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Charge-Step Polarography

SIR: The theory and practice of electroanalysis by the coulometric method in the 10^{-5} – $10^{-7}M$ range have been described in detail (1–7). The potential of an electrode is initially set at the foot of the I – E curve for the substance to be analyzed. A charge is applied to the electrode in a few microseconds or less to bring the potential to a value on the plateau of the I – E curve. The general equation describing the open-circuit potential-time variation after the termination of charge injection for a diffusion limited reaction at a stationary plane electrode is:

$$E = \frac{\pm 2nFC^{\circ}D^{1/2}}{\pi^{1/2}C_d} t^{1/2} = \frac{\lambda C^{\circ}t^{1/2}}{C_d}$$

where C° and D are the bulk concentration (mol/cm³) and diffusion coefficient (cm²/sec) of the electroactive species, C_d is the double-layer capacitance of the electrode, and λ is $\pm 2nFD^{1/2}/\pi^{1/2}$. Hence concentrations of substances involved in the electrode reaction can be determined from the slope of a plot of ΔE vs. $t^{1/2}$. In this method the difficulty of the double layer charging current which prevents the application of ordinary polarography to trace analysis is entirely avoided. Also measurements are made at times during which no appreciable net current passes through the cell so that no corrections for ohmic potentials are needed during the measurement or in interpreting the results. Thus, the experimental difficulties encountered when using the low electrolyte concentrations necessary in nonaqueous solvents and desirable in trace analysis are greatly reduced. Despite these demonstrated advantages of the coulometric method for trace analysis, reported applications of this method have been rare. This is perhaps due to the lack of selectivity of the method, the need to replot real time data on a root-time scale for accurate measurement, and the relative inconvenience of the hanging mercury drop electrode (HMDE) compared to the dropping mercury electrode (DME).

A technique is described in this note which combines the advantages of the coulometric technique with the simplicity of operation and selectivity of dc polarography. A DME is held at a potential where there is essentially zero faradaic current. Late in the life of the drop, a small charge is quickly added to the electrode. Points on the resulting potential-time curve are acquired by a small digital computer which calculates the slope and intercept (E at $t = 0$) for the first portion of the E vs. $t^{1/2}$ curve. The experiment is then repeated on the next drop using a somewhat larger charge. When the charge is sufficient to polarize the electrode at a potential approaching the polarographic $E_{1/2}$ for a substance in solution, the slope will increase. Further increases in charge which bring the electrode potential to the polarographic plateau region of the substance should yield an increased, but potential-independent, slope value. In other words, a plot of the slope vs. intercept values for incrementally increasing charge steps applied to successive drops at a DME should resemble a smoothed dc polarogram and allow both qualitative and quantitative analysis of mixtures of elec-

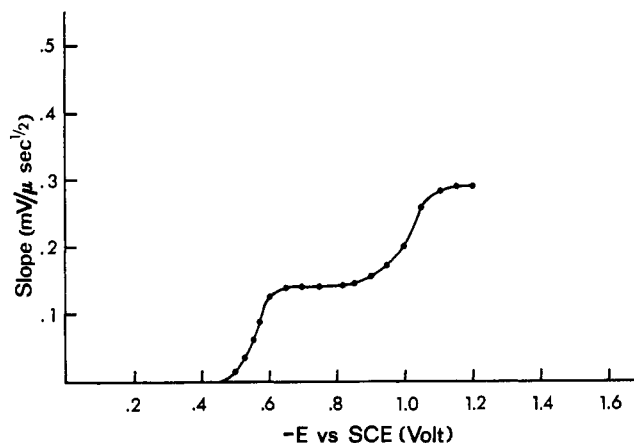


Figure 1. Charge-step polarogram of Cd²⁺ and Zn²⁺, $5 \times 10^{-6}M$ each

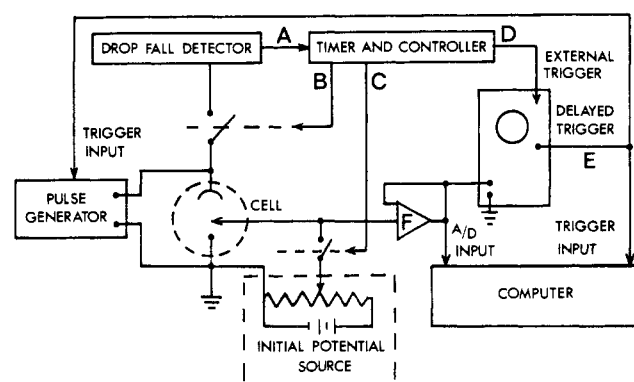


Figure 2. Block diagram of the charge-step polarograph

troactive substances. Such a curve for a mixture of $5 \times 10^{-6}M$ Cd²⁺ and $5 \times 10^{-6}M$ Zn²⁺ is shown in Figure 1. For the reasons discussed above, this method should have significant advantages over other polarographic techniques when using low concentrations of electroactive species in dilute solutions of supporting electrolyte.

The instrumentation used consisted of a Tektronix 535A oscilloscope with a type W plug in, an Intercontinental Instruments Model PG-33 pulse generator, and a PDP8/I computer with an A/D converter and 8K of memory. The logic circuitry was designed using Heath EU801 modules and logic cards. A DME was used with the system rather than a HMDE. To overcome the problem of synchronization of the DME with the experiments, a drop fall detecting circuit was used. To minimize the effects of drop growth, the experiment was initiated late in the life of a drop and data were obtained over a period of time that was short enough to neglect the change in area of the drop. This particular experimental design allowing the use of a DME rather than a HMDE has the advantages of very reproducible electrode areas, ease of automation, and relative freedom from impurity adsorption effects.

A block diagram of the components of the system and their interconnections is shown in Figure 2. The sequence of events will be described beginning with the end of drop life, after the charge step has been added and the potential-time data recorded. At this time in drop life control lines B and C

- (1) P. Delahay, *Anal. Chim. Acta.*, **27**, 90 (1962).
- (2) *Ibid.*, p 400.
- (3) P. Delahay, *ANAL. CHEM.*, **34**, 1267 (1962).
- (4) P. Delahay and Y. Ide, *ibid.*, p 1580.
- (5) P. Delahay, *ibid.*, p 1662.
- (6) A. Aramata and P. Delahay, *ibid.*, **35**, 1117 (1963).
- (7) P. Delahay and Y. Ide, *ibid.*, p 1119.

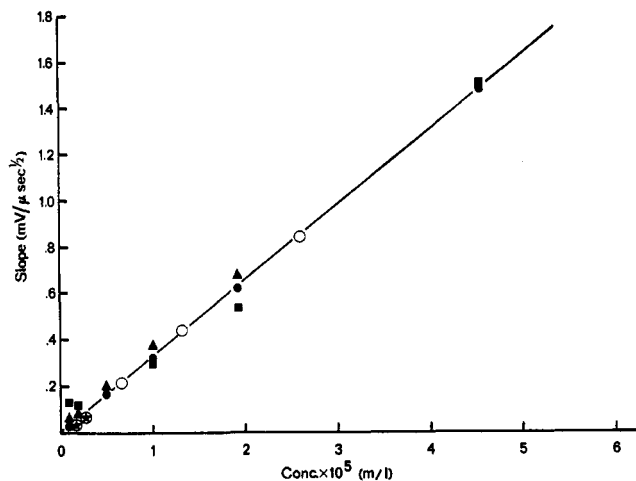


Figure 3. Relationship between reducible ion concentration and measured limiting slope

- Cd^{2+} alone
- Cd^{2+} with Zn^{2+}
- ★ Zn^{2+} alone
- Zn^{2+} with Cd^{2+}
- ▲ Ni^{2+}

cause their respective switches to be closed. Thus the drop-fall detector and the initial potential source are connected to the cell. At the time of drop fall the detector sends a pulse to input *A* of the timer and controller. This resets and starts a precision timer constructed of a 1-MHz crystal oscillator and digital scaler and causes control line *B* to remove the drop fall detector from the cell. While the drop is growing, it is held at the potential set by the initial potential source.

When the timer reaches the desired time in drop life (accurate to $\pm 1 \mu\text{sec}$), control line *C* causes the initial potential source to be disconnected and a pulse at line *D* is generated to trigger the oscilloscope sweep for visual monitoring of the decay curve. After a very short delay to allow the oscilloscope sweep to begin, a pulse is generated at *E*, the delayed trigger output of the oscilloscope. This pulse is used to trigger the pulse generator to add the charge and to trigger the data acquisition by the computer. The rate at which data are acquired is determined by a programmable clock in the computer. Typically a data point was acquired every millisecond, for 50 msec. It was necessary to measure the slope within about 2% of the drop life at the time of the pulse, to avoid distortion of the E vs. $t^{1/2}$ curve due to the increasing capacitance of the DME caused by the continuously increasing area.

Exactly one second after the pulse at *D*, control lines *B* and *C* cause the drop fall detector and the initial potential source to be reconnected to the cell. The measurement cycle is now complete and the sequence can be repeated when the drop fall is detected if desired. Provisions have been made in the computer program to allow ensemble averaging of the relaxation data acquired over a preselected number of runs. After the experimental run or runs have been completed, the data are corrected for the injection time and a least squares analysis is performed on the data to obtain an intercept and a slope proportional to the square root of time.

A series of measurements were made using zinc, cadmium, and nickel ions at various concentrations between $1 \times 10^{-6}M$ and $5 \times 10^{-5}M$ in a supporting electrolyte of $1 \times 10^{-2}M$ KCl, to verify the proportionality between concentration

Table I. A Comparison of Steepness of Charge-Step Polarographic Waves of Several Ions and a Reversible Polarographic Wave

Species	$E_{3/4} - E_{1/4}$, mV
$5 \times 10^{-6}M \text{ Cd}^{2+}$	-60
$5 \times 10^{-6}M \text{ Zn}^{2+}$	-60
$5 \times 10^{-6}M \text{ Ni}^{2+}$	-90
Reversible species in polarography	$-56/n$

and measured slope. The slopes of the various metal ions were obtained in potential regions where the reduction of the particular ion was diffusion controlled and are shown in Figure 3. Also shown in Figure 3 are the results from experiments run on solutions containing a mixture of zinc and cadmium ions in solution. As expected from Equation 1, the measured slope is proportional to the concentration. Because the electrode capacitance and metal ion diffusion coefficients are of nearly the same value, the proportionality constants for each of the metal ions measured are the same within experimental error. Assuming a capacitance of $20 \mu\text{F}/\text{cm}^2$ and a diffusion coefficient of $7.1 \times 10^{-6} \text{ cm}^2/\text{sec}$ the predicted value of λ/C_d is $30 \times 10^3 \text{ V sec}^{-1/2} M^{-1}$ compared with a measured value of $32 \times 10^3 \text{ V sec}^{-1/2} M^{-1}$.

Background corrections, which had been obtained by making measurements on solutions containing only supporting electrolyte, were applied to the slopes obtained before plotting. The background slope corrections, caused primarily by reduction of oxygen not stringently eliminated from the solutions, became comparable with the slopes for the metal ions at concentrations of the order of $10^{-6}M$. With an improved cell and technique, the oxygen interference can probably be reduced (8).

Since one of the advantages expected to be achieved by using this technique was the ability to use low concentrations of supporting electrolyte, a series of experiments was performed to ascertain the effect, if any, of varying the concentration of supporting electrolyte at a constant concentration of electroactive species. For this series of measurements, a cadmium concentration of $1 \times 10^{-5}M$ in KCl electrolytes with concentrations of 1×10^{-1} , 1×10^{-2} , 1×10^{-3} , $1 \times 10^{-4}M$ was used. At these concentrations, the values for the slopes that were obtained on the plateau region agreed to within 3% with each other and to the expected value of the slope calculated from Equation 1. It should be noted that the lowest concentration of supporting electrolyte used, $1 \times 10^{-4}M$, does not represent the lower limit for this technique, but rather indicates the lowest limit that could be reached with the maximum voltage of the pulse generator used.

In all of the above experiments, measurements were made at small enough increments of charge and over an extended enough initial potential range to give detailed information about the shape of the waves obtained. It should be recognized that a linear ΔE vs. $t^{1/2}$ curve is not expected when the rate of discharge of the double layer is partially charge-transfer controlled and/or when the electrode potential change during the measured discharge causes a significant change in the surface concentration of the active substance. Such conditions will exist during the rising portion of a charge-step polarogram, but not on the plateau. Plotting the slope and intercept values of a ΔE vs. $t^{1/2}$ curve fit is justified analytically since quantitative information is obtained only from the

(8) F. Smith and P. Delahay, *J. Electroanal. Chem.*, **10**, 435 (1965).

plateau regions. The shape of the wave affects only the selectivity of the technique. In these measurements, only the first 30 to 50 mV of the decay curve were used in the data analysis in order to improve the sharpness of the half-wave region. The slope or sharpness of the wave was essentially independent of the supporting electrolyte concentration, if anything becoming slightly more sharp at lower supporting electrolyte concentration. The waves obtained for cadmium and zinc were similar in shape (Figure 1) while, as would be expected, nickel which is less reversible than zinc or cadmium gave a more drawn out wave. The sharpness of the waves (a measure of the resolving power of the technique) measured as $E_{3/4} - E_{1/4}$ is compared in Table I for the charge-step polarograms of several ions and a reversible conventional polarographic wave.

The experimental results presented in this note demonstrate the feasibility of this technique for rapid, automated analysis of low concentrations of electroactive species in very dilute concentrations of supporting electrolyte. Further work is under way to improve the instrumentation and more

completely automate the experiment in order to obtain the entire slope *vs.* intercept curves automatically. With these further improvements, more complete measurements can be made to determine the experimental limits for the accuracy, sensitivity, and selectivity of the technique and to determine the lower practical limit for the supporting electrolyte concentration.

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Modified Method for Spectrophotometric Determination of Monoamine Oxidase Activity

SIR: Current work in our laboratory (1, 2) dictated the necessity for an expedient method to assay various biological preparations for monoamine oxidase (MAO) activity. We attempted to adapt the method described by Deitrich and Erwin (3), which monitors the production of *p*-dimethylaminobenzaldehyde using *p*-dimethylaminobenzylamine as a substrate for MAO, to our needs. Many of our preparations contained relatively little MAO activity and a large amount of cellular debris (*i.e.*, rat brain stem homogenates), making it impossible to follow the reaction spectrophotometrically because of settling of the debris in the cuvette. The use of a solubilizer (*i.e.*, Lubrol or Triton) was abandoned for fear of altering the (possible) allosteric organization of the preparation. Therefore, it was decided, after a suitable incubation period, to deproteinize the incubation mixture by acidification and remove the debris by centrifugation. Incubation mixtures consisted of enzyme [0.5 ml of rat brain stem homogenate, 100 mg (wet weight)/ml] and 15 μ moles of *p*-dimethylaminobenzylamine, all in 0.1M potassium phosphate buffer pH 7.5, in a total volume of 1 ml. Samples were incubated for 1 hour at 30 °C, and the reaction was stopped by the addition of 1 ml of 3N HCl. All samples were then centrifuged at 15,000 \times g for 15 minutes. Absorbance was determined using a Zeiss PMQ II spectrophotometer. When the supernatant fluid from the acidified incubation medium was assayed at 355 nm for the product, very little absorbance was observed. However, when the pH of an aliquot of the supernatant fluid was adjusted to pH 7.0, strong absorbance was observed (see Table I). Figure 1 illustrates the absorbance spectrum of both *p*-dimethylaminobenzylamine and *p*-dimethylaminobenzaldehyde at pH 7.6 and in 1N HCl. No anomalous

Table I. Effect of Acid on Absorbance Due to *p*-Dimethylaminobenzaldehyde^a

Absorbance at 355 nm		
Aliquot in 1N HCl		Aliquot at pH 7.0
1. 0.008		0.336
2. 0.006		0.354
3. 0.014		0.368

^a Samples for assay were prepared as described in the text. One ml of the supernatant fluid was taken from each sample and these aliquots were divided into two groups. In the first group, 1 ml of water was added to the sample while the pH of the second group was adjusted to 7.0 by the addition of 0.35 ml of 5N KOH and 0.65 ml 1M potassium phosphate pH 7.0. Controls contained enzyme incubated without substrate.

peaks were detected when the amine and aldehyde were present together at pH values of 7.6, 1.9, and in 1N HCl, thus indicating the absence of interaction, such as Schiff base formation, between the two types of molecules. Figure 2 illustrates the pH dependence of absorbance due to *p*-dimethylaminobenzaldehyde. It can be seen that at pH values below 2.7, the absorbance at 355 nm due to this compound decreases, and another peak at 245 nm becomes prominent. These changes are probably a result of the protonation of the dimethylamine substituent on the benzaldehyde affecting the resonance pattern by inductive effects (4). The pK_a value for this protonation was calculated to be approximately 2.0 by the method described by Kappe and Armstrong (5).

The assay procedure was also utilized for the assay of a partially purified monoamine oxidase preparation. Rat liver mitochondria prepared by the method of Whittaker (6)

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