# A System for Automated Polarographic Analysis

L. F. CULLEN, M. P. BRINDLE, and G. J. PAPARIELLO▲

**Abstract** □ A sensitive and potentially highly versatile automated polarographic procedure was developed. A polarographic analyzer is interfaced with a continuous-flow system which completely removes interfering dissolved oxygen. Inclusion of a peristaltic valve in the manifold design allows an extended sampling period at steady-state conditions, at the same time increasing the productive rate of the system by a factor of two. High-resolution scans of the sample stream with a multifunctional polarographic analyzer set to monitor diffusion current while the applied potential is varied have the capability of quantitating electroactive compounds in the presence of decomposition products or formulation components. The capabilities of the method were demonstrated by collecting data on tablet and capsule formulations of lorazepam, a 1,4-benzodiazepine. Fifteen samples per hour can be assayed with a relative standard deviation of ±1.4% at the 0.5-mg. lorazepam level. The method is applicable to unit-dose analysis of lorazepam in the 0.5–6-mg./dose range.

**Keyphrases** □ Polarography—automated system and procedure, analysis of lorazepam tablets and capsules □ Lorazepam tablets and capsules—analysis, automated polarographic procedure

In recent years, polarographic techniques have been finding wide application in pharmaceutical analysis. Requests for a large number of assays to assist in evaluating the pharmaceutical acceptability and chemical stability of various dosage forms for new investigational drugs prompted the development of an automated polarographic method of analysis for tablet and capsule formulations.

Previously reported automated techniques employing polarized electrodes in continuous-flow systems (1–3) have been restricted to the measurement of diffusion current at a constant potential due to limitations imposed by the use of conventional continuous-flow dynamics. However, it is necessary to monitor diffusion current as a function of varying applied potential when quantitating electroactive compounds in the presence of decomposition products or formulation components that interfere in the fixed potential technique. In designing a completely automated analytical system capable of producing authentic polarograms, several unique problems in continuous-flow analysis were encountered.

The automatic analyzer system[1] described was developed following a systematic consideration of the capabilities and limitations of standard automated modules. The technique interfaces a multifunctional polarographic analyzer with a continuous-flow system employing sophisticated design features. The capabilities of the method were demonstrated by collecting data on tablet and capsule formulations of lorazepam [7-chloro-5-(*o*-chlorophenyl)-1,3-dihydro-3-hydroxy-2*H*-1,4-benzodiazepin-2-one]. This potentially versatile analytical system expands the capabilities of automated analysis to include polarography, a well-established electrochemical technique.

## EXPERIMENTAL

**Apparatus**—A standard automatic analyzer[1] system was employed consisting of the following modules: (*a*) a SOLIDprep sampler, programmed at 15 samples/hr. with a sample aspiration stage set at 40–90 units; (*b*) proportioning pumps[2] (three required); (*c*) a continuous filter, speed 2; (*d*) a peristaltic valve[3]; (*e*) a polarographic analyzer equipped with a mechanical drop timer[4]; (*f*) a circulating constant-temperature unit[5]; (*g*) a 25.4-cm. (10-in.) linear potentiometric strip-chart recorder[6]; and (*h*) transmission lines (Solvaflex) and proportioning tubes.

The dimensional details of the polarographic flow cell used in this investigation were presented by Lento (1). To prevent deterioration from the predominantly nonaqueous supporting electrolyte considered, the cell was machined from a transparent 4.44-cm. (1.75-in.) rod[7].

**Supporting Electrolyte**—The supporting electrolyte consisted of 0.1 *M* sodium acetate and 0.1 *M* acetic acid in methanol–water (8:2, v/v). In the preparation of the supporting electrolyte, reagent grade materials were used without further purification.

**Nitrogen**—Prepurified grade nitrogen[8] was used in the deaeration unit.

**Automated Methodology**—A flow diagram indicating the arrangement of the apparatus employed in this system is presented in Fig. 1. Figure 2 is a schematic wiring diagram of the necessary synchronization control circuits.

In performing the analyses, standards are placed on the sample plate followed by samples of intact or powdered tablets or capsules. At the end of a series of 20 samples, additional standards are placed on the sample plate. Samples are introduced into the SOLIDprep unit and dispersed in the methanolic sodium acetate–acetic acid supporting electrolyte. Following dissolution of the drug, the sample is aspirated into the flow system and automatically filtered to remove insolubles. To prevent mechanical obstruction at the tubing connections from insoluble excipient materials, a decantation trap is placed between the SOLIDprep unit and the proportioning pump.

After passage through the continuous filter module, the filtrate is segmented with nitrogen and pumped to the inlet manifold of the "steady-state extension unit." The extension unit employs a differential flow rate principle and synchronized valve control to prolong the duration of the sample's steady state. From the outlet manifold of the steady-state extension unit, the expanded sample stream is directed to the inlet of the continuous deaerator. Within this unit, a high velocity stream of methanol-saturated nitrogen is employed to transform the sample into a thin, turbulent liquid film. Entrained by the gas stream, the film is transported rapidly through the deaeration coil, which provides the surface area necessary for efficient oxygen removal. Thermostatic control of the purging gas is provided

[1] AutoAnalyzer, Technicon Corp., Tarrytown, N. Y.

[2] Model I, Technicon Corp.
[3] Catalog No. 167-A002-01, Technicon Corp.
[4] Model PAR-174, Princeton Applied Research Corp., Princeton, N. J.
[5] Catalog No. 66590, Precision Scientific Co., Chicago, Ill.
[6] Beckman model 1005, Beckman Instruments, Inc., Fullerton, Calif.
[7] Solvent-resistant Homalite CR-39, Homalite Corp., Wilmington, Del.
[8] Matheson Co., East Rutherford, N. J.

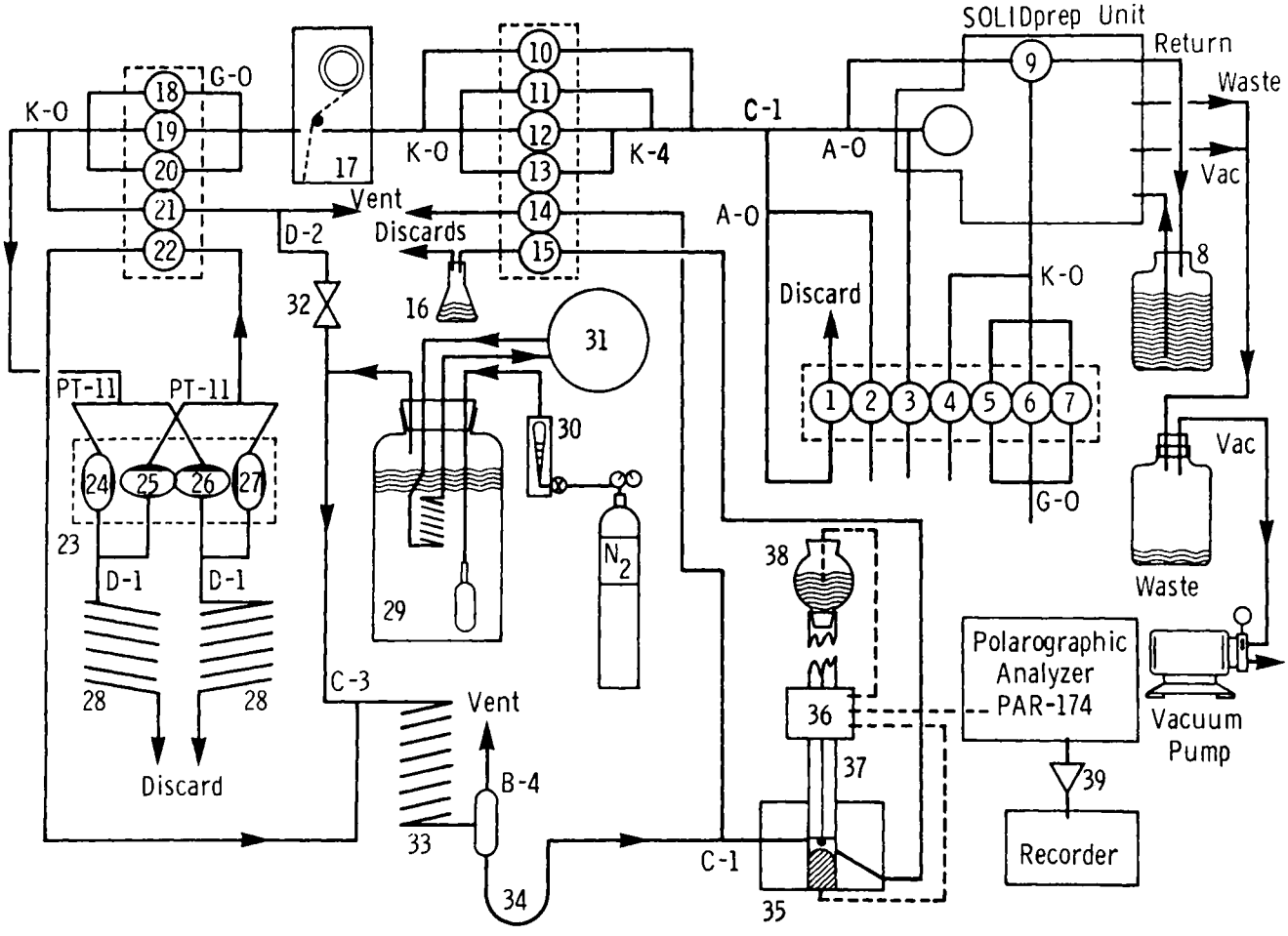


**Figure 1**—*Automated flow diagram. Key: 1, 0.56 ml./min.; 2, 0.42 ml./min. water; 3, 0.56 ml./min. air; 4, 3.39 ml./min. air; 5, 6, 7, 3.39 ml./min. supporting electrolyte; 8, reservoir-supporting electrolyte; 9, SOLIDprep wash distribution valve; 10, 11, 12, 3.39 ml./min. sample; 13, 1.69 ml./min. sample; 14, 2.02 ml./min. debubbler; 15, 2.02 ml./min. flow cell; 16, mercury trap; 17, continuous filter module; 18, 3.39 ml./min. sample; 19, 2.42 ml./min. sample; 20, 3.39 ml./min. sample; 21, 0.56 ml./min. nitrogen; 22, 2.42 ml./min. sample; 23, peristaltic valve module; 24, 0.063-cm. (0.025-in.) i.d. manifold tubing; 25, 0.038-cm. (0.015-in.) i.d. manifold tubing; 26, 0.063-cm. (0.025-in.) i.d. manifold tubing; 27, 0.038-cm. (0.015-in.) i.d. manifold tubing; 28, sample storage coil, 12.2 m. (40 ft.), 2.4 mm. i.d.; 29, gas saturation apparatus; 30, gas flowmeter; 31, circulating constant-temperature bath; 32, nitrogen flow-limiting orifice, 0.038-cm. (0.015-in.) i.d. pulse suppressor; 33, deaeration coil, 14-turn 3.4-mm. i.d. mixing coil; 34, 6-cm. glass "U", 2 mm. i.d.; 35, polarographic flow cell; 36, mechanical drop timer; 37, mercury capillary, 9 cm. (Sargent-Welch S-29419); 38, mercury reservoir; and 39, signal attenuator, 10 v.–100 mv.*

to protect the electrolyte system from evaporational losses. The sample is withdrawn from the outlet connection of the deaerator and transported to the polarographic flow cell. Segmenting nitrogen is also drawn from the purging unit into the sample effluent for the purpose of providing a secondary deaeration stage in which residual dissolved oxygen is removed from the sample stream.

The polarographic analyzer and recorder are synchronized to initiate a scan at the moment the sample attains steady state at the flow cell. The instrument is used in the current-sampled d.c. polarographic mode (4) with the mechanical drop timer operating at 1 drop/sec. For lorazepam, the polarographic scan is conducted at a rate of 5 mv./sec., starting at an initial potential of −0.86 v. and continuing to −1.76 v. Calculations are made through comparison of the diffusion currents observed with standards and solid dosage formulation samples.

## RESULTS AND DISCUSSION

**Manifold Design Considerations**—In the development of an automated continuous-flow system that employs a scanning analytical technique, such as polarography, it is necessary to meet design criteria not ordinarily encountered in conventional automated methodologies. Since a scan may be conducted only while the sample concentration at the detector is held constant, it is necessary to achieve steady states of appreciable duration within the flow system. This requirement presents a considerable design problem in automated manifolds operating at rapid assay rates.

The elapsed time required for a single polarographic determination is expressed by the equation:

$$t_p = \frac{\Delta E}{R} \qquad \text{(Eq. 1)}$$

where $t_p$ is the scan duration in seconds, $\Delta E$ is the width of the voltage range to be studied in millivolts, and $R$ is the voltage scan rate expressed in millivolts per second.

The extent of the potential region that must be scanned is determined by the positions of the polarographic waves characteristic of the compounds under investigation and the electrolyte system employed. Both the rate at which the scan is conducted and the polarographic mode utilized affect the definition of the recorded polarogram (5). With a given polarographic mode, the maximum permis-

**Table I**—Comparison of Polarographic and Quantitative TLC Analyses of Intentionally Degraded Lorazepam Samples

| Sample Treatment | Percent of Initial | |
|---|---|---|
| | Polarographic Method | TLC Method |
| Stored at 25° for 47 months | 99 | 98 |
| Stored at 60° for 1 month | 94 | 95 |
| Stored at 75° for 2 weeks | 76 | 77 |

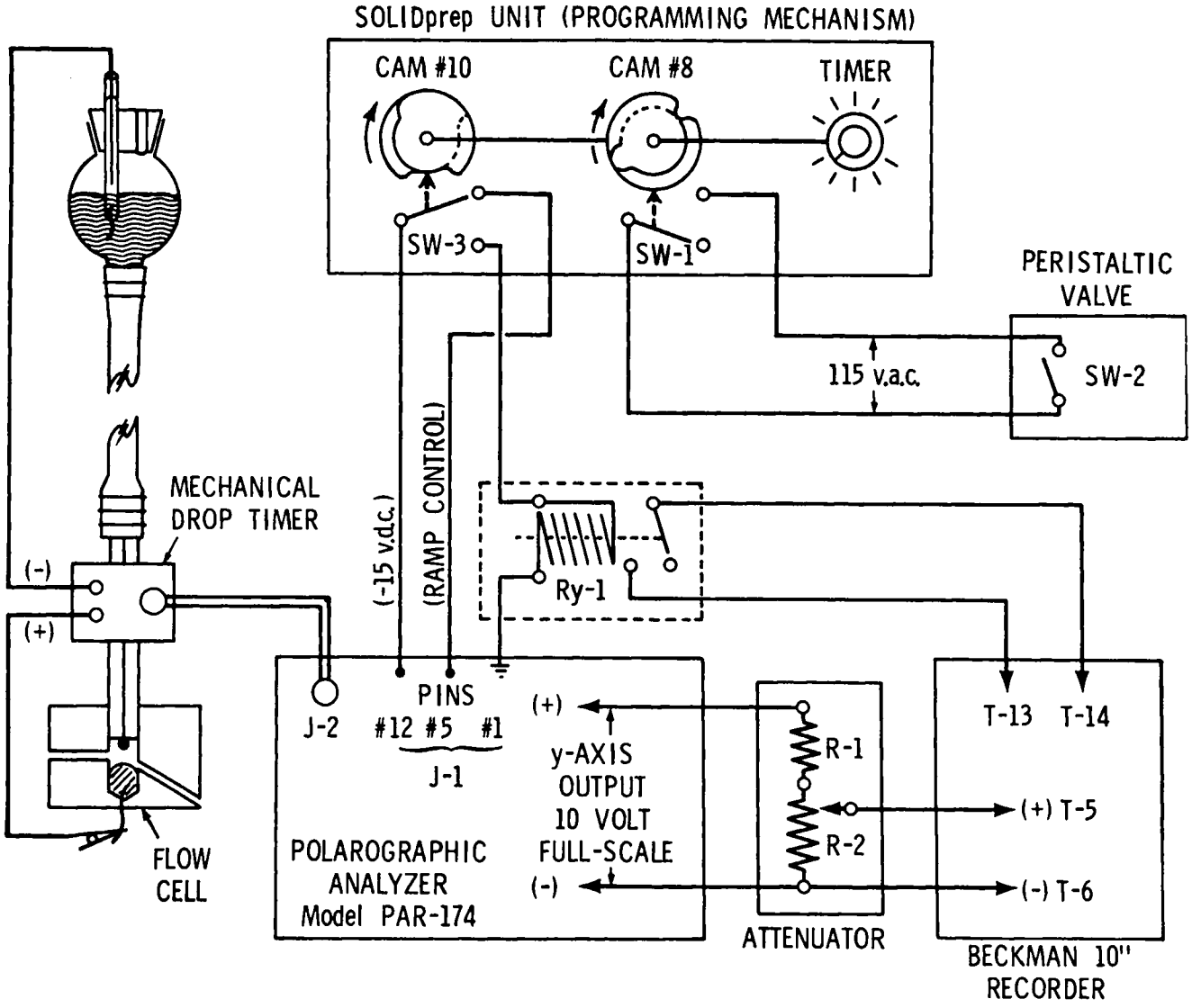


**Figure 2**—*Schematic wiring diagram. Key: SW-1, cam-actuated microswitch 8 (SOLIDprep unit); SW-2, manual index switch (peristaltic valve module); SW-3, cam-actuated microswitch 10 (SOLIDprep unit); T-5, T-6, recorder input terminals (100 mv. full-scale sensitivity); T-13, T-14, recorder remote control terminals; Ry-1, recorder control relay (Allied Electronics No. 41C7676); R-1, precision resistor, 900 kohm ± 1%; R-2, 10-kohm 10-turn helipot; J-1, accessory connector plug (PAR-174); and J-2, cell/drop timer connector plug (PAR-174).*

sible scan rate depends on the precision and resolution required for the specific determination of interest. Through the conditions imposed on the values of $\Delta E$ and $R$ (Eq. 1), the minimum acceptable steady-state duration is controlled by the requirements of the particular assay.

In the quantitation of lorazepam, for example, it was found necessary to scan a voltage region of 900 mv., *i.e.*, from −0.86 to −1.76 v. *versus* mercury pool electrode. For the desired level of resolution, the maximum scan rate was determined experimentally to be 5 mv./sec. By using Eq. 1, the minimum permissible steady-state period, *i.e.*, the scan duration, was calculated to be approximately 3 min. However, because of normal diffusion in the flow system, the sample aspiration stage must be somewhat longer than the required steady-state period.

A study of the diffusional characteristics of the automated polarographic system revealed that 1 min. is required for the transition between successive steady states within the flow cell. This study was conducted by monitoring diffusion current *versus* time at an applied potential of −1.5 v. and observing the transients that resulted when an alternating series of lorazepam standards and empty sample cups were placed in the SOLIDprep sample tray. Thus, to supply the required 3 min. of steady state, a sample aspiration period of at least 4 min. is necessary.

The requirement for protracted aspiration imposes a severe limitation on the available assay rate of a conventional automatic analyzer system employing the SOLIDprep unit for sample dissolution. The required sample homogenation time and instrument design limitations restrict the sample aspiration stage of the SOLIDprep operating cycle to a maximum of 50% of the programmed cycle period. Therefore, with a 4-min. aspiration period, the minimum cycle duration is 8 min. This assay rate, *i.e.*, 7.5 samples/hr., would not have offered a significant advantage over manual polarographic procedures.

**Steady-State Extension**—The steady-state extension unit (Fig. 1) was developed to overcome the assay rate limitations encountered with conventional automated systems. This unit provides a twofold increase in assay rate with no loss of sensitivity or resolution.

Operationally, a peristaltic valve module is employed to permit selective collection and release of steady-state material. The valve is programmed to route the inlet sample stream to one of the storage coils, while the other coil provides sample to the outlet manifold. With each successive valve cycle, the inlet and outlet channels alternate and the flow directions in the storage coils reverse. The valve cycle is synchronized to coincide with the termination of a sample steady state at the extension unit inlet. The closing of an inlet channel causes the steady-state portion of the sample stream to be selectively retained at the upper end of the associated storage coil. The simultaneous opening of the coil's outlet channel releases this ma-

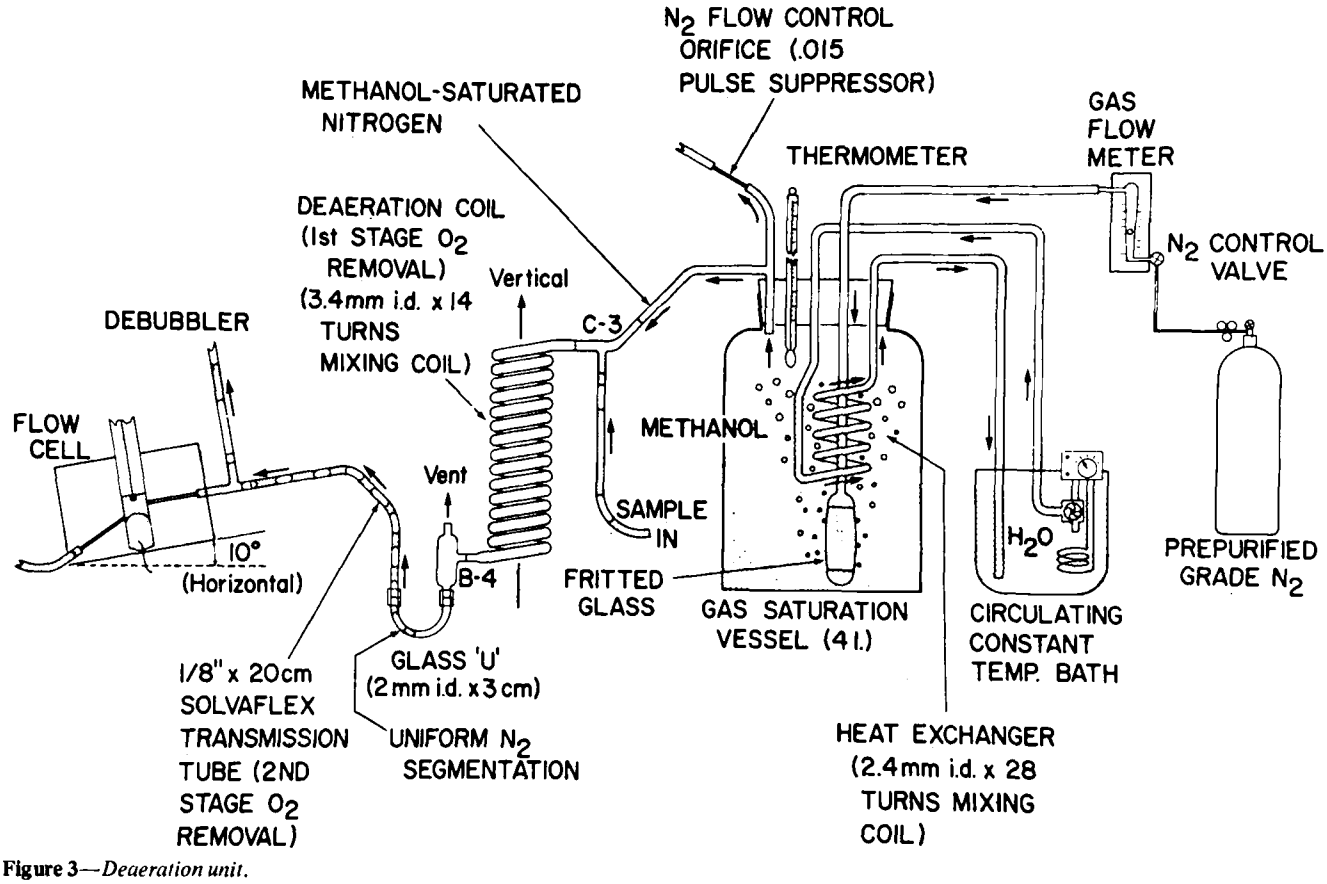


**Figure 3**—*Deaeration unit.*

terial to the extension unit's outlet manifold. The alternating collection and release of sample in conjunction with the inlet-outlet flow rate differential provide the desired steady-state extension. The required synchronization is accomplished through electrical interconnection of the valve's indexing switch with the programming mechanism of the SOLIDprep unit (Fig. 2).

A detailed description of the extension unit's operational principles, capabilities, and various applications will be published in the near future.

**Deaeration Unit**—For polarographic applications demanding high sensitivity and precision, it is essential to exclude dissolved oxygen from the electrode environment. This requirement is not easily satisfied in continuous-flow systems. Additionally, the low solubility of the model compound, lorazepam, in aqueous media complicated the deaeration problem by necessitating the use of a predominantly methanolic supporting electrolyte. Existing techniques for the continuous removal of oxygen from automatic analyzer streams (1, 3) and chromatographic column effluents (6–8) would not fulfill the requirements of the automated polarographic system. Generally, these methods are intended for use in aqueous solvent streams and are not designed to handle the exceptionally high oxygen solubility encountered in the supporting electrolyte of interest. Furthermore, the reported deaeration methods make no provisions to protect volatile solvents from excessive evaporation. Those techniques intended specifically for chromatopolarographic applications are not designed for use in segmented automatic analyzer streams and usually require the fabrication of sophisticated apparatus.

The continuous-flow deaerator (Fig. 3) was developed to meet the stringent performance criteria of this automated polarographic procedure. The apparatus consists entirely of standard automatic analyzer fittings and readily available laboratory equipment. The unit's effectiveness was evaluated at sample flow rates of 1–5 ml./min. for both air-segmented and nitrogen-segmented streams of supporting electrolyte. Within this range, oxygen removal was found to be essentially quantitative; *i.e.*, no detectable contribution from oxygen was observed in the residual current at a full-scale current sensitivity of 0.5 μamp. The nitrogen flow rate in each case was adjusted to yield uniform segmentation of the sample stream at the outlet of the deaerator, corresponding to nitrogen flow rates of 1–2 l./min. This high level of performance suggests that the deaerator will effectively remove oxygen from any common polarographic electrolyte. The unit's small hold-up volume, about 0.5 ml., and rapid sample transit, about 20 sec., minimize diffusional mixing in the flowing stream.

The volatile nature of the supporting electrolyte and the rapid nitrogen flow rates within the deaerator necessitated thermostatically controlled presaturation of the purging gas. The circulating constant-temperature bath is adjusted to maintain nitrogen temperatures at the saturation vessel outlet of 2 ± 1° above the ambient temperature of the laboratory.

**Instrument Synchronization**—The operation of the automated system depends upon precise synchronization of the steady-state extension unit and the polarographic analyzer with the flowing sample stream. To eliminate the possibility of synchronization-drift problems, which could result from the use of separate timing mechanisms, both the peristaltic valve module and the polarographic analyzer were interfaced with the programming mechanism of the SOLIDprep unit. The two cam-operated microswitches (Fig. 2) are accessory switches within the SOLIDprep programmer and were employed to provide common time-base control of all necessary synchronization signals.

The external circuit between terminals 5 and 12 on the accessory connector of the polarographic analyzer controls the scanning functions of the instrument. When this circuit is closed, the applied cell potential reverts to the selected initial value and the instrument is placed in a standby mode. Opening of the circuit initiates the voltage scan. The constant −15-v. signal available at terminal 12 is also employed to activate the relay connected to the remote control terminals, 13 and 14, of the recorder. Cam 10 is adjusted to initiate the voltage scan at the beginning of sample steady state within the polarographic cell. Cam 8 is adjusted to provide a momentary closure in the peristaltic valve's control circuit as the terminal portion of a sample steady state enters the extension unit's inlet manifold.

**Current-Sampled d.c. Polarography**—High polarographic resolution requires the use of an electrode drop rate that is rapid in comparison to the scan rate, *i.e.*, the individual drops are closely spaced along the polarogram's potential axis. Consequently, it is desirable to utilize a drop rate that is also more rapid than is commonly

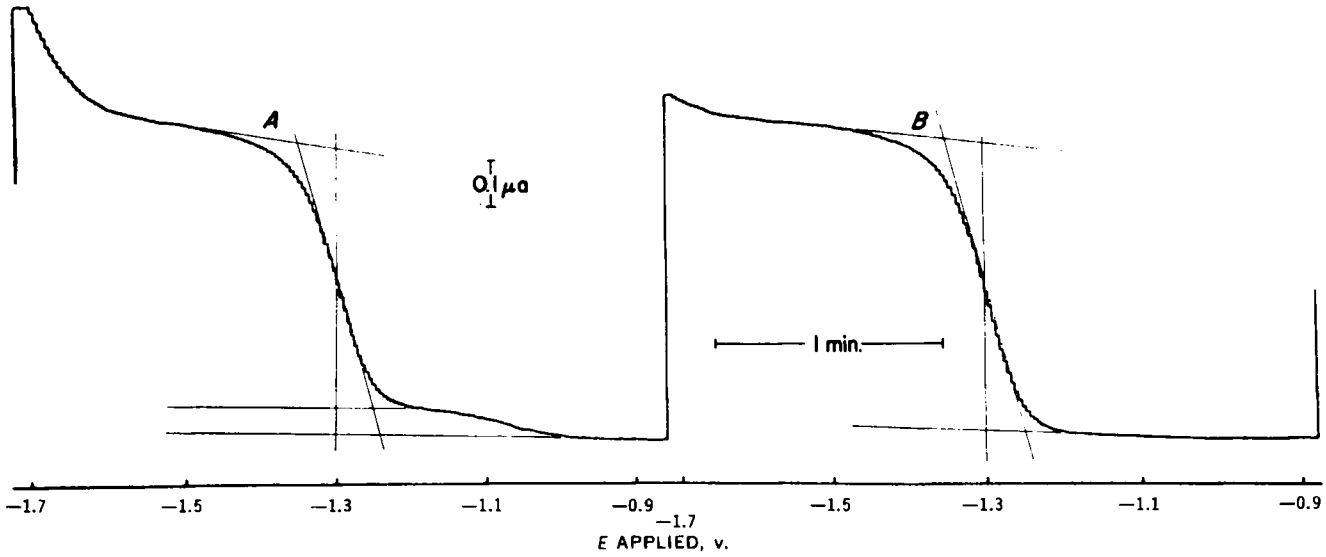


**Figure 4**—*Reproduction of lorazepam polarograms at the 2-mg. level and 15-sample/hr. assay rate. Key: A, intentionally degraded sample; and B, lorazepam reference standard.*

employed in manual procedures. However, dropping mercury electrodes that display suitably short natural drop periods at the electrocapillary maximum tend to become unstable at moderately negative applied potentials. For this reason, it is not practical to depend on natural drop-fall when operating at high drop rates (9).

To provide the required drop rate, this system employs an electrode with a long natural drop period (2.5 sec.) in conjunction with a mechanical drop timer which induces drop separation at precisely timed, selected intervals of 1 sec. Just prior to the mechanical removal of the drop, the current flowing in the cell is sampled electronically. An output proportional to this current is stored and presented to the recorder until the cell is again sampled electronically 1 sec. later, just prior to removal of the next mercury drop. When using this current-sampled d.c. mode, a peak-current polarogram is obtained in which all drop-induced fluctuations are removed electronically without introducing the waveform distortions characteristic of damping circuits. This polarographic mode is particularly well suited to automated applications since the smooth, well-defined waveforms may be rapidly and easily interpreted.

Typical polarograms of intact and intentionally degraded lorazepam, generated by the automated system at an assay rate of 15 samples/hr., are reproduced in Fig. 4. The fine definition obtained with the current-sampled d.c. presentation is apparent. The quiet residual current baseline is indicative of the low noise level achieved in the system. In the polarogram of the thermally degraded sample of lorazepam, the wave associated with the decomposition product appears at an $E_{1/2}$ of −1.1 v. and the wave at $E_{1/2}$ of −1.3 v. corresponds to the intact material. The excellent separation between these waves permits precise quantitation, by the established extrapolation technique described by Meites (9), of intact lorazepam.

**Suitability as Analytical Method**—Specificity of the method for analysis of the model compound, lorazepam, in the presence of its thermal degradation products was demonstrated by comparing analytical values of intentionally degraded samples to those obtained by quantitative TLC. In the TLC procedure, the lorazepam was separated from its degradation products on a silica gel chromatographic plate using a development solvent system of benzene–dioxane–ethanol (5:4.4:0.6). A zone of the silica gel encompassing the intact lorazepam spot was scraped from the plate. The lorazepam was eluted from the adsorbent with 95% ethanol and subsequently assayed by a UV spectrophotometric procedure to obtain a quantitative value. Since there is good agreement between the values by the two techniques (Table I), it is concluded that the automated polarographic procedure is stability indicating.

The linearity of the diffusion current–concentration relationship

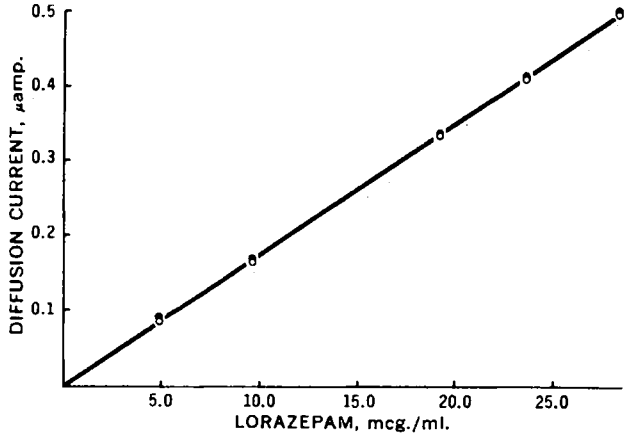


**Figure 5**—*Relationship of diffusion current to lorazepam concentration.*

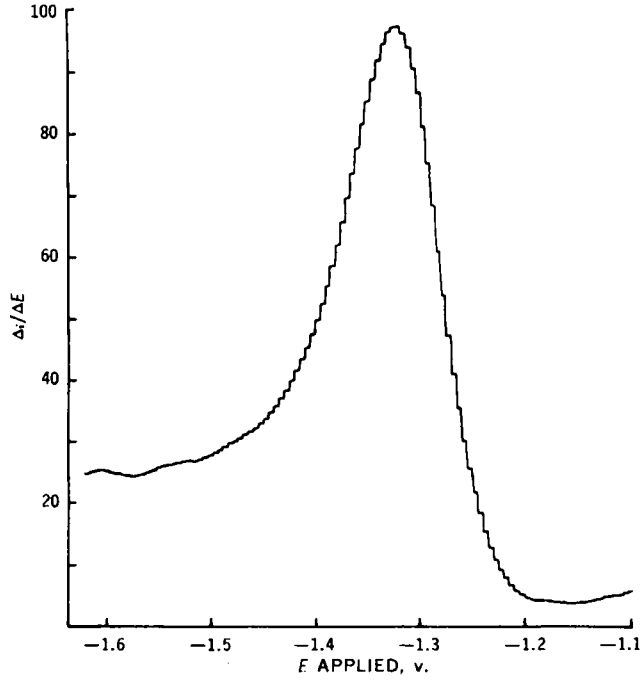


**Figure 6**—*Reproduction of a constant-amplitude differential pulse polarogram of lorazepam at the 2-mg. level and 15-sample/hr. assay rate.*

was shown by analyzing standards of lorazepam, yielding concentrations in the homogenization vessel in the range of 5–30 mcg./ml. ($1.5–9.5 \times 10^{-5}$ *M*) (Fig. 5). This concentration range corresponds to lorazepam levels of 0.25–6.0 mg. in the finished product, with the SOLIDprep unit delivering a fixed volume between 50 and 200 ml. of diluent. The actual measured lower limit of sensitivity is 0.05 mg./sample, corresponding to $3 \times 10^{-6}$ *M* in the flow cell.

Both the precision and accuracy of this technique were determined by adding known quantities of lorazepam in the 0.5–2.0-mg. range to tablet excipient mixtures and measuring the percentages recovered. Twelve replicate assays at the 0.5-, 1.0-, and 2.0-mg. lorazepam/tablet levels produced relative standard deviations of ±1.4, ±1.4, and ±1.2%, respectively, with recoveries of 99% of the theoretical amount present in all three cases.

The effect of common inert tablet components on this automated polarographic procedure when applied to lorazepam was investigated to uncover any possible interfering material. In this study, inactive component–lorazepam ratios of 200:1 for lactose, 100:1 for talc and microcrystalline cellulose[9], and 50:1 for calcium sulfate, magnesium stearate, stearic acid, potassium polacrilin[10], methylcellulose[11], and starch were evaluated. No interference was experienced from these materials at these levels.

**Further Applications**—Preliminary investigations indicate that the automated method is readily adaptable to a wide variety of polarographic assay procedures. The use of a multifunctional polarographic analyzer permits the system to be operated in other polarographic modes. A constant-amplitude differential pulse polarogram of lorazepam obtained by the automated technique is presented in Fig. 6. This polarographic mode of operation offers increased sensitivity and expands method capabilities by permitting the resolution of closely spaced polarographic waves.

[9] Avicel.
[10] Amberlite IRP-88.
[11] Methocel.

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# Simultaneous Semiautomated Determination of Pentaerythritol Tetranitrate or Mannitol Hexanitrate and Phenobarbital in Tablets


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**Abstract** □ A semiautomated spectrophotometric method for the simultaneous determination of pentaerythritol tetranitrate or mannitol hexanitrate and phenobarbital in single tablets is described. The organic nitrate ester component is assayed by a colorimetric procedure involving the diazotization of *p*-chloroaniline with nitrite formed by alkaline hydrolysis with tetramethylammonium hydroxide and coupling of the resultant compound with *N*-1-naphthylethylenediamine. The intensity of the color is measured at 570 nm. for pentaerythritol tetranitrate and at 613 nm. for mannitol hexanitrate. Phenobarbital is determined by UV absorption at 241 nm. after extraction into chloroform followed by extraction into aqueous base. The effect of one component on the assay results of the other is reported. Results from the semiautomated method are in agreement within ±3% with those from USP and NF methods. The coefficients of variation for the semiautomated procedure are 1.60, 0.68, and 1.24% for pentaerythritol tetranitrate, mannitol hexanitrate, and phenobarbital, respectively.



**Keyphrases** □ Pentaerythritol tetranitrate or mannitol hexanitrate and phenobarbital tablets—simultaneous spectrophotometric analysis □ Mannitol hexanitrate or pentaerythritol tetranitrate and phenobarbital tablets—simultaneous spectrophotometric analysis □ Phenobarbital and pentaerythritol tetranitrate or mannitol hexanitrate tablets—simultaneous spectrophotometric analysis □ Colorimetry—analysis, pentaerythritol tetranitrate or mannitol hexanitrate in tablets with phenobarbital □ UV spectrophotometry—analysis, phenobarbital in tablets with pentaerythritol tetranitrate or mannitol hexanitrate


Pentaerythritol tetranitrate and mannitol hexanitrate are both organic nitrate esters believed capable of coronary dilation. Their onset of action is slower and their duration much longer than nitroglycerin. They are used therapeutically in the prophylaxis of attacks of angina pectoris.

Phenobarbital, due to its sedative effect, has also been shown to have value in the prevention of angina pectoris attacks. It is not uncommon, therefore, to find pharmaceutical preparations containing both phenobarbital and one of the organic nitrate drugs as active ingredients. The purpose of this study was to find a suitable