Pulse Polarographic Determination of Disulfiram

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Abstract □ A pulse polarographic method for the determination of bis(diethylthiocarbamoyl) disulfide (disulfiram) in tablets is described. The pulse polarographic method is more sensitive than d.c. polarography, and the peak-shape readout is inherently easier to identify and quantitate. An aqueous, ethanolic acetate buffer is used rather than the more commonly used aqueous, ethanolic ammonia buffer because interference by diethyldithiocarbamate is eliminated. A mechanism for the electrode process is proposed which involves reaction of the disulfiram with mercury drop to form the insoluble mercuric salt, which then undergoes reduction at the electrode surface.

Keyphrases □ Disulfiram tablets—analysis, pulse polarography using a dropping mercury electrode □ Pulse polarography—analysis, disulfiram tablets

Disulfiram[1], bis(diethylthiocarbamoyl) disulfide (I), has been used for the treatment of alcoholism. It produces a sensitivity to alcohol by interfering with the normal metabolic degradation of alcohol in the body, resulting in an increased acetaldehyde concentration in the blood.

Several methodologies, particularly colorimetry and polarography, have been used to determine disulfiram. Domar *et al.* (1) described a colorimetric method based on chelation with copper. Yoshida *et al.* (2) extended the chelation reaction to a variety of metals and organic solvents with considerable success. Tompsett (3) further modified the Domar *et al.* (1) method to determine I in blood and urine.

Several polarographic methods have been described for the determination of I. Gregg and Tyler (4) and Belilskaya (5) described the conventional d.c. polarographic behavior of I. Taylor (6) described an analytical method for I in synthetic rubber based on cathode-ray polarography. Brand and Fleet (7) found that the dimethyl analog of I could be determined by a.c. polarography at levels as low as 10^{-6} *M*.

This report describes the application of pulse polarography to the determination of I and diethyldithiocarbamate (II). Both the dropping mercury electrode (DME) and the rotating glassy graphite electrode (RGE) are used. An analytical method for I in tablets is presented based on derivative pulse polarography and the DME. The use of an acetate buffer in 50% (v/v) aqueous ethanol rather than the ammonia-buffered aqueous ethanol previously used (4–7) provides a considerable improvement in specificity.

[1] Antabuse, Ayerst Laboratories.

EXPERIMENTAL

Apparatus[2]—Both the RGE and DME were used. The glassy graphite rod was purchased[3], and the electrode was constructed according to the procedure described by Plock (8). A synchronous rotator[4] was used to rotate the electrode at a constant 600 r.p.m. The DME had the following characteristics (open circuit) in acetate and ammonia buffers: $m = 5.78 \times 10^{-4}$ g. sec.$^{-1}$ and $m = 8.53 \times 10^{-4}$ g. sec.$^{-1}$, respectively. A saturated calomel and a platinum wire were used as the reference electrode and auxiliary electrode, respectively.

Stock solutions of I and II were prepared in ethanol at a level of 0.2 mg./ml. and diluted with the appropriate alcoholic buffer to the desired concentrations. All solutions were deaerated with commercial grade nitrogen which was presaturated with solvent. The solutions were prepared fresh daily.

Reagents—Alcohol USP was used for triturating the tablets and as solvent for the supporting electrolyte. The following were also used: glacial acetic acid[5] meeting USP specifications; sodium acetate[6]; ammonium hydroxide[5] meeting ACS specifications, assay 58% NH_4OH; ammonium chloride[5] meeting ACS specifications; diethyldithiocarbamic acid, sodium salt[7]; and disulfiram, house reference standard, assay 99.8%.

The acetate buffer consisted of 0.5 *N* NaOAc and 0.5 *N* HOAc, pH 4.5. The ammonia buffer consisted of 2 *N* NH_4OH and 0.2 *N* NH_4Cl, pH 10.5. Both buffers gave no electrochemical response in the potential range of interest. The supporting electrolyte solution was prepared by mixing an equal volume of alcohol USP with each buffer. The solutions were degassed with nitrogen prior to use. A liter of this solution required 1 hr. of rapid bubbling to eliminate oxygen interference.

Analytical—*Standard Preparation*—An amount of I weighing 20 mg. was dissolved in 100 ml. of alcohol USP and diluted to volume. Four, six, and eight milliliters of this solution were pipeted into separate 100-ml. volumetric flasks and diluted to volume with the supporting electrolyte solutions.

Spiked Placebo Preparation—Spiked placebos were prepared by adding about 40 mg. of I (accurately weighed) to an appropriate amount of the placebo so that the ratio of I to excipients was the same as that found in the tablet formulation.

Sample Preparation—The average single tablet weight of 10 tablets was determined and recorded. The tablets were ground, and an amount of tablet powder equivalent to 40 mg. of I was weighed and transferred with the aid of about 100 ml. of ethanol to a 200-ml. volumetric flask. The flask was shaken for 30 min. and diluted to volume with ethanol. A 6.0-ml. aliquot was pipeted into a 100-ml. volumetric flask and diluted to volume with the supporting electrolyte.

An aliquot of the standard or sample solution was transferred to the polarographic cell. The solution was degassed for 1 min. before immersing the DME and for 1 min. more after immersing the DME and other electrodes.

[2] Polarograms were obtained using a Princeton Applied Research polarographic analyzer and recorder, model 171, equipped with a mechanical drop knocker, model 172.
[3] Tokai Electrode Mfg. Co., Ltd.
[4] Sargent-Welch, catalog number S-76485.
[5] Mallinckrodt.
[6] Fisher certified.
[7] Eastman Organic Chemicals.

Table I—Pulse Polarographic Determination of I in Spiked Formulations

Spiked Placebo	Input, mg.	Found, mg.	Percent Recovery
1	519.1	514.9	99.2
2	537.2	544.2	101.3
3	496.9	500.2	100.7
4	511.6	511.6	100.0
5	541.8	547.1	100.9

The following settings were made on the polarograph: scan rate, 2 mv./sec.; scan range, 3.0 v.; starting potential, −0.3 v.; drop time, 2 sec.; current range, 10–20 μamp.; pulse amplitude, 100 mv.; operating mode, derivative pulse; and current readout, differential.

Scans were started at −0.3 v. and continued to −0.8 v. A peak was observed with a maximum at −0.55 v. *versus* saturated calomel electrode (SCE). The heights of the peaks were measured, and a calibration curve was constructed with peak height as the abscissa and weight of I as the ordinate.

The sample potency was calculated as follows:

$$\text{mg./tablet} = (A)(33.3) \times \text{(average tablet weight/sample weight)} \quad \text{(Eq. 1)}$$

where A = total number of milligrams of I in the supporting electrolyte solution as read from the calibration curve corresponding to the measured peak height.

RESULTS AND DISCUSSION

Recoveries from spiked placebos are listed in Table I. The mean recovery is 100.4% at levels of 500 mg./tablet. The standard deviation and coefficient of variation expressed as percentages are both 0.9. The data were collected over a period of 2 months. A calibration curve (see *Experimental*) was constructed for each determination. A placebo with no added I was also analyzed. No interferences were observed in the potential range of interest.

No special precautions were taken to control the cell's temperature since a temperature study indicated that the peak height did not vary significantly over a range of 20–30°.

The data presented in Table II illustrate that the correlation between the iodimetric and polarographic procedures is quite good.

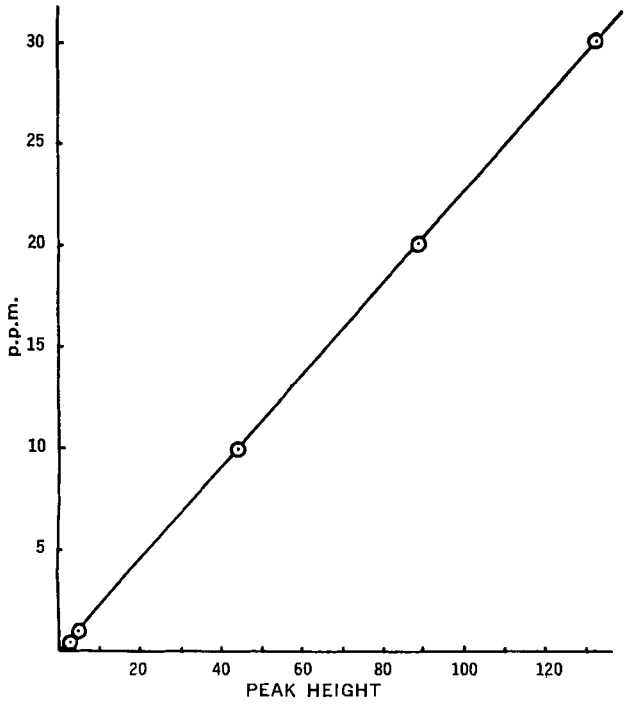


Figure 1—*Concentration* versus *peak height. Compound I in acetate–ethanol buffer.*

Table II—Comparison of the Iodimetric and Pulse Polarographic Methods for Determining I in Tablets

Sample	Iodimetric, mg./Tablet	Polarographic, mg./Tablet
1	496	496
2	494	493
3	254	264
4	250	264
5	502	494
6	499	494

A rectilinear relationship exists between peak height and concentration over a range of 0.5–30 p.p.m. (Fig. 1). These concentrations are below the sensitivity range of conventional d.c. polarography; therefore, the derivative pulse mode with differential readout was used. This afforded about a 100-fold increase in sensitivity. At concentrations greater than 30 p.p.m., a second wave appears at a slightly more positive potential. This observation concurs with conclusions previously reached by Gregg and Tyler (4), who studied electrocapillary curves which clearly indicated adsorption. Brand and Fleet (7) also studied the adsorption phenomenon using cyclic voltammetry and a.c. polarography; in general, they agreed that the more negative wave is adsorption controlled and the more positive is diffusion controlled. A more detailed treatment of the adsorption process will be the subject of a future report.

The superiority of the acetate–ethanol system over the ammonia–ethanol system is illustrated in Fig. 2. Whereas II (which is a conceivable impurity since it is the starting material for the preparation of I) is electroactive at the same potential as I in the basic medium (Fig. 2*b*), it gives no response at all in the acidic system (Fig. 2*a*). In acid the carbamate immediately decomposes to carbon disulfide and diethylamine (9).

Although Gregg and Tyler (4) found that the polarographic behavior of I and II in basic medium fulfilled the criteria of Nernstian reversibility, they failed to recognize that the anodic wave was the result of the catalytic oxidation of mercury to form the mercury complex (9).

Polarograms obtained for I in ammonia buffer (Fig. 3) with the RGE show a reduction halfwave potential of −1.20 v. *versus* Ag/AgCl (or −1.24 v. *versus* SCE). This reduction potential is 0.74 v.

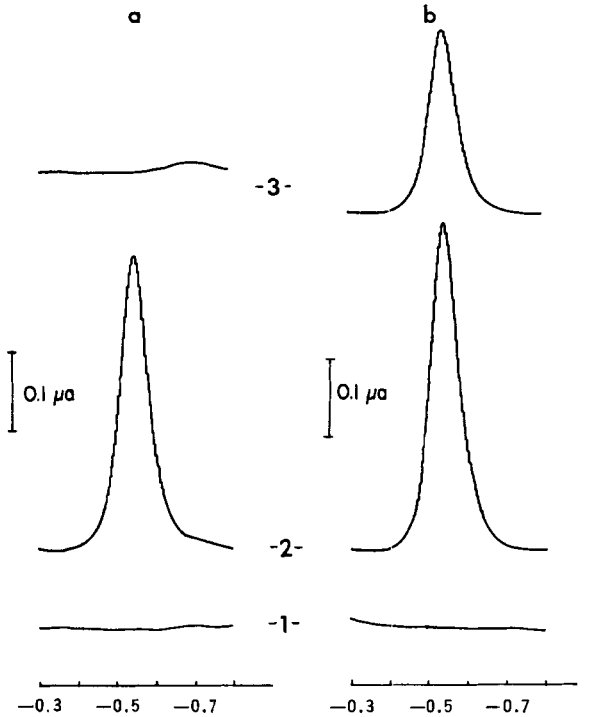


Figure 2—*Polarograms of I and carbamate anion II in acetate and ammonia buffers. Key: a, acetate-ethanol buffer; b, ammonia-ethanol buffer; 1, blank; 2, I in corresponding buffer (10 mcg./ml.); and 3, carbamate anion II in corresponding buffer (10 mcg./ml.).*

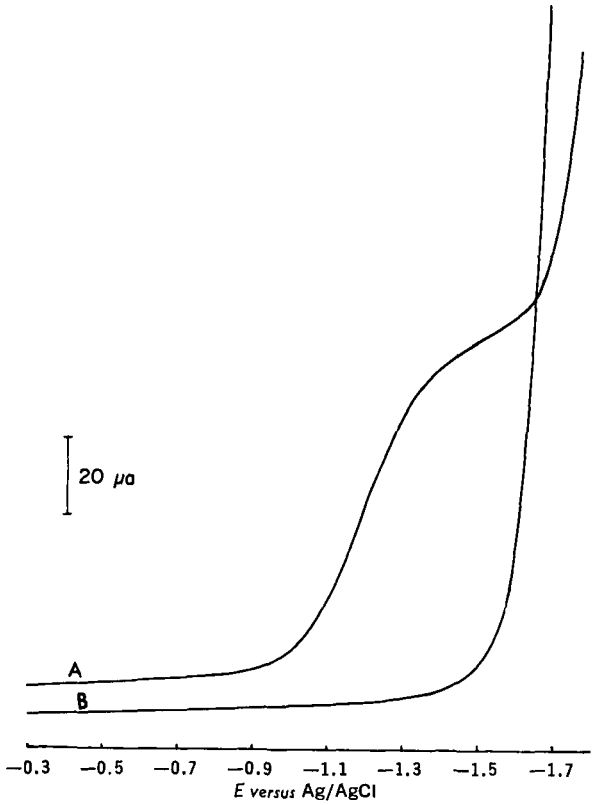


Figure 3—*D.C. polarogram of I in ammonia buffer obtained with the RGE. Key: A, I (300 mcg./ml.); and B, blank.*

more cathodic than the reduction potential observed in the same medium with the DME. In addition, it has long been accepted that the adsorption wave appears second when the reactant is adsorbed on the mercury drop (4), a fact established for disulfiram (4, 7) and observed in this laboratory. Both of these observations suggest a mechanism which involves chemical interaction of I with the mercury prior to reduction.

In acetate buffer the reduction occurs too close to the background discharge to be clearly discernible when the RGE is used.

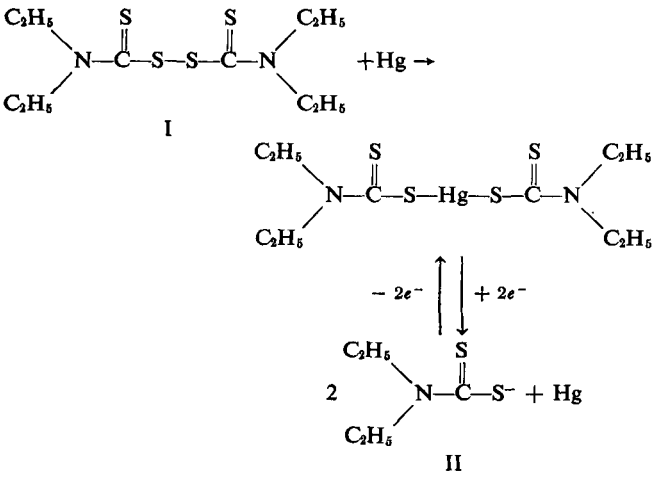
Previous authors (9) also established that the anodic wave observed with II is not due to the oxidation of the substance to the corresponding disulfide but rather to the formation of a mercury complex with concomitant oxidation of mercury.

The available evidence, therefore, is consistent with the mechanisms shown in Scheme I.

Scheme I not only accounts for the reversible electrochemical behavior (4) but is consistent with the experience of a number of workers with other disulfide compounds such as 8,8′-diquinolyl-disulfide (10) and cystine–cysteine systems (11).

CONCLUSIONS

The preliminary precision and accuracy data and the enhancement of specificity using pulse polarography indicate that it is the most suitable technique for determining I in the presence of diethyldithiocarbamate when an acidic buffer is used. Linearity of the adsorption wave may be obtained when the concentration of I is in the range of 0.5–30 p.p.m. Results obtained using the RGE indicate that the nature of the reduction at the DME involves a chemical reaction with the mercury surface.



Scheme I

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