Polarographic Method of Analysis for Pharmaceuticals

By A. FRANCIS SUMMA

Polarographic methods for the determination of acetazolamide, chlorothiazide, and nitrofurantoin, and their application to pharmaceutical dosage forms, are described. These methods are satisfactory for the assay of pharmaceutical preparations official in U.S.P. XVI. The polarographic methods have the desired accuracy and precision for routine control procedures.

SINCE the introduction of polarography by Heyrovsky in 1920, there have been a number of reports in the literature concerning its application to pharmaceutical dosage forms (1–5). As with other new analytical techniques, there has been a tendency among some of the more enthusiastic supporters to be uncritical of polarographic methods and to use a polarographic procedure whether or not some other more satisfactory method existed. Some of the polarographic analyses suggested in the literature are quite complex and do not compare favorably with simpler colorimetric, volumetric, and spectrophotometric methods available. A polarographic approach should be considered whenever new and more accurate methods of analysis for pharmaceuticals are being sought. For many pharmaceutical analytical problems, polarography may well offer a method that has advantages over all other techniques.

There are some official pharmaceutical preparations for which the present method of analysis does not provide for the separation of excipients prior to analysis. In some cases, the compound cannot be separated from its excipients by partition between immiscible solvents and there is no other convenient physical or chemical means of separation. These excipients may interfere with a particular assay and lead to erroneous results. This is especially a danger in an ultraviolet spectrophotometric assay. In some cases, excipients will not interfere with the polarographic analysis, for polarography will produce a wave which is specific for a given compound with an electroreducible or oxidizable group. Acetazolamide, chlorothiazide, and nitrofurantoin were chosen as examples.

EXPERIMENTAL

Apparatus

The polarographic waves were obtained with the Sargent model XXI recording polarograph. The cells used were either large (20-ml. sample volume) or small (3-ml. sample volume) H-type cells containing a saturated calomel electrode (S.C.E.) separated from the sample compartment by an agar plug and fritted-glass diaphragm. The electrode capillary delivered 1.136 mg. of mercury per second at a column height of 45 cm. with a drop-time of 4.20 seconds.

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Acetazolamide Tablets

Acetazolamide produces a polarographic wave having a half-wave potential ($E_{1/2}$) of -0.518 volts in 0.1 N hydrochloric acid solution *vs.* S.C.E. The diffusion current was found to be linear with concentration in the range between 8×10^{-5} M and 4×10^{-4} M.

Procedure.—Weigh and finely powder not less than 20 acetazolamide tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg. of acetazolamide, transfer to a 100-ml. volumetric flask, and dissolve in about 40 ml. of boiling water. Heat the mixture on a steam bath for 15 minutes, cool to room temperature, add water to volume, and mix. Filter a portion of this solution, discarding the first 10 ml. of filtrate, and then pipet a 3-ml. aliquot of the filtrate, accurately measured, to a 25-ml. volumetric flask, add 2 ml. of 1 N hydrochloric acid, dilute to volume with water, and mix. Transfer a portion of this solution to an electrolytic cell, place in a constant temperature bath regulated at 25.0 ± 0.5° and remove the dissolved oxygen by aerating the solution with nitrogen for approximately 10 minutes. Insert the electrolytic cell under the capillary tip of the dropping mercury electrode assembly having a drop time of 4 to 5 seconds. Trace the polarogram from -0.20 to -0.75 volt vs. S.C.E. at a sensitivity which will give a suitable curve. Extrapolate the residual curve and draw parallel to it a line through the diffusion current plateau. Measure the height of the diffusion wave as the difference between the residual current and the diffusion current plateau. Calculate the quantity in mg. of $C_4H_6N_4O_3S_2$ in the portion of the tablets taken by the formula $C\,(I_{d\,\text{sample}}/I_{d\,\text{std.}})$ in which $I_{d\,\text{sample}}$ is the diffusion current value of the sample, C is the concentration of the standard solution, and $I_{d\,\text{std.}}$ is the diffusion current value of U.S.P. Acetazolamide Reference Standard determined similarly in a solution of the same composition containing about 60 mcg. in each ml.

Chlorothiazide Tablets

Chlorothiazide produces a polarographic wave having a half-wave potential ($E_{1/2}$) of -1.528 volts in an equimolar solution of 1 N ammonium chloride solution and 1 N ammonium hydroxide solution *vs.* S.C.E. The diffusion current was found to be linear

with concentration in the range between 1.2×10^{-4} M and 6×10^{-4} M.

Procedure.—Weigh and finely powder not less than 20 chlorothiazide tablets. Weigh accurately a portion of the powder equivalent to 100 mg. of chlorothiazide and transfer to a 100-ml. volumetric flask. Add 20 ml. of dimethylformamide, agitate for 5 minutes, and make to volume with an equimolar solution of 1 N ammonium chloride solution and 1 N ammonium hydroxide solution. Pipet a 3-ml. aliquot of this solution, measured accurately, to a 25-ml. volumetric flask and make to volume with distilled water. Transfer a portion of this solution to an electrolytic cell, place in a constant temperature bath regulated at 25.0 ± 0.5°, and remove the dissolved oxygen by aerating the solution with nitrogen for approximately 10 minutes. Insert the electrolytic cell under the capillary tip of the dropping mercury electrode assembly having a drop time of 4 to 5 seconds. Trace the polarogram from −1.25 to −1.75 volts *vs.* S.C.E. at a sensitivity which will give a suitable curve. Extrapolate the residual current curve and draw parallel to it a line through the diffusion current plateau. Measure the height of the diffusion wave as the difference between the residual current and the diffusion current plateau. Calculate the quantity, in mg., of $C_7H_6ClN_3O_4S_2$ in the portion of the tablets taken by the formula $C\,(I_{d\ \text{sample}}/I_{d\ \text{std.}})$, in which $I_{d\ \text{sample}}$ is the diffusion current value of the sample, C is the concentration of the standard solution, and $I_{d\ \text{std.}}$ is the diffusion current value of U.S.P. Chlorothiazide Reference Standard determined similarly in a solution of the same composition containing about 125 mcg. in each ml.

Nitrofurantoin Oral Suspension

Nitrofurantoin produces a polarographic wave having a half-wave potential ($E_{1/2}$) of −0.384 volt in an equimolar solution of 1 N ammonium chloride solution and 1 N ammonium hydroxide solution *vs.* S.C.E. The diffusion current was found to be linear with concentration in the range between 8×10^{-5} M and 4.8×10^{-4} M.

Procedure.—Pipet accurately an amount of nitrofurantoin oral suspension equivalent to approximately 50 mg. of nitrofurantoin into a 100-ml. volumetric flask. Add 20 ml. of dimethylformamide, agitate for 5 minutes, and make to volume with an equimolar solution of 1 N ammonium chloride solution and 1 N ammonium hydroxide solution. Pipet a 3-ml. aliquot of this solution, accurately measured, into a 25-ml. volumetric flask. Add 0.10 ml. of a 0.1% gelatin solution and make to volume with distilled water. Transfer a portion of this solution to an electrolytic cell, place in a constant temperature bath regulated at 25.0 ± 0.5°, and remove the dissolved oxygen by aerating the solution with nitrogen for approximately 10 minutes. Insert the electrolytic cell under the capillary tip of the dropping mercury electrode assembly having a drop time of 4–5 seconds. Trace the polarogram from −0.20 to −0.70 volt *vs.* S.C.E. at a sensitivity which will give a suitable curve. Extrapolate the residual current curve and draw a line parallel to it through the diffusion current plateau. Measure the height of the diffusion wave as the difference between the residual current and the diffusion current plateau. Calculate the quantity, in mg., of $C_8H_6N_4O_5$ in the portion of the suspension taken by the formula $C\,(I_{d\ \text{sample}}/I_{d\ \text{std.}})$, in which $I_{d\ \text{sample}}$ is the diffusion current value of the sample, C is the concentration of the standard solution, and $I_{d\ \text{std.}}$ is the diffusion current value of U.S.P. Nitrofurantoin Reference Standard determined similarly in a solution of the same composition containing about 60 mcg. in each ml.

Nitrofurantoin Tablets

Procedure.—Weigh and finely powder not less than 20 nitrofurantoin tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg. of nitrofurantoin, and transfer to a 100-ml. volumetric flask. Proceed as directed in the assay for nitrofurantoin oral suspension, beginning with "add 20 ml. of dimethylformamide." Calculate the quantity in mg. of $C_8H_6N_4O_5$ in the portion of the tablets taken by the formula $C\,(I_{d\ \text{sample}}/I_{d\ \text{std.}})$, in which $I_{d\ \text{sample}}$ is the diffusion current value of the sample, C is the concentration of the standard solution, and $I_{d\ \text{std.}}$ is the diffusion current value of U.S.P. Nitrofurantoin Reference Standard determined similarly in a solution of the same composition containing about 60 mcg. in each ml.

DISCUSSION

The intersection-point method of measuring the diffusion current, as shown in Figs. 1, 2, and 3, was used for all polarographic waves. When the diffusion current so measured was plotted against concentration, a straight line was obtained (Fig. 4). Each point on the graph represents an average of three determinations. The experimental error by this method of analysis was found to be ±1%. It was felt that the greatest source of error in the analyses resulted from the measurement of the diffusion current height from the polarographic wave.

The results obtained by both polarography and U.S.P. XVI methods of analysis for each dosage form are listed in Table I. The table shows that

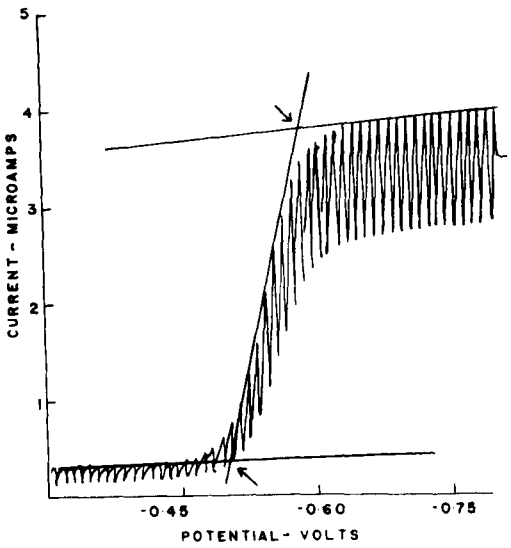

Fig. 1.—Acetazolamide polarogram in 0.1 N hydrochloric acid solution.

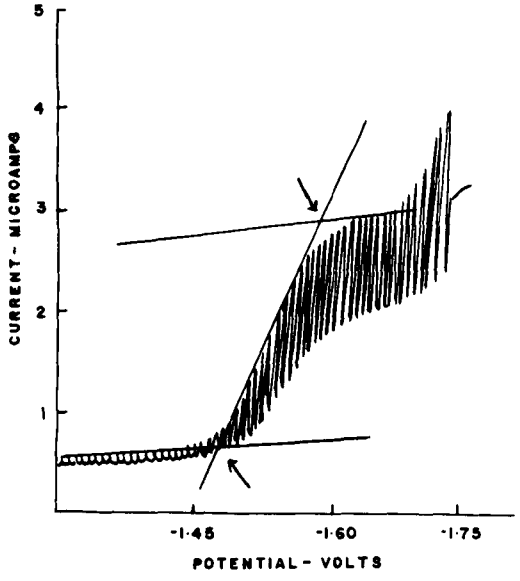

Fig. 2.—Chlorothiazide polarogram in equimolar ammonium chloride-ammonium hydroxide solution.

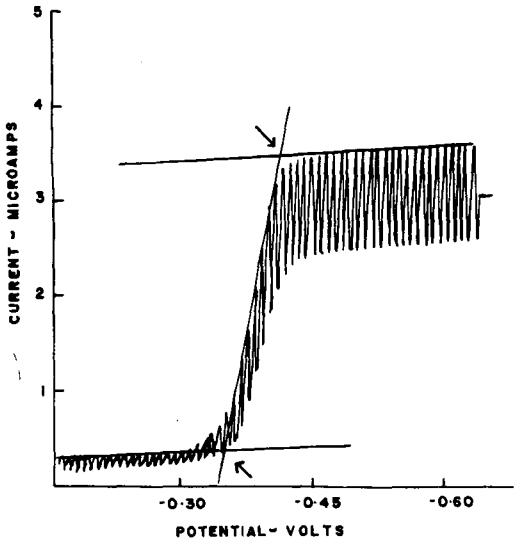

Fig. 3.—Nitrofurantoin polarogram in equimolar ammonium chloride-ammonium hydroxide solution.

the methods are in good agreement and that the results obtained by the polarographic method show greater precision than those obtained by the U.S.P. XVI method. In all cases, excipient material present in the commercially available preparations did not interfere with the polarographic and U.S.P. analysis and, therefore, no attempt was made to separate them prior to analysis. The average volume occupied by the undissolved tablet excipients was approximately 0.05 ml. so that the error introduced by leaving the tablet excipient in the solution is less

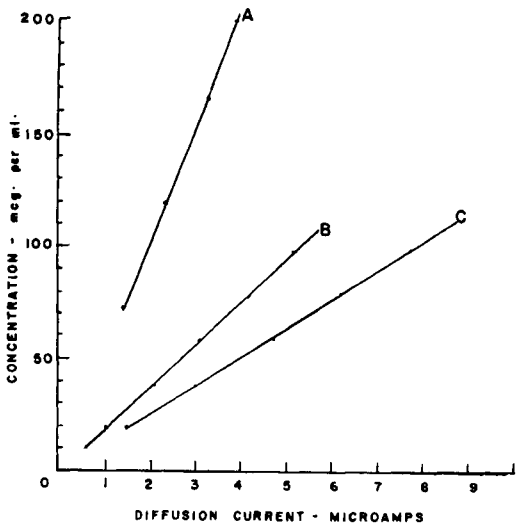

Fig. 4.—Plot of diffusion current *vs.* concentration for chlorothiazide (A), acetazolamide (B), and nitrofurantoin (C) solutions.

TABLE I.—RESULTS OF POLAROGRAPHIC AND U.S.P. XVI ANALYSES

Sample	% of Declared	
	Polarography	U.S.P. XVI
Acetazolamide tablets, 250 mg.	96.8	96.5
	96.8	97.2
	96.9	97.0
Chlorothiazide tablets, 25 mg.	102.4	101.3
	103.1	102.1
	103.3	...
	103.0	...
Nitrofurantoin tablets, 25 mg.	96.8	96.9
	96.6	96.1
	96.2	97.1
	95.7	96.3
Nitrofurantoin oral suspension	95.9	98.0
	96.3	97.4
	...	96.9[a]
	...	97.1[a]
Simulated nitrofurantoin oral suspension	99.2	99.4[a]
	99.2	99.7[a]

[a] U.S.P. XVI method modified by centrifuging sample after filtration.

than 0.2%, considerably less than the errors involved in measuring the diffusion current.

No difficulties were encountered with the polarographic analysis for acetazolamide, chlorothiazide, or nitrofurantoin in tablets and, therefore, outside of the development of a quantitative procedure no further investigation was undertaken. However, it was found that with the U.S.P. XVI spectrophotometric assay for nitrofurantoin oral suspension, a strong background was produced. This background can be attributed to the colloidal suspension produced by the Veegum present in the formulation which is not eliminated by simple filtration. The results obtained by the U.S.P. XVI method (Table I) for nitrofurantoin oral suspension are somewhat higher than those obtained by polarography. Modification of the U.S.P. XVI method by centrifuging the sample after filtration produced results which agreed more closely.

To determine whether the U.S.P. XVI or the polarographic method was at fault, a simulated nitrofurantoin oral suspension was prepared. Again, as shown in Table I, the polarographic and the modified U.S.P. XVI methods are in good agreement. It can be concluded that the spectrophotometric method is more susceptible to interference from extraneous materials than the polarographic method.

SUMMARY

Samples of acetazolamide, chlorothiazide, and nitrofurantoin were reduced at the dropping-mercury electrode. Their diffusion currents were found to be linear with concentration in the ranges investigated and therefore can be used for a quantitative polarographic assay.

A 0.1 *N* hydrochloric acid solution was found suitable for the polarographic determination of acetazolamide. A solution of dimethylformamide, 1 *N* ammonium chloride solution, and 1 *N* ammonium hydroxide solution were found suitable for the polarographic determination of both chlorothiazide and nitrofurantoin.

Similar experimental conditions were maintained throughout each assay to keep the variables at a minimum. The temperature was held within $\pm 0.5°$ by a constant temperature bath.

Polarographic analyses of dosage forms were compared with U.S.P. XVI analyses and proved to be quite satisfactory.

REFERENCES

(1) Brezina, M., and Zuman, P., "Polarography in Medicine, Biochemistry, and Pharmacy," Interscience Publishers, Inc., New York, N. Y., 1958.
(2) Heyrovsky, J., *Cusopis Ceskoslov. Lekarnictra*, **14**, 285 (1934).
(3) Marsh, M. M., and Hilty, W. W., *Anal. Chem.*, **24**, 271 (1952).
(4) Mashiko, Y., *J. Pharm. Soc. Japan*, **72**, 18(1952).
(5) Matsumoto, K., *Study of Polarography*, **2**, 10(1952).

Metal Chelates of Oxazolidinones as Central Nervous System Stimulants

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In an attempt to determine the effect of chelation upon central nervous system activity, a series of metal chelates have been prepared of 2-imino-5-phenyl-4-oxazolidinone, 2-imino-5,5-diphenyl-4-oxazolidinone, and 2-imino-5-*p*-biphenyl-4-oxazolidinone, using Cu^{++}, Ni^{++}, Mg^{++}, and Fe^{+++} ions. Spectral and analytical evidence has indicated a 1:1 ratio of metal to oxazolidinone in the respective chelates of all four metals. The magnesium chelate of 2-imino-5-phenyl-4-oxazolidinone exhibited the selective central nervous system stimulating characteristics of the parent compound and it also provided two therapeutic advantages, an earlier onset of action and a relatively shorter span of activity.

In 1913 Traube (1) reported the synthesis of a series of oxazolidinones, but it was not until 1956 that Schmidt (2) called attention to the central stimulant activity of one of the compounds, 2-imino-5-phenyl-4-oxazolidinone. In 1957 Lienert and Janke (3) in a further study of the same compound showed an activity similar to one parameter of caffeine activity, namely central nervous system stimulation, without the objectionable side-effects of the alkaloid.

The presence of a center for chelation in the oxazolidinone molecule suggested that chelates could be prepared. The possibility then exists that metal chelates of an oxazolidinone might provide a water-insoluble compound with more desirable pharmacological activity. This then would be a start in a study of the effects of chelation on drugs which must cross the blood-brain barrier. Accordingly, various oxazolidinones were reacted with a number of metal salts capable of forming chelates, and several metal chelates have been prepared.

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DISCUSSION

In order to determine the influence of substitution on the central nervous system stimulant activity of the oxazolidinone molecule, the following oxazolidinones were prepared.