KINETIC FLUORIMETRIC DETERMINATION OF TRACES OF SULFIDE BASED ON
ITS INHIBITORY EFFECT ON THE OXIDATION OF PYRIDOXAL NICOTINYLDRA-
ZONE BY POTASSIUM PEROXODISULFATE CATALYZED BY SILVER (I)

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An indirect kinetic fluorimetric method for the determination of traces of sulfide, based on its inhibitory effect on the oxidation of pyridoxal nicotinyldrazone by potassium persulfate is described. The reaction is followed spectrofluorimetrically by measuring the rate of change of the fluorescence intensity ($\lambda_{\text{ex}} = 365 \text{ nm}$, $\lambda_{\text{em}} = 450 \text{ nm}$). The calibration graph (percentage inhibition vs. sulfide concentration) is linear in the $10\text{--}80 \text{ ng S}^{2-} \text{ ml}^{-1}$ range. The interferences of the method are described.

INTRODUCTION

In recent years, kinetic methods of analysis based on catalytic rates modified¹⁾²⁾ by inhibition or activation have been developed for determining several chemical species in solution. Both aspects, inhibition and activation, have been applied to enzymatic as well as nonenzymatic catalysis.

The development of sensitive analytical methods for sulfide is of great interest owing to the extreme toxicity of this ion caused by its great ability to coordinate with many metals in the human metabolism. Sulfides are also often present in industrial effluents and it is necessary to ensure the absence of hydrogen sulfide in such industrial waste water because it is a catalyst poison.

Several fluorimetric methods for the determination of sulfide ions have been described in the bibliography. One of these methods is based on the inhibitory effect of sulfide ions on the enzymatic reaction between the homovanillic acid and hydrogen peroxide in the presence of peroxidase³⁾, giving 2,2'-dihydroxy-3,3'-dimethoxybiphenyl-5,5'-diacetic acid.

The oxidation of thiamine to thiocrome by oxidizing agents such as permanganate is inhibited by the sulfide ion. Based on this effect a fluorimetric method for the determination of the sulfide ($< 20 \text{ g l}^{-1}$) has been proposed⁴⁾.

Various fluorimetric methods for the determination of sulfide based on substitution reactions of ligands have also been described. In some of these methods, the sulfide ion inhibits the fluorescence of various systems, e.g. di(acetoxymercury) fluorescein ($1\text{--}10 \text{ ng ml}^{-1}$)⁵⁾⁻¹¹⁾, tetra(acetoxymercury) fluorescein (20 ng ml^{-1})¹²⁾⁻¹⁶⁾ and 3,4,5,6-tetrachlorofluorescein-Hg(II) ($0\text{--}4 \text{ } \mu\text{g ml}^{-1}$)¹⁷⁾. Another method

depends on the demasking of quinoline-8-ol-5 sulfonic acid by sulfide from the non-fluorescent potassium bis (5-sulfoxino) palladium (II). The liberated quinoline-8-ol-5-sulfonic acid then coordinates with the magnesium ion present to form a fluorescent chelate, which is a measure of the amount of sulfide present¹⁸⁾.

Ludwig and coworkers¹⁹⁾ have proposed a photometric kinetic method for the determination of sulfide ($0.2\text{--}0.9\text{ }\mu\text{g ml}^{-1}$) using the indicatory sulfanilic acid-peroxodisulfate reaction, catalyzed by silver(I) and inhibited by sulfide.

The oxidation of pyridoxal nicotinyldrazone (PNH) with potassium peroxodisulfate catalyzed by silver (I) has been previously described and applied to the kinetic determination of silver(I)²⁰⁾. The sulfide ion produces an inhibitory effect on this catalyzed reaction, probably due to its interaction with the silver (I). Other ions such as CN^- , $\text{S}_2\text{O}_3^{2-}$, SCN^- , and I^- also show this inhibitory effect under the same conditions. In this paper, the inhibitory effect of the sulfide ion on the $\text{PNH/K}_2\text{S}_2\text{O}_8/\text{Ag(I)}$ system is studied and a kinetic fluorimetric method for the determination of this ion is described.

This method is comparable in sensitivity and selectivity to the procedures described above and its reproducibility is excellent.

EXPERIMENTAL

Apparatus and reagents

A Perkin Elmer spectrofluorimeter MPF-43A was used, fitted with a device for kinetic measurements and with 1-cm quartz cells. The cell compartment of the spectrofluorimeter was thermostated by circulating water. A set of standard fluorescence polymer samples were used daily to adjust the spectrofluorimeter to compensate for changes in source intensity. A Radiometer pHM-26 digital pH meter with a combined glass-calomel electrode was used.

A silver solution (0.998 g l^{-1}) was prepared from silver nitrate and standardized by potentiometric titration with potassium chloride. A standard sulfide solution (0.987 g l^{-1}) was prepared from sodium sulfide and standardized by potentiometric titration with a silver nitrate standard solution. Working solutions were prepared daily by suitable dilution.

Pyridoxal nicotinyldrazone was synthesized from pyridoxal hydrochloride and nicotinic acid hydrazide²¹⁾.

All solvents and reagents were of analytical reagent grade.

Procedure for determination of sulfide.

Optimum working conditions were chosen so that the difference between the catalyzed reaction and the inhibited reaction was the maximum. The fluorescence intensity-time curves were recorded with a solution containing 200 ng ml^{-1} of silver (I), 0.3 ml of PNH solution (0.05%) in ethanol, 4 ml of 2 M ammonia-ammonium chloride buffer of pH 10.5, 1 ml of 0.2 M potassium peroxodisulfate solution and a known amount of sample solutions needed to give a final sulfide concentration between 10 and 80 ng ml^{-1} , which was diluted to 10 ml with distilled water. A similar solution was prepared containing no sulfide (catalyzed reaction). The intensity of the fluorescence emitted ($\lambda_{\text{ex}} = 365\text{ nm}$, $\lambda_{\text{em}} = 450\text{ nm}$) was measured exactly 3 min after the addition of the sample.

Fig. 1 Inhibitory effect of S^{2-} ion on the pyridoxal nicotinyldiazotization/peroxo-disulfate/silver(I) system
 Curves I-IV : Excitation spectra; Curves I'-IV' : Emission spectra;
 I, I' : PNH; II, II' : $PNH/S_2O_8^{2-}$; III, III' : $PNH/S_2O_8^{2-}/Ag(I)$; IV, IV' : $PNH/S_2O_8^{2-}/Ag(I)/S^{2-}$; Experimental conditions : 4.41×10^{-5} M PNH, 2×10^{-2} M $K_2S_2O_8$, 200 ng ml^{-1} Ag(I), 0.8 M ammonia-ammonium chloride (pH 10.5) and 50 ng ml^{-1} of sulfide.

The reaction rates were calculated from the slopes of the fluorescence intensity-time curves. The percentage of inhibition was calculated as :

$$\% \text{ inhibition} = \frac{(\Delta I_F / \text{min})_{\text{sample}}}{(\Delta I_F / \text{min})_{\text{blank}}} \times 100 \quad (I)$$

where: $(\Delta I_F / \text{min})_{\text{sample}}$ = Difference between the initial rate of the catalyzed reaction and the initial rate of the inhibited reaction.

$(\Delta I_F / \text{min})_{\text{blank}}$ = Difference between the initial rate of the catalyzed reaction and the initial rate of the uncatalyzed reaction (without silver (I)).

The concentration of sulfide is determined from the calibration plot of percentage inhibition vs. concentration of sulfide.

RESULTS AND DISCUSSION

Inhibitory effect of the sulfide on the $PNH/S_2O_8^{2-}/Ag(I)$ system

The sulfide ion retards the catalytic activity of silver (I) and so can be determined indirectly.

Under working conditions in the presence of silver (I) the sulfide ion interacts with this ion producing the partial inhibition of the catalytic effect of silver (I).

The fluorescence spectra of PNH, $\text{PNH/S}_2\text{O}_8^{2-}$, $\text{PNH/S}_2\text{O}_8^{2-}/\text{Ag(I)}$ and $\text{PNH/S}_2\text{O}_8^{2-}/\text{Ag(I)/S}^{2-}$ systems in an ammoniacal buffer solution are shown in Fig. 1. In the absence of oxidizing agent (curves I, I') the obtained spectra are the characteristics of PNH ($\lambda_{\text{ex}} = 300 \text{ nm}$, $\lambda_{\text{em}} = 365 \text{ nm}$). In the presence of potassium peroxodisulfate the reagent is oxidized and the spectra show different maxima (curves II, II'), ($\lambda_{\text{ex}} = 365 \text{ nm}$, $\lambda_{\text{em}} = 455 \text{ nm}$). This oxidation reaction is catalyzed by traces of silver (I) (curves III, III'). In the presence of the sulfide ion, however, the fluorescence maxima intensity become less (curves IV, IV'). All the spectra were recorded 40 min after the preparation of the samples. The results obtained show the inhibitory of sulfide ion.

The rate of change of fluorescence intensity for the sample with the catalyzed reaction against a similar sample containing sulfide is proportional to the sulfide concentration.

Effects of reaction variables

The influence of temperature on the reaction rate was studied in the 30-60 °C range. Between 30 and 50 °C the inhibitory effect rises when increasing the temperature and it remains constant in the 50-60 °C range. The greatest inhibitory effect is obtained in this range of temperature (Fig. 2a). The activation energy was found to be 13.25 kcal per mole.

The inhibitory effect of sulfide increases linearly when increasing the silver (I) concentration in the 50-125 ng ml^{-1} range. For silver (I) concentrations of over 225 ng ml^{-1} the inhibitory effect of sulfide decreases (Fig. 2b). A 200 ng ml^{-1} concentration of silver (I) was selected as the optimum value for maximum inhibition.

To study the effect of pH on the inhibitory reaction, all the samples were prepared in 0.75 M ammonia solution and the pH was adjusted by hydrochloric acid and sodium hydroxide. The results obtained were similar to those obtained in the study for the catalytic reaction, where the optimum pH is in the 10.3-10.8 range. A pH of 10.5 ammonia-ammonium chloride buffer was chosen for the experimental procedure.

The effect of the buffer concentration was studied in the 0.2-1.4 M range. The inhibitory effect of sulfide increases linearly with a rise of the buffer concentration from 0.2 to 0.7 M but it remains constant between 0.7 and 1.4 M. A 0.8 M buffer concentration was selected.

As the PNH is dissolved in ethanol, the effect of this solvent on the system was studied. The ethanol concentration (between 3 to 17 percent v/v) causes an exponential decrease of the reaction rate when increasing the percentage of ethanol.

Potassium chloride and potassium nitrate have been tested at different concentrations (0.03-0.15 M) to study the effect of the ionic strength on the inhibitory effect of sulfide. For values of ionic strength of less than 0.05 M, the rate does not change in the presence of these salts. In the 0.05-0.15 range the rate of reaction decreases exponentially.

Fig. 2 Effect of temperature (a), silver (I) concentration (b), pyridoxal nicotinylhydrazone concentration (c), and potassium peroxodisulfate concentration (d) on the inhibitory effect of sulfide

$$\Delta \tan \alpha = \tan \alpha_{\text{inhibited reaction}} - \tan \alpha_{\text{catalyzed reaction}}$$

The effect of the PNH concentration was studied in the 1.47×10^{-5} – 1.03×10^{-4} M range. The inhibitory effect of sulfide remains constant up to the reagent concentration of 5×10^{-5} M. In a higher reagent concentration, this effect decreases (Fig. 2c). A 4.4×10^{-5} M solution of PNH was selected.

The effect of the oxidizing agent concentration on the inhibitory reaction was studied in the $(0.5\text{--}4) \times 10^{-2}$ M range (Fig. 2d). It increases linearly with a rise of the concentration of the oxidizing agent in the $(0.5\text{--}1) \times 10^{-2}$ M range. When the oxidizing agent concentration is higher, the inhibitory effect does not change. A 0.02 M solution of potassium peroxodisulfate was selected.

Rate equation

According to the kinetic study carried out, the following equation is suggested for the rate of oxidation of PNH by potassium peroxodisulfate catalyzed by silver and inhibited by sulfide:

$$v_o = \frac{-d(\text{PNH})_{\text{ox}}}{dt} = k - k'(S^{2-})$$

where $(\text{PNH})_{\text{ox}}$ is the concentration of the oxidized reagent, K' is the conditional rate constant of the inhibited reaction and K is the conditional rate constant for the catalyzed and uncatalyzed reactions.

KINETIC DETERMINATION OF SULFIDE

The concentration of sulfide is determined from a calibration plot of percentage of inhibition, calculated from the expression (I), versus concentration of the inhibitor ion. The $\Delta I_F/\text{min}$ values were obtained from the straight portion of the curves (initial rates). The percentage of inhibition was calculated as indicated in the experimental section.

The calibration graph is linear in the $10\text{--}80 \text{ ng ml}^{-1}$ range of sulfide ions. The relative standard deviation for $50 \text{ ng S}^{2-} \text{ ml}^{-1}$ was 1.19% ($n=11$, $p=0.05$).

The fixed time and variable time methods were also applied and the determination ranges of sulfide were the same to those for the initial rate method. For the fixed time method, a time of 6 min was selected. The relative standard deviation for $50 \text{ ng S}^{2-} \text{ ml}^{-1}$ was 0.90% ($n=11$, $p=0.05$). For the variable time method, the inverse of the time necessary to obtain a fluorescence intensity of 25% was measured. The relative standard deviation for $50 \text{ ng S}^{2-} \text{ ml}^{-1}$ was 1.95% ($n=11$, $p=0.05$).

For the three methods, the inhibition percentage was 100% when the sulfide concentration was 80 ng ml^{-1} . The initial rate and fixed time methods are recommended.

Effect of foreign ions

The maximum tolerable concentrations of several ions in the determination of sulfide were established (Table 1). The initial rate method was applied. Many ions did not affect the determination of S^{2-} when they were present in amounts of over 100 fold. Only five ions (Ni(II), Au(III), Os(VIII), Mn(II) and I^-) among sixty one ions tested cause interferences at equal concentrations to sulfide. The inter-

Table 1 Effect of various ions on sulfide determination at a concentration of 50 ng ml^{-1}

Tolerance ratio Ion/ S^{2-}	Ion added
100	K(I), Na(I), Th(IV), Fe(III), Fe(II), Cu(II), Cr(III), W(VI), Cd(II), Sn(II), Ca(II), Se(II), Zr(IV), Ba(II), Pb(II), Mo(VI), Bi(III), Hf(IV), Ti(IV), Sr(II), Rh(IV), EDTA, tartrate, citrate, Br^- , F^- , NO_2^- , NO_3^- , ClO_3^- , ClO_4^- , CO_3^{2-} , $\text{C}_2\text{O}_4^{2-}$, SO_3^{2-} , BrO_3^- , VO_3^- , PO_4^{3-} , MoO_4^{2-} , $\text{B}_4\text{O}_7^{2-}$
50	CrO_4^{2-} , IO_3^- , Pd(II)
20	$\text{UO}_2(\text{II})$, Pt(IV), CN^- , IO_4^-
1	$\text{S}_2\text{O}_3^{2-}$, SCN^- , AsO_2^- , Ce(IV), MnO_4^- , Be(II), Co(II), Al(III), In(III), Mg(II), Zn(II)

ferences of gold (III), manganese (II), and osmium (VIII) are due to the catalytic effect of these ions. Nickel (II) interferes because it forms a colored complex, and the iodide ion causes an inhibitory effect similar to the sulfide.

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