Simultaneous Polarographic Determination of Bisulfite and Iproniazid in Gel Formulations

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Abstract ☐ A polarographic method is described for the simultaneous determination of sodium bisulfite and iproniazid in gel formulations. The standard deviation for the bisulfite determination was ±0.00$_3$% and the precision ts at the 95% confidence level was ±0.00$_5$%. The standard deviation for the iproniazid determination was ±0.03% and the precision ts at the 95% confidence level was ±0.07%.

Keyphrases ☐ Iproniazid–bisulfite—simultaneous polarographic determination ☐ Bisulfite–iproniazid—simultaneous polarographic determination ☐ Polarography—simultaneous determination of iproniazid and bisulfite in gel formulations

Due to the pharmaceutical importance of isoniazid and its derivatives, the analysis of this class of compounds has received considerable attention in the literature. The polarographic and spectrophotometric behavior of these compounds was investigated (1), and it was found that isoniazid exhibited two polarographic reduction waves in the pH 1.5–7 range. At pH >8, these waves merged to a single wave. By using the sum of both polarographic waves, isoniazid in tablets was determined with a precision of ±1.0% (1). No interference from excipients was noted. By using UV spectrophotometry, isoniazid in tablets was measured with a precision of about 1–2% (1).

A photometric procedure for the determination of iproniazid and other hydrazide derivatives, based on a reaction with molybdic acid in an acidic medium to form a red complex, also was reported (2). While the method is specific and sensitive, the concentration of acid is critical for the development of maximum color intensity.

Many methods have been reported which are based on the quantitative oxidation of the hydrazine moiety using bromine (3), iodine (4, 5), iodate (6), and nitrite (7). Recently, a titrimetric method for isoniazid and its derivatives was reported (8), which is based on the oxidation of isoniazid with excess chloramine T and back-titration. These methods all lack specificity, however, particularly when other reducing substances are present.

The most frequently used methods for the determination of bisulfite and sulfite are oxidation and subsequent determination as sulfate and direct oxidation with iodine (9). As with methods for hydrazine derivatives, these methods are also subject to interferences from other reducing substances. To avoid this problem in the determination of sulfite, a polarographic procedure was developed (10) based on the conversion of sulfite to sulfur dioxide and subsequent polarographic determina-

tion of the sulfur dioxide. The method was applied to the determination of sulfite in sewage water; however, both sulfide and thiosulfate were sources of interference.

Drug formulations containing bisulfite, as an antioxidant, and isoniazid or its derivatives do not readily lend themselves to present compendial (5) methods since simultaneous oxidation of both species occurs. The present article describes a polarographic method for the simultaneous determination of bisulfite and iproniazid which eliminates the problem of mutual interference often encountered when these species are present in the same formulation.

EXPERIMENTAL

Apparatus—A polarographic assembly[1] and a recorder[2] were used in conjunction with a dropping mercury electrode and a silver–silver chloride reference electrode (Ag/AgCl) to obtain the current voltage curves.

A digital pH meter[3] was used for all pH measurements.

The dropping mercury electrode characteristics, m and t, were determined in 0.25 M ammonium chloride solution at a potential of −1.0 v. versus Ag/AgCl and at a mercury column height of 56 cm.; m was 3.60 mg./sec. and t was 2.68 sec.

Reagents and Solutions—Triple-distilled mercury[4] was used in all polarographic experiments. Demineralized water was used to prepare all solutions. This water was prepared by passing ordinary distilled water through a demineralizer[5].

A pH 8 buffer was prepared by mixing 100 ml. of a stock solution containing phosphoric acid[6], acetic acid[7], and boric acid[7], each at a concentration of 0.04 M, with 62.5 ml. of 0.2 N sodium hydroxide[8] solution.

A 10% hydrochloric acid solution was prepared by diluting 236 ml. of concentrated hydrochloric acid[9] to 1000 ml. with demineralized water.

A stock reference standard solution of sodium metabisulfite[10] was prepared by accurately weighing approximately 40 mg. of sodium metabisulfite into a 100-ml. volumetric flask and diluting to volume with demineralized water. Ten milliliters of this solution was then diluted to 100 ml. with 40 ml. of buffer and approximately 50 ml. of demineralized water.

A stock reference standard solution of iproniazid phosphate was prepared by accurately weighing approximately 60 mg. of iproniazid phosphate into a 100-ml. volumetric flask and diluting to volume with 40.0 ml. of buffer and 60 ml. of deaerated demineralized water.

Procedure—Approximately 2 g. of the gel sample was transferred to a tared 50-ml. volumetric flask and weighed. Twenty milliliters of deaerated buffer was added, and the sample was shaken for approximately 30 min. to ensure extraction of the active substance

Table I—Polarographic Determination of Bisulfite in 1% Iproniazid Gel

Sample	Bisulfite Found[a]
1	0.06_1
2	0.05_7
3	0.06_2
4	0.06_5
5	0.06_2
6	0.06_3
7	0.06_1
8	0.05_7
9	0.06_5
10	0.06_1
11	0.06_2
12	0.06_5
13	0.05_9
14	0.06_1
15	0.06_5
16	0.06_5
17	0.06_1
18	0.06_1
Average	0.06_2
Standard deviation	$\pm 0.00_2$
Precision[b]	$\pm 0.00_5$

[a] Theoretical bisulfite = 0.10%. [b] ts for 17 degrees for freedom at the 95% confidence level.

from the gel matrix. The sample was then diluted to volume with deaerated demineralized water. Ten milliliters of this solution was pipeted into the polarographic cell and deaerated with prepurified tank nitrogen[11] for approximately 5 min. The nitrogen flow was then reversed to the solution surface, and 1 ml. of 10% hydrochloric acid was added and mixed into the solution. The polarogram for bisulfite as sulfur dioxide was immediately recorded using a voltage span of from 0 to −1.0 v. versus Ag/AgCl, a mercury column height of 56.0 cm., and a current sensitivity of 0.02 μa./mm. When the polarogram was completed, the solution was vigorously deaerated with nitrogen to eliminate the sulfur dioxide formed from the bisulfite, and a residual current curve was obtained using the same polarographic conditions.

Polarograms for iproniazid were recorded using the same solution. The instrument conditions were the same, except that the current sensitivity was 0.2 μa./mm.

Polarograms were also recorded for the sodium metabisulfite and iproniazid phosphate reference standards using the same instrument conditions.

Calculations—The diffusion currents were measured by standard procedures (11). The percent bisulfite in the formulation was calculated as follows:

$$\text{percent bisulfite} = \frac{(i_s)(C_{st})(5)(1.095)}{(i_{st})(\text{weight sample, grams})} \quad \text{(Eq. 1)}$$

where:

i_s = diffusion current (microamperes) for sample
C_{st} = concentration of standard (milligrams per milliliter)
5 = dilution factor and conversion to percent
1.095 = conversion factor for metabisulfite to bisulfite
i_{st} = diffusion current (microamperes) for standard

The percent iproniazid in the formulation was calculated as follows:

$$\text{percent iproniazid} = \frac{(i_s)(C_{st})(5)(0.6465)}{(i_{st})(\text{weight sample, grams})} \quad \text{(Eq. 2)}$$

where:

i_s = diffusion current (microamperes) for sample
C_{st} = concentration of standard (milligrams per milliliter)
5 = dilution factor and conversion to percent

[11] Union Carbide Corp., Linde Division.
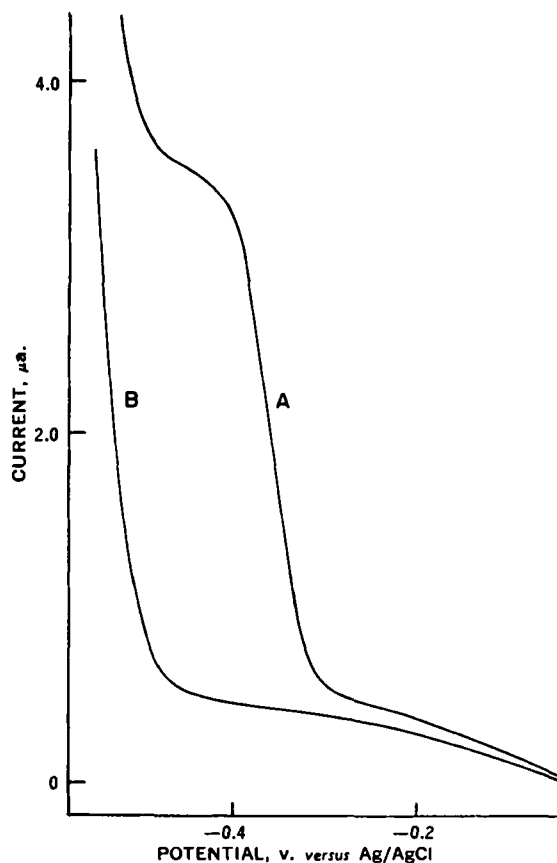
Figure 1—*Polarograms for bisulfite determination. Key: A, typical reduction wave for sulfur dioxide; and B, typical residual current curve obtained after deaeration.*

0.6465 = conversion factor for iproniazid phosphate to base
i_{st} = diffusion current (microamperes) for standard

RESULTS AND DISCUSSION

Polarographic Determination of Bisulfite—In acidic media, the gel sample exhibited three reduction waves. The first wave, which occurred at −0.38 v. versus Ag/AgCl (Fig. 1), may be attributed to the reduction of sulfur dioxide liberated from the bisulfite stabilizer under acidic conditions (Scheme I).

$$HSO_3^- + H^+ \rightarrow SO_2 + H_2O$$
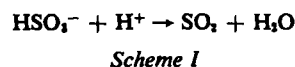
Scheme I

According to Kolthoff and Miller (12), the resulting sulfur dioxide is reduced in a single two-electron step to sulfoxylic acid (Scheme II).

$$SO_2 + 2H^+ + 2e^- \rightarrow H_2SO_2$$
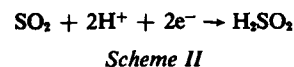
Scheme II

The metabisulfite standard, under the same solution conditions, is readily converted to bisulfite (Scheme III), which subsequently reacts as shown in Schemes I and II.

$$S_2O_5^{-2} + H_2O \rightarrow 2HSO_3^-$$

Scheme III
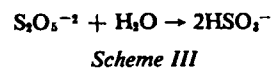
The polarographic results obtained for the bisulfite stabilizer are presented in Table I. The average for 18 determinations was $0.06_2\%$ with a standard deviation of $\pm 0.00_2\%$ and a precision (ts) for 17 degrees of freedom at the 95% confidence level of $\pm 0.00_5\%$.

The obvious discrepancy in bisulfite added (0.1%) and bisulfite found is most likely due to the unavoidable losses of bisulfite during formulation. While the final formulation is a neutralized gel
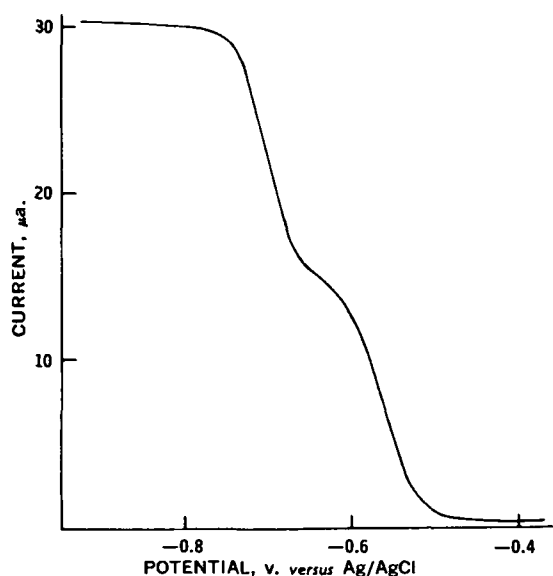
Figure 2—*Typical polarogram for iproniazid.*

medium, the pH of the formulation is acidic until the final step in the preparation due to the presence of iproniazid phosphate. This acidic pH would most likely cause losses of bisulfite as SO_2 (Scheme I).

Polarographic Determination of Iproniazid—The two additional reduction waves, observed at -0.54 and -0.72 v. versus Ag/AgCl in an acidic medium (Fig. 2), are due to the reduction of iproniazid (1). Although the half-wave potentials for the sulfur dioxide wave and the first reduction wave for iproniazid are separated by only 160 mv., vigorous deaeration of the solution after measurement of the sulfur dioxide wave eliminates any possible interference from sulfur dioxide in the subsequent measurement of the diffusion current for iproniazid.

Polarograms for the bulk drug at various concentrations indicated that the total current for the two reduction waves was proportional to concentration in the 0.05–0.6-mg./ml. range. A plot of diffusion current, i_d, versus concentration, milligrams per milliliter, was linear with a slope equal to 30 μa. ml./mg.

In contrast to the polarographic results obtained for bisulfite, the results obtained for iproniazid indicated that the determination was dependent on the extraction time used to free the hydrazide from the gel matrix. Initially, samples were extracted by shaking for approximately 5 min. prior to scanning on the polarograph. The average assay value for eight determinations was 0.95% with a standard deviation of ±0.06% and a precision (ts) for seven degrees of freedom at the 95% confidence level of ±0.13% (Table II). When the extraction time was increased to approximately 30 min., the average for 10 determinations was 0.98% with a standard deviation of ±0.03% and a precision (ts) for nine degrees of freedom at the 95% confidence level of ±0.07% (Table II).

CONCLUSIONS

The advantages of the described method over procedures for bisulfite and hydrazine derivatives that are based on direct oxidation with iodine are twofold. The primary advantage is that it eliminates the mutual interference problem between bisulfite and iproniazid which may be encountered in methods based on oxidation. Secondly, the present method allows both the active hydrazine substance and

Table II—Effect of Extraction Time on Percent Iproniazid Found

| Extracted 5 min. | | Extracted 30 min. | |
Sample	Percent Found[a]	Sample	Percent Found[a]
1	0.89	1	0.99
2	0.84	2	1.00
3	1.00	3	1.02
4	0.99	4	0.96
5	0.99	5	0.97
6	0.96	6	0.97
7	0.95	7	0.99
8	0.98	8	0.99
		9	0.98
		10	0.96
Average	0.95		0.98
Standard deviation	±0.06		±0.03
Precision	±0.13[b]		±0.07[c]

[a] Theoretical iproniazid = 1.00%. [b] ts for seven degrees of freedom at the 95% confidence level. [c] ts for nine degrees of freedom at the 95% confidence level.

the stabilizer used in the formulation to be determined in the same sample preparation. As a result, this procedure significantly reduces the overall time required for the dosage form analysis when both the active substance and the antioxidant must be determined.

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