pharmacokinetics of I in humans is underway, and the results will be reported later.

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Differential Pulse Polarographic Determination of Colchicine

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Received December 4, 1979, from the *Food and Drug Administration, Department of Health, Education, and Welfare, 850 Third Avenue, Brooklyn, NY 11232.* Accepted for publication June 4, 1980.

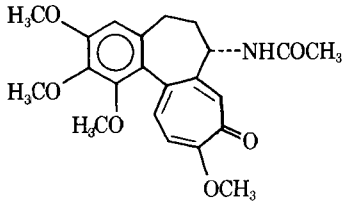
Abstract □ A differential pulse polarographic method for the analysis of colchicine-containing dosage forms is described. The reduction of the tropolone carbonyl is useful for quantitative analysis in that the relationship of the colchicine concentration to the current is linear over the concentration range of 0 to ~50 μg/ml, with a slight negative deviation at higher concentration. The procedure involves no preliminary treatment, is simple and specific, and is applicable to the assay of composite tablets, individual tablets, and injectable solutions. Polarography is conducted on a solution of colchicine in pH 1.81 Britton–Robinson buffer with 0.01% alkylphenoxy polyethoxyethanol. The quantitative analysis is achieved using the method of standard addition. A relative standard deviation of 3.2% was obtained for tablets. The results agree with those obtained using the USP XIX method.

Keyphrases □ Colchicine—analysis by differential pulse polarography □ Polarography, differential pulse—analysis of colchicine

Colchicine (I), an alkaloid obtained from various species of *Colchicum*, is used for the suppression of gout. The USP XIX assay for colchicine tablets involves extraction with chloroform followed by a spectrophotometric determination. The quantity of the drug specified to be taken for the assay is 3 mg. Since the usual tablet strength is 0.5 mg, this amount is equivalent to six tablets. For a content uniformity test, the method needs to be scaled down sixfold to accommodate a single tablet. As a result, the aqueous phase volume, as well as the organic volumes used for extractions, becomes less than optimum, so that difficulties with the procedure may be encountered.

Colchicine has a tropolone ring which bears a reducible carbonyl group, similar to an aromatic aldehyde that was determined by classical direct-current polarography in Britton–Robinson or McIlvane buffer (1–3). Reduction of colchicine at a dropping mercury electrode was studied at various pH levels by Sartori and Guadiano (4). These investigators reported that below pH 8, two reduction waves are obtained; the potential of the first wave becomes more negative with increasing pH and reaches that of the second wave at pH ~8. Above pH 8, there is only one wave, whose potential does not vary with pH and corresponds to the formation of a secondary alcohol. This reduction is irreversible (4).

However, the sensitivity of direct-current polarography is limited due to the large capacitance current contribution to the Faradaic current. This difficulty is reduced in differential pulse polarography. This paper describes the application of differential pulse polarography to the determination of colchicine in dosage forms with the goal of developing a general method applicable to the assay of composite tablets as well as individual tablets and injection solutions.



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EXPERIMENTAL

Apparatus and Polarographic Conditions—A polarographic analyzer[1] equipped with a drop timer[2] in conjunction with a three-electrode system was used for polarographic determinations. The electrodes were a dropping mercury electrode, a saturated calomel electrode, and a platinum wire auxiliary electrode. The drop timer was set at 1 sec, and the height of the mercury column was 70 cm. Other conditions were: current range, 2–5 μamp; pulse amplitude, 50 mv; and scan rate, 5 mg/sec. The potential range scan was from −0.7 to −1.2 v. All polarograms were recorded on an *x–y* recorder[3].

Reagents and Chemicals—All chemicals were reagent grade unless otherwise specified. The standard was colchicine USP[4].

Supporting Electrolyte Solution—One liter of solution was prepared to contain 0.04 *M* acetic acid, 0.04 *M* phosphoric acid, and 0.04 *M* boric acid in water (pH 1.81). Different pH buffers were prepared for the pH *versus* polarographic behavior study by mixing 100 ml of the supporting electrolyte solution with the required volumes of 0.2 *M* NaOH and checking with the pH meter[5].

Standard Colchicine Solution—Colchicine standard, 25 mg, was accurately weighed and dissolved with supporting electrolyte solution in a 25-ml volumetric flask and then diluted to volume with the same solvent.

Surfactant Solution—The surfactant solution was 0.5% alkylphenoxy polyethoxyethanol in water[6].

[1] Model 174, Princeton Applied Research Corp., Princeton, N.J.
[2] Model 174/70, Princeton Applied Research Corp., Princeton, N.J.
[3] Omnigraphic model 2200-3-3, Houston Instruments, Austin, Tex.
[4] City Chemical Corp., New York, N.Y.
[5] Zeromatic SS-3, Beckman Instruments, Fullerton, Calif.
[6] Triton X-100, Rohm & Haas, Philadelphia, Pa.

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Table I—Composite Tablet Assay of Commercial Samples by the Differential Pulse Polarographic Method and by the USP XIX Method

Colchicine Dosage Form	Amount Declared	Differential Pulse Polarography[a] Amount Found	Differential Pulse Polarography[a] Percent of Claim	USP XIX[a] Amount Found	USP XIX[a] Percent of Claim
Tablets	0.60 mg/tablet	0.59 mg/tablet	99.3	0.60 mg/tablet	100.0
Tablets	0.50 mg/tablet	0.50 mg/tablet[b]	100.0	0.49 mg/tablet	98.0
Injection	1 mg/2 ml	1.04 mg/2 ml	104.0	1.04 mg/2 ml	104.0
Tablets	0.648 mg/tablet	0.590 mg/tablet	91.0	0.582 mg/tablet	89.8

[a] Single determinations. [b] Mean value of six determinations; *RSD* = 3.2%.

Procedure—*Composite Tablet Assay*—Twenty tablets were weighed accurately, and the average weight per tablet was determined. The tablets were ground thoroughly in a mortar, and a portion of the powder containing ~0.5 mg of colchicine was weighed and transferred to a 50-ml volumetric flask. About 20 ml of supporting electrolyte solution was added, and the flask was stoppered and shaken for ~5 min. Then 1 ml of surfactant solution was added, and the solution was diluted to volume with supporting electrolyte solution and mixed.

Content Uniformity—The same procedure was used as for the composite tablet assay, except that an intact tablet was used as the sample.

Polarographic Procedure—Ten milliliters of the solution to be assayed was pipetted into the dry polarographic cell, and the solution was deaerated for 5 min with nitrogen and then polarographed under the described conditions. With a micropipet, 100 μl of the standard solution was added, and the solution was deaerated for 1 min and polarographed exactly as before. Peak heights were measured that occurred at ~−0.88 v *versus* a saturated calomel electrode. The milligrams of colchicine in the sample was calculated from:

$$\text{mg/tablet or mg/ml of injection solution} = \frac{A}{(B \times 1.01) - A} \times 0.1 \times \frac{50}{10} \times \frac{1}{C} \quad \text{(Eq. 1)}$$

where A is the peak height of the sample, B is the peak height of the sample plus the standard, 1.01 is the concentration factor for the dilution,

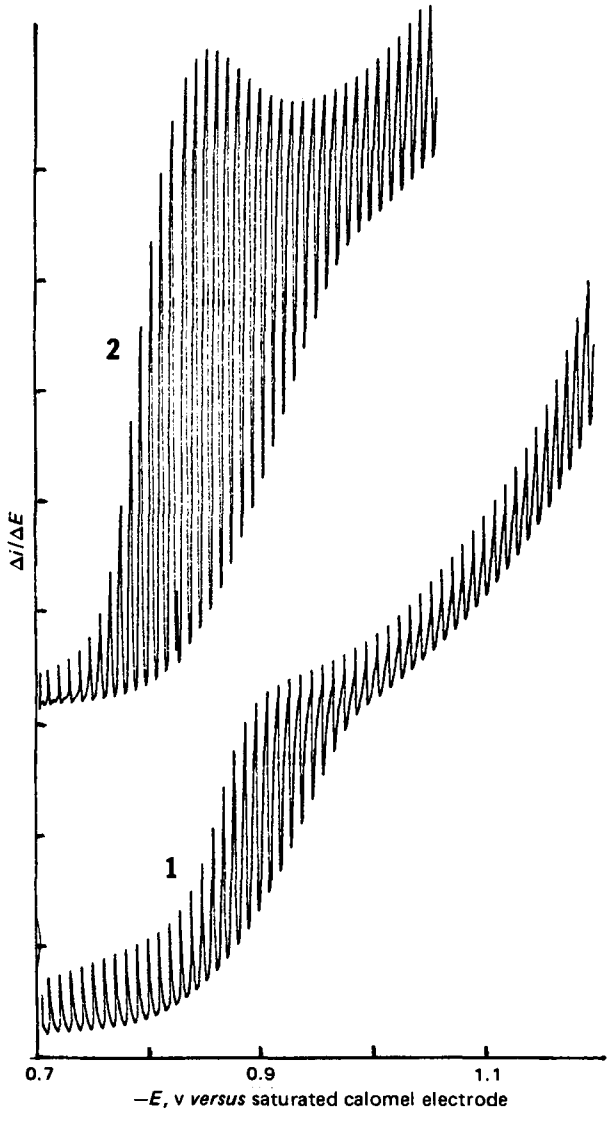

Figure 1—*Direct-current polarograms of colchicine in Britton–Robinson buffer (pH 1.81). Key: 1, 10 μg/ml; and 2, 20 μg/ml.*

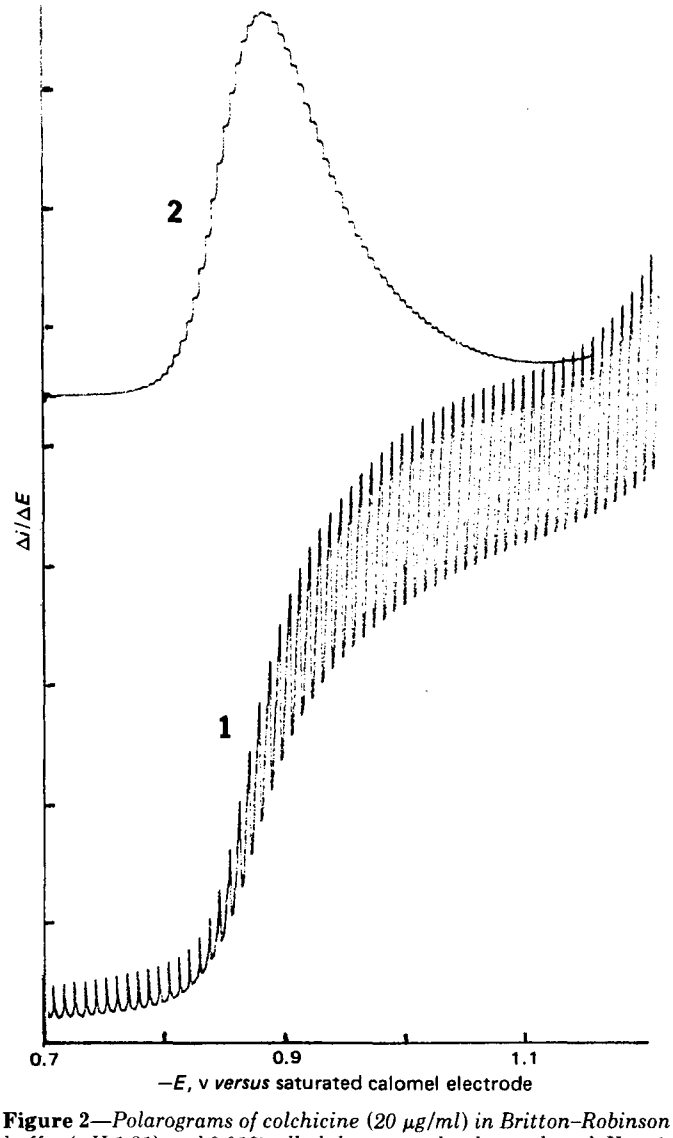

Figure 2—*Polarograms of colchicine (20 μg/ml) in Britton–Robinson buffer (pH 1.81) and 0.01% alkylphenoxy polyethoxyethanol. Key: 1, direct-current mode; and 2, differential pulse polarographic mode.*

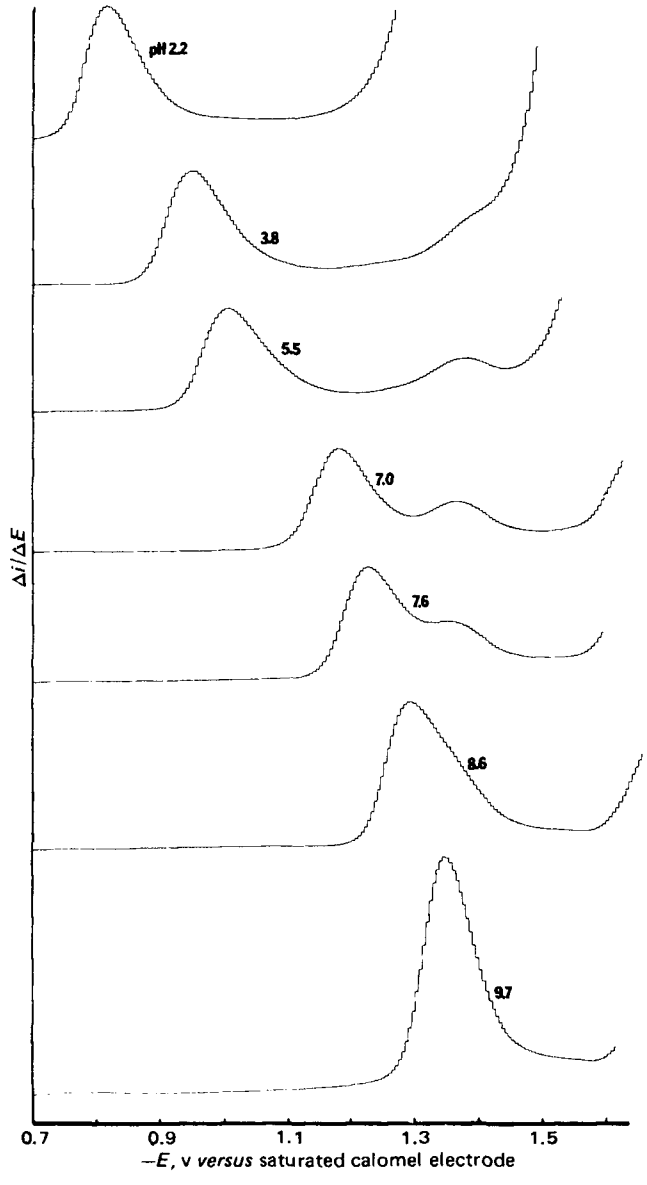

Figure 3—*Differential pulse polarographic behavior of colchicine (20 μg/ml) as a function of pH.*

and C is the number of tablets or milliliters of injection solution. In the composite tablet assay, C is the weight of the sample divided by the average weight per tablet; in the individual tablet assay, $C = 1$.

RESULTS AND DISCUSSION

When the initial differential pulse polarograms in Britton–Robinson buffer (pH 1.81) at colchicine concentrations of 10–50 μg/ml were recorded, there was a pronounced concentration-related peak potential shift in the anodic direction. The effect of adsorption was suspected and then was confirmed by running polarograms in the direct-current mode and observing the presence of a maximum (Fig. 1). Addition of 0.01% alkylphenoxy polyethoxyethanol (Fig. 2) to the test solution eliminated the maximum and almost all of the peak potential shift.

The general polarographic behavior of colchicine is similar to that of aromatic aldehydes. For example, the data for benzaldehyde (5) indicate that the mechanism for its reduction involves the formation of a free radical followed by dimerization (first wave) and the production of benzyl alcohol (second wave). A similar mechanism may be operative during the reduction of colchicine. The differential pulse polarographic behavior of colchicine as a function of pH is shown in Fig. 3.

Table II—Content Uniformity Analysis of 0.5-mg Colchicine Tablets by Differential Pulse Polarography

Tablet	Amount Found, mg/tablet
1	0.50
2	0.50
3	0.54
4	0.50
5	0.50
6	0.50
7	0.48
8	0.49
9	0.47
10	0.50
Average	0.50

The electrochemical properties of colchicine are useful for its quantitative measurement in pharmaceutical dosage forms. Quantitation is achieved by a method of standard addition to compensate for differences in sample matrixes.

A linear relationship between colchicine concentration and electrochemical behavior ($\Delta i/\Delta E$) was established from the following data points (micrograms per milliliter *versus* microamperes): 11.2, 1.08; 22.4, 2.23; 33.6, 3.29; and 44.8, 4.35. The line showed an intercept of 0.020 μamp, a slope of 0.0971 (μamp ml)/μg, and a correlation coefficient of 0.9998. The line showed a slight negative deviation from linearity at higher concentrations.

Four commercial preparations were analyzed using the proposed method and the USP XIX procedure (6). The results are given in Table I. An estimate of the precision of the differential pulse polarographic method obtained by analyzing one sample six times gave a relative standard deviation of 3.2%. Ten individual tablet assay results for 0.5-mg tablets ranged from 0.47 to 0.54 mg/tablet (Table II), with an average of 0.50 mg/tablet. The same value was obtained for the composite tablet assay.

The differential pulse polarographic assay presented here is rapid and sensitive and is specific for analyzing colchicine-containing preparations. Moreover, the same procedure is applicable for the assay of individual tablets. The results of the differential pulse polarographic method are in excellent agreement with those obtained using the more involved USP XIX assay.

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ACKNOWLEDGMENTS

Presented at the APhA Academy of Pharmaceutical Sciences, Kansas City meeting, November 12, 1979.

The authors thank Dr. Thomas Medwick, Food and Drug Administration, New York, N.Y., and Rutgers University, New Brunswick, N.J., and Dr. Cesar A. Lau-Cam, Food and Drug Administration, New York, N.Y., and St. John's University, Jamaica, N.Y., for their assistance in the preparation of this paper.