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ACKNOWLEDGMENTS AND ADDRESSES

Received January 14, 1975, from the *School of Pharmacy, University of Montana, Missoula, MT 59801*

Accepted for publication April 29, 1975.

Supported in part by the U.S. National Science Foundation under the SFC, Grants GF 38851 and GF 39207.

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Polarographic Determination of Isosorbide Dinitrate

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Abstract □ Isosorbide dinitrate and two isomeric isosorbide mononitrates are shown to be polarographically reducible in aqueous sodium perchlorate or potassium chloride solution. The current, measured at -1.6 v, versus the saturated calomel electrode is proportional to the concentration of organic nitrate in the 10–100- μ g/ml range. Inorganic nitrate produces no interference. However, the mononitrate isomers produce an additive response to isosorbide dinitrate and cannot be determined individually. The polarographic method is applicable to single-tablet assay for content uniformity determination, with precision and accuracy comparable to automated colorimetric analysis. Comparison is made between replicate analyses obtained polarographically and by IR and automated colorimetric analysis for six different commercially available formulations. The polarographic determination is sensitive, specific for nitrate esters, precise, requires little sample preparation, and utilizes relatively inexpensive apparatus.

Keyphrases □ Isosorbide dinitrate—polarographic analysis, compared to IR and automated colorimetric methods, six commercial formulations □ Polarography—analysis, isosorbide dinitrate, compared to IR and automated colorimetric methods, six commercial formulations

Since the discovery that organic nitrate esters are reducible at the dropping mercury electrode, several polarographic methods have been developed for such pharmaceutically important compounds as nitroglycerin and pentaerythritol tetranitrate. Nitrate esters are reduced polarographically in a two-electron, pH-independent process to yield nitrite ion and the corresponding alcohol (1). The polarographic determination of nitroglycerin and other polynitrate esters, including pentaerythritol tetranitrate, was reported (2, 3).

Polarographic procedures have several advantages over other procedures for determining nitrate esters of pharmaceutical interest. The principal advantages are sensitivity, specificity, speed of analysis, and the moderate cost of the apparatus. Samples generally can be analyzed without prior separation or other sample preparation, since excipients usually do not interfere. This procedure results in a considerable saving in time, particularly when large numbers of single tablets must be analyzed to establish content uniformity.

For example, a procedure was developed for determining content uniformity for nitroglycerin sub-

Table I—Current–Concentration Relationships for Isosorbide Dinitrate^a

Concentration, μ g/ml of Dinitrate	Current, μ amp
10	0.92
20	1.66
30	2.46
40	3.44
50	4.26
60	4.94
70	5.80
80	6.82
90	7.53
100	8.33

^aMicrograms per milliliter of dinitrate = $12.0 \times \mu\text{amp} - 0.41$, and $r = 0.9995$.

lingual tablets in which single tablets are introduced into the polarographic cell and are analyzed in the presence of tablet excipients such as lactose, mannitol, and corn starch (4). Lactose and mannitol had no effect on the polarographic reduction. Although corn starch produced a slight increase in the current–concentration ratio, this increase was negligible at concentrations normally found in sublingual tablets.

There are several procedures for analyzing isosorbide dinitrate (I). A colorimetric procedure was described based on the hydrolysis of the nitrate ester and the determination of the nitrate ion produced using *vic-m*-xylenol (5). In a study of the dissolution rates of isosorbide dinitrate tablets, a UV absorption spectrophotometric method was utilized for assay

Table II—Current–Concentration Relationship for the Two Isosorbide Mononitrates

Micrograms per Milliliter of Mononitrate	Equivalent Concentration of Dinitrate, μ g/ml	Current for <i>exo</i> -Mononitrate ^a , μ amp	Current for <i>endo</i> -Mononitrate ^b , μ amp
16.2	10	0.96	0.95
32.4	20	1.78	1.75
48.6	30	2.72	2.64
64.8	40	3.53	3.45
81.0	50	4.37	4.34

^aMicrograms per milliliter of *exo*-mononitrate = $18.89 \times \mu\text{amp} - 1.88$, and $r = 0.9997$. ^bMicrograms per milliliter of *endo*-mononitrate = $19.12 \times \mu\text{amp} - 1.65$, and $r = 0.9999$.

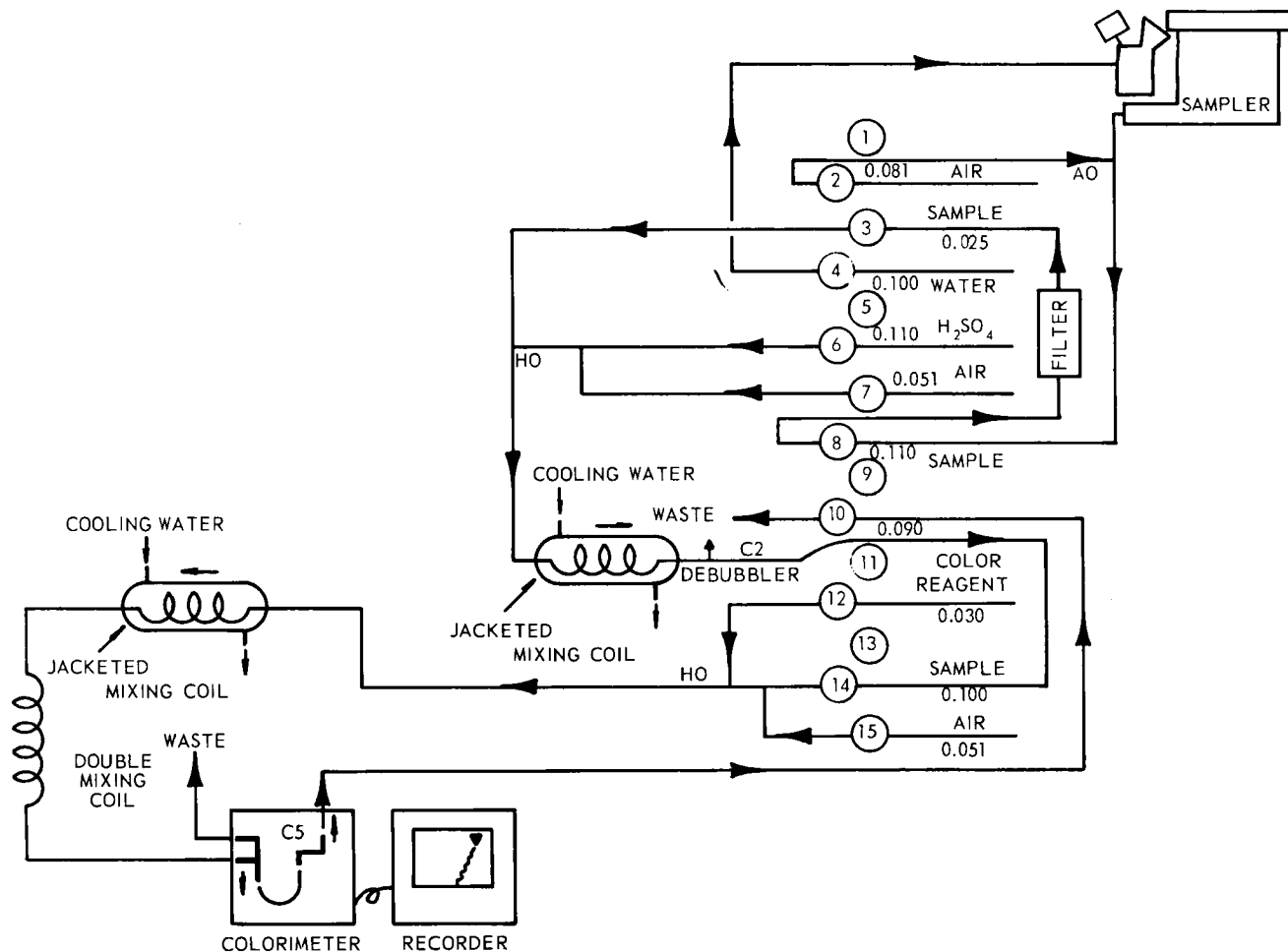


Figure 1—Automated analyzer flow diagram for determining nitrate content of single tablets. The H_2SO_4 is 85% (v/v) sulfuric acid, and the color reagent is 4 g of *vic-m*-xylenol in 100 ml of acetic acid and 100 ml of sulfuric acid. Absorbance is measured at 498 nm with a 50-mm tubular flowcell. Tubing sizes and fittings are given.

purposes (6). Excellent agreement was found between results obtained by IR analysis and colorimetric analysis for both synthetic mixtures and commercial 5- and 10-mg tablets (7). Several GLC procedures have been described for the analysis of nitrate esters (8–12) including isosorbide dinitrate. Recently, isosorbide dinitrate in tablets was analyzed using a polarographic procedure (13).

This article discusses the polarographic behavior of isosorbide dinitrate and the two isosorbide mononitrate esters. Isosorbide dinitrate can be determined polarographically in various dosage forms with comparable precision to that obtained by IR analysis and automated analysis methods, particularly in the single-tablet assays required for content uniformity.

EXPERIMENTAL

Polarographic Analysis—Analyses were performed using a polarograph¹ with jacketed cells maintained at $25 \pm 0.1^\circ$. The supporting electrolyte consisted of 0.1 M $NaClO_4$ ², and 0.005% gelatin was used as a maximum suppressor. Sodium perchlorate was chosen for the initial polarographic studies because the wave for organic nitrates begins to form at around -0.1 v and by using perchlorate ion one can work at more positive potentials without in-

terfering anodic waves. Dissolved oxygen was removed by vigorously bubbling nitrogen through the sample in the cell for approximately 5 min.

For bulk assays and single-tablet assays, potassium chloride was substituted for sodium perchlorate for reasons of convenience and economy. The whole tablet was dissolved (or dispersed) in approximately 100 ml of 0.1 N KCl using a blender, and the solution was diluted to 125 ml with the electrolyte solution. The current, measured at -1.6 v, versus the saturated calomel electrode (SCE) was proportional to the concentration of organic nitrate. The concentration was calculated from a calibration curve prepared using pure isosorbide dinitrate or by comparison with a single standard.

Automated Analysis—The flow diagram³ for assaying single tablets is shown schematically in Fig. 1. The tablet was disintegrated in a solid sampler, with the water (70 – 80°) diluent delivered at a rate to give 0.05 mg/ml of organic nitrate. A water sample was required as a wash between standards and samples to reduce carryover. The aqueous suspension was then filtered, and the filtrate was reacted with 85% (v/v) sulfuric acid to hydrolyze the organic nitrate to inorganic nitrate.

Acid-resistant tubing was used for the delivery of sulfuric acid and the color reagent as well as for the sample stream after hydrolysis and color development. After about 16 hr of operation, the tubing had to be replaced. The inorganic nitrate was then reacted with *vic-m*-xylenol⁴ reagent (4 g of *vic-m*-xylenol in 100 ml of acetic acid and 100 ml of sulfuric acid). After cooling and mixing, the yellow color was measured photometrically using a 498-nm filter.

IR Analysis—Because the IR analysis is not as sensitive as ei-

¹ Metrohm Polarecord E261, Brinkmann Instruments.

² Fisher Scientific Co., Pittsburgh, Pa.

³ AutoAnalyzer, Technicon Instruments Corp.

⁴ Eastman Organic Chemicals, Rochester, N.Y.

Table III—Analysis of Synthetic Mixtures of Isosorbide Dinitrate, the Two Mononitrates, and Potassium Nitrate by Polarographic Analyses

Isosorbide Dinitrate, mg	<i>exo</i> -Isosorbide Mononitrate, mg	<i>endo</i> -Isosorbide Mononitrate, mg	Potassium Nitrate, mg	Dinitrate Calculated, mg	Dinitrate Found, mg
10	—	—	8.57 ^a	10	9.86
5	8.10 ^b	—	—	10	10.1
5	—	8.10	—	10	10.2
—	8.10	8.10	—	10	10.4
5	8.10	8.10	4.28 ^b	10	14.9

^a Equivalent to 10 mg of dinitrate in nitrate content. ^b Equivalent to 5 mg of dinitrate in nitrate content.

Table IV—Comparison of Polarographic (POL) and IR Methods for Isosorbide Dinitrate Representative Portions of Powdered Tablets^a

	2.5 mg Sublingual		5 mg Sublingual		5 mg Oral		10 mg Oral		10 mg Oral with Phenobarbital	
	POL	IR	POL	IR	POL	IR	POL	IR	POL	IR
	2.48	2.55	5.01	5.00	5.18	4.99	10.3	10.2	9.94	9.95
	2.57	2.53	4.87	4.83	5.01	5.08	10.3	10.3	10.1	9.90
	2.53	2.45	5.08	4.88	5.11	4.87	10.4	10.2	9.97	9.95
	2.51	2.51	4.93	4.95	5.08	4.91	10.2	10.2	10.1	10.1
	2.51	2.45	4.97	5.00	5.09	4.91	10.5	10.3	10.0	10.0
	2.46	2.46	4.93	4.97	5.11	4.99	10.2	10.4	9.95	9.90
	2.55	2.51	5.03	4.93	5.15	5.03	10.3	10.5	10.0	10.0
	2.51	2.51	5.00	4.82	5.03	5.00	10.3	10.3	10.1	9.95
	2.48	2.45	4.89	4.79	5.12	4.89	10.5	10.2	9.97	9.95
	2.51	2.47	5.01	4.93	5.08	4.99	10.3	10.4	9.95	10.1
Mean	2.51	2.49	4.97	4.91	5.10	4.97	10.3	10.3	10.0	9.98
SD	0.033	0.037	0.066	0.076	0.051	0.067	0.106	0.105	0.066	0.071
RSD, %	1.3	1.5	1.3	1.5	1.0	1.3	1.0	1.0	0.66	0.71

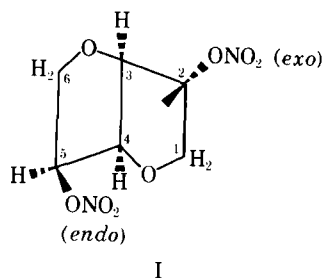
^a Results expressed as milligrams per average tablet weight.

ther the polarographic or automated method, sufficient tablets to yield approximately 70 mg of isosorbide dinitrate were needed. The tablets were pulverized and extracted with chloroform. After filtering and concentrating, an IR spectrum⁵ was run on the chloroform extract. The net absorbance at 1650 cm⁻¹ is proportional to isosorbide dinitrate concentration and was compared with similarly prepared standards. Phenobarbital, if present, does not interfere with the analysis.

Standards—Isosorbide dinitrate⁶, used for reference purposes and for the investigation of polarographic behavior, was purified by recrystallization from an aqueous ethanol solution. Its purity was established by elemental and TLC analyses.

Isosorbide mononitrates were prepared by the alkaline hydrolysis of the dinitrate. The two isomers were separated by either preparative liquid chromatography or countercurrent extraction and recrystallization from an ethanol-ethyl acetate solution. Isosorbide dinitrate and the 2- and 5-isosorbide mononitrates were well separated on silica gel G plates⁷, using trichloroethylene-acetone (4:1), and were detected with diphenylamine-sulfuric acid spray reagent. This reagent produces an intense blue color with nitrates and other oxidizing substances.

The pharmaceutical dosage forms analyzed were actual production lots of commercially available formulations⁶.



⁵ Beckman IR-8 spectrophotometer.

⁶ Stuart Pharmaceutical Division, ICI United States, Inc., Wilmington, Del.

⁷ Analtech Inc., Newark, Del.

RESULTS AND DISCUSSION

As shown in Fig. 2, isosorbide dinitrate is reduced at the dropping mercury electrode, producing a rather broad polarogram with a half-wave potential of -0.45 v versus the SCE. Since isosorbide dinitrate contains two nonequivalent nitrate ester groups at the 2- (*exo*) and 5- (*endo*) positions, the broad shape of the wave is probably due to the overlap of two waves arising from the reduction of each of the two nonequivalent groups. The polarographic behavior of the 2- and 5-isosorbide mononitrate esters tends to confirm this theory since the former has a half-wave potential of -0.38 v and the latter has a half-wave potential of -0.46 v. It is consistent that the *exo*-isomer should be more easily reduced than the sterically hindered *endo*-isomer.

The current plateau in the region from -0.8 to -1.8 v provides a large potential range in which the current is independent of voltage and proportional to the concentration of isosorbide dinitrate. For analytical work, the current measurement was normally made at -1.6 v. The current-concentration relationship for isosorbide dinitrate in the range of 10–100 μ g/ml is given in Table I. For comparison, similar data were obtained for the mononitrate esters, and one can see from Table II that the current values for the mononitrates correspond with those for equivalent amounts of the dinitrate ester. The equations given at the bottom of each table are calculated by the least-squares method. The correlation coeffi-

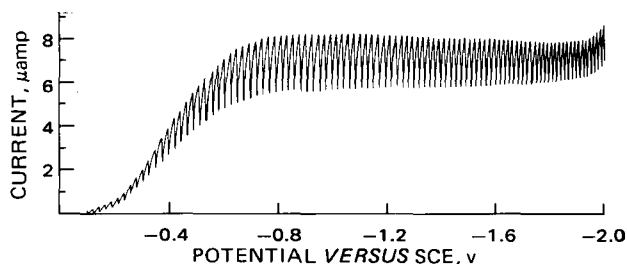


Figure 2—Polarogram of 100 μ g of isosorbide dinitrate/ml in 0.1 M NaClO₄ and 0.005% gelatin. The mononitrate esters produce polarograms similar in appearance.

Table V—Comparison of Polarographic (POL) and Automated Analysis (AA) Methods for Isosorbide Dinitrate Content Uniformity Single-Tablet Assay^a

	2.5 mg Sublingual		5.0 mg Chewable		5.0 mg Sublingual		5.0 mg Oral		10 mg Oral		10 mg Oral with Phenobarbital	
	POL	AA	POL	AA	POL	AA	POL	AA	POL	AA	POL	AA
	2.50	2.30	4.90	4.98	4.75	5.08	4.97	5.08	10.2	9.50	9.9	9.8
	2.51	2.30	4.96	4.85	4.83	4.84	5.18	4.74	10.4	9.71	10.5	9.6
	2.52	2.30	4.98	4.92	4.95	4.97	5.00	4.84	10.6	9.44	10.3	9.7
	2.56	2.25	4.92	4.85	5.14	4.62	4.86	5.00	10.3	9.71	11.1	9.3
	2.52	2.22	4.97	4.93	4.92	4.65	5.06	5.00	10.3	9.90	10.0	9.3
	2.46	2.25	5.12	4.88	4.87	4.80	4.90	5.08	10.0	10.1	10.0	8.9
	2.51	2.23	4.86	4.81	4.88	4.93	4.90	5.08	10.0	10.1	10.4	9.2
	2.62	2.25	4.87	4.79	5.14	4.90	5.05	5.13	10.0	10.2	10.2	8.8
	2.47	2.26	4.86	4.73	4.90	5.08	4.98	4.96	10.0	10.2	9.8	9.0
	2.47	2.27	4.92	4.78	4.90	4.81	5.08	5.10	9.8	10.1	10.2	8.8
Mean	2.51	2.26	4.94	4.85	4.93	4.87	5.00	5.00	10.2	9.90	10.2	9.2
SD	0.048	0.029	0.078	0.077	0.124	0.157	0.098	0.125	0.241	0.287	0.374	0.369
RSD, %	1.9	1.3	1.6	1.6	2.5	3.2	2.0	2.4	2.9	2.9	3.7	4.0

^a Results expressed as milligrams per tablet.

cients in each case are better than 0.999, indicating a linear relationship between current and concentration.

Synthetic mixtures were prepared which included isosorbide dinitrate, the two isosorbide mononitrates, and potassium nitrate in various combinations and in equivalent amounts. The presence of inorganic nitrate in the form of potassium nitrate produced no interference. However, equivalent mixtures of the dinitrate and the two mononitrates yielded values proportional to the organic nitrate content. Although the mononitrates cannot be determined individually in the presence of the dinitrate, the total organic nitrate content, calculated as isosorbide dinitrate, can be determined (Table III).

Table IV gives 10 replicate analyses for each of five dosage forms of isosorbide dinitrate. Representative portions of powdered tablets were analyzed by both polarographic and IR methods. The agreement was excellent with respect to both accuracy and precision, with relative standard deviations in the range of 1.0–1.5%. The results are expressed as milligrams of isosorbide dinitrate per average tablet weight.

Table V shows a comparison of the polarographic analysis of single tablets compared with single-tablet assays using the automated analysis procedure. Ten individual tablets were assayed, representing actual production lots of six common dosage forms of isosorbide dinitrate: 2.5 mg sublingual, 5.0 mg sublingual, 5 mg chewable, 5 mg oral, 10 mg oral, and 10 mg oral with phenobarbital. The agreement between the polarographic method and the automated analysis method was generally good; however, the results appear not to be as precise as the previous results for the assay of representative portions of powdered tablets. This result is indicative of the tablet-to-tablet variations in isosorbide dinitrate content and is a measure of the content uniformity.

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ACKNOWLEDGMENTS AND ADDRESSES

Received November 22, 1974, from the *Chemical Research Department and the †Stuart Pharmaceutical Division, ICI United States Inc., Wilmington, DE 19897

Accepted for publication April 30, 1975.

Presented at the Pharmaceutical Analysis and Control Section, APHA Academy of Pharmaceutical Sciences, New Orleans meeting, November 1974.

The authors are grateful for the support provided by the Analytical Section of the Chemical Research Department and the Product Development Section of the Stuart Pharmaceutical Division of ICI United States Inc., particularly for the assistance provided by Mr. Paul H. Nason.

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