed, and a polarogram was recorded (B). By

Table 3. Results of the determination of glycolaldehyde in ethylene glycol
OPD: 2×10^{-2} M, Condensation: 25°C, 60 min

Sample No.	Glycolaldehyde contents, %	
	Polarographic	Titrimetric*
1	0.298	0.409
	0.297	0.409
	0.298	0.410
2	0.143	
	0.130	
	0.140	
	0.160	
	0.140	

* Hydroxylamine titration method.

comparing the two polarograms, (A) and (B), the glycolaldehyde content in the original sample was calculated. Some of the results obtained are presented in Table 3, together with those obtained by the titration method.[18] Since sometimes a condensation product of glyoxal gave a small oxidation wave[9] in the early stages of a condensation reaction under the present experimental conditions, the limiting currents of the condensation products of glycolaldehyde with OPD were measured 60 min after the addition of OPD in order to avoid any interference by glyoxal. On the other hand, when the determination of glycolaldehyde was carried out by the titration method, glyoxal interfered seriously with the determination of glycolaldehyde.

Alcohols (methanol, ethanol, and ethylene glycol), carbohydrates (ribose, glucose, fructose, and sucrose), and α-dicarbonyl compounds (glyoxal, methylglyoxal, and biacetyl) do not interfere with the determination of glycolaldehyde and glyceraldehyde. Though acetone and chloride ion show an anodic wave under the present experimental conditions, they do not cause any interference when present in concentrations less than 3×10^{-2} M. Since the condensation products of such monoaldehydes as formaldehyde, acetaldehyde, propionaldehyde, and butyraldehyde with OPD behave similarly at the dropping mercury electrode, these substances interfere with the determination of glycolaldehyde and glyceraldehyde.

The authors wish to express their thanks to Dr. Masanosuke Takagi, for his helpful discussions and suggestions during this work. They are also indebted to Mr. Mitsutoshi Ochi for his assistance in the experimental work.

18) S. Siggia, "Quantitative Organic Analysis *via* Functional Groups," Wiley, New York (1963), p. 73.