In most instances the use of inert powders did not stabilize capsules stored in open containers. However, in most cases it was found unnecessary to add any inert powder in prescriptions for capsules containing deliquescent drugs, provided that the capsules were dispensed in airtight glass containers. Hence it is imperative to use an airtight container, such as a screw-top glass capsule vial, in dispensing capsules containing deliquescent substances.

In case of capsules containing deliquescent substances, the presence of powder adhering on the outside of the capsule hastens deterioration.

REFERENCES

(1) Husa, William J., and Becker, Charles H., JOUR. A. PH. A., 29 (1940), 78.

(2) Hilton, S. L., *Amer. Drug.*, 86 (1932), No. 3, 94.

(3) Hilton, S. L., JOUR. A. PH. A., 22 (1933), 130.

(4) Husa, William J., and Webb, Herbert M., *Ibid.*, 26 (1937), 905.

(5) Morrison, S. W., *Ibid.*, 18 (1929), 142.

A Micro-Colorimetric Method for the Determination of Copper in Ampules of Iron, Arsenic and Copper

By Roland A. Bosee and Paul Fehder†*

INTRODUCTION

In the course of control analysis of ampule products containing organic combinations of iron, arsenic and copper, used in the treatment of hypochromic anemias, a reliable method for separating and estimating micro-quantities of copper was devised and corroborated. The original procedure worked out by a former associate[1] was based on a micro-colorimetric method (1) for determining traces of copper in blood and milk. This procedure was modified by a simple preliminary treatment to separate the copper-ion from the organic compounds of iron and arsenic. The copper was determined by the development of the cupric sulfocyanate-pyridine complex, soluble in chloroform and possessing a characteristic green color.

* Director of Laboratories, Endo Products, Inc., New York, N. Y.

† Analytical Department, Endo Products, Inc., New York, N. Y.

[1] Daniel Brawley, Analytical Department, Endo Products, Inc., N. Y.

EXPERIMENTAL

Procedure.—Measure an exact aliquot of the sample solution equivalent to approximately 0.40 or 0.45 mg. of copper, into a small beaker, add 0.5 cc. of concentrated hydrochloric acid and dilute with distilled water to a volume of about 25 cc. Saturate the solution with hydrogen sulfide at room temperature until the mixture turns milky and a deposit of copper sulfide and sulfur is formed. Filter through a small retentive filter paper (C. S. & S. No. 595). Wash the residue with several portions of hot distilled water. Reject the filtrate and washings which contain the iron and arsenic.

Dissolve the copper sulfide by pouring several portions of boiling 25% nitric acid into the filter. Collect the filtrate (containing cupric nitrate) in a 100-cc. beaker. Wash the filter several times with hot distilled water, collecting the washings in the beaker containing the filtrate. Evaporate the filtrate, at first on a hot plate to a small volume, and finally on a steam-bath. Keep the beaker covered with a ridged watch glass during the evaporation to avoid contamination with dust or cinders. To the residue of cupric nitrate, add 5 cc. of concentrated hydrochloric acid and again evaporate to dryness on a steam-bath.

Dissolve the cupric chloride residue in several drops of $N/1$ hydrochloric acid. A clear solution should be formed. Add 2 cc. of distilled water and transfer the mixture to a 25-cc. glass-stoppered volumetric flask. Rinse the beaker with four 2-cc. portions of distilled water and transfer the rinsings to the flask. The total volume of solution should not exceed 10 cc.

Prepare a copper standard solution by measuring exactly 5 cc. of a Standard Copper Solution, containing 0.1 mg. Cu in each 1 cc. of solution, into a second 25-cc. glass-stoppered volumetric flask. Add to this flask the same number of drops of $N/1$ hydrochloric acid as added to the cupric chloride residue. Dilute with distilled water to the same volume (not more than 10 cc.). The Standard Copper Solution is prepared by dissolving exactly 392.8 mg. of clear, *uneffloresced* crystals of reagent grade cupric sulfate, $CuSO_4.5H_2O$, in exactly 1000 ml. of distilled water.

Now add 2 drops of phenolphthalein T.S. to both flasks and titrate each with $N/1$ sodium hydroxide to a pink end-point. Following this, add to each flask, in succession, exactly 10 cc. of chloroform, 1 cc. of glacial acetic acid, 1 cc. of 10% potassium sulfocyanate solution and 15 drops of pyridine, medicinal grade. Dilute both flasks to the mark with distilled water, stopper and agitate thoroughly. When the chloroform fractions containing the dissolved copper

sulfocyanate-pyridine complex separate, transfer each mixture to dry separatory funnels. Permit the chloroformic and aqueous layers to separate completely. Drain about 4 cc. of each of the chloroformic fractions into individual colorimetric comparison cups of a Duboscq colorimeter. Compare the green colors as to relative intensity. Take an average of ten readings rapidly.

$$\frac{\text{Reading of standard (mm.)} \times \text{0.5 mg. of Cu}}{\text{Reading of sample (mm.)} \times \text{Volume of sample taken for assay}} = \text{mg. Cu per 1 ml. sample}$$

Discussion.—The method presented above has given consistently accurate results with average deviations of plus 3.7% and minus 2.0% from the theoretical quantities of copper assumed to be present. Aside from experimental and manufacturing errors, the possibility of some exsiccation in the copper salts used in the manufacture of these ampule products may be a contributing factor to such deviations. The following tables are compiled from the assay reports of the different lots of ampules prepared during the year 1939, and are presented to prove the accuracy of the method:

Table I.—Copper Content of Ampule No. 175-A[a]

Lot No.	Copper Content, in mg., per 10 Cc. Ampule
10,875	0.314
10,986	0.309
11,200	0.313
11,260	0.313
11,446	0.307
11,536	0.291
11,666	0.284
11,822	0.294
11,987	0.296
12,038	0.308
12,129	0.298
12,197	0.307
12,345	0.298
Average	0.3025

Table II.—Copper Content of Ampule No. 175[a]

Lot No.	Copper Content, in mg., per 5 Cc. Ampule
10,897	0.147
10,925	0.158
11,003	0.166
11,283	0.155
11,504	0.152
11,599	0.146
11,764	0.145
11,805	0.155
11,985	0.161
12,030	0.154
12,233	0.145
Average	0.153

[a] The theoretical quantity of copper is 0.300 mg. Cu per 10 cc. Ampule Solution.

Table III.—Copper Content of Ampule No. 345[a]

Lot No.	Copper Content, in mg. per 2 Cc. Ampule
11,263	0.420
11,388	0.418
11,558	0.417
11,986	0.410
Average	0.416

Table IV.—Copper Content of Ampule No. 345-B[a]

Lot No.	Copper Content in mg., per 1 Cc. Ampule
10,882	0.198
11,218	0.210
11,583	0.202
Average	0.203

[a] The theoretical quantity of copper is 0.200 mg. Cu per 1 cc. Ampule Solution.

It has been observed in some instances that the shade of green color shown by the copper sulfocyanate-pyridine complex produced by the sample is a trifle more yellow than that shown by the standard copper solution complex. Traces of iron or sulfur alter the shade of green color. However, the intensities can be definitely compared without difficulties. Several attempts to obtain comparable readings by treating the standard copper solution aliquot by the same procedure used in separating and extracting the copper in the ampule sample produced inconsistent and low results.

SUMMARY

1. A suitable method for estimating micro-quantities of copper in ampules of iron, arsenic and copper has been devised, based on a micro-colorimetric method employed in biochemistry for estimating traces of copper in blood and milk (1).

2. The results of thirty-one determinations indicate that the method is sufficiently accurate and reliable to warrant its use in estimating micro-quantities of copper salts in medicinal preparations.

REFERENCE

(1) Hawk and Bergeim, "Practical Physiological Chemistry," 10th Edition (1931), page 468.

Book Reviews

Biological Products, by LOUIS GERSHENFELD, P.D., B.Sc., Ph.M. Publishers, Romaine Pierson, New York. 236 pages, price $4.00.

The author is professor of Bacteriology and Hygiene at the Philadelphia College of Pharmacy and Science. He has prepared the work with a purpose to give information on the preparation, manufacture, uses and other essentials of bacteriology and their practical application; to serve students as a guide for their studies, and for those who carry on laboratory work. It grew out of the need of the author in his field and gives particularly useful information on antitoxins, serums, vaccines for those in the allied public health professions. The book is illus-