A Spectrophotometric Method for the Determination of Sulphate and Organic Sulphur on the Micro- and Ultramicroscale

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Among the numerous methods proposed for the determination of sulphate, those based on its precipitation in the form of benzidine sulphate are becoming increasingly important¹⁻³. The method presented below belongs to this category.

In earlier methods the isolated benzidine sulphate has been treated in various ways; it has been titrated with alkali¹ and with permanganate⁴, or treated in different ways to permit colorimetric determination⁵⁻⁷, and it has even been oxidized, the resulting carbon dioxide being then determined manometrically⁸. The present method is based on the fact that benzidine and its salts strongly absorb ultraviolet light. The complete procedure is as follows: the sulphate is precipitated as benzidine sulphate, is isolated, dissolved in hydrochloric acid, and diluted to a certain volume, and its absorption measured in a spectrophotometer. The salts of benzidine have a distinct absorption maximum⁹ at 2500 Å, so strong that the optimal range of operation with 1 cm cells is 1-15 mg of benzidine sulphate/l. The precipitate is crystalline and easily filtered, but is of such low density that it is not very suitable for centrifugation. Its solubility in water is 98 mg/l at room temperature¹⁰ and the precipitation cannot, therefore, be made from a highly diluted solution. Strong acidity raises the solubility further¹¹, one l of 2.8 *N* hydrochloric acid dissolving 1.93 g of benzidine sulphate at 25°, but it is most advantageous to work with slightly acid solutions. Powerful oxidizing agents such as free halogens, nitrite, chromate, and hydrogen peroxide, and some ions giving slightly-soluble benzidine salts, such as cyanoferrate II and III, oxalate, and, to a certain extent, phosphate, have an interfering influence. Further information on the precipitation of benzidine sulphate is to be found in the literature¹⁻³.

The considerable solubility in water of the benzidine sulphate prevents the method from being, *mutatis mutandis*, directly applicable to ultramicro quantities. The solubility is best reduced by using alcoholic solutions (Table 1).

Table 1. The solubility of benzidine sulphate in the system ethanol + water.

% Ethanol	mg benzidine sulfate/l
0 (10)	98
50	10.1
95	1.16

Acetone is unsuitable because of its absorption of ultraviolet light. The benzidine hydrochloride reagent must be neutral, as even the small excess of mineral acid which is present in the normal reagent seems to dissolve some of the precipitate. The precipitation must be carried out in hot solution.

Some results obtained by applying the micro- as well as the ultramicro method to standard solutions are given in Fig. 1. A solution of 515.3 mg of carefully dried ammonium sulphate in 250 ml of water was used as standard. This solution contains 0.5 mg of sulphur/ml. For the ultramicro method this solution was diluted to 5 μ g of sulphur/ml, and suitable quantities were pipetted and evaporated as described below.

I. THE MICRO METHOD

Apparatus: 1) A spectrophotometer for ultraviolet light; 2) A few small Büchner-funnels of about 1.5–2 cm diameter; 3) One or more 1 l measuring flasks; 4) Slow filter paper.

Reagents: 1) Benzidine hydrochloride: 6.7 g of benzidine + 20 ml of about 6 *N* hydrochloric acid are diluted to 1 l, allowed to stand until the benzidine is dissolved, and filtered if necessary. The solution gradually becomes unusable because of mold growth¹²; 2) 0.2 *N* acetic acid; 3) 50 % ethanol; 4) Hydrochloric acid, one part of conc. acid + three parts of water.

The sample to be analysed should be free from interfering substances, almost neutral, and 0.1–0.01 *N* with respect to sulphate. A suitable volume is 1–10 ml containing 0.1–1.5 mg of sulphur. The solution is acidified slightly with 0.25–2 ml of acetic acid and 0.5–2 ml of benzidine hydrochloride reagent is added. One ml of 0.1 *N* sulphate (= 1.6 mg of sulphur) requires about 2 ml of reagent. The precipitate is allowed to stand for 10–30 min. and is then filtered and eventually washed with a few ml of 50 % ethanol. The whole funnel is placed in 200 ml of warm hydrochloric acid. Any precipitate remaining in the precipitation vessel is likewise dissolved in this solution. After some cautious stirring, the precipitate is dissolved and the funnel and filter paper are removed and rinsed with water. The solution is poured into a measuring flask, diluted to 1 l with water, and measured spectrophotometrically at 2 500 Å. The solvent is used as blank solution, for hydrochloric acid absorbs ultraviolet light to a certain extent. The accuracy is 1–2 %.

Fig. 1. Standard curve for the determination. Precipitate dissolved in hydrochloric acid.
Wave length 2 500 Å; 1 cm cells.

● Values obtained by the micromethod; dilution to 1 litre.

× Values obtained by the ultramicromethod; dilution to 25 ml.

II. THE ULTRAMICRO METHOD

Apparatus: 1) The filtration is best carried out through fine glass wool *e.g.* Pyrex Brand in the apparatus shown in Fig. 2, and 2) 1–2 simple pipettes with fine tips for small drops of about 0.02 ml; 3) A measuring vessel, volume 25 ml.

Reagents: 1) Neutral benzidine hydrochloride solution: 500 mg of benzidine + 5.5 ml of 0.1 *N* hydrochloric acid + 100 ml of water are heated to boiling, cooled and filtered; 2) 0.2 *N* acetic acid; 3) 50 % ethanol saturated with benzidine sulphate; 4) Hydrochloric acid, one part of conc. acid + nine parts of water.

The sample to be analysed, containing 1–25 µg of sulphur in the form of a neutral sulphate solution, is evaporated, dissolved in 1–2 ml of 95 % ethanol, and acidified with one small drop of acetic acid. The sulphate is precipitated with benzidine hydrochloride, of which about one small drop is taken for each µg of sulphur. The latitude is some 3–12 drops/5 µg but smaller for larger quantities of sulphur. The precipitation is carried out in hot solution. After about 15–30 min. the solution is filtered, and the precipitate washed, at first with 50 % ethanol saturated with benzidine sulphate, and then with 95 % ethanol. The precipitate is dissolved in hydrochloric acid, transferred to the measuring vessel, diluted to 25 ml with hydrochloric acid, and measured spectrophotometrically. The accuracy is slightly lower than that of the micro method.

Fig. 2. Apparatus for filtration.

The method can also be used for the determination of sulphur in organic material, which must first be completely oxidized. The destruction is generally best carried out by the method of Carius, in a small sealed tube of about 0.5 cm diameter. The nitric acid is evaporated, and the remaining traces of free acid must be carefully neutralized, which is best done by means of ammonia.

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