METHOD OF ANALYSIS FOR SULFANILAMIDE.*

BY R. W. TOWNE AND R. M. HITCHENS.[1]

Coincident with the rapid growth of sulfanilamide therapy, many methods for the analysis of this compound have arisen. Numerous of these pertain to detection and estimation in blood, spinal fluid and the like. In addition, there is need for simple, accurate methods of estimating the purity of the sulfanilamide itself as marketed. "New and Non-Official Remedies" (1) offers several such means. Their melting-point determination is a rapid and reasonably accurate criterion of purity. Likewise, their microanalysis for sulfur allows an estimation of purity with a permissible limit of 98.4 to 101.6 per cent which is rather a wide range for an official assay procedure. More nearly quantitative is their volumetric diazotization assay with sodium nitrite, although this method suffers from the slowness of the reaction and the smallness of the sample. It has an allowable limit of 99.0 to 100.5 per cent. Unfortunately these last two methods are microchemical in nature and require special laboratory facilities and technique not available in much of the trade.

Shulek and Boldizar (2) have developed an assay by bromination which gives results within 0.5 per cent, tending to be high. Work done in this laboratory indicates that the results may be as much as 5 per cent high if room temperature is construed to be 25° C.

The methods used in this laboratory for the assay of sulfanilamide are in nature simple and rapid. They involve the physical method of crystallizing-point determination, plotting a time-temperature curve, which is sensitive to 0.1 mol. per cent impurity, and the "New and Non-Official Remedies" diazotization procedure, performed potentiometrically using a platinum electrode on a macro sample in the presence of potassium bromide as a catalyst, and accurate to 0.1 per cent. The potentiometer may be replaced by starch-iodide indicator with little, if any, loss in accuracy.

The two methods are outlined in detail below:

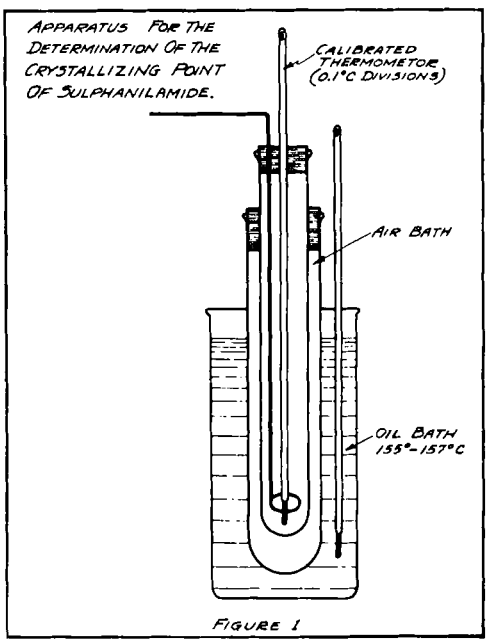

FIGURE 1

METHOD I. DETERMINATION OF CRYSTALLIZING POINT.

The apparatus for crystallizing point determinations (Fig. 1) consists of a test-tube with stirrer, a slightly larger tube for an air jacket, a vessel for an oil bath, and a precision calibrated thermometer graduated in 0.1° divisions.

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Sufficient sample is placed in the inner tube to produce a 3-inch layer of molten sulfanilamide. The tube is then fitted with a glass ring stirrer and the precision thermometer and suspended in an air jacket within an oil-bath maintained at 157–160° C. The contents of the tube are stirred slowly as the temperature falls. The temperature is noted each minute until the crystallizing plateau has been reached and passed.

NOTE: The temperature must not be permitted to rise above 175° C. when melting the sample. After solidification the contents of the tube are remelted and the determination repeated.

METHOD II. ASSAY BY POTENTIOMETRIC DIAZOTIZATION.

Reagents: (1) Hydrochloric acid, A. R. (2) 0.5*N* sodium nitrite solution, standardized against pure anthranilic acid and pure sulfanilic acid. (3) Potassium bromide, A. R.

PROCEDURE.

Place 3.4 Gm. of the sample, accurately weighed, in a 250-cc. beaker; add 20 cc. of concentrated hydrochloric acid and 80 cc. of water. Cool to 0–5° C. Add a few crystals of potassium bromide and about 20 cc. of chipped ice. Place the beaker in an ice-bath. Insert this mixture under a mechanical stirrer and the electrode system, bright platinum-solution-0.1*N* KCl-AgCl-Ag. Place the burette tip beneath the surface of the solution and add about 38 cc. of 0.5*N* sodium nitrite solution taking approximately two minutes for the addition. From this point read the potential after each 0.05 cc. addition of reagent. Calculate the difference in potential for each addition. The largest change in potential occurs at the end-point. This end-point occurs at about 425–525 mv.

Calculation:

$$\text{Per cent sulfanilamide} = \frac{\text{cc. } 0.5N\ NaNO_2 \times 0.0860 \times 100}{\text{Weight of sample}}$$

NOTE: When approaching the end-point, wait a few seconds before adding each increment of reagent as the potential is slow to reach a steady value. This difficulty disappears at once after the end-point is reached.

VERIFICATION OF PROPOSED METHODS.

Pure sulfanilamide was prepared by recrystallizing a commercial sample from 50 per cent alcohol and from water in succession, pulverizing and drying *in vacuo*. The purity was checked by taking the crystallizing point after each crystallization and as there was no increase, the material was assumed to be pure.

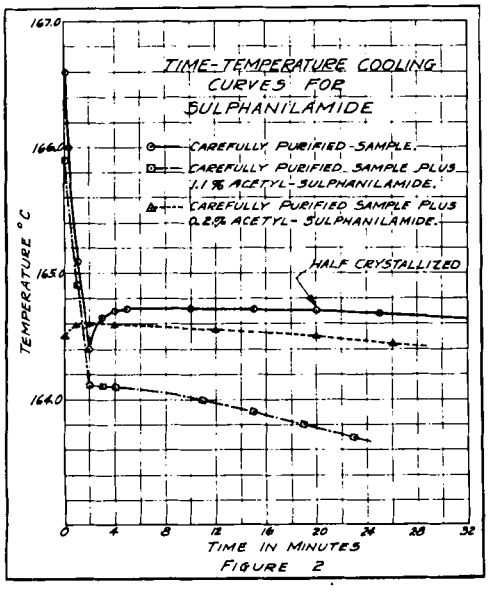

FIGURE 2

This was verified by careful crystallization point determinations, the time-temperature curve for one of which is shown in Fig. 2. The extreme flatness of this curve during crystallization is ample evidence that this material is pure. Within the limits of error of reading the thermometer, there is no drop in crystallizing point during solidification of over half the sample.

The sensitiveness of the crystallizing point method to the presence of small amounts of impurities was checked by adding to the sample varying amounts of acetyl sulfanilamide, a likely impurity in commercial sulfanilamide, and making new time-temperature curves. Figure 2 shows clearly that the addition of 0.2 mol per cent acetyl sulfanilamide not only lowers the crystallizing point 0.1° C., but also produces a time-temperature curve with a distinct downward slope. The

addition of a larger amount, 1.2 mol per cent, produces the same effect to a proportionately greater degree. Evidently the crystallizing point determination is sensitive to 0.1 mol per cent of impurities soluble in sulfanilamide. The same pure sample assayed by Method II was found to have a purity of 100.0 per cent. The abruptness of the change in potential at the end-point is readily seen in Fig. 3. The differential curve, in which changes in potential per cc. of reagent are the ordinates, brings this out even more forcibly (Fig. 4).

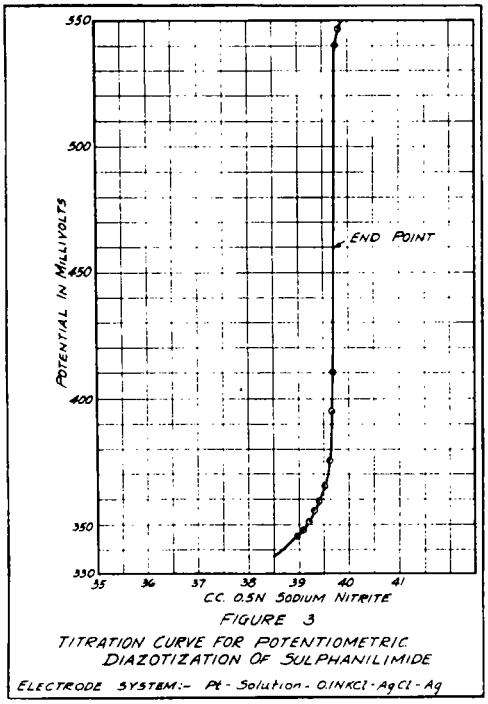

FIGURE 3

TITRATION CURVE FOR POTENTIOMETRIC DIAZOTIZATION OF SULPHANILIMIDE

ELECTRODE SYSTEM:- Pt - Solution - 0.1NKCl - AgCl - Ag

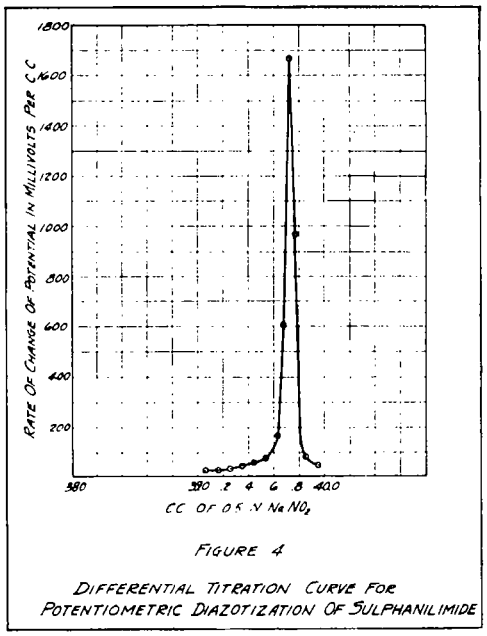

FIGURE 4

DIFFERENTIAL TITRATION CURVE FOR POTENTIOMETRIC DIAZOTIZATION OF SULPHANILIMIDE

Several commercial samples of sulfanilamide were analyzed by the two methods, the results being shown in Table I.

TABLE I.—ANALYSIS OF COMMERCIAL SULFANILAMIDES.

Sample.	Crystallizing Point, ° C.	Assay by Potentiometric Diazotization, ° C
1*	164.7	100.0
2	164.7	100.0
3	164.5	99.9
4	164.6	100.0
5	164.7	100.0
6	164.6	99.9
7	164.3	99.7
8	164.6	99.9

* Pure sulfanilamide.

CONCLUSIONS.

The two methods described for the assay of sulfanilamide are both rapid and accurate. The crystallizing point method will detect as little as 0.1 mol per cent of soluble impurities including isomers and other amino compounds. The diazotization titration is likewise accurate to 0.1 per cent. The two methods combined are a precise method for estimating the purity of sulfanilamide.

REFERENCES.

(1) "New and Non-Official Remedies," page 452 (1938).
(2) Shulek, E., and Boldizar, I., *Z. Anal. Chem.*, 108, 396–400(1937).

THE VOLATILE OIL OF COMPTONIA ASPLENIFOLIA.*

BY ROMULUS DE NICOLA AND E. V. LYNN.[1]

The so-called sweet fern, which grows prolifically in many regions of Massachusetts, is *Comptonia asplenifolia, Aiton* (*Myricaceæ*). It is found in sterile soil from Nova Scotia to Saskatchewan and southward to North Carolina and Tennessee. All parts of the plant contain a resinous substance, but the characteristic odor is apparently due to the volatile oil found chiefly in the leaves.

In 1890 Schimmel & Co. obtained[2] 0.08 per cent of an oil with a specific gravity of 0.926. Later Braun distilled[3] 0.02 per cent of an oil with a specific gravity of 0.8945 and a slight levorotation. He fractionated the oil and reported the presence of aldehydes and esters but identified no specific compounds.

Our material was gathered in the summer of 1938 in Massachusetts and New Hampshire and was identified by Dr. Youngken. Upon drying in the air it lost a total of 63.14 per cent. Distillation of 130 pounds of the fresh leaves and stems gave a total of 31.5 Gm. of oil, a yield of 0.054 per cent, or 0.14 per cent on the dry basis. It was yellowish brown in color, with a cinnamon-like odor, a specific gravity of 0.9154 at 20°/20° C., an index of refraction of 1.4870 at 20° C. and a congealing point of about 5° C. The acid value was 14.04, ester value 13.30, and saponification value after acetylization 58.66. The color prevented determination of optical rotation.

The acids, extracted by sodium carbonate, were distilled and titrated (Du Claux method) to give values of 6.6, 6.6 and 7.5. Theoretical for formic, acetic and propionic are 4, 7 and 12, respectively. From the acid mixture could be extracted by ether a small amount of substance which was precipitated with silver nitrate. The ignited precipitates left residues of 43.21 and 33.70 per cent of silver, indicating acids with molecular weights of 142.9 and 213.4, respectively.

The phenolic fraction, extracted by alkali, amounted to 0.24 per cent. From it no bromide or phenylurethane could be obtained, and other reactions indicated that the substance consisted chiefly of lactone.

The original oil gave no reaction with Schiff's reagent or with semicarbazide hydrochloride which would indicate that the oil contains no aldehydes nor ketones.

Distillation at 18 mm. pressure yielded 20 per cent below 70° C., probably terpenes. No crystalline nitrosochloride could be obtained, although the mixture was cooled greatly.

The fraction between 70° and 150° C. amounted to 35 per cent. It was found to contain cineol by converting to the iodol compound melting at 110° C. Borneol could not be identified.

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[1] Massachusetts College of Pharmacy, Boston, Mass.

[2] Report October, 1890, page 61.

[3] JOUR. A. PH. A., 15, 336–337 (1926).