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A COLORIMETRIC METHOD FOR THE DETERMINATION OF THIOSULFATE

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In a recent study[1] on the formation of thiosulfate in biological systems, the classical iodometric method for the determination of this compound was attempted. However, it was found inapplicable to some of these systems, as in these cases a sharp end-point could not be obtained, due to interfering reducing compounds. Other existing methods for the determination of thiosulfate appeared too laborious and time-consuming[2,3]. When looking for a better method the possibility of converting thiosulfate to thiocyanate and determining the latter colorimetrically with ferric ions, was considered. The conversion of thiosulfate to thiocyanate can be accomplished by heating with cyanide for 30 minutes on a steam bath[4], but it has now been found that this conversion occurs much more rapidly and at room temperature in the presence of cupric ions. The present communication presents a simple colorimetric method for the determination of thiosulfate, based on this procedure.

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MATERIALS AND METHODS

Reagents

0.10 M potassium cyanide; 0.10 M cupric chloride; ferric nitrate reagent: 100 g $Fe(NO_3)_3 \cdot 9H_2O$ and 200 ml 65 % nitric acid and distilled water to 1000 ml. All compounds were commercial A.R. except sodium tetrathionate which was prepared according to GILMAN *et al.*[5].

Procedure

The sample can be used directly for analysis if free from buffering compounds. It must otherwise be brought to the blue color for thymolphthaleine, which can be used as an internal indicator. To 4.2 ml of the sample, containing up to 1.5 μ-equivalent of thiosulfate, 0.50 ml potassium cyanide and, after mixing, 0.30 ml cupric chloride are added. A precipitate is slowly formed, which dissolves when 0.5 ml ferric nitrate reagent is added. It is important, that the cupric chloride be well mixed with the sample immediately after its addition, as otherwise a precipitate forms which is not completely dissolved by the ferric nitrate reagent. The absorbancy at 460 mμ is then determined with a suitable instrument. The Coleman Junior and the Beckman Model DU spectrophotometers were used alternatively in the present work. The value obtained is corrected for a "blank", obtained by adding to another aliquot of the sample first the ferric nitrate reagent, followed by potassium cyanide and cupric chloride. 1 μ-equivalent of thiosulfate gives a corrected absorbance value in a 1 cm cuvette in the Beckman spectrophotometer of about 0.58. The blank value should be less than 0.010. The method thus permits the determination of 0.05 μ-equivalents of thiosulfate in a final volume of 5 ml.

EXPERIMENTAL

The effect of several variables on the conversion of thiosulfate to thiocyanate has been studied. Preliminary experiments demonstrated that thiosulfate in dilute solutions could be quantitatively converted to thiocyanate if cyanide was added before the copper. If the cupric ions were added first, the yield was only 50–60%, and if the copper and cyanide solutions were mixed before adding them to the sample the yield was only about 10%. No conversion was obtained when the cupric ions were replaced by silver, cuprous or ferric ions. The reaction between thiosulfate, cyanide and cupric ions was completed within 15 seconds and thus no time-effect could be demonstrated. The effect of cyanide and copper ion concentrations was then investigated: the results are shown in Fig. 1.

From this figure it is evident that there is a certain relationship between the cyanide and copper concentration at 100% conversion. Increase in both cyanide an copper ion concentration causes a decrease in the color given by the ferric-thiocyanate complex. The reaction conditions 0.01 M CN^- and 0.006 M Cu^{2+} were chosen for the determination procedure. The effects of acids and bases on the reaction are shown in Table I. From these results it is evident that maximum conversion occurs around pH 9–11, the yield being decreased at lower and higher pH-values. The relationship between thiosulfate concentration and absorbancy under the conditions selected for the thiosulfate determination is shown in Fig. 2. Exactly the same absorbance values were obtained when thiosulfate was replaced by thiocyanate, demonstrating that quantitative conversion of thiosulfate to thiocyanate had occurred.

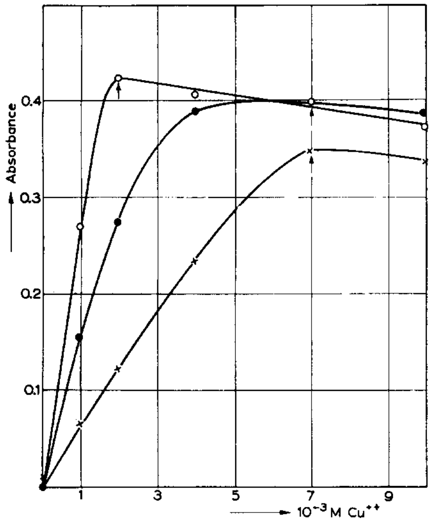

Fig. 1

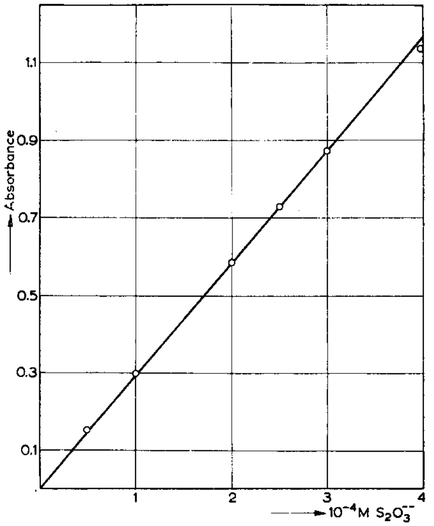

Fig. 2

Fig. 1. Influence of copper concentrations on color intensity produced by thiosulfate. The curves have been determined at cyanide concentrations 0.005 *M* (○), 0.010 *M* (●) and 0.020 *M* (×). Thiosulfate concentration $2 \cdot 10^{-4} M$. Concentrations refer to conditions before addition of ferric nitrate reagent. Arrows indicate where complete conversion of thiosulfate to thiocyanate occurred (determined by replacing thiosulfate by thiocyanate in the test). Absorbance determined with a Coleman Junior spectrophotometer using round cuvettes of 10 mm diam.

Fig. 2. Effect of thiosulfate concentration on absorbance. Standard test conditions. Absorbance determined with a Beckman DU spectrophotometer using 1 cm cuvettes.

TABLE I

ACID-BASE EFFECT ON COLOR DEVELOPMENT OF THIOSULFATE

The test contained $2 \cdot 10^{-4} M$ thiosulfate, 0.01 *M* KCN, 0.06 *M* $CuCl_2$ and HCl or NaOH as indicated. Other conditions as in Fig. 1.

Concentration of acid or base	*Absorbance*
Standard test	0.290
HCl 0.0025 *M*	0.290
HCl 0.0050 *M*	0.288
HCl 0.0075 *M*	0.227
HCl 0.0100 *M*	0.067
NaOH 0.0050 *M*	0.263
NaOH 0.0100 *M*	0.155

INTERFERENCES

Positive interference is of course given by preformed thiocyanate, but this is included in the blank value and does not cause any difficulty if not present in large amount. If desired, preformed thiocyanate can be separately determined by carrying out another type of blank determination in which the sample is omitted. Positive interference is also given by polythionates[7], which react spontaneously with cyanide, giving thiocyanate and in some cases thiosulfate. If not present in too large an excess over the thiosulfate, a correction for the presence of polythionate may be obtained by adding cyanide to the sample and determining on an aliquot the thiocyanate formed after complete cyanolysis of the polythionate*. The thiosulfate, which does not spontaneously react with cyanide at room temperature, is then determined in the cyanolysed sample by the addition of cupric ions and ferric nitrate reagent. When the polythionate is trithionate the determination made in this manner represents the true preformed thiosulfate present in the sample. However, when tetra-, penta- or hexathionate are present, the value must be corrected for thiosulfate formed from the polythionate according to the following equation:

$$S_nO_6^{2-} + (n-3)CN^- + OH^- \longrightarrow (n-3)CNS^- + S_2O_3^{2-} + HSO_4^-$$

The reaction of trithionate and cyanide proceeds as follows without thiosulfate formation:

$$S_3O_6^{2-} + CN^- + OH^- \longrightarrow CNS^- + SO_3^{2-} + HSO_4^-$$

It is apparent that a correction for the presence of polythionate cannot be made if more than one of these substances is present or if the identity of the substance is unknown. The behavior of tetrathionate under the conditions of the assay procedure has been studied. It was found that the cyanolysis of $2 \cdot 10^{-4}$ *M* tetrathionate in 0.01 *M* cyanide was a first order reaction with a rate constant of 0.4 sec^{-1} at 20°C. When cupric ions followed by ferric nitrate reagent were added to a mixture of tetrathionate and cyanide, the amount of thiocyanate formed was dependent on the time of contact between tetrathionate and cyanide. The maximum value corresponded to two moles of thiocyanate per mole of tetrathionate according to the theory, as the thiosulfate formed in the cyanolysis is converted to thiocyanate by the cupric ions. Extrapolation of the data to zero time of cyanolysis indicated that cupric ions do not catalyze the cyanolysis of tetrathionate. Consequently, when thiosulfate is determined in the presence of tetrathionate, it is important that the latter should be allowed to react completely with cyanide before the addition of cupric ions. Otherwise variable results are obtained. A reaction time of 30 minutes is suggested at ordinary laboratory temperatures.

Positive interference is also given by sulfide ion, which was found to be completely converted to thiocyanate under the test conditions. However, sulfide can easily be precipitated with cadmium ions (which are preferred to zinc, see below). Other thiol compounds also interfere to some extent owing to their colored iron complexes, but are also precipitated with cadmium ions.

Negative interference is given by all compounds which interfere in the colorimetric determination of thiocyanate by ferric ions. Thus phosphate at 0.01 *M* concentration was found to decrease the thiocyanate color 25% under the test conditions and interfered with the determination of thiosulfate to the same extent. The effect of zinc ions was different. At 0.01 *M* concentration zinc inhibited the conversion of thiosulfate to thiocyanate 60% without significantly affecting the color given by thiocyanate. As cadmium ions at the same concentration gave only 10% interference, they are to be preferred for precipitation of interfering thiol compounds. Negative interference is also given by compounds that reduce the cupric ion concentration. 0.01 *M* sulfite gave 30% interference, but was without effect at 0.001 *M* concentra-

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* A colorimetric method for the determination of tetrathionate based on this procedure has recently been published[6].

tion. No effect on the assay was observed with 0.01 M bicarbonate or 0.1 M ammonia-ammonium chloride buffer of pH 10.4.

DISCUSSION

The reaction mechanism for the conversion of thiosulfate to thiocyanate by cyanide and cupric ions is not known. The fact that cupric ions oxidize thiosulfate to tetrathionate and the latter spontaneously gives thiocyanate with cyanide, suggests that tetrathionate is an intermediate. However, the cyanolysis of tetrathionate is a comparatively slow reaction, whereas the reaction between thiosulfate, cyanide and cupric ions occurs instantaneously. Accordingly the formation of tetrathionate cannot explain the reaction. Cupric ions also react with cyanide giving free cyanogen. As it has been mentioned, if the copper and cyanide are mixed before addition to the sample, the conversion of thiosulfate to thiocyanate is not quantitative, although the formation of cyanogen could be expected to be the same as when copper is added to the sample in the presence of cyanide. Therefore the formation of cyanogen cannot explain the reaction mechanism. The latter probably involves the formation of a copper-thiosulfate-cyanide complex, which rapidly decomposes, giving thiocyanate.

The present method was also tried out in the determination of thiosulfate in urine, at concentrations normally present. The sensitivity appeared to be sufficient for this purpose, but it was found that other substances, normally occurring in urine, gave such a high absorbancy in the presence of ferric ions, that the method was not applicable. It may be applicable to urine and blood determinations when increased concentrations of thiosulfate are present, as in clearance and extracellular space studies. Since existing methods[8] appear to be adequate, no experiments in this direction have been carried out.

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SUMMARY

A colorimetric procedure for the determination of thiosulfate in concentration as low as $1 \cdot 10^{-5} M$ is described. This method is based on the conversion of thiosulfate to thiocyanate in the presence of cyanide and cupric ions, followed by determination of thiocyanate as the iron complex.

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