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Polarographic Study with a Microelectrode Past which an Electrolyte is Flowing[1]

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Microelectrodes other than dropping mercury have been studied with renewed interest in recent years in order to devise practical applications for which the dropping mercury electrode is not suited[3,4] and to go beyond its limitations in the positive potential range.

A very careful study of the behavior of a variety of platinum wire electrodes, stationary and rotating, was made by Laitinen and Kolthoff,[5,6] who pointed out some of the difficulties encountered in their use. They chose as most satisfactory for general use an electrode composed of a small piece of platinum wire sealed into a glass tube. With this wire electrode a "steady state" diffusion current could soon be obtained, which was least subject to irregular periodic fluctuations. This "steady state" diffusion current depends on a state of uniform and reproducible convection in the solution immediately surrounding the microelectrode. Hence, it is necessary to protect the cell from all thermal and mechanical disturbances. In order to get significant results with a wire microelectrode, one must wait two minutes or longer after applying a given voltage until the current becomes steady. Thus the preparation of current–voltage curves becomes very tedious. The use of a rotating wire microelectrode eliminates this disadvantage and, with high speeds of rotation (1800 r. p. m.), increases the sensitivity of the method. Whether the attainment of a "steady state" with this electrode is fast enough to make it suitable for automatic (polarographic) recording is not stated by Laitinen and Kolthoff.[6] They consider the rotating electrode to have but limited applicability since, in all cases investigated, the electrode reactions are accompanied by pronounced polarization effects. Theoretical current–voltage curves, calculated on the assumption that concentration polarization is the only kind of polarization, deviated greatly from the experimental curves.

As might be expected, essentially the same difficulties were encountered in the present study when an electrolyte was made to flow past a stationary microelectrode. However, the automatic polarographic recording of current–voltage curves obtained with it brought out some additional information. The results presented here are more qualitative than quantitative since the purpose of this investigation was mainly to evaluate the possible usefulness of such a "by-pass" electrode. Further, more accurate and detailed studies, which lend themselves better for mathematical analysis, are contemplated.

The work with this "by-pass" electrode was begun with the hope that (a) it would contribute to our understanding of electrode reactions in which equilibrium conditions can no longer be established and (b) it would make available a simple method of analysis of flowing systems. From the standpoint of the physiologist this latter possibility is of great importance because then the by-pass electrode could, if inserted into the bloodstream, provide an immediate indication of the *availability of material* in the blood to a given tissue. To find this at present one must, first, draw blood for a determination of the concentration of a given material and, secondly, measure the rate of blood flow through the vessel. As will be shown below, the limiting current found on the current–voltage curve obtained with the by-pass electrode is a function of both the rate of flow and the concentration of electroactive material. Hence this new method shows promise for physiological studies.

Experimental

Several metals were tried as electrode materials but only the findings obtained with a platinum microelectrode will be reported here. Its construction and operation will become apparent from Fig. 1. The solution under study[7] flows from the flask, A, under a constant head of pressure (usually 1 m.) to the microelectrode M. In analyses of solutions containing atmospheric oxygen, the Mariotte-flask principle is employed to maintain a constant pressure, by keeping the stopcocks F and V closed and T open to the air. When oxygen interferes, an indifferent gas is introduced at G for thirty to sixty minutes before the analysis, with the vent V open. During the analysis the stopcock V is closed and the same gas displaces the solution at a pressure which is maintained constant by the water seal S. Additions to the flask can be made through the funnel F while vent V is open. After passing the microelectrode M, the solution bathes a saturated potassium chloride–agar agar

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[2] Present address: Department of Physiology, Syracuse University, College of Medicine, Syracuse, N. Y.

[3] L. R. Blinks and R. K. Skow, *Proc. Natl. Acad. Sci.*, **24**, 420 (1938).

[4] P. W. Davies and F. Brink, *Am. J. Physiol.*, **133**, P257 (1941).

[5] H. A. Laitinen and I. M. Kolthoff, THIS JOURNAL, **61**, 3344 (1939), and *J. Phys. Chem.*, **45**, 1061 (1941).

[6] H. A. Laitinen and I. M. Kolthoff, *J. Phys. Chem.*, **45**, 1079 (1941).

[7] To avoid the need for large quantities of solution an apparatus has now been designed in which an atomizer and gas lift are combined to circulate about 50 ml. of solution automatically. This circulating system can be operated with any indifferent gas or air under pressure. A description of it will appear elsewhere.

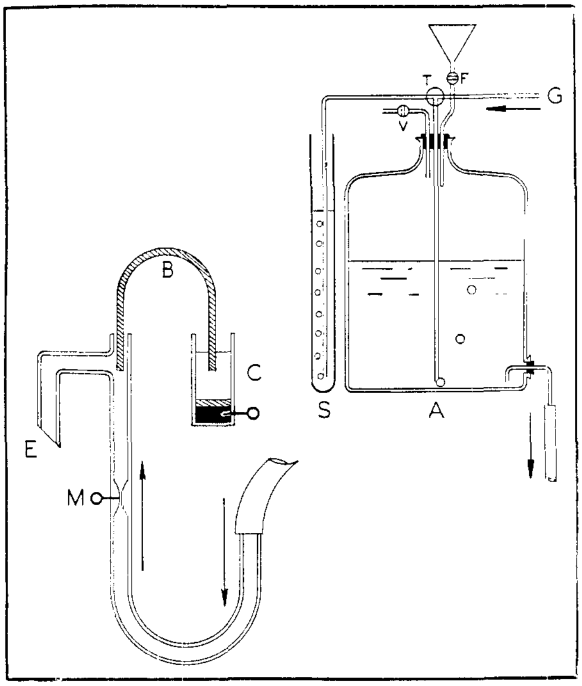

Fig. 1.—Diagram of the by-pass electrode and the device for maintaining a constant flow of de-oxygenated solution.

bridge, B, before overflowing at E. The bridge establishes contact with the non-polarizable reference electrode, a saturated calomel half-cell, C.

The microelectrode consists of a piece of 26-gage platinum wire, sealed into a glass tube the constricted part of which is about 1 mm. long and 0.4 mm. in diameter. The exposed area of the wire is approximately 0.3 sq. mm. and thus considerably smaller than the wire electrodes of Laitinen and Kolthoff with areas of 6–7 sq. mm.

This very small area was thought desirable to keep the current, *I*, small in order to avoid complications due to *IR*[8] when the resistance, *R*, becomes large, as is likely to be the case in measurements in the blood stream.

The duration of the electrolyses was so short and the flow of current so minute in these experiments that deposition of mercury from the calomel half-cell onto the microelectrode, as described by Giguère and Lauzier,[9] never was encountered. It is known that the liquid junctions of such a system will have but little effect (about 1 mv.) on the potentials measured.[10] All experiments were carried out at room temperature which did not vary by more than ±0.5° for comparative tests.

The polarograph used was a Nejedly instrument, Model VIII; the galvanometer had a maximal sensitivity of 2.1×10^{-9} amp./mm./m., and a special shunt reduced this sensitivity, *S*, to desired values while the critical damping resistance was kept constant. The driving motor of this polarograph was governor controlled so that the speed with which the applied voltage was altered could be maintained constant. In the experiments which are reported here this speed was 320 mv. per minute. Reversing gears on the polarograph made it possible to change quickly from an increasing to a decreasing applied voltage and *vice versa*. The voltage increment between abscissas is indicated on the published polarograms. Potentials are calculated from the abscissa marked with a circle as a point of reference for each curve. This point corresponds to the potential of the S.C.E.; to the right of it potentials become more negative and to the left of it more positive. A horizontal line indicates the position of the galvanometer at rest; current above this line (positive current) represents a reduction, and below this line (negative current) an oxidation at the microelectrode. To make it possible to distinguish overlapping curves on the polarogram in some of the curves (interrupted curves) the light illuminating the galvanometer was flashed on and off at regular intervals by means of a commercial sign flasher of 100-watt capacity.

Reproducibility.—As could be expected, the reproducibility of current–voltage curves is excellent as long as the experimental conditions are identical. Not only is it important in these studies to maintain constant the temperature and the rate of flow of the solution past the electrode and to record while the applied voltage is altered in the same direction and at the same rate, but also one must be certain of the same pretreatment of the electrode. This is demonstrated in Fig. 2 which shows two sets of reproducible curves obtained with a solution of 0.17 *M* ferric chloride in 0.1 *N* hydrochloric acid, all recorded from left to right. Each of the three uninterrupted curves was preceded by an *anodic* polarization of the electrode for two minutes at ± 1.8 v. (*vs.* S.C.E.), while each of the three interrupted curves was preceded by a *cathodic* polarization of the same

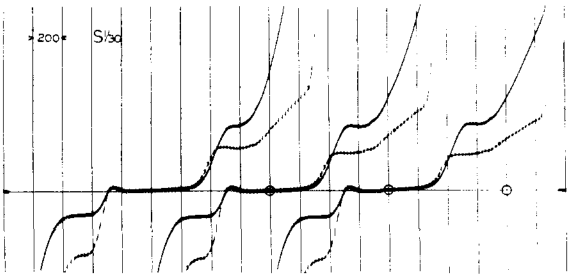

Fig. 2.—Polarogram of 0.17 *M* ferric chloride in 0.1 *N* hydrochloric acid. Polarization from left to right. Each uninterrupted curve is preceded by anodic polarization for 2 minutes at +1.8 v. (*vs.* S. C. E.). Each interrupted curve is preceded by cathodic polarization for two minutes at −0.6 v. (*vs.* S. C. E.).

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[8] O. H. Müller, "The Polarographic Method of Analysis," *J. Chem. Education*, Easton, Pa., 1941, p. 76f.

[9] P. A. Giguère and L. Lauzier, *Can. J. Research*, **B23**, 223 (1945).

[10] D. A. MacInnes, "The Principles of Electrochemistry," Reinhold Publishing Corp., New York, N. Y., 1939, p. 230.

duration at −0.6 v. (*vs.* S.C.E.). This cathodic prepolarization resulted in still smaller cathodic waves and larger anodic waves when the polarogram was recorded from right to left. Omission of the pre-polarization, as a rule, spoils the reproducibility of the records; the limiting currents then may be anywhere between the two extremes described. Undoubtedly adsorption of the reaction products at the electrode is in part responsible for this phenomenon since it is not of equal magnitude with different substances.

Reduction of Oxygen.—As could be anticipated on the basis of earlier work with platinum microelectrodes, dissolved oxygen is reduced at the platinum by-pass electrode and gives rise to a limiting current (see Fig. 3). Polarization toward more negative potentials (from left to right on the polarogram) results in a wave with a slow initial start followed by a steep rise and a fairly level plateau. Polarization in the opposite direction produces a more symmetrical S-shaped curve with a well rounded maximum (the interrupted curves of Fig. 3). Whenever such oxygen curves interfere with an analysis, they can be eliminated readily either by bubbling an inert gas through the solution, or by chemical removal of the dissolved oxygen.

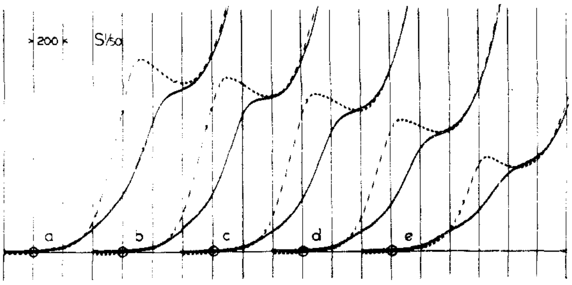

Fig. 3.—Polarogram of oxygen contained in 0.1 *N* potassium chloride in equilibrium with air. Rates of flow: (a) 0.429, (b) 0.374, (c) 0.314, (d) 0.238, and (e) 0.132 ml./second.

Reversibility of the Electrode Reaction.—In studies with the dropping mercury electrode the identity of the half-wave potentials in the reduction of an oxidant and in the oxidation of the corresponding reductant in the same well buffered solution serves as a simple criterion for the reversibility of the electrode reaction.[11] The hydroquinone–quinone system[12] is probably the most typical example. This system and all other organic systems which have so far been tested, fail to exhibit such reversibility at the by-pass electrode. However, an inorganic system, the ferrous–ferric system in acid solution, behaves in a reversible manner as is illustrated in Fig. 4. Note that the half-wave potentials obtained in the reduction of ferric chloride (curves a), are identical with those obtained in the oxidation of ferrous chloride (curves c). The same half-wave potential was also found when a mixture of equal volumes of these solutions was analyzed (curves b), and a smooth, single wave resulted which is partly cathodic and partly anodic. The half-wave potentials of the set of curves shown on the left side of Fig. 4 are +0.447 v. while those shown on the right side of Fig. 4 are +0.480 v. (*vs.* S.C.E.). The nature of this difference has not yet been fully ascertained; it is, in part, due to a lag in the response of the galvanometer, since the curves were recorded in different directions (indicated on the polarogram). The average of the two values gives us a potential of $E_h = +0.708$ v. in fair agreement with published data.[13]

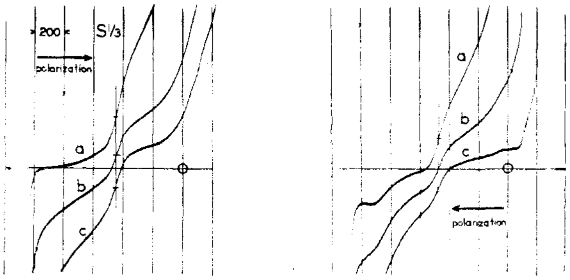

Fig. 4.—Polarogram illustrating a perfectly reversible reaction at the by-pass electrode: (a) 0.017 *M* ferric chloride in 1 *N* hydrochloric acid; (b) a mixture of equal volumes of (a) and (c); (c) 0.017 *M* ferrous chloride in 1 *N* hydrochloric acid.

Organic Oxidation–Reduction Systems.—In order to learn why organic oxidation–reduction systems fail to behave reversibily at the by-pass electrode, the quinone–hydroquinone system was investigated in greater detail, since it behaves ideally at the dropping mercury electrode, *i. e.*, shows no sign of any anomalous waves.[14] Its behavior at the by-pass electrode is shown in the polarogram reproduced in Fig. 5, which was obtained with a sulfuric acid solution of *p*H 2, containing approximately equal quantities of quinone and hydroquinone.[4] The reproducibility

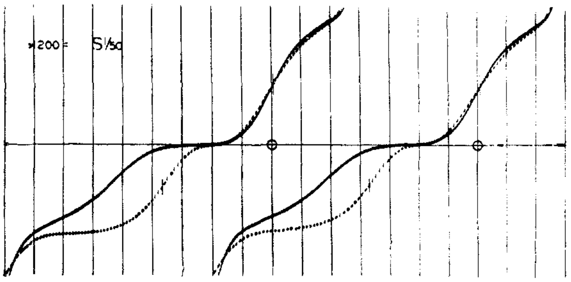

Fig. 5.—Polarogram illustrating reproducibility of curves obtained with approximately equal quantities of quinone and hydroquinone in a sulfuric acid solution of *p*H 2. Continuous curve shows "forward" polarization, interrupted curve shows "backward" polarization.

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[11] O. H. Müller, *Cold Spring Harbor Symposia Quant. Biol.*, **7**, 59 (1939).

[12] O. H. Müller and J. P. Baumberger, *Trans. Electrochem. Soc.*, **71**, 181 (1937).

[13] S. Popoff and A. H. Kuntz, THIS JOURNAL, **51**, 382 (1929).

[14] O. H. Müller, *Trans. Electrochem. Soc.*, **87**, 441 (1945).

of such curves is excellent, as may be seen by comparing the two sets shown. The *continuous* curves represent polarization from left to right (henceforth called "forward" curves); a short anodic polarization preceded the drawing of these curves. Polarizations in the opposite direction are shown as *interrupted* curves ("backward" curves); they were drawn immediately upon the completion of the forward curves. In comparing these forward and backward curves, note that the cathodic portions, *i. e.*, the waves which indicate reduction of quinone overlap fairly well, whereas the anodic portions, *i. e.*, the waves which indicate oxidation of hydroquinone are widely separated. Still more striking is the fact that instead of a single S-shaped anodic-cathodic wave, two waves are obtained which separate the oxidative from the reductive reactions. In this respect the curves look very similar to those obtained with the dropping mercury electrode for quinhydrone in the absence of a buffer or in the presence of an unsatisfactory buffer.[15,16]

The conditions illustrated by Fig. 5 were purposely chosen in such a way that slowness in the reaction of a buffer could not be a complicating factor. The dissociation and association of the quinhydrone molecules and ions must therefore be considered as the most important factor responsible for the separation of the anodic and cathodic waves. Naturally the effect of pre-polarization also contributes to this separation because the products of the electrode reaction may be absorbed. While a theoretical treatment of the observed facts will be reserved for a subsequent paper, it should be mentioned here that the wave of a backward curve representing the oxidation of hydroquinone can be expressed by the equation

$$E = \frac{RT}{2F}\ln\frac{I}{I_l - I} + \frac{2RT}{F}\ln 2I + \frac{2RT}{F}\ln\frac{I}{I_l - I}$$

where I_l is the limiting current corrected for residual current. This equation has been derived as a close parallel to one established for unbuffered hydroquinone curves obtained with the dropping mercury electrode.[11,16]

Inasmuch as the rates of dissociation and association of hydroquinone molecules and ions were inadequate to permit establishment of equilibrium conditions during a reaction at the by-pass electrode, it seemed likely that the dissociation and association of the components of McIlvaine's phosphate-citric acid buffer would also be slow enough under these conditions to add complications. That this is indeed the case is shown in Fig. 6 where the forward and backward curves fail to overlap in their cathodic portions as well. An analysis of the cathodic portions of the curves has not been attempted because even in the case of the dropping mercury electrode the unbuffered cathodic curves are not completely explained.[16]

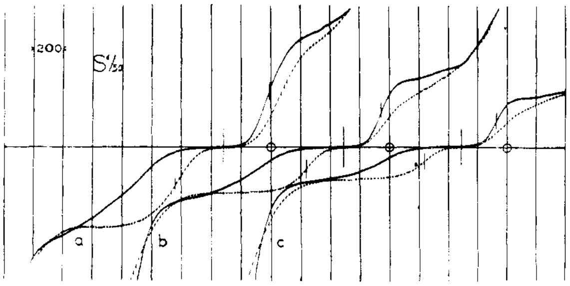

Fig. 6.—Polarogram showing effect of rate of flow of solution of quinhydrone in McIlvaine buffer of *p*H 3.4. Rates of flow: (a) 0.158, (b) 0.032 and (c) 0.015 ml./sec. Note separation of cathodic "forward" and "backward" curves.

The mean of the two half-wave potentials for the two waves of the backward curves of Fig. 5 gives a value corresponding very nearly to the theoretical quinhydrone potential at the *p*H of the solution. In Fig. 6, similarly, the correct potential of the system at the *p*H of the solution is found as the mean of two half-wave potentials. In this case, however, those parts of the curves will have to be selected which are least influenced by pre-polarization, *i. e.*, the cathodic portion of the forward curves and the anodic portion of the backward curves. The separation of the half-wave potentials of the anodic and cathodic waves increased with the concentration of quinhydrone in a manner perfectly analogous to the findings with this system at the dropping mercury electrode in unbuffered solutions.[15]

Knowing the satisfactory behavior of a stationary platinum electrode in quinhydrone for the determination of *p*H, *i. e.*, the quinhydrone electrode, one could expect that a decrease in flow past the by-pass electrode would decrease the separation of the anodic and cathodic half-wave potentials. That this is so may be seen from Figs. 6 and 7.

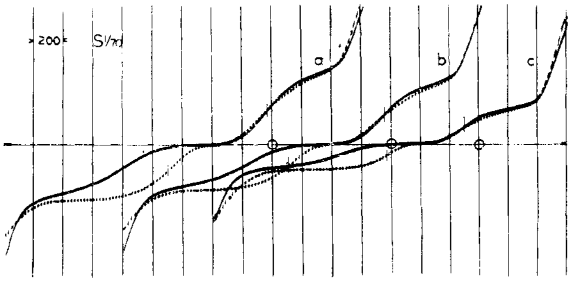

Fig. 7.—Polarogram showing effect of rate of flow on solution from Fig. 5. Rates of flow: (a) 0.145, (b) 0.093, and (c) 0.024 ml./sec. Note overlapping of cathodic "forward" and "backward" curves.

However, not all of the deviation from the theoretical potential value is due to the motion of the solution since the separation of anodic and cathodic waves still exists if the flow is stopped entirely (see Fig. 8). The cause for this is the relatively high rate with which the polarograph changes

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[15] O. H. Müller, THIS JOURNAL, **62**, 2434 (1940).

[16] O. H. Müller, "Polarography," in A. Weissberger "Physical Methods of Organic Chemistry," Vol. II, Interscience Publishers, Inc., New York, N. Y., 1946.

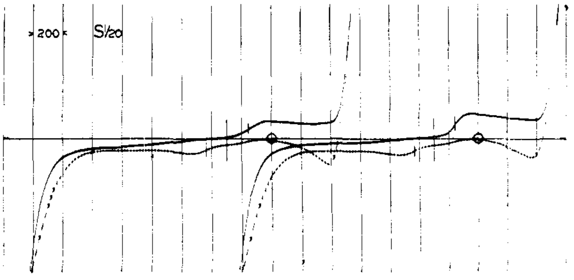

Fig. 8.—Polarogram of solution from Fig. 6 with flow stopped.

the applied voltage. The anodic and cathodic waves became fused into a single anodic–cathodic curve with the correct half-wave potential, when, in the absence of any flow, enough time (at least two minutes) was allowed for the establishment of equilibrium between each alteration in the applied voltage. This is in agreement with the observations of Laitinen and Kolthoff.[5]

Effect of Concentration on Limiting Current.—If all conditions of measurement are kept constant, the limiting current obtained in a given reaction is proportional to the concentration of the reacting material. For instance, in the oxidation of hydroquinone in 0.7 N sulfuric acid, the equation $I_l = 1.10 \times 10^{-3}\ C$ represents the observations with an average and a maximum variation of 0.10×10^{-6} and 0.15×10^{-6} amp., respectively, over the concentration range of 5×10^{-4} to $9 \times 10^{-3}\ M$ (see Fig. 9). As in studies with the dropping mercury electrode, it is necessary to correct all such current measurements for any residual current at the potential at which the limiting current is measured.

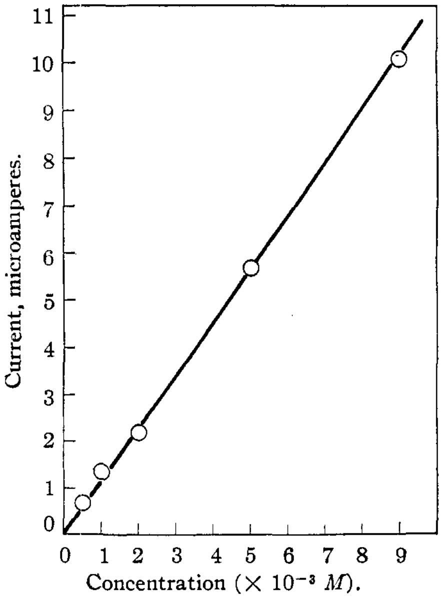

Fig. 9.—Limiting currents at by-pass electrode obtained with hydroquinone in 0.7 N sulfuric acid (corrected for residual current), plotted *vs.* concentration.

Effect of Rate of Flow on Limiting Current.—The effect of flow on the limiting currents obtained with the by-pass electrode is illustrated by Figs. 3, 6, 7 and 8. When the results were plotted graphically, as in Fig. 10, it was found that a linear relationship existed between the logarithm of flow and the limiting current. The slopes of the straight lines are identical for the same substances even in different media but they vary with different materials, apparently in the same order as their diffusion coefficients. For instance, in the case of oxygen, quinone, and hydroquinone, shown in Fig. 10, the slopes are 5.3, 2.3 and 1.7, respectively, while the corresponding diffusion coefficients (determined polarographically[17]) are 26.0, 8.6 and 7.4×10^{-6}sq. cm./sec.

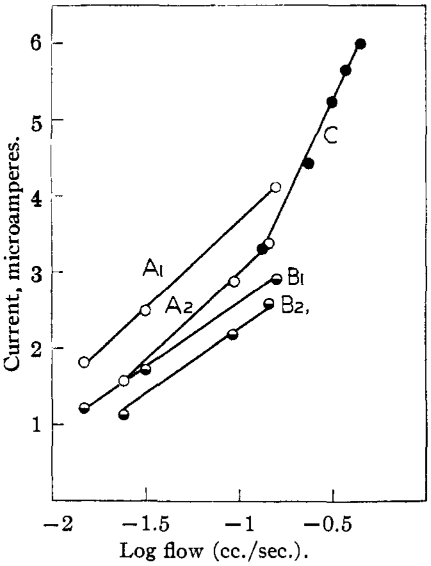

Fig. 10.—Graph showing relationship between limiting currents (corrected) and the logarithm of the rate of flow past the by-pass electrode. A_1A_2 and B_1B_2 refer to the values for quinone and hydroquinone, respectively, in different media and at different concentrations (from Figs. 6 and 7); C refers to the values for oxygen from Fig. 3.

Conclusions

Although the experimental evidence presented here is quite sketchy and covers a wide variety of conditions, it has established certain facts on the basis of which a few conclusions may be drawn about possible applications of this new type of electrode.

First, the foregoing data have demonstrated clearly that it is possible to obtain reproducible results with the by-pass electrode if certain experimental conditions are kept perfectly constant. These conditions have been defined.

The results further indicate that an empirical application of the by-pass electrode can be made to the determination of electroactive constituents in a fluid circulating at a constant rate. In addi-

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[17] I. M. Kolthoff and J. J. Lingane, "Polarography," Interscience Publishers, Inc., New York, N. Y., 1941.

tion, the new electrode should make it possible to determine from the polarographic curve the rate of flow past the electrode if the concentration of the reacting material is known. Both of these methods should prove of considerable value in industry, especially if continuous records are wanted. In this respect the application of the cathode-ray oscillograph[18] should be of advantage.

If the rate of flow and the concentration of the electroactive material are both unknown, an analysis by means of the by-pass electrode would only give the rate at which this material reaches the electrode. This type of analysis is of particular importance in physiological studies where one needs to know the rate at which a given component of the blood reaches the tissues. If the blood normally contained no suitable reactant for this study, one could conceivably introduce a satisfactory, non-toxic substance into the blood stream. Investigations of this nature are planned in this laboratory.

Besides empirical applications of this new technique, some applications in theoretical chemistry suggest themselves. The results obtained so far indicate that in order to have conditions of equilibrium at the by-pass electrode, the state of the electroactive material in solution is of primary importance. Apparently only substances which can exist as free ions in both the oxidized and the reduced states will give rise to a reversible wave. The ferric and ferrous ions in acid solution serve as an example. Additional information obtained with the ferro-ferricyanide system, which will be reported in a subsequent publication, seems to confirm this viewpoint. The results obtained with quinhydrone in strongly acid solutions indicate that the rates at which the hydroquinone ion associates with the hydrogen ions of the solution must be too slow to establish reversible conditions at the by-pass electrode. The additional results with quinhydrone in a buffer suggest that even the dissociation of the latter is too slow to maintain constant the *p*H at the electrode surface.

Thus the by-pass electrode may possibly serve as a tool in the investigation of the reaction rates which are too fast to be measured with conventional techniques.

Summary

A new polarographic technique is described in which a stationary microelectrode sealed into the constricted part of a glass tube is polarized while the solution under analysis is flowing past it.

The results are reproducible as long as the following conditions are kept constant: (a) rate of flow of solution past the electrode, (b) rate and direction of change of the applied voltage, (c) temperature, (d) pretreatment of the electrode, (e) kind and concentration of the supporting electrolyte.

Oxygen is reduced at this electrode and, if its presence interferes with the analysis of other constituents, must be removed from the solution. A set up suitable for this purpose is described.

If both components of an oxidation–reduction system are present as free ions in solution, the electrode reaction is perfectly reversible as indicated by the identity of the half-wave potentials of the corresponding anodic and cathodic waves. This is not the case when an acid–base equilibrium is involved or when McIlvaine's buffer is used to maintain constant the *p*H at the electrode surface. To account for this effect, it is postulated that the rates of dissociation and association of weak acids and bases are not sufficiently fast to maintain equilibrium during the electrode reaction.

Under proper conditions the limiting currents obtained with the by-pass electrode are a linear function of (a) the concentration of the reacting substance and (b) of the logarithm of the rate of flow.

Possible applications of this new technique are pointed out.

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[18] J. Heyrovsky, *Chem. Listy*, **35**, 155 (1941).