Colorimetric Determination of Procainamide in Injectable Preparations

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Abstract □ A simple, rapid colorimetric method is described which determines procainamide in commercially available, injectable solutions. It is based on complexing the drug with cupric ion and then measuring the absorption at 380 nm. The reaction is fast and the metal–drug ratio in the complex is 1:1. The pH optimum for maximum reaction is 4.0–4.5 which is maintained by an acetate buffer. Linear standard curves were obtained from 1.52 to 6.06 mg/mL. Analysis of 29 commercial samples resulted in an average of 97.3% of the label claim.

Keyphrases □ Procainamide—colorimetric determination, injectable preparations, cupric ion complexing □ Colorimetry—procainamide, injectable preparations, cupric ion complexing

Procainamide (I) is an antiarrhythmic agent used to treat cardiac disorders. Dosages and plasma concentrations as well as half-life and ultimate fate have been the subject of previous studies (1, 2). All of these studies require an analytical method for the analysis of I, and there exists an obvious need for such a method in manufacturing and quality control.

A large volume of literature exists on the determination of I in pharmaceutical and biological systems. One of the most favored techniques is HPLC (3–5), although gas chromatography (6), TLC (7), spectrophotofluorometry (8, 9), and even polarography (10) have been used. Some of the claimed advantages in specificity are not realized (11), and some of the methods are quite tedious and time-consuming.

Spectrophotometric procedures which involve diazotization and coupling reactions have been reported (12, 13). A colorimetric procedure for I that requires less manipulation and fewer reactions would be useful and more convenient. The text describes such a colorimetric method for I based on the formation of a complex between procainamide and cupric ion. This colorimetric method for the drug is shown to have sufficient sensitivity and adequate specificity, especially for relatively pure samples. The suitability of this colored complex for analytical purposes, and the application of this colorimetric method are also described.

EXPERIMENTAL SECTION

Materials—Procainamide hydrochloride[1] (II), *N*-acetylprocainamide[1], procaine hydrochloride[1], sulfuric and acetic acids[2] (reagent grade), sodium hydroxide[2] (50% solution), iron(III) nitrate[2], cobalt(II) chloride[2], and sodium acetate[2] (ACS grade), and chlorides[2] of copper(II), nickel(II), and chromium(III) were obtained commercially. The injectable vials[3] were obtained through local retail outlets.

Preliminary work was done on a recording UV-visible light instrument[4], but later analytical work was carried out using a single-beam spectrophotometer[5]. pH measurements were made on an instrument[6] which was standardized daily with commercially prepared biphthalate buffers.

Complex Formation—Screening of various metal ions for potential use was carried out by adding a few drops of ~2M solutions of the various metal ions (cobalt(II), nickel(II), chromium(III), copper(II) chlorides, and iron(III) nitrate) to ~1 mL of a 10-mg/mL solution of II in a spot plate. The only visible color change occurred with cupric ion; a green color became apparent on addition of the metal.

Spectra of solutions of II were obtained from 330–550 nm both in the presence and absence of the cupric ion, as shown in Fig. 1. Using the absorption generated at 380 nm, the formula of the complex was obtained by the continuous variations method (14). The absorption at 380 nm was maximized at $X_{Cu} = 0.5$, where X_{Cu} is the mole fraction of cupric ion.

pH Dependence of the Complex—The optimum pH for the complex was established by mixing 0.10 M solutions of II and cupric chloride, adjusting to a measured pH of 1.5 with concentrated sulfuric acid, and then gradually neutralizing the solution with dropwise addition of 10% NaOH, after measurement of absorbance at 380 nm. A plot of absorbance *versus* pH (Fig. 2) shows a maximum at pH 4. Increasing the pH >4.3–4.6 causes a white to light blue precipitate to form; therefore, a pH of 4.0 was selected for further studies.

Linearity Studies—A pH 4.0 acetate buffer (prepared by addition of sodium acetate to a 1 M solution of acetic acid) was used as the solvent for these studies. A series of solutions of the complex in acetate buffer were prepared; the solutions each contained a constant, excess level (10.5 mg/mL) of the cupric ion, and varying (1.52, 3.04, 4.55, and 6.06 mg/mL) amounts of II. This series of solutions was prepared by mixing three solutions in various ratios: 37.87 mg/mL II, 131.1 mg/mL cupric ion, and the buffer. Measurements of the absorbances of these solutions and their respective concentrations are shown in Table I.

Analysis of Injectable Solutions—A series of standards of II were prepared that are similar to those in the linearity studies; the absorbances were measured and a least-square line prepared from the data. Concurrently, 100 mg/mL commercial samples were analyzed by withdrawing 1 mL of the liquid, mixing with 4 mL of the 131.1 mg/mL cupric ion solution, and diluting to 50 mL with pH 4 acetate buffer. The absorbances of these solutions were also determined at 380 nm, and their concentrations calculated from the slope and intercept of the least-square line described above. As shown in Table II, recoveries varied from a low of 88.4% to a high of 107.9% based on label claims.

RESULTS AND DISCUSSION

Cupric ion complexed instantly with the procainamide molecule, the 1:1 coordination resulting in a visible absorption with a maximum at 380 nm. The complex formed most completely at a pH value of 4.0–4.5; a precipitate formed near the upper pH limit. Attempts to form the complex at pH 5.0 gave a dark blue precipitate which dissolved on long standing. The curve shape shown in Fig. 2 was reproduced by later experiments in which II in water (pH 4.5) was acidified, as opposed to the acid to neutral direction reported in the *Experi-*

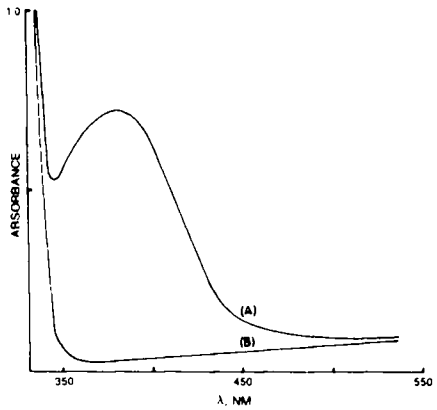

Figure 1—*Visible spectrum of II in presence (A) and absence (B) of the cupric ion.*

[1] ICN K & K Labs, Plainview, N.Y.
[2] Fisher Scientific Co., Fair Lawn, N.J.
[3] Pronestyl; E. R. Squibb & Sons, Inc., Princeton, N.J.
[4] Model 618-900; Perkin-Elmer Corp., Norwalk, Conn.
[5] Spectronic 70; Bausch & Lomb, Rochester, N.Y.
[6] Model 7; Corning Glass Works, Corning, N.Y.

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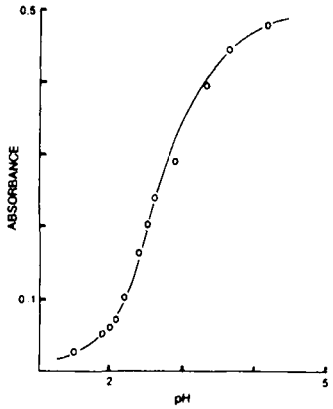

Figure 2—*380 nm Absorption of the cupric ion–procainamide complex as a function of pH.*

mental Section. The anion present with copper did not affect complex formation and the sulfate salt was used routinely in later work.

An acetate buffer, pH 4.0, was found to be an excellent medium for analytical work; however, unbuffered solutions, as well as those buffered with biphthalate, were also tried and found to give linearly increasing absorbance at 380 nm with increasing levels of II. Biphthalate, however, repressed complex formation (as evidenced by the lower-than-expected absorbances), probably by competing with II for the cupric ion. Repetition of the above experiment showed that the concentration of acetate buffer was not critical; however, for reproducible results, the pH must be kept constant. This is most easily accomplished by preparing and using large quantities of buffer. It was also discovered, in later experiments, that the amount of excess copper was not critical; but, a minimum of two-threefold excess was used throughout. This observation indicates that the equilibrium constant for formation is very favorable and, thus, well-suited for an analytical technique.

The reported experiment on the analyses of injectable vials was carried out on four different days; each repetition gave a linear standard curve, and recoveries of the samples (all were guaranteed 100 mg/mL) varied from a low of 88.4% to a high of 107.9% on the results from 29 different samples. Between sets of samples there was a slight day-to-day variation in recovery; within a set the results were remarkably consistent (the highest *SD* within a set was 2.6%, the lowest was 0.5%, based on recovery figures). The variation between sets can be attributed to slight pH differences in different batches of buffer, therefore, it is suggested that large amounts of buffer be made to promote uniformity.

To address the question of the specificity of this procedure, a spot plate test of procaine hydrochloride and *N*-acetylprocainamide showed that the color is not unique to procainamide. Procaine gave the color, the *N*-acetylprocainamide did not. Procaine is the most chemically similar drug available, so the presence of color, not altogether surprising, was the most stringent test possible. *N*-Acetylprocainamide was selected because it is chemically similar to I, and, in addition, is the major metabolite in humans (15). The involvement of nitrogen atoms in coordination with copper is also suggested by the lack of color with the *N*-acetyl compound. The lack of absolute specificity was not viewed as a serious drawback, since use in laboratory was limited to the injectable sample only.

Table I—380 nm Absorbance Values of Varying Concentrations of the Copper–Procainamide Complex

Conc. of Procainamide, mg/mL	Absorbance
0	0.063
1.52	0.388
3.04	0.705
4.55	1.01
6.06	1.28

Table II—Concentrations of Pronestyl Injectables Calculated from Absorbance Data

Sample Number	Conc., mg/mL	Sample Number	Conc., mg/mL
1	102	16	89.5
2	91.2	17	96.8
3	89.0	18	93.6
4	89.2	19	99.6
5	89.7	20	96.0
6	90.7	21	88.4
7	89.0	22	88.5
8	99.3	23	104.4
9	100.5	24	107.1
10	101.4	25	104.6
11	107.5	26	106.8
12	102.6	27	107.9
13	92.5	28	106.5
14	100.9	29	105.7
15	88.8		

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