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The Polarographic Behavior of the Condensation Products of o-Phenylenediamine with Biacetyl and with Methylglyoxal, and Its Application to the Determination of Biacetyl and Methylglyoxal

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Both the condensation product of *o*-phenylenediamine (OPD) with methylglyoxal and that with biacetyl produce two diffusion-controlled reduction waves, each corresponding to a one-electron transfer, at pH values below 3 ($E_{1/2}$: -0.220 V and -0.332 V *vs.* SCE for the methylglyoxal product, and -0.270 V and -0.447 V for the biacetyl product respectively, at pH = 1.5), or a single well-defined reduction wave corresponding to a two-electron transfer at pH values above 3 ($E_{1/2}$: -0.831 V and -0.902 V respectively, at pH = 9.0). At pH values below 8, it has also been observed that the reduction product with the two-electron wave shows an ill-defined reduction wave ($E_{1/2}$: -1.15 V and -1.07 V respectively, at pH = 5.0) at a little more positive potential than that of the hydrogen ion discharge. During the course of the controlled-potential electrolysis at -1.0 V *vs.* SCE at a pH value of about 9, it has been observed that the anodic wave of the reduction product with the same half-wave potential as the above reduction wave grows at the expense of the reduction wave (probably because of the quinoxaline formed by the condensation), while, though the reduction wave decreases with the electrolysis at -0.7 V, no anodic wave due to the oxidation of the electrolytic reduction product is obtained at pH values lower than 6. A linear relationship between the limiting current of the condensation product and the concentration ($0.5—20\times10^{-4}$ M) of methylglyoxal or biacetyl has been obtained under the following conditions, which are recommended for the practical determination: 3—10 for pH, $(1—2)\times10^{-2}$ M for the concentration of OPD, 25°C for the temperature, and 20—60 min for the condensation time. The method was also used to determine the purity of a methylglyoxal prepared in the laboratory and that of a commercial biacetyl.

It has been reported by several authors that biacetyl[1-7] and methylglyoxal[8-10] show two reduction waves at the dropping mercury electrode, and that the first wave can be applied to the determination of biacetyl[5-7] and methylglyoxal.[8-10] However, the reduction wave is partially reaction-controlled in nature; hence, the limiting current varies with the temperature and with the pH of the electrolytic solution, and also decreases with time in the alkaline region due to the instability of biacetyl and methylglyoxal. Experimental conditions must, therefore, be strictly controlled if this reduction wave is to be used for the determination of biacetyl and methylglyoxal.

The present authors have previously reported on the reduction wave of the condensation product of glyoxal with *o*-phenylenediamine (OPD) in various buffer solutions, and on the application of the reduction wave to the polarographic determination of glyoxal.[11]

On the other hand, it has been known that biacetyl[1] and methylglyoxal[9] are condensed with OPD, and that the condensation products produce the reduction wave at the dropping mercury electrode.

The present paper will describe the polarographic behavior of biacetyl and methylglyoxal in various buffer solution containing excess OPD, and a method of analyzing biacetyl and methylglyoxal by using these reduction waves.

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Experimental

Materials and Reagents. Methylglyoxal was prepared according to the method of Riley, Morley and Friend[12] by oxidizing acetone with hydrogen peroxide and selenium dioxide. Biacetyl, a commercial product, was purified by fractional distillation[13]; the fraction with a bp of 88.0—88.5°C was used. Aqueous stock solutions of methylglyoxal and biacetyl were prepared to be about 10^{-2} M, a concentration checked by the titration method.[14,15] Methylquinoxaline and dimethylquinoxaline were prepared according to the methods of Jones and McLaughlin[16] and Bost and Towell[17] respectively. OPD was a product for chromatography from E. Merck AG.; the stock solution (about 0.1 M) was prepared fresh daily by dissolving OPD in redistilled water. All the other chemicals were of a reagent grade or equivalent. No maximum suppressor was used. As for the buffer solutions, Britton-Robinson buffer (BR) was mainly used, while for checking the effect of the components of the buffer solution, McIlvaine buffer (McII) and a phosphate buffer (Phos) were also employed.

Apparatus and Procedure. The polarograph, thermostat, and pH-meter were the same instruments as were used in a previous work.[11] The characteristics of the capillary used were: $m=1.143$ mg/sec and $t=4.00$ sec/drop in the buffer solution (pH, 4.0) when the height of the mercury reservoir was 71 cm and when the applied potential was 0 V *vs.* SCE at 25°C. For the temperature control, the modified H-type cell used in the previous work[11] was employed. The accuracy of the temperature control was ±0.1°C. A saturated calomel electrode was used as the reference electrode.

The experimental procedure was as follows: a given volume (usually 1 to 4 m*l*) of a stock solution, either methylglyoxal or biacetyl, was placed in a 20 m*l* volumetric flask; a given volume (usually 1 to 4 m*l*) of an aqueous solution (about 0.1 M) of OPD and 10 m*l* of an appropriate buffer solution were then added, after which the solution was made up to the mark with redistilled water. A portion 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, polarograms were recorded in the usual way.

Controlled-potential Electrolysis. The controlled-potential electrolysis of methylquinoxaline and dimethylquinoxaline was carried out by means of the same procedure and the same apparatus as have been described in the previous paper.[11]

Results and Discussion

Reduction Waves of Biacetyl and Methylglyoxal. Though it has been reported by several authors[1-3] that biacetyl shows two reduction waves in neutral media, in the present studies a single reduction wave (Fig. 1, c) was observed in a McIlvaine buffer solution and in a phosphate buffer solution (pH, 2.0—11.0); this is in accordance with the observation by Harrison.[4] On the other hand, methylglyoxal showed the two reduction waves (Fig. 1, f and g) in the same buffer solutions; the polarographic behavior of the first wave coincides with that obtained by Krupicka and Novak,[10] while the half-wave potential of the second wave almost coincides with that obtained by Mackinney and Temmer.[8]

The reduction waves of methylglyoxal and biacetyl are partially reaction-controlled currents: the slopes

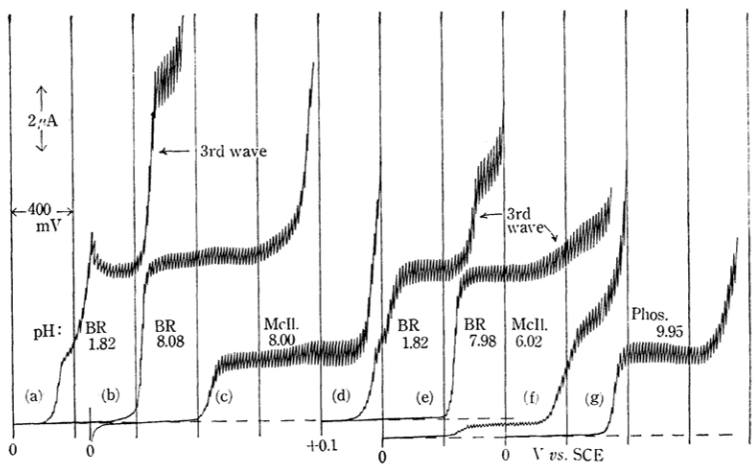

Fig. 1. Polarograms of biacetyl and methylglyoxal with or without OPD at 25°C.
Biacetyl: (a)—(c), 1×10^{-3} M; Methylglyoxal: (d), (e), 1×10^{-3} M; (f), (g), 5×10^{-3} M; OPD: (a), (b), (d), (e), 1×10^{-2} M; Condensation: 50 min

12) H. L. Riley, J. F. Morley and N. A. C. Friend, *J. Chem. Soc.*, **1932**, 1875.
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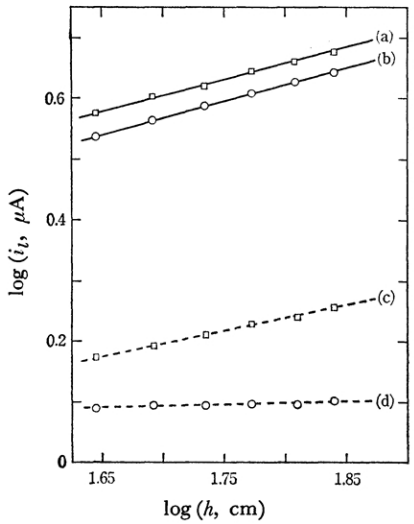

Fig. 2. Relationship between $\log i_l$ and $\log h$ at 25°C.
Biacetyl: (a), (c), 1×10^{-3} M;
Methylglyoxal: (b), 1×10^{-3} M; (d), 4×10^{-3} M
(a), (b): with 1×10^{-2} M OPD in BR buffer (pH, 9.0), condensation, 60 min, (c), (d): without OPD in McIlvaine buffer (pH, 5.0).

of the plots[18] of the logarithm of the wave height (i_l) against the logarithm of the height of the mercury reservoir (h) are 0.0235 for methylglyoxal and 0.430 for biacetyl (Fig. 2, c and d), and the temperature coefficients of the reduction waves are 9.5 and 4.9%/deg respectively in McIlvaine buffer solution (pH, 5.0).

Reduction Waves of the Condensation Products of Methylglyoxal and Biacetyl with OPD. A well-defined reduction wave was obtained when methylglyoxal and biacetyl were treated with OPD in appropriate buffer solutions by the procedure described above, as may be seen in Fig. 1 (a, b, d, and e). The reduction wave was a diffusion-controlled current: the slopes in Fig. 2 (a and b) for the condensation products of methylglyoxal and biacetyl with OPD are 0.505 and 0.495 respectively, and both reduction waves have the small temperature coefficient of 1.6%/deg.

Figure 3 shows the change in the limiting currents of the condensation products with the pH when the concentrations of methylglyoxal, biacetyl, and OPD were kept constant and only the pH of the mixture was changed. The limiting currents of the condensation products were measured 45 min after the addition of OPD at 25°C, since they increase with time during the first 10 min for methylglyoxal and during the first 20 min for biacetyl. Under the present experimental conditions, the limiting currents of the two-electron reduction of the condensation products remain almost constant, except for the splitting of the reduction wave into two waves at pH values lower than about 3. At pH values lower than 8, an ill-defined and irreversible reduction wave (Fig. 1, third wave) was also observed at a little more positive potential than that of the hydrogen ion discharge; it showed a maximum value at pH values between 4—5 and disappeared at pH values above 8.

The changes in the half-wave potentials of the condensation products with the pH value are presented in Fig. 4.

Since the same behavior was also observed in the experiments using methylquinoxaline[16] and dimethylquinoxaline,[17] the condensation products are

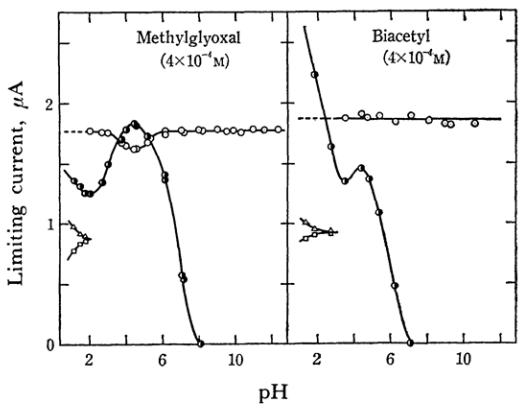

Fig. 3. Effect of pH on the reduction waves of the condensation product with 1×10^{-2} M OPD at 25°C.
Condensation: 45 min; △—1st, □—2nd, ◑—3rd and ○—main wave (1st+2nd)

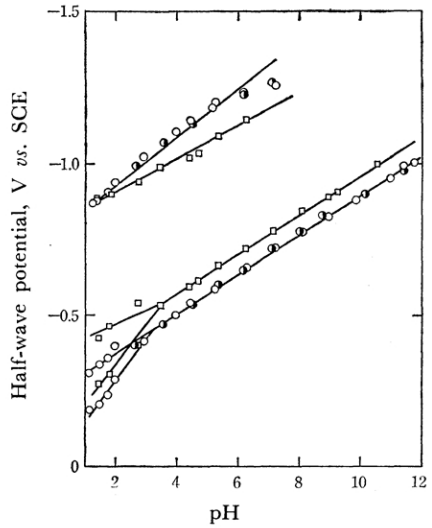

Fig. 4. pH-dependence of the half-wave potential of the condensation product at 25°C.
○, ◑—methylglyoxal, □—biacetyl; ○, □—BR buffer, ◑—McIlvaine and phosphate buffer

18) W. B. Swann, W. M. McNabb and J. F. Hazel, *Anal. Chim. Acta*, **28**, 441 (1963).

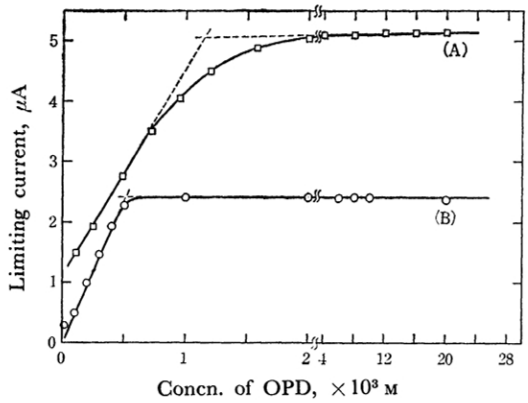

Fig. 5. Effect of the concentration of OPD in BR buffer (pH, 9.0) at 25°C.
(A): 1.15×10^{-3} M biacetyl, (B): 5.20×10^{-4} M methylglyoxal; Condensation: 150 min

probably quinoxaline derivatives, described in the previous paper.[11]

The effects of the components of the buffer solutions on the reduction wave of the condensation products were checked, but no appreciable change was observed.

Figure 5 shows the relationships between the limiting currents and the concentration of OPD in a BR buffer with a pH value of 9.0 at 25°C when the concentrations of methylglyoxal and biacetyl were kept constant. From Fig. 5, it can be concluded that the reaction of methylglyoxal and biacetyl with OPD takes place stoichiometrically.

Figure 6 shows the change in the limiting current of the condensation product with the time elapsing after the addition of OPD to an electrolytic solution containing methylglyoxal and a BR buffer with a pH value of 9.0, with the concentrations of methylglyoxal and OPD kept constant and the experimental temperature changed. With the rise in the temperature, the growing velocity of the wave increased; in other words, the time required for the

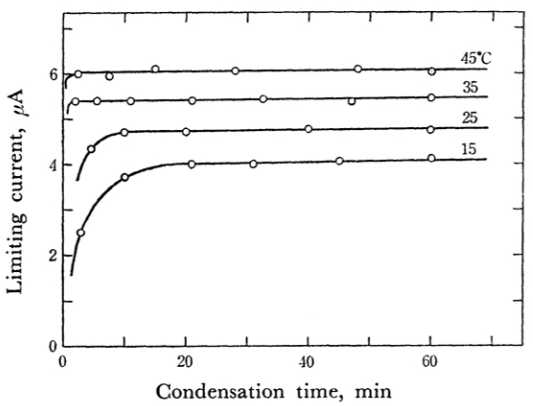

Fig. 6. Effect of the temperature in BR buffer (pH, 9.0).
Methylglyoxal: 1.07×10^{-3} M, OPD: 1×10^{-2} M

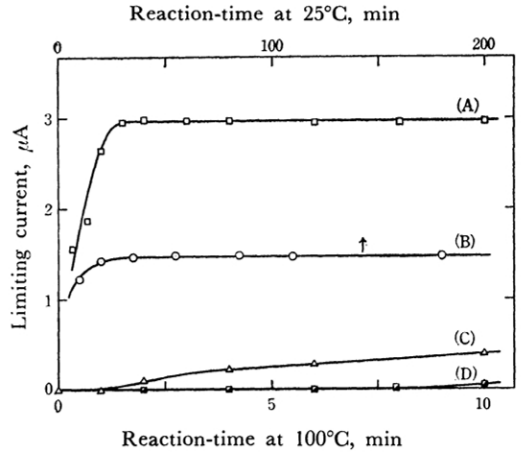

Fig. 7. Effect of the reaction-temperature on the condensation of biacetyl with 1×10^{-2} M OPD in BR buffer (pH, 9.1). All polarograms were recorded at 25°C.
(A) with biacetyl and 1×10^{-2} M sodium sulfite at 100°C
(B) with biacetyl and without sodium sulfite at 25°C
(C) without biacetyl and sodium sulfite at 100°C
(D) without biacetyl and with 1×10^{-2} M sodium sulfite at 100°C

condensation was shortened. Similar behavior was also observed with biacetyl which had been freshly purified by distillation.

Figure 7 shows the change in the limiting current of the condensation product of biacetyl, which might be partially polymerized,[19] when the condensation reaction was carried out either at 25°C or at 100°C in a BR buffer with a pH value of 9.0. At the reaction temperature of 100°C, a small reduction wave ($E_{1/2}$: -0.7——0.9 V *vs.* SCE), which seems to be due to phenazine[20,21] produced by the oxidation of OPD with dissolved oxygen, was observed in the absence of biacetyl. Therefore, to remove the dissolving oxygen, a given volume (usually 1—2 m*l*) of a 0.1 M aqueous solution of sodium sulfite was added to the reaction mixture. The mixture was then transferred to a test tube with a ground glass stopper, kept in boiling water for a given time, and then cooled quickly with ice water. After that, the polarograms were recorded in the usual way at 25°C.

At the reaction temperature of 25°C, the limiting current of the condensation product increased for the first 20 min and then remained almost constant for a long time, while at 100°C, not only was the growing velocity of the reduction wave much faster, but also the wave height, which was recorded at

19) R. M. Cresswell, W. R. D. Smith and H. C. S. Wood, *J. Chem. Soc.*, **1961**, 4882.
20) R. C. Kaye and H. I. Stonehill, *ibid.*, **1952**, 3240.
21) Y. Asahi, *Yakugaku Zasshi* (*J. Pharm. Soc. Japan*), **80**, 679 (1960).

25°C, was higher than those obtained when the entire reaction at 25°C. For the freshly-distilled biacetyl, the difference in the wave height of the condensation product with the reaction temperature described above was not observed. These results seem to indicate, that at the reaction temperature of 25°C, free biacetyl alone in the sample reacts with OPD, while at 100°C, since polymerized biacetyl is converted to free biacetyl by thermal disintegration, all the biacetyl reacts completely with OPD.

Controlled-potential Electrolysis. During the course of the controlled-potential electrolysis, it was observed that dimethylquinoxaline and methylquinoxaline, the condensation products of biacetyl and methylglyoxal with OPD, showed a behavior similar to that observed with quinoxaline,[11] except that the reduction product was dissolved more easily in an aqueous solution. With the electrolysis at a fixed potential of -1.0 V *vs.* SCE and at a pH value of 9.2, the anodic wave which is due to the oxidation of the electrolytic-reduction product grew at the expense of the reduction wave, and the electrode reaction was reversible because the half-wave potential of the anodic wave was identical with that of the reduction wave, as may be seen in Fig. 8 (a, b, and c). After the electrolysis was completed at the pH value of 9.2, the pH value of the electrolytic solution was changed to 4.5 by means of the addition of acetic acid. It was observed that the anodic wave due to the reduction product then immediately disappeared, while a new reduction wave, which seemed to be the third reduction wave of quinoxalines, appeared, as may be seen in Fig. 8 (d). At pH values lower than 6, though the reduction wave of quinoxalines decreased with the electrolysis at a fixed potential of -0.7 V, while at the same time the electrolytic solution became yellow, the anodic wave by the reduction products was not observed. The third wave of quinoxalines in Fig. 1, was not influenced by the electrolysis (Fig. 8, e, f, and g). After the electrolysis had been completed, the reduction wave due to the quinoxalines was obtained again simply by bubbling air through the electrolytic solution, because the electrolytic reduction product is easily oxidizable.

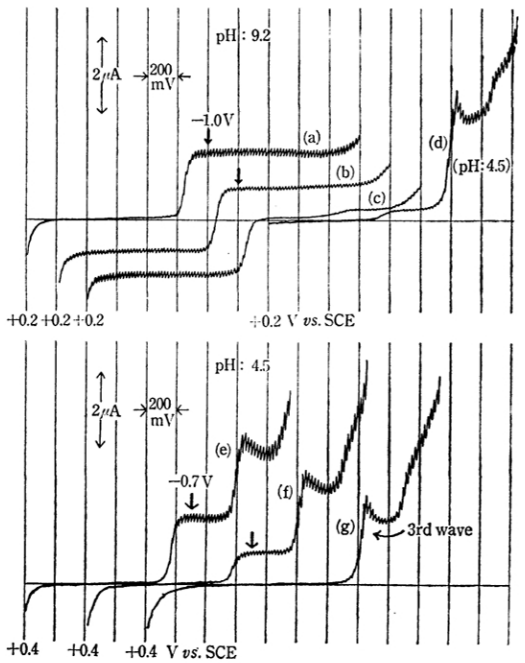

Fig. 8. Controlled-potential electrolysis of dimethylquinoxaline in BR buffer at room temperature about 20°C.
(a), (e), before electrolysis; (b), (f), after 15; (c), (g), 45 min; (d), pH, 4.5 [(c)+acetic acid]; Dimethylquinoxaline: 5×10^{-4} M

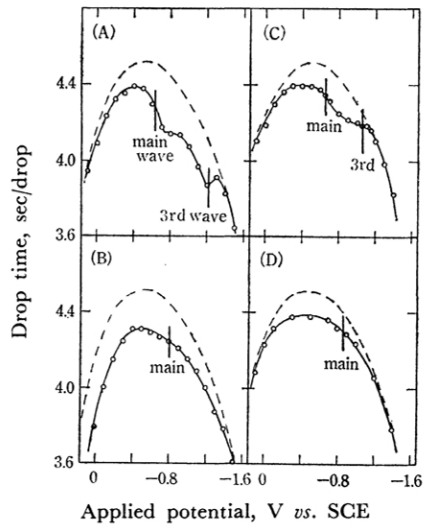

Fig. 9. Electrocapillary curves in BR buffer containing 1×10^{-2} M OPD with or without glyoxals at 25°C.
(A), (B), methylglyoxal; (C), (D), biacetyl
(A), (C), pH, 5.0; (B), (D), pH, 9.0
—— with 5×10^{-4} M glyoxals; - - - without glyoxals

Figure 9 show the electrocapillary curves in a BR buffer containing excess OPD, with or without glyoxals (methylglyoxal and biacetyl). At a pH value of about 5, in which the third wave (see Fig. 1) is obtained, the adsorption of the reduction product was observed in the potential range between the main and the third wave, but no adsorption was observed at pH values above 8, in which no third wave is obtained.

From the results described above, the reduction product seems to be comparatively stable at pH values of about 9, but it becomes unstable at pH values of less 6. In the acidic range, the reduction product is transformed into another substance which is unoxidizable at the dropping mercury electrode, but oxidizable by the dissolving oxygen. This substance may cause the capillary-active properties

which were described above in connection with the electrocapillary curves.

Since the wave height of dimethylquinoxaline or methylquinoxaline is almost equal to that of quinoxaline[11] at the same concentration, it may be deduced that the electrode reaction for dimethylquinoxaline and methylquinoxaline requires two electrons.

Application to the Determination of Methylglyoxal and Biacetyl. On the basis of the above results, the following conditions may be recommended as the most practical conditions for the determination of methylglyoxal and biacetyl using the reduction wave of the condensation product with OPD: 3—10 for the pH range, $(1—2)\times10^{-2}$ M for the concentration of OPD, 25°C for the temperature, and 10—60 min for the condensation time. If the sample is partially polymerized, the total amounts may also be determined by the following procedure: a reaction mixture (pH, 8—10) containing $(1—2)\times10^{-2}$ M OPD and $(1—2)\times10^{-2}$ M sodium sulfite is kept in boiling water for 3—10 min and then quickly cooled with ice water. After that, polarograms are recorded in the usual way at 25°C. Under these conditions a linear relation is obtained between the concentration of methylglyoxal and biacetyl and the limiting current of their condensation products in the range of $(0.5—20)\times10^{-4}$ M, as is shown in Fig. 10.

For applying the present OPD method to the determination of methylglyoxal in a reagent prepared in the authors' laboratory according to the method of Riley, Morley and Friend,[12] the following procedure was employed: 0.5—2 g of a sample was transferred to a 1-*l* volumetric flask and diluted to the mark with distilled water. An aliquot of the diluted sample was treated with OPD under the conditions described above. Polarograms were recorded in the usual way at 25°C, and the methylglyoxal content was calculated by comparison with the calibration curve under the same conditions. The results obtained are listed in Table 1, together with those obtained by the titration method[14] based on the Cannizaro reaction. The results obtained by the two methods are in good agreement with each other.

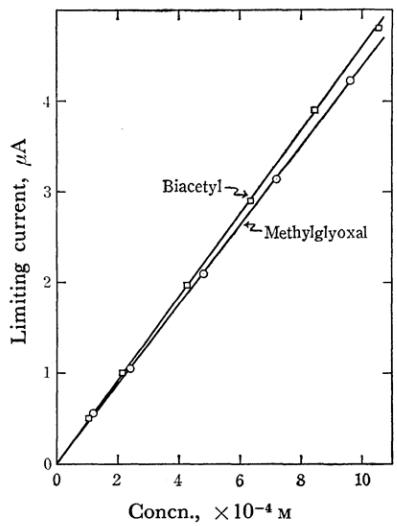

Fig. 10. Linear relationship between the limiting currents of the condensation products and the concentration of biacetyl and methylglyoxal in BR buffer (pH, 9.0) at 25°C.
OPD: 1×10^{-2} M, Condensation: 50 min

Table 1. The results of the determination of methylglyoxal
OPD: 1×10^{-2} M, pH, 9.0, 25°C, 45 min

Exp. No.	Methylglyoxal content, %	
	Polarographic	Titrimetric*
1	70.4	69.7
2	72.0	
3	72.0	
4	70.4	
5	70.8	
6	71.9	69.1
7	71.4	
8	72.3	
9	70.9	
10	71.4	

* Determination by the titration method[14] based on Cannizaro's reaction.

As another application, the determination of the purity of a commercial biacetyl was made under the conditions described above. The biacetyl contents were calculated by means of the standard addition method.[11] The results obtained are presented in Table 2, together with those obtained by the titration method.[15] For the biacetyl purified by distillation, it was observed that the results obtained at the reaction temperature of 100°C are in good agree-

Table 2. The results of the determination of biacetyl in commercial products
OPD: 1×10^{-2} M, pH, 9.1

Sample	Biacetyl content, %		
	Polarographic[a]		Titrimetric[b]
	25°C, 50 min	100°C, 5 min	
A[c]	99.9_7	98.7_8	98.9_9
B[d]	99.5_0	101.1_3	97.1_2
C	39.1_0	87.2_1	68.2_8
D	87.5_6	98.1_2	90.0_3
E	52.2_6	67.6_9	66.0_9

a) All polarograms were recorded at 25°C.
b) Hydroxylamine titration method,[15] 70°C, 20 min.
c) Purified by distillation from D.
d) Purified by distillation from C.

ment with those obtained at 25°C, and also with that obtained by the titration method. For commercial products of biacetyl, however, the results obtained at 100°C did not coincide with those obtained at 25°C. Therefore, the variation in the results with the reaction temperature seems to be due to the amount of polymerized biacetyl, such as the dimer or the trimer, which is easily decomposed to biacetyl by thermal disintegration at 100°C. The results obtained by the titration at 70°C were higher than those obtained by the polarographic OPD method at 25°C and lower than those obtained polarographically at 100°C. The discrepancy may be due to the different conditions of measurements; polymerized biacetyl may disintegrate at elevated temperature. The initial degree of polymerization may also differ considerably in the commercial products.

The presence of the alcohols such as methanol, ethanol, and ethylene glycol did not interfere with the determination of methylglyoxal and biacetyl. Formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, and acetone showed the anodic waves*1 characteristic of monocarbonyl compounds under the present experimental conditions, but they do not give reduction waves, whereas glyoxals do. Therefore, the presence of the monoaldehydes and monoketone did not interfere with the determination of methylglyoxal and biacetyl. At a reaction temperature of 25°C, though glycolaldehyde and glyceraldehyde gave the anodic waves*1 which have been described in the case of monoaldehyde, they did not give the reduction waves. Accordingly, the presence of these aldehydes did not interfere at all with the determination of methylglyoxal and biacetyl. However, at a reaction temperature of 100°C, these aldehydes gave reduction waves ($E_{1/2}$: -0.8—-0.9 V *vs.* SCE, at pH 10) which seem to be due to the quinoxaline derivatives produced by condensation with OPD. Therefore, the presence of these aldehydes interferes with the determination of methylglyoxal and biacetyl.

Since the condensation products of methylglyoxal, biacetyl, and glyoxal[11] with OPD behaved similarly at the dropping mercury electrode, the presence of any of these substances may be said to interfere with the determination of the others.

The authors wish to express their thanks to Professor Dr. Sôzaburo Ono for his kind advice and encouragement, and to Dr. Masanosuke Takagi for his helpful discussions and suggestions during this work.

*1 The details of this kind of observation will be given elsewhere.