The method reported here is simple and reliable and could be easily modified for studying all gelatin capsule dosage forms. The diffusivities and activation energies calculated from the data obtained by this method are consistent with those obtained from diffusion studies of similar systems (6, 7). The activation energies calculated for the five water-soluble dyes studied fall into two distinct groups (Table II). The first group includes Yellow No. 5, Yellow No. 6, and Red No. 4, which have similar activation energies ranging from 25.1 to 25.7 kcal/mole. The energies for these three dyes were found to be statistically indistinguishable by testing the homogeneity of the regression coefficients for their Arrhenius slopes. The second group includes Red No. 2 and Green No. 3 which have activation energies of 34.8 and 36.0 kcal/mole, respectively. The activation energies for these two dyes were also statistically indistinguishable.

The dyes in the first group, with lower activation energies, contain two sulfonate groups per molecule; those in the second group, with higher activation energies, contain three sulfonate groups per molecule. It will be necessary, however, to conduct various additional studies on a larger number of dyes before attempting to establish a relationship between the activation energies of these compounds and their structural variations.

## REFERENCES


(1) W. H. Hansen, A. A. Nelson, and O. G. Fitzhugh, *Toxicol. Appl. Pharmacol.*, **5**, 16(1965).

(2) S. E. Sheppard, R. C. Houck, and C. Dittman, *J. Phys. Chem.*, **46**, 158(1942).

(3) T. Vickerstaff, "The Physical Chemistry of Dyeing," 2nd ed., Interscience, New York, N.Y., 1954, pp. 136, 137.

(4) A. J. Kapadia, W. L. Guess, and J. Autian, *J. Pharm. Sci.*, **53**, 720(1964).

(5) W. Jost, "Diffusion in Solids, Liquids, Gases," Academic, New York, N.Y., 1952, p. 22.

(6) T. Vickerstaff, "The Physical Chemistry of Dyeing," 2nd ed., Interscience, New York, N.Y., 1954, pp. 160, 161.

(7) D. Powell, J. Nematollahi, W. L. Guess, and J. Autian, *J. Pharm. Sci.*, **58**, 843(1969).


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# Differential Pulse Polarographic Analysis of Everninomicin Complex in Fermentation Broth


**PETER KABASAKALIAN[x], SAMI KALLINEY, and ANITA WESTCOTT**



**Abstract** □ A direct, sensitive, and fast (15 min) method for following the laboratory fermentation course of everninomicins is described. It replaces the 20-hr elapsed-time agar-diffusion bioassay method.



**Keyphrases** □ Everninomicin complex—differential pulse polarographic analysis in fermentation broth □ Polarography, differential pulse—analysis, everninomicin complex in fermentation broth □ Fermentation broths—differential pulse polarographic analysis of everninomicin complex


The production of everninomicin complexes by fermentation was described previously (1, 2). To optimize the yield, a fast, reliable analytical method should be used to follow the course of fermentation. Since the components of an everninomicin complex (*e.g.*, B, C, and D) possess an electroactive group, the nitro group (3), they are amenable to polarographic analysis (4). A more recent advance, differential pulse polarography, would be ideally suited for this purpose due to its sensitivity (5) and larger linear range.

## EXPERIMENTAL

The broth (5.00 ml) was transferred to a 50-ml volumetric flask and diluted to volume with acetonitrile. The suspension was mixed well and left for a few minutes to allow the insoluble matter to settle, thus permitting the antibiotic to be separated from the mycelium. The supernate (5.00 ml) was mixed with an equal volume of 0.01 *M* phosphate buffer of pH 7.0. This solution was placed in the sample compartment of a small polarographic H-cell along with the dropping mercury electrode; in the other compartment was a normal calomel electrode, used both as a reference and as a counter-electrode. The cell compartments were separated by an agar plug and a fritted-glass diaphragm. The chloride ion in both the calomel cell and the agar plug was furnished by 1 *N* tetramethylammonium chloride[1]. Both compartments of the H-cell were sealed with rubber stoppers. A small annulus was made around the dropping mercury electrode to allow the capillary to move to dislodge the mercury drops and to permit the escape of nitrogen.

The solution to be analyzed was deoxygenated using nitrogen. Before being passed through the cell, the nitrogen was passed through a gas washing bottle containing a solvent similar to that of the solution. While polarograms[2] were being run, nitrogen was passed over the solution.

A drop time of 1 sec, a scan rate of 2 mv/sec, and a modulation amplitude of 100 mv were kept constant throughout the analysis. Potentials were scanned from −0.6 to −1.4 v (normal calomel electrode). The current scale was 2 μamp.

## RESULTS AND DISCUSSION

A typical differential pulse polarogram of the broth yielded peaks at a potential corresponding to the reduction of everninomicin D. No other peak was observed in the vicinity (Fig. 1). The peak currents of the broth samples as a function of the fermentation time are shown in Fig. 2. These results are in good agreement with the everninomicin production curve shown in Fig. 4 of Ref. 2, which was determined by the bioassay method.

This assay lacks specificity since it is essentially a functional group assay responding to the nitro group present in everninomicin complex. This lack of specificity is also present in the bioas-

[1] Southwestern Analytical Chemicals, Austin, TX 78767

[2] A Princeton Applied Research model 170 electrochemistry system in conjunction with a model 172A drop timer (Princeton Applied Research Corp., Princeton, NJ 08540) was used to obtain the differential pulse polarograms.

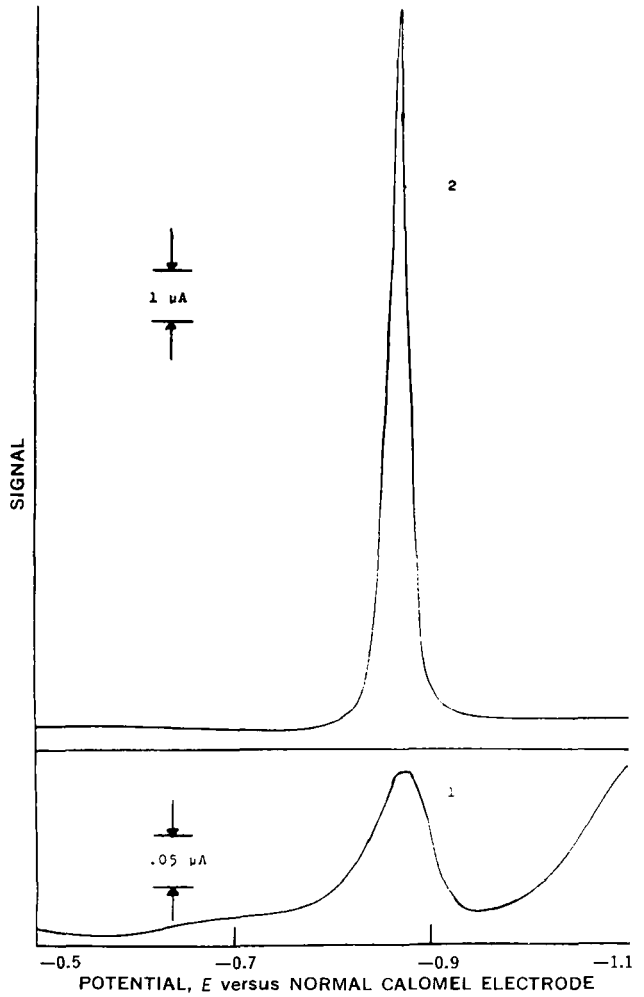


**Figure 1**—*Differential pulse polarograms of: (1) a 140-hr fermented broth sample, and (2) everninomicin D standard (0.099 mg/ml). The following conditions were employed: acetonitrile–phosphate buffer solution (0.005 M, pH 7.0); drop time, 1 sec; modulation amplitude, 100 mv; and scan rate, 2 mv/sec.*

say procedure (2), which cannot differentiate the various everninomicins. However, the linear relationship between the polarographic current and everninomicin concentration makes the polarographic method a more precise one. The polarographic method is the most efficient way to follow the fermentation. However, a bioassay of the final product is still essential to assure the potency of the product.

The polarographic current determined can be converted into equivalent units of everninomicin D (the predominant everninomicin) bioactivity per milliliter by running an assay with the appropriate concentration of everninomicin D in the unfermented broth. With the experimental conditions given, 1.00 μamp of current represents 150 units of everninomicin D per milliliter. The

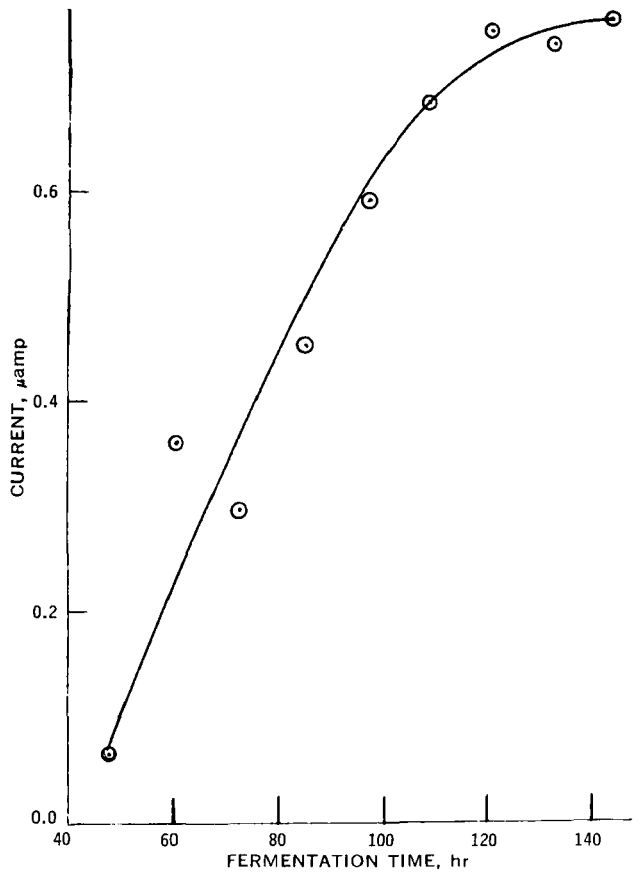


**Figure 2**—*Differential pulse polarogram of the fermentation of everninomicin. The following conditions were employed: acetonitrile–phosphate buffer solution (0.005 M, pH 7.0); drop time, 1 sec; modulation amplitude, 100 mv; scan rate, 2 mv/sec, and* $E_{peak} = -0.85$ *v (normal calomel electrode).*

everninomicin D unit here is the same as that given by Wagman *et al.* (2).

## REFERENCES

(1) M. J. Weinstein, G. M. Luedemann, E. M. Oden, and G. H. Wagman, *Antimicrob. Ag. Chemother.*, **1964**, 24.

(2) G. H. Wagman, G. M. Luedemann, and M. J. Weinstein, *ibid.*, **1964**, 33.

(3) A. K. Ganguly, O. Z. Sarre, and H. Reimann, *J. Amer. Chem. Soc.*, **90**, 7129(1968).

(4) I. M. Kolthoff and J. J. Lingane, "Polarography," vol. II, 2nd ed., Interscience, New York, N.Y., p. 746.

(5) J. B. Flato, *Anal. Chem.*, **44**, 75A(1972).

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