and should be suitable for single- or multiple-dose pharmacokinetic or bioavailability studies.

REFERENCES

(1) L. C. Mark, H. J. Kayden, J. M. Steele, J. R. Cooper, I. Berlin, E. A. Rovenstein, and B. B. Brodie, *J. Pharmacol. Exp. Ther.*, **102**, 5 (1951).

(2) J. Koch-Weser and S. W. Klein, *J. Am. Med. Assoc.*, **215**, 1454 (1971).

(3) S. Bellet, S. E. Zeeman, and S. A. Hirsh, *Am. J. Med.*, **13**, 145 (1952).

(4) J. Koch-Weser, S. W. Klein, L. L. Foo-Canto, J. A. Kastor, and R. W. Desanatis, *N. Engl. J. Med.*, **281**, 1253 (1969).

(5) J. M. Sterling and W. G. Haney, *J. Pharm. Sci.*, **63**, 1448 (1974).

(6) J. Dreyfuss, J. T. H. Bigger, Jr., A. J. Cohen, and E. C. Schreiber, *Clin. Pharmacol. Ther.*, **13**, 366 (1972).

(7) A. J. Atkinson, Jr., M. Parker, and J. Strong, *Clin. Chem.*, **18**, 643 (1972).

(8) J. Sterling, S. Cox, and W. G. Haney, *J. Pharm. Sci.*, **63**, 1744 (1974).

(9) D. M. Ottonstein, *J. Chromatogr. Sci.*, **11**, 136 (1973).

(10) K. J. Simons and R. H. Levy, *J. Pharm. Sci.*, **64**, 1967 (1975).

(11) J. Meola and M. Vanko, *Clin. Chem.*, **18**, 579, 643 (1972).

(12) E. Karlsson, L. Molin, B. Norlander, and F. Sjoqvist, *Br. J. Clin. Pharmacol.*, **1**, 467 (1974).

(13) R. F. Adams, F. L. Vandmark, and G. Schmidt, *Clin. Chim. Acta*, **69**, 515 (1976).

(14) K. Carr, R. L. Woosley, and J. A. Oates, *J. Chromatogr.*, **129**, 363 (1976).

(15) J. Dreyfuss, J. J. Ross, Jr., and E. C. Schreiber, *Arzneim.-Forsch.*, **21**, 948 (1971).

(16) R. A. Majors, *Anal. Chem.*, **44**, 1722 (1972).

(17) C. Graffner, G. Johnsson, and J. Sjögren, *Clin. Pharmacol. Ther.*, **17**, 414 (1975).

Noninvasive Polarographic Measurement of Drug Dissolution

M. R. HACKMAN ˣ **and M. A. BROOKS**

Received July 18, 1977, from the *Department of Biochemistry and Drug Metabolism, Hoffmann-La Roche Inc., Nutley, NJ 07110.* Accepted for publication October 14, 1977.

Abstract □ Polarographic analysis was applied successfully to dissolution studies and content uniformity assessment of both capsules and tablets, using a dropping mercury electrode with the modified Levy beaker method. The described noninvasive technique places the polarographic sensor probe directly into the dissolution flask and thus simplifies dissolution measurement by eliminating transfer lines and pumps typically required with the invasive (sampling) mode of analysis. A continuous sampling flowcell with polarographic detection was also evaluated for invasive measurements. Continuous dissolution profiles and content uniformity were determined for chlordiazepoxide, trimethoprim, ornidazole, and isoniazid, using the invasive and noninvasive sampling modes. Results obtained for these drugs showed excellent precision with both sampling techniques. In addition, excellent correlation to UV spectrophotometric data was obtained.

Keyphrases □ Polarography, noninvasive—applied to drug dissolution and content uniformity assessment of various capsules and tablets □ Dissolution, drug—noninvasive polarographic study of various capsules and tablets □ Content uniformity—noninvasive polarographic assessment of various capsules and tablets

The dissolution rate of a drug is determined by measuring the amount dissolved per unit of time. These measurements are obtained conventionally by two invasive sampling techniques: (*a*) discrete sampling of the medium in the dissolution flask, followed by spectrophotometric analysis; and (*b*) continuous sampling and measurement by pumping of the dissolution medium through a UV spectrophotometer. A proposed means of obtaining dissolution data is *via* direct or noninvasive measurement by placement of an analyzing sensor directly into the dissolution flask. Spectrophotometric measurement using the noninvasive technique is not possible because of the lack of a spectrophotometric probe. Other limitations of a spectrophotometric method include disturbances caused by the stirring action, the presence of excipients, and a limited linear dynamic range.

Voltammetric analysis, which has been well documented for assays of drugs in dosage forms (1, 2) and biological fluids (3, 4), can be applied to both invasive and noninvasive dissolution measurements. The only prerequisite is that the drug undergo voltammetric reduction or oxidation in the dissolution medium. Voltammetric analysis is ideally suited for drug dissolution measurements since the technique has a wide linear dynamic range. In addition, these assays are specific for the compound of interest in the presence of excipients. The method also permits the analysis of content uniformity on the same sample after dissolution. Feher *et al.* (5) reported the use of voltammetry for the continuous invasive measurement of the dissolution of aminopyrine and promethazine by oxidation using a silicone rubber-based graphite electrode. Gaglia *et al.* (6) recently reported the noninvasive voltammetric analysis of the dissolution of nitroglycerin tablets by reduction of the nitro group at a rotating platinum electrode.

The use of a polarographic sensor, the dropping mercury electrode (DME), for drug dissolution measurement is being described for the first time. The dropping mercury electrode, when used either in a flowcell (continuous invasive measurement) or directly in the dissolution flask (noninvasive measurement), has the distinct advantage over other voltammetric sensors of presenting a constantly renewable surface for analysis. Therefore, it is not subject to a buildup of interferences during dissolution.

Polarography was used in this study to measure the dissolution rate of four drugs, chlordiazepoxide (I), or-

Table I—Drug Dissolution and Formulation Content of Compounds Assayed by Polarographic Measurement

Compound	Chemical Name	Empirical Formula	Molecular Weight	Melting Point	Standard/ Sample Weight	Amount of Gelatin Required, mg
I[a]	7-Chloro-2-methylamino-5-phenyl-3*H*-1,4-benzodiazepine 4-oxide	$C_{16}H_{14}ClN_3O$	299.71	236–236.5°	8.92[b]	60.0[c]
II	2-Methyl-5-nitro-1*H*-imidazole-1-(3-methoxy-2-propanol)	$C_7H_{10}ClN_3O_3$	219.5	77–78°	500	25.0
III	2,4-Diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine	$C_{14}H_{18}N_4O_3$	290.33	199°	100	0
IV	4-Pyridinecarboxylic acid hydrazide	$C_6H_4N_3O$	137.15	171–173°	100	0

[a] Formulated as the hydrochloride salt. [b] This weight is equivalent to the amount of chlordiazepoxide contained in 10 mg of chlordiazepoxide hydrochloride. [c] Capsule contained 40 mg of gelatin; 20 mg of additional gelatin was required.

nidazole (II), trimethoprim (III), and isoniazid (IV), by both noninvasive and invasive techniques. The reduction current was monitored at either the peak reduction potential (E_p) for differential pulse polarography or at the limiting current plateau for sampled dc polarography. Upon completion of the dissolution rate measurement, formulation content was measured by performing polarographic scans of applied potential *versus* current.

EXPERIMENTAL

Reagents—The dissolution medium was 0.1 *N* HCl prepared by mixing 16.7 ml of concentrated (12 *N*) HCl (ACS reagent grade) in 2000 ml of distilled deionized water.

The stock gelatin solution (50 mg/ml) was prepared by weighing 5.00 g of gelatin USP and dissolving it in 100 ml of boiling 0.1 *N* HCl by stirring. The hot solution was then cooled and stored in a glass-stoppered bottle.

Standards of I–IV are indicated in Table I.

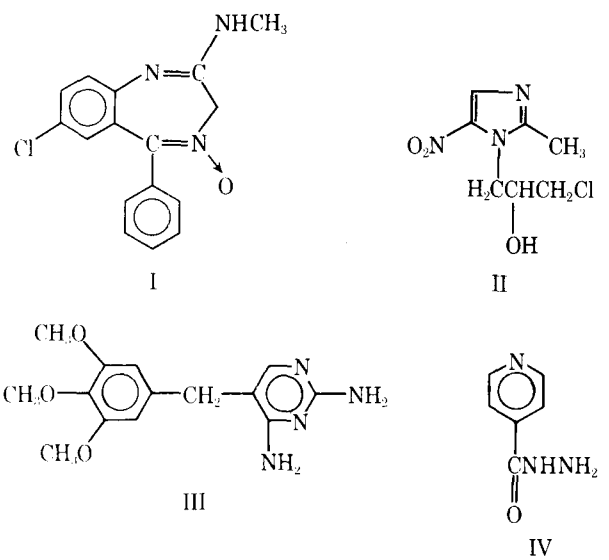
Apparatus—The Levy–Hayes beaker dissolution apparatus (7) was modified by replacing the beaker with a specially constructed round-bottom flask. The flask was designed so that 350 ml of fluid would occupy 75% of the rounded area. It was 12.5 cm in overall height (4.5-cm neck and 8.0-cm rounded area) with a 9-cm diameter mouth and a diameter of 10.5 cm at the midpoint of the rounded area. Thermoregulation was achieved using a 0–100° contact thermometer[1] connected to a relay control box[2] using a 750-w flexible immersion heater[3]. The bath stirrer was a variable speed stirring motor[4] with a 25.4-cm three-blade polyethylene paddle.

The polarograph[5] was used in conjunction with a drop timer[6]. The display was achieved through the use of an *x–y* recorder mainframe[7] with a precision dc switching/time base module[8] for the *x*-axis and a precision dc switching module[9] for the *y*-axis.

Deaeration was accomplished by flowing prepurified nitrogen[10] through a coarse-porosity micro filter stick[11]. Special electrodes and flowcells were used with the two modes of polarographic drug dissolution.

Noninvasive Polarographic Equipment—A tapered dropping mercury electrode (indicator electrode) was prepared by drawing out a 30-cm length of dropping mercury electrode capillary (6–12 sec)[12] to approximately 50% of its outer diameter and cutting it into two pieces at the narrowest part. A 20-cm length of 22-gauge platinum wire[13] was used as the auxiliary electrode. The reference electrode was a saturated calomel electrode[14] (SCE). The correct placement of the three electrodes in the special dissolution flask for noninvasive measurements is shown in Fig. 1.

Invasive Polarographic Equipment—The reference electrode was identical to that used in the noninvasive mode. The electrode, along with the sample line probe[11] (coarse-porosity filter stick) and return lines to the flowcell, was placed in the upper portion of the dissolution flask. The flowcell (Fig. 2) was a 55-mm glass tube (4 mm i.d.) to which was attached a 15-mm glass side arm (8 mm i.d.) at an angle of 60° containing the dropping mercury electrode [10-cm length of capillary tubing (2–5 sec)][15]. A glass connector[16] was attached to the upper portion of the flowcell *via* a polyvinyl chloride tubing sleeve, which served as an inlet for the dissolution medium. This same connector allowed for venting of trapped



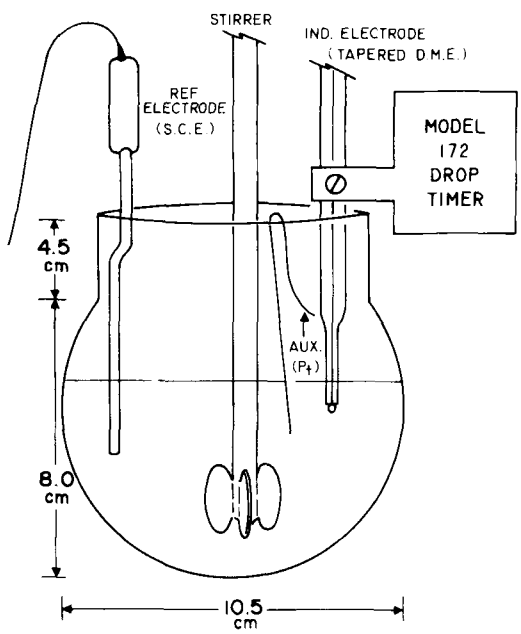


Figure 1—*Dissolution flask with electrode placement for noninvasive polarographic determination.*

[1] Catalog No. 22-3088/02, Ace Scientific, Linden, N.J.
[2] Catalog No. 22-3365/01, Ace Scientific, Linden, N.J.
[3] Catalog No. 18-6420/05, Ace Scientific, Linden, N.J.
[4] Catalog No. 22-1800/01, Ace Scientific, Linden, N.J.
[5] Model 174, Princeton Applied Research Corp., Princeton, N.J.
[6] Model 172, Princeton Applied Research Corp., Princeton, N.J.
[7] Houston-Omnigraphic model 2200, Houston Instruments, Bellaire, Tex.
[8] Houston-Omnigraphic model 6, Houston Instruments, Bellaire, Tex.
[9] Houston-Omnigraphic model 3, Houston Instruments, Bellaire, Tex.
[10] Matheson Gas Products, East Rutherford, N.J.
[11] Catalog No. JM-5385-S, SGA Scientific, Bloomfield, N.J.
[12] Catalog No. S-29417, Sargent-Welch, Springfield, N.J.
[13] B&S.
[14] Catalog No. 39178, Beckman Instruments, Mountainside, N.J.
[15] Catalog No. S-29419, Sargent-Welch, Springfield, N.J.
[16] Catalog No. 116-0202P03, Technicon Instruments, South Plainfield, N.J.

Table II—Instrument Parameters for Polarographic Measurement of Drug Dissolution and Formulation Content

Compound	Mode	Measuring Potential, v *versus* SCE	Time Base Rate, sec/in.	Scan Range, v	Current Range, μamp	
					Noninvasive	Invasive
I	Differential pulse	−0.600	100	−0.450 to −0.800	20	10
II	Differential pulse	−0.070	200	+0.075 to −0.300	500	500
IV	Differential pulse	−0.685	100	−0.300 to −0.850	200	500
III	Sampled dc	−1.300	200	−1.000 to −1.450	20	50

air bubbles in the flowcell through the capillary arm, which was fitted with a polyethylene nipple[17]. A 3-ml syringe, fitted with a length of 1.59-mm (0.0625-in.) polyvinyl chloride tubing, was placed over the nipple for removal of trapped air bubbles.

A mercury separator, which served as a collector for used mercury and as an outlet for the dissolution medium, was attached to the lower portion of the flowcell *via* another polyvinyl chloride sleeve. The separator was constructed from a 4.76-mm ($^3/_{16}$-in.) i.d. Y-tube, which was modified by removal of the three nipples and by sealing in a 22-gauge platinum wire[13] in the lower arm. Polyvinyl chloride tubing, 4.76 mm ($^3/_{16}$ in. i.d.), was attached to the lower arm of the mercury separator and closed off *via* a tubing clamp. This clamp allowed for removal of excess mercury from the flow system. The mercury level in the lower arm of the Y-tube was maintained slightly above the platinum wire electrode, producing a mercury pool as the auxiliary electrode.

A single-speed peristaltic pump[18] with a 2.79-mm (0.110-in.) (3.9 ml/min) polyvinyl chloride pump tube was used[19]. The connections from the dissolution flask to the flowcell and back to the flask are outlined in Fig. 3. All tubing to the flowcell was narrow bore polyethylene [0.58 mm (0.023 in. i.d.)]. The return line to the dissolution flask was polyethylene tubing [1.59 mm (0.0625 in. i.d.)].

Instrumental Parameters—The polarograph was operated in either the differential pulse or sampled dc mode (Table I), using a scan rate of 5 mv/sec with a 0.5-sec drop time. In the differential pulse mode, the pulse modulation amplitude was −50 mv. The measuring potential, current range, and time base were adjusted to the required setting (Table II), and the low pass filter was not used. The capillary characteristics were: the standard dropping mercury electrode at 22°, m = 2.39 mg/sec, $m^{2/3}\,t^{1/6}$ = 1.593; and the tapered dropping mercury electrode at 22°, m = 0.907 mg/sec, $m^{2/3}\,t^{1/6}$ = 0.8345, and at 37°, m = 1.25 mg/sec, $m^{2/3}\,t^{1/6}$ = 1.032.

Noninvasive Procedure—*Standard*—A 300-ml aliquot of 0.1 *N* HCl containing the required amount of gelatin was added to the dissolution flask and was thermally equilibrated to 37°. During this interval (approximately 5 min), the solution was deaerated with nitrogen by means of the micro filter stick. After deaeration, the required weight of standard was dissolved in the medium with the aid of the stirrer. The stirrer was adjusted to 50 rpm, and the appropriate polarographic measuring potential was set. Complete dissolution was noted by a current plateau lasting at least 5 or 10 min at a recorder rate of 100 or 200 sec/2.54 cm, respectively. After dissolution, the stirrer was turned off, the recorder was converted to the x–y mode, and a polarographic scan was made.

Formulation—The dissolution flask was drained using a water aspirator, and the flask was rinsed several times with distilled water. New dissolution medium was added to the flask, thermally equilibrated, and deaerated as already described. The recorder was reconverted to the time base mode, which was engaged simultaneously with the dropping of the tablet (or capsule) into the dissolution medium. (To ensure that total immersion of the capsule occurred, a paper clip was placed around the capsule as a weight.) The dissolution kinetic curve was monitored until a current plateau was measured. The initial potential was set, and the polarographic scan was recorded using the x–y mode.

Invasive Procedure—The procedure was identical to the noninvasive procedure except that after the dissolution medium was thermally equilibrated, the pump was turned on and the entire flow system was filled. In addition, any air bubbles present were removed by pulling air from the vent (Fig. 2) with a 3-ml syringe fitted with a length of 1.59-mm (0.0625-in.) polyvinyl chloride tubing. All measurements of both dissolution and formulation content in this mode were made with the stirrer operational.

After completion of the analysis of the standard and while the pump was running, the return line was removed from the dissolution flask and placed into a waste receptacle, such as a 125-ml erlenmeyer flask. The dissolution flask was then rinsed several times with distilled water, allowing enough time for the rinse to run through the entire flow system. Upon final rinsing, the lines and flowcell were dried by pumping with air. After the lines were dry, the return line was replaced in the dissolution flask, a new dissolution medium was added, and the analysis of the drug in the dosage form was carried out.

Calculation of Percent Dissolved at t_{25}, t_{50}, and t_{75}—The heights from the extrapolated baseline to the current plateau for the standard (I_{100}, standard) and sample (I_{100}) were measured. These two heights will be virtually equal if the drug in the dosage form is completely dissolved. Based on the value of I_{100}, the time at 25, 50, and 75% dissolution was determined by extrapolating to the x-axis. [In the invasive mode, the delay time, the time for the dissolved sample to traverse the distance from the flask to the detector (experimentally determined to be 72 sec) must be subtracted from the t_{25}, t_{50}, and t_{75} times of dissolution.] An example of this type of calculation is shown for the invasive determination of ornidazole in Fig. 4.

Calculation of Content Uniformity Using Differental Pulse Po-

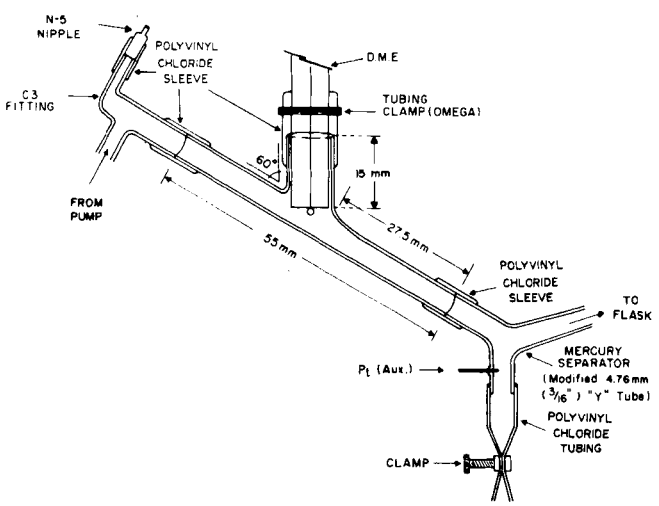


Figure 2—*Flowcell for invasive polarographic measurement.*

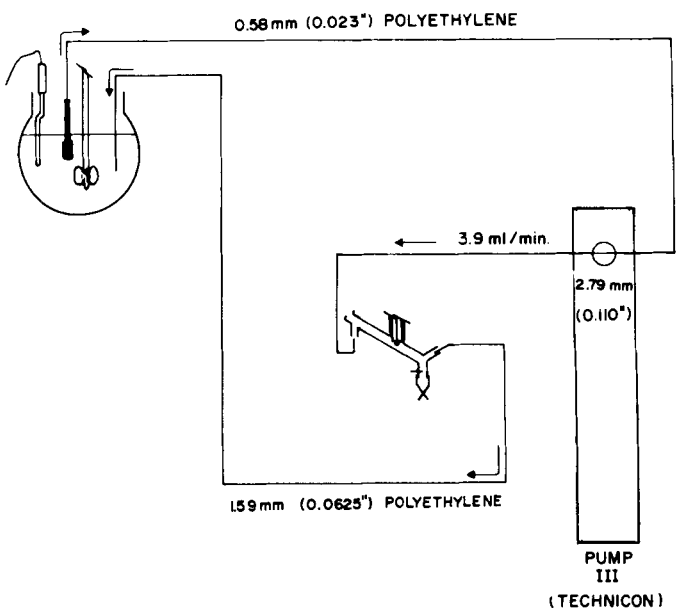


Figure 3—*Flow diagram for invasive measurement.*

[17] Catalog No. 116-0002PO1, Technicon Instruments, South Plainfield, N.J.
[18] Catalog No. 133-A014, Technicon Instruments, South Plainfield, N.J.
[19] Catalog No. 190-0427P16, Technicon Instruments, South Plainfield, N.J.

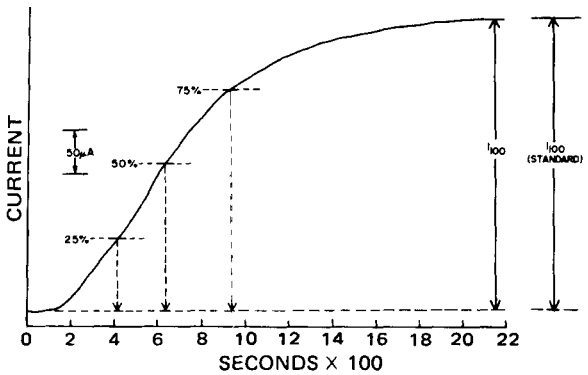


Figure 4—*Typical calculation for determination of dissolution times for ornidazole.*

larography—The measurement of the peak height, H, in centimeters was converted to a current, I, in microamperes, using the following relationship (Fig. 5A):

$$I = \frac{H}{25.4} \times \text{current range} \qquad \text{(Eq. 1)}$$

The currents of the standard, I_s, and the unknown, I_u, were determined, and the ratio of the currents to the known standard concentration, C_s, was used to determine the unknown concentration, C_u, by the following relationship:

$$C_u = \frac{C_s}{I_s} \times I_u \qquad \text{(Eq. 2)}$$

In a similar manner, the concentration was determined for the sampled dc curve. The plateau height was calculated as the difference from the measuring potential to the extrapolated baseline (Fig. 5B).

RESULTS AND DISCUSSION

Special equipment, except the tapered dropping mercury electrode, is not necessary for the noninvasive polarographic measurement of drug dissolution. The electrode modification was necessary to decrease the natural drop time to greater than 0.5 sec at 37°, as required for mechanical dislodgment.

In contrast, both the invasive polarographic method and the spectrophotometric method require special pumping equipment, flowcell (Fig. 2), and sample lines (Fig. 3) to measure drug dissolution. In this mode, it is critical that all three electrodes be immersed in the continuous stream of the dissolution medium to ensure electrical conductivity. Thus, it is essential that all air bubbles be removed from the lines at the vent in the upper end of the flowcell. If this condition is not met, a large potential, resulting from a break in the feedback loop of the potential generating circuitry, will be applied across the indicator and reference electrodes; the system will go into electrical overload, with possible damage to the electrodes.

Changes in conductivity also were experienced when mercury was permitted to leave the flowcell and enter the dissolution flask. To trap the mercury falling from the dropping mercury electrode, a mercury separator was incorporated into the lower end of the flowcell. This device

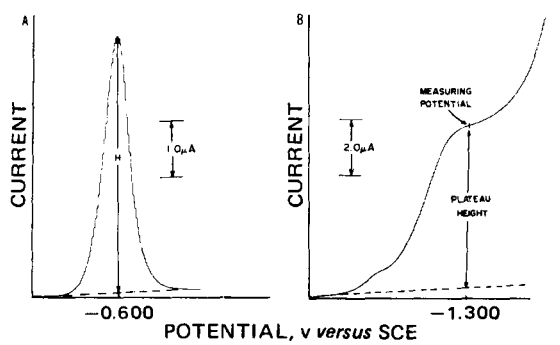


Figure 5—*Typical calculation for formulation content. Key: A, differential pulse polarogram of chlordiazepoxide; and B, sampled dc polarogram of trimethoprim.*

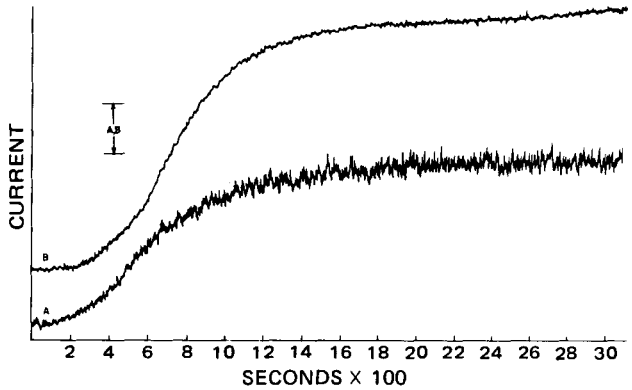


Figure 6—*Typical dissolution kinetic curve for a capsule containing 8.92 mg of chlordiazepoxide. Key: A, 20 μamp and noninvasive; and B, 10 μamp and invasive.*

collected all spent mercury and could be easily drained through the tubing attached to the lower arm of the separator. If long dissolution times are encountered (≃1 hr), this side arm may be drained during the assay.

The reduction of a species at the dropping mercury electrode takes place at the surface of the mercury drop. Therefore, anything that affects that surface, such as stirring, particles, or surface-active agents, will affect the resulting signal. In the noninvasive mode, the sensing electrode is in close proximity to the stirrer, so effects from stirring are significant. The currents measured during the process are a sum of currents due to migration resulting from the stirring as well as natural diffusion. The removal of the stirrer from the immediate vicinity of the electrode (Fig. 1) reduces irregular migration due to the vortex action of stirring and greatly diminishes noise on the drug dissolution curve.

A stirring rate of 50 rpm maintains this noise level at a minimum to allow for measurement of as little as 8.56 mg of chlordiazepoxide (Fig. 6A). A stirring rate of 100 rpm produces an unacceptably low signal to noise ratio, which prevents measurement of low concentrations (10–50 mg), but it can be used with formulations containing levels of active ingredients in excess of 100 mg. The collision of suspended particles with the dropping mercury electrode introduces only slight disturbances to the reduction signal. The determination of formulation content in the noninvasive mode is performed without stirring after completion of the dissolution of the drug. A more stable signal results without the stirring action, so a higher degree of precision and accuracy is obtained. This effect can be attributed to the elimination of the vortex action of the stirrer and/or sedimentation of the particulate matter.

The current measured in the invasive mode is controlled by a laminar type of flow of material past the electrode surface, which shows little turbulance and is virtually free of noise. Particles that might block the flowcell or disrupt the flow are excluded *via* the use of the filter stick. Thus, measurement of drug dissolution can be carried out at both 50 and 100 rpm and with formulations below 10 mg. The resultant dissolution curves obtained in the invasive mode (see Fig. 6B for the determination of chlordiazepoxide) are easier to interpret than from the noninvasive mode. In the invasive mode, formulation content measurement is performed with the stirrer operational and the dissolution medium contin-

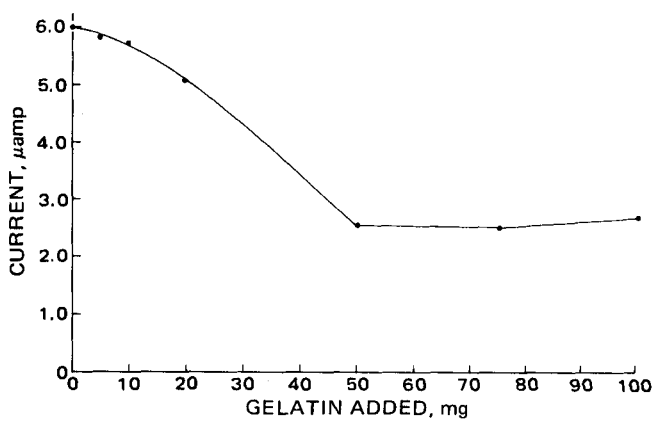


Figure 7—*Effect of gelatin on the polarographic reduction of chlordiazepoxide.*

Table III—Dissolution Characteristics: Mean Time (Minutes ± *SD*) for 25, 50, and 75% Dissolution (t_{25}, t_{50}, and t_{75}, Respectively)

Formulation	Noninvasive				Invasive			
	t_{25}	t_{50}	t_{75}	n[a]	t_{25}	t_{50}	t_{75}	n
I	8.56 ± 0.76	13.1 ± 1.7	18.5 ± 2.9	7	6.00 ± 0.61	9.17 ± 1.4	15.1 ± 2.8	4
II	6.83 ± 0.55	11.3 ± 1.8	19.4 ± 3.2	4	6.00 ± 1.0	10.6 ± 1.5	17.2 ± 2.4	4
III	6.20 ± 0.77	13.1 ± 1.6	24.4 ± 2.7	5	9.3 ± 2.3	17.5 ± 3.2	29.8 ± 6.0	6
IV	3.83 ± 0.50	6.46 ± 0.82	8.23 ± 1.0	6	5.23 ± 1.1	7.10 ± 1.0	9.98 ± 1.1	5

[a] Number of replicate samples assayed.

Table IV—Determination of Formulation Content

Compound	Invasive		Noninvasive	
	% of Claim ± *SD*	n[a]	% of Claim ± *SD*	n
I	97.7 ± 3.2	5	99.5 ± 3.3	7
II	96.6 ± 1.1	5	99.4 ± 3.0	8
III	98.2 ± 2.8	6	97.9 ± 0.74	5
IV	92.1 ± 2.0	5	90.9 ± 1.7	6

[a] Number of replicate samples assayed.

uously pumped through the flowcell to ensure electrical continuity.

Compounds I–IV were selected for this experiment because they contained different reducible functional groups, thus demonstrating the utility of the polarographic method over a wide range of concentrations (10–500 mg/sample), and because they were completely soluble in the dissolution medium.

Compound I was formulated as a 10-mg gelatin capsule[20]. Chlordiazepoxide has three electrochemically reducible functional groups—*viz.*, the 1,2- and 4,5-azomethine and N^4-oxide bonds (8). The reduction of the 4,5-azomethine bond, which is the most sensitive and easily measured, was used for drug dissolution studies. The gelatin in capsules is a natural surfactant and will affect the dropping mercury electrode. Small amounts of gelatin (approximately 0.003%) are widely used for suppression of surface effects on the mercury drop called maxima (9). Since these same surfactants can also suppress the polarographic reduction curve, their concentration in drug dissolution determinations must be held constant.

The effect of adding varying amounts of gelatin, from 5 to 100 mg, on the reduction current of 8.92 mg of chlordiazepoxide was determined (Fig. 7). The data indicated that the addition of gelatin resulted in a progressive decline in the reduction current until a total of 50 mg was added; a constant plateau was noted in the region of 50–75 mg of gelatin added. Since the No. 2 capsule used in the chlordiazepoxide formulation weighed about 40 mg, an additional 20 mg of gelatin was added to ensure constant suppression. Consequently, a total of 60 mg of gelatin was added to the dissolution medium for the measurement of standards of chlordiazepoxide.

Compound II was formulated as a 500-mg tablet. The compound demonstrates polarographic activity due to the reduction of the nitro functional group (10). At the high concentration encountered for the analysis of these tablets, a polarographic maximum was noted (9). This phenomenon was demonstrated in the form of exaggerated and distorted differential pulse polarographic peaks, which invalidated peak height measurement. The addition of 25 mg of gelatin eliminated peak distortion and simplified peak height measurement.

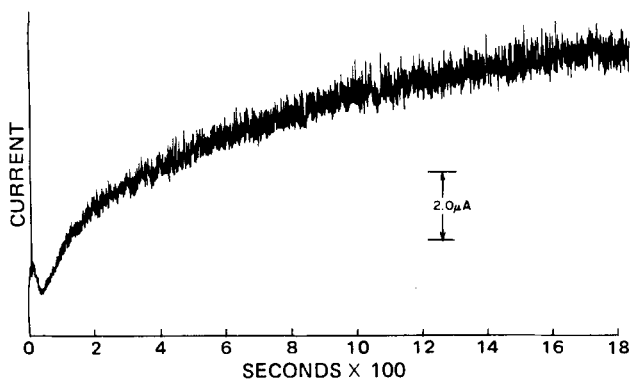


Figure 8—*Dissolution curve of trimethoprim.*

[20] Librium is the hydrochloride salt of chlordiazepoxide.

Compound III was formulated as a 100-mg tablet. The compound demonstrates polarographic activity due to the reduction of the azomethine groups in the pyrimidine ring (11). The use of differential pulse polarography for the assay of this compound is not feasible because the reduction is a two-step process in which the close proximity of the reduction potentials results in overlapping peaks. By using sampled dc polarography, a current is obtained that equals the summation of the two-step reduction process and yields a signal proportional to concentration.

The drug dissolution curve for trimethoprim employing both the invasive and noninvasive modes demonstrated a characteristic dip at the beginning of the dissolution curve (Fig. 8). The phenomenon was not noted with the assay of a placebo. Although no definite explanation for this phenomenon is known, it may be related to large changes in concentration that occur at the onset of the assay, with resultant surface effects at the dropping mercury electrode. The shape of the curve did not invalidate drug dissolution measurements made by extrapolating the baseline from the lowest portion of the dip.

Compound IV also was formulated as a 100-mg tablet. The polarographic reduction yields four peaks (Fig. 9). The first is due to the reduction of the hydrazide moiety, and the second and third are due to the stepwise reduction of the carbonyl group (12). The fourth peak is probably due to the reduction of the azomethine bond in the pyridine ring. The choice of the second peak (E_p = −0.685 v *versus* saturated calomel electrode) for analytical measurement was based upon the ease of measurement at that peak potential as well as its higher relative sensitivity.

Table III indicates that both the noninvasive and invasive method of polarographic drug dissolution yielded essentially the same values for the four drugs tested. The tablet to tablet variability (standard deviation) using either technique gave similar results.

All four formulations were tested for drug content. Comparisons were made with standard data on potency such as the content uniformity specifications outlined in the USP. All four formulations fell well within the content uniformity specifications as shown in Table IV. Formulation content measurements were also performed on the same samples of II and IV by polarography (noninvasive) and UV spectrophotometry (discrete sampling). The UV analysis of II and IV required dilution by factors of 1:100 and 1:25, respectively, to bring the absorbance values within the usable range of the spectrophotometer. This requirement further demonstrates the utility of the wide linear dynamic range of the polarographic method, which requires no dilutions to measure these concentrations. Table V indicates that essentially identical results are obtained using either technique.

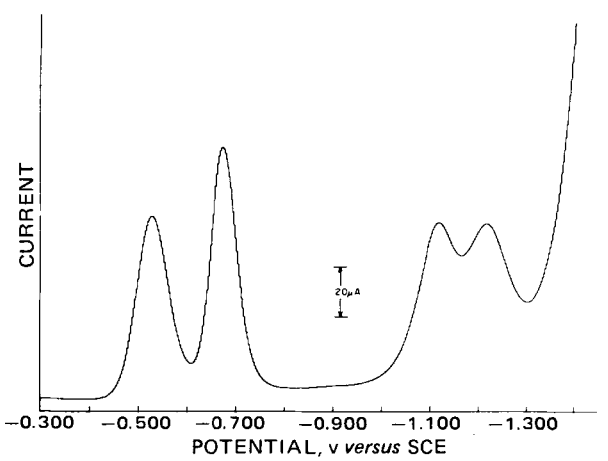


Figure 9—*Differential pulse polarogram of isoniazid (333 μg/ml in 0.1 N HCl).*

Table V—Formulation Content Determined by UV Spectrophotometry and Differential Pulse Polarography

Tablet	Concentration by UV, mg	Concentration by Differential Pulse Polarography, mg
Orindazole		
1	501.9	507.0
2	518.2	519.2
3	498.5	502.4
Mean ± *SD*	506.4 ± 11.3	509.5 ± 9.2
Isoniazid		
1	93.56	92.50
2	97.69	96.25
3	96.75	95.88
Mean ± *SD*	96.0 ± 1.77	94.9 ± 1.26

CONCLUSION

Polarographic measurement of drug dissolution and formulation content of four different drug formulations demonstrated the utility of polarography to provide such data with high accuracy and precision. The technique provides for measurement of compounds with varied chemical structures contained in formulations covering a wide range of concentrations and in the presence of excipients. The tapered dropping mercury electrode served as an excellent sensor for noninvasive measurements, thereby precluding the use of flowcells, transfer lines, and pumps as well as simplifying the calculation of data. Invasive measurements were also made by incorporating the dropping mercury electrode into a specially designed flowcell to obtain continuously sampled polarographic data.

REFERENCES

(1) P. Zuman and M. Brezina, in "Progress in Polarography," vol. II, P. Zuman and I. M. Kolthoff, Eds., Interscience, New York, N.Y., 1962.

(2) H. Hoffmann and J. Volke, in "Electroanalytical Chemistry," vol. 10 in the series "Advances in Analytical Chemistry and Instrumentation," H. W. Nurnberg, Ed., Wiley, London, England, 1974.

(3) J. A. F. de Silva and M. A. Brooks, in "Drug Fate and Metabolism," vol. 2, E. R. Garrett and J. L. Hirtz, Eds., Dekker, New York, N.Y., 1978.

(4) M. A. Brooks, J. A. F. de Silva, and M. R. Hackman, *Am. Lab.*, **5** (9), 23 (1973).

(5) Z. Feher, G. Nagy, K. Toth, and E. Pungor, *Analyst*, **99**, 699 (1974).

(6) C. A. Gaglia, Jr., J. J. Lomner, B. L. Leonard, and L. Chafetz, *J. Pharm. Sci.*, **65**, 1691 (1976).

(7) G. Levy and B. A. Hayes, *N. Engl. J. Med.*, **262**, 1053 (1960).

(8) M. R. Hackman, M. A. Brooks, J. A. F. de Silva, and T. S. Ma, *Anal. Chem.*, **46**, 1075 (1974).

(9) L. Meites, in "Polarographic Techniques," 2nd ed., Interscience, New York, N.Y., 1965.

(10) M. A. Brooks, L. D'Arconte, and J. A. F. de Silva, *J. Pharm. Sci.*, **65**, 112 (1976).

(11) M. A. Brooks, J. A. F. de Silva, and L. M. D'Arconte, *Anal. Chem.*, **45**, 263 (1973).

(12) Y. P. Kitaev and G. K. Budnikov, *Bull. Acad. Sci. USSR, Div. Chem. Sci.*, **1967**, 535.

Functional Group Contribution of Bile Salt Molecules to Partitioning of a Quaternary Ammonium *N*,*N*-Dimethyl Derivative of Propranolol

H. K. LEE ˣ, Y. W. CHIEN, T. K. LIN, and H. J. LAMBERT

Received July 28, 1977, from *Searle Laboratories, G. D. Searle & Co., Skokie, IL 60076.* Accepted for publication October 12, 1977.

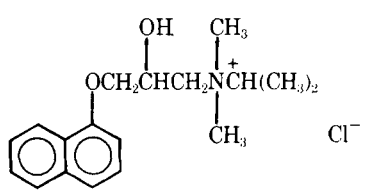
Abstract □ A quaternary ammonium *N*,*N*-dimethyl derivative of propranolol was extracted from pH 7.4 phosphate buffer into 1-octanol as ion-pairs with 12 different bile salts. The binding number, n, and the extraction constant, K_e, were determined. To obtain group contribution values of the bile salt molecule from the ion-pair extraction data, multiple linear regression analysis by the Free–Wilson technique was applied. The results showed that the fundamental premise of the functional group's contribution to the ion-pair extraction is valid. The functional groups of counterions contribute to the partitioning of the ammonium compound independently and additively in this system.

Keyphrases □ Bile salt molecules—functional group contribution to ion-pair partitioning of quaternary ammonium derivative of propranolol □ Quaternary ammonium salts—*N*,*N*-dimethyl derivative of propranolol, ion-pair partitioning, functional group contribution of bile salt molecules □ Ion-pair partitioning—*N*,*N*-dimethyl derivative of propranolol, functional group contribution of bile salt molecules

The effect of the structure of the pairing ion on quaternary ammonium compounds has received considerable attention (1). Partitioning of organic salts or complexes was reported to be influenced by the molecular weight of the organic ions, the branching effect of the aliphatic amine cations, and the organic solvent (2, 3).

A comprehensive group contribution study based on partition coefficient measurement was presented (4). From the consistency of the thermodynamic data, it was concluded that ion-pair extraction equilibria provide a feasible method for determining group contribution. The use, with some limitations, of ion-pair extraction data to obtain group contribution values was demonstrated (5).

The *N*,*N*-dimethyl derivative of propranolol chloride, *N*,*N*-dimethyl-1-isopropylamino-3-(1-naphthyloxy)-2-propanol chloride (I), has antiarrhythmic activity without significant β-blocking and local anesthetic effects (6). In general, quaternary ammonium compounds are absorbed poorly from the GI tract because of their cationic nature (7). This poor absorption might be circumvented if the cationic nature of the onium head could be masked by ion-pair formation. The present investigation studied the interaction between I and bile salt molecules by the group



I