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Polarographic Assay of Glyceryl Trinitrate Sublingual Tablets for Content Uniformity

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Abstract □ A polarographic method of assaying glyceryl trinitrate in sublingual tablets is presented. The method is direct, rapid, and free from interference by nitrate and nitrite ions. Its sensitivity is sufficient to permit the analysis of single tablets with a precision of ±1%. Polarographic analysis of pharmaceutical preparations gives results comparable to but more precise than those obtained by the current BP and USP methods.

Keyphrases □ Glyceryl trinitrate sublingual tablets—analytic method □ Polarography—analysis □ Analyses, comparison—glyceryl trinitrate tablets □ Colorimetric analysis—spectrophotometer □ IR spectrophotometry—analysis

Current methods of assaying glyceryl trinitrate in pharmaceutical preparations are based on the following techniques: reduction of nitrogen to ammonia determined subsequently by titration (1); IR spectrophotometry (2); acid hydrolysis to nitrate ion and subsequent spectrophotometric determination of nitrated phenoldisulfonic acid (3); and alkaline hydrolysis to nitrite ion followed by diazotization and spectrophotometric determination (4). The first two techniques require 5 mg. of glyceryl trinitrate per determination and hence are unsuitable for the analysis of single sublingual tablets, which usually contain 0.3–0.6 mg. of drug. The nitration method is indirect and subject to interference from nitrate ion. The diazotization method is likewise indirect, and subject to interference from nitrite ion.

In order to overcome these shortcomings, a polarographic method was developed. The reduction of nitrate esters at the dropping mercury electrode has been studied by several authors (5–7). In aqueous ethanolic solution a well-developed single wave, independent of pH in the range of 3 to 13, was observed. The products of the reduction were the parent alcohol and nitrite ion. It was deduced that the reduction was diffusion-controlled and irreversible with two electrons being consumed with each nitrate group.

EXPERIMENTAL

Reference Standard—USP glyceryl trinitrate reference standard was used. Each 100 mg. of standard was labeled to contain 9.25 mg. of glyceryl trinitrate in a diluent of lactose.

Polarographic Solvent—Eight hundred milliliters of 2-propanol was mixed with 100 ml. of 1.0 *N* tetramethylammonium chloride and 100 ml. alkaline buffer (0.10 *N* in NH_4Cl and NH_4OH). The polarogram of the solvent, recorded daily under conditions analogous to those of the samples, was examined for waves due to impurities. This polarogram subsequently served as the blank (Fig. 1).

Apparatus—A polarograph[1] with synchronous drop controller[2] was used for all polarographic determinations. Measurements of potential were obtained with a silver/silver chloride electrode and then expressed relative to the saturated calomel electrode (8). The solution in the salt bridge was replaced daily. Unless otherwise noted, the following polarographic parameters were used: drop time, 0.2 sec; temperature, 34.8 ± 0.1°; scan speed, 0.4 v./min.; scan range, 0.0 to −2.0 v.; sensitivity, 5×10^{-8} A/mm.; and damping, nil. Currents were measured at the midpoint of the oscillations.

A spectrophotometer[3] was used to measure absorbance in the visible range. A spectrometer[4] was used in the IR range. A conductivity bridge[5] was used to determine conductivities and a meter[6] to obtain pH measurements.

Method of Assay—*Single Tablets*—Place a tablet into the polarographic cell and powder carefully with a glass rod. Add 10.0 ml. of solvent for each 0.6 mg. of glyceryl trinitrate. Thoroughly mix for 30 sec. Remove the glass rod, add a small magnetic stirring bar, and couple the cell to the polarograph. Deoxygenate the sample mixture with pure nitrogen (saturated at room temperature with solvent) while stirring for a period of 25 min. (Stirring must be sufficiently vigorous to maintain the solid phase in motion.) Record the polarogram from 0.0 to −2.0 v.

Transfer an accurately weighed quantity of the reference standard equivalent to approximately 6 mg. of glyceryl trinitrate to a 100-ml. volumetric flask. Make up to volume with solvent, add a magnetic stirring bar, and agitate (with occasional inversion) for 20 min. Transfer an aliquot (of the same volume as that used for the analysis of the sample) to the polarographic cell, add a small stirring bar, and

[1] Metrohm model E261, Herisou, Switzerland.
[2] Metrohm model E354.
[3] Beckman model DU-2, Beckman Instruments, Inc., Fullerton, Calif.
[4] Perkin-Elmer model 221, Norwalk, Conn.
[5] Industrial Instruments model RC-16B, Beckman Instruments, Inc.
[6] Metrohm model E300.

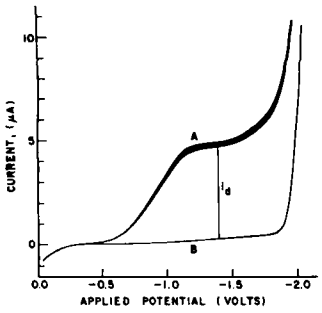

Figure 1—*Polarogram of glyceryl trinitrate and determination of diffusion current* (i_d). *Curve* A, *glyceryl trinitrate, 0.688 mg./10 ml.; Curve* B, *pure solvent.*

couple the cell to the polarograph. Deoxygenate with stirring as indicated above. Record the polarogram.

Determine the diffusion currents at −1.39 v. saturated calomel electrode (S.C.E.) (Fig. 1) and calculate the weight of glyceryl trinitrate per tablet.

Composite Samples—Weigh and finely powder not less than 20 glyceryl trinitrate tablets. Weigh accurately a portion of the powder, equivalent to about 0.06 mg. glyceryl trinitrate/ml. of cell volume, and transfer to the polarographic cell. Proceed as outlined for single tablets. Weigh, and assay several additional portions.

Comparison of Analytical Procedures—Three commercial preparations of sublingual glyceryl trinitrate tablets were chosen for comparative assay by polarography, the USP procedure (2), and the BP procedure (3). Composite assays were done by each method and for each preparation. In addition, 10 single tablets were analyzed by the polarographic procedure.

For the composite assays of each preparation, sufficient tablets to contain at least 50 mg. glyceryl trinitrate were weighed and finely ground. The powder was stored in the dark in glass-stoppered bottles. Weighed aliquots (containing 5 mg. of drug) of each of the powdered samples were analyzed during each of three successive weeks by each of the three analytical procedures. Concurrently two calibration values were obtained for each method with the aid of the reference standard. In the polarographic procedure, the aliquots were added to the polarographic cell followed by 50 ml. of solvent and a stirring bar. The BP procedure was modified by using the USP reference standard (in place of KNO_3) to facilitate the comparison of the results with those of the other methods. Due to the large sample size, 25 ml. glacial acetic acid was used instead of 5 ml. as specified in the BP method. Measurements were made at 405 mμ.

RESULTS AND DISCUSSION

Solvent and Cell Characteristics—The apparent pH of the solvent was found to be 8.7 at 25° and its specific conductance 0.0015 ohms/cm. at 35° and 1,000 c.p.s. The resistance of the polarographic cell was found to be approximately 1,500 ohms. This value produces a negligible error in the measurement of diffusion currents. The polarogram of the solvent did not exhibit any waves other than those due to the electrolyte and the buffer (Curve B of Fig. 1).

Table I—Diffusion Current/Concentration Ratio and Half-Wave Potential as Functions of Concentration of Glyceryl Trinitrate

Concn. of Glyceryl Trinitrate, mg./ml.	Mercury Dropping Time and Respective Diffusion Current and Half-Wave Potential: t = 0.2 sec.[a] R,[c] μamp./(mg./ml.)	$E_{0.5}$, v. *versus* S.C.E.	t = 2.1 sec.[a, b] R,[c] μamp./(mg./ml.)	$E_{0.5}$, v. *versus* S.C.E.
0.00708	51	−0.90	—	—
0.01132	50	−0.90	84	−0.80
0.02123	52	−0.91	—	—
0.02867	53.5	−0.91	91.4	−0.80
0.05035	53.6	−0.90	92.2	−0.79
0.0708	54.8	−0.91	—	—
0.1398	54.3	−0.93	94.2	−0.79
0.1415	55.0	−0.93	—	—
0.2123	55.1	−0.93	—	—
0.2323	55.3	−0.92	94.2	−0.81

[a] Maximum suppressor, 0%. [b] t = 2.13 sec. and m = 2.77 mg./sec. measured at −1.39 v. (S.C.E.). Dampening, six units. [c] R, the diffusion current/concentration ratio at −1.39 v.

Table II—Reproducibility of the Diffusion Current/Concentration Ratio for Glyceryl Trinitrate[a]

Week	R,[b] μamp./(mg./ml.)	$E_{0.5}$, v. *versus* S.C.E.
1	57.8	−0.90
2	58.2	−0.91
3	58.2	−0.93
4	59.4	−0.92
5	57.0	−0.91
6	58.1	−0.91
7	58.2	−0.91

[a] Concentration of glyceryl trinitrate, 0.047 mg./ml. [b] Drop time, 0.2 sec., at −1.39 v. (The values of R differ from those of Table I due to a change in capillaries.)

Linearity of the Calibration Curve—Whitnack *et al.* (6) observed a virtually linear relationship between the concentration of glyceryl trinitrate and the diffusion current in a similar solvent system. The data of Table I show that over a wide range, the calibration curve is not quite linear; however, when the samples and standards are of the same approximate concentration, this effect is negligible. Table II indicates a satisfactory week-to-week reproducibility of the ratio of the current to concentration. The relative standard deviation was 1.3%.

Half-Wave Potential—Whitnack *et al.* (6) found that the half-wave potential, $E_{0.5}$ remained virtually constant in buffered solutions with apparent pH in the range 4.0 to 11.1. Similarly, with a series of buffered solutions of apparent pH 9, they found $E_{0.5}$ to be virtually independent of concentration; however, when the solvent was unbuffered or quite basic (pH 12.8), $E_{0.5}$ had a more positive value.

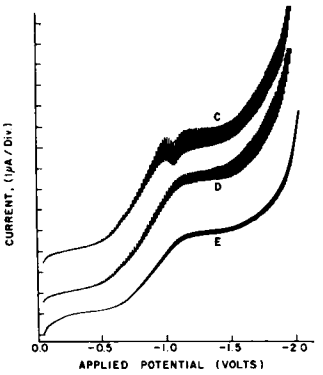

Figure 2—*Effect of alkyl phenoxy polyethoxy ethanol (surfactant) and drop time on the polarogram of glyceryl trinitrate (0.555 mg./10 ml.). Curve* C, *drop time, 2.6 sec.; surfactant, 0%. Curve* D, *drop time, 2.6 sec.; surfactant, 0.002%. Curve* E, *drop time, 0.2 sec.; surfactant, 0%.*

In the present study, as shown in Table I, no significant change in $E_{0.5}$ was observed with changing concentration. With a forced drop time of 0.2 sec., $E_{0.5}$ was found to be 0.1 v. more negative than it was with a natural drop time of 2.1 sec.

Maxima and Their Suppression—As shown by Curve C of Fig. 2, the polarogram of glyceryl trinitrate obtained with a drop time of 2.6 sec. exhibits a maximum at −1.0 v. On the other hand, as shown by Curves D and E, the polarogram obtained either with 0.002% alkyl phenoxy polyethoxy ethanol[7] in the solvent, or with a drop time of 0.2 sec., exhibited no maximum. Gelatin and methyl red (0.002%) also suppressed the maximum but the low solubility of gelatin in alcoholic solutions and the reducibility of methyl red make the use of these substances less desirable. As can be seen from Figs. 1 and 2, the diffusion current can be measured over a wider potential range if maxima are not present. Hence a short drop time or the presence of alkyl phenoxy polyethoxy ethanol is desirable.

Interference from Excipients—The solvent and operating temperature were chosen in order to insure a high solubility for glyceryl

[7] Triton X-100, Rohm & Haas, Philadelphia, Pa.

Table III—Effect of Excipients on the Diffusion Current/Concentration Ratio for Glyceryl Trinitrate[a]

Lactose		Mannitol		Cornstarch	
Amt., mg./ml.[b]	R,[c] μamp./ (mg./ml.)	Amt., mg./ml.	R, μamp./ (mg./ml.)	Amt., mg./ml.	R, μamp./ (mg./ml.)
0.4	59.4	0.0	57.0	0.0	58.1
16.4	59.4	4.1	56.7	20.3	59.4
20.8	59.2	11.1	56.9	31.1	60.2
25.4	59.4	20.0	56.9	38.2	60.2
—	—	26.8	56.7	—	—

[a] Samples contained 0.047 mg./ml. glyceryl trinitrate and 0.4 mg./ml. dissolved lactose with additional excipient (which apparently remained undissolved) added as indicated. [b] This amount includes the lactose originating as diluent in the reference standard. [c] Drop time, 0.2 sec., at −1.39 v.

Table IV—Relative Increase in Diffusion Current Resulting from the Presence of Sodium Nitrite

% by Wt. of $NaNO_2$ Relative to Glyceryl Trinitrate[a]	% Increase in Diffusion Current at Several Applied Potentials,[b] −1.34 v.	−1.39 v.	−1.44 v.
58	0.2	0.4	0.8
350	1	3	5
1250	2	5	10

[a] Concentration of glyceryl trinitrate, 0.047 mg./ml. [b] Drop time, 0.2 sec.

Table V—Assay of Glyceryl Trinitrate Tablets

Brand[a]	Polarographic Assays of 10 Individual Tablets, % Mean[b]	SD[c]	Polarographic[b]	Composite Assays, % USP XVII[b]	BP, 1963[b]
X	105	3	104, 103, 104 (mean 104)	103, 110, 91 (mean 101)	100, 95, 104 (mean 100)
Y	109	1.2	106, 106, 104 (mean 105)	117, 100, 105 (mean 107)	109, 104, 103 (mean 105)
Z	75	4	70, 72, 70 (mean 71)	84, 61, 78 (mean 74)	67, 60, 60 (mean 62)

[a] The pharmacopeial designation and label claim of each were: Brand X(BP), 0.6 mg./tablet; Brand Y (BP), 0.5 mg./tablet; and Brand Z (USP) 0.32 mg./tablet. [b] In terms of label claim. [c] In terms of amount present. (The standard deviation of the mean equals the standard deviation of the individual assays/$\sqrt{10}$.)

trinitrate while maintaining a suitable electrical conductivity. Since most tablet excipients are insoluble in this solvent, the excipients are unlikely to alter the solvent's polarographic properties. The presence of undissolved excipient in the cell had no apparent effect.

Table III shows the effect of three common excipients on the diffusion current/concentration ratio for glyceryl trinitrate. The ratio is unaffected by the presence of lactose or mannitol, but increases by 1% for each 10 mg. of cornstarch/ml. (The polarogram of the solvent was unaffected by the addition of cornstarch.) Since sublingual tablets of glyceryl trinitrate generally yield a solution containing much less than 10 mg./ml. cornstarch, this effect is considered negligible.

Nitrate and Nitrite—Nitrate and nitrite ions may be present in sublingual tablets as impurities and decomposition products. No change in diffusion current (drop time, 0.2 sec.) was produced by the addition of nitrate (2.4 mg. $LiNO_3$/ml.) to a standard solution of glyceryl trinitrate (0.05 mg./ml.). As shown in Table IV, nitrite will not interfere with the assay if its concentration is less than that of glyceryl trinitrate.

Analytical Results—The results of the polarographic analysis of 10 single tablets of each of the three brands are shown in Table V along with those of the composite assays by the polarographic, USP, and BP methods. Although good agreement is apparent between means obtained by each analytical procedure with each formulation, superior reproducibility was achieved with the polarographic method. Undoubtedly, greater reproducibility could be achieved by the USP and BP procedures with more frequent usage, but the many manipulations required for these methods, in comparison with the polarographic method, makes them more susceptible to error. Moreover, each polarographic determination requires only 15 min. of the analyst's time (assuming dissolution and deoxygenation do not represent lost time). Where measurements at a single potential are sufficient, the time required may be as short as 5 min.

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