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Micellar-mediated Extractive Spectrophotometric Determination of Hydrogen Sulfide/Sulfide through Prussian Blue Reaction: Application to Environmental Samples

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A sensitive surfactant-mediated extractive spectrophotometric method has been developed, based on the reaction of ferric iron with sulfide to form ferrous iron and its subsequent reaction with ferricyanide to form Prussian Blue, to quantify trace levels of hydrogen sulfide/sulfide in environmental samples. The method obeys Beer's law in the concentration range 2 – 10 μg of sulfide in 25 mL of aqueous phase with molar absorptivity (ε) of 3.92×10^4 L mol^{-1} cm^{-1}. The colored species has been extracted into isoamyl acetate in the presence of a cationic surfactant *i.e.* cetylpyridinium chloride, to enhance the sensitivity of the method with ε value 5.2×10^4 L mol^{-1} cm^{-1}. The relative standard deviation has been found to be 0.69% for 10 determinations at 4 μg of sulfide and the limit of detection was 0.009 μg mL^{-1}. The interference from common anions and cations has been studied. The proposed method has been applied to the determination of residual hydrogen sulfide in the laboratory fume hood as well as ambient atmospheric hydrogen sulfide in the vicinity of open sewer lines after fixing the analyte in ionic form using suitable trapping medium.

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Introduction

The detection of sulfide or hydrogen sulfide has gained importance within the analytical community as a consequence of its high toxicity. Hydrogen sulfide acts as a cellular poison through the deactivation of aerobic respiration, with death typically occurring through asphyxiation. Lethal doses depending upon the exposure can range from 300 – 1000 ppm and suggests an arguably greater toxicity than that posed by hydrogen cyanide at similar levels.[1,2] The main industrial sources of hydrogen sulfide are kraft-pulp mills, petroleum refineries, gasification of coal, meat processing plants and sewage treatment plants.[3] From the environmental point of view, sulfide is one of the most important parameters to monitor in water matrices due to its high toxicity for aquatic organisms. The sulfide anion is an important constituent of aqueous systems wherever microbial colonies flourish, whether in environmental[4] or in physiological contexts.[5] Sulfide is produced in natural waters from the oxidation of organic matter and the reduction of sulfate ions.[6,7] Other important contributors to such releases are the leather and paper processing industries.[8,9] Sodium sulfide has been extensively used in the removal of hair from animal hides with processing liquors containing sulfide ion concentration up to 2000 ppm.[1] The determination of sulfide in water provides an important parameter for understanding redox processes in the aquatic environment and for maintaining water quality. Control of sulfide concentration in wastewater treatment is an important phenomenon in different stages of the process.[10] Due to the high reactivity of sulfide, sample manipulation must be minimized in order to avoid the losses of sulfide by air oxidation or volatilization.[11] All the aforementioned cases mean that there is a pressing need for the development of simple and sensitive methods for the determination of sulfide at trace level concentrations.

Sulfide analysis is well represented in different branches of analytical science and can be grouped into three broad categories: titrimetric, chromatographic and spectroscopic. Ultraviolet/Visible spectroscopy retains significant analytical value in terms of simplicity, selectivity and sensitivity. Among the several spectrophotometric methods, methylene blue method is the most popular and standard procedure for the determination of sulfide at trace level.[12–16] Several modifications have been reported based on the same principle with different degrees of sensitivity for sulfide quantification. Among them, multi syringe flow injection analysis, solid-phase extraction and solid phase reflectometry techniques are significant ones in recent years.[17,18] Very few methods have been reported based on the reduction property of hydrogen sulfide/sulfide for its quantification. Among them, 1,10-phenanthroline and neocuproine methods are well known.[19,20] The phenanthroline method suffers from interference of sulfite and nitrite, which are commonly present along with the dissolved sulfide in water bodies.

Herein we report a new method for the estimation of sulfide/hydrogen sulfide based on its reaction with ferric iron and its subsequent Prussian Blue complex formation in the presence of ferricyanide in acidic condition. Micellar-mediated extraction of the color complex into organic solvent enhances the sensitivity of the method by lowering the detection limit.

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Experimental

Apparatus

Absorbance measurements were made using a Shimadzu scanning spectrophotometer (Model UV-3101PC) with 1 cm quartz cuvettes. All pH measurements were carried out using a Control Dynamics digital pH meter (Model APX 175). A Miclins peristaltic pump (Model pp 30) with suitable suction devices was used for sampling of hydrogen sulfide from ambient air. All reagents were analytical grade and were used without further purification. Distilled water was used throughout the experiment

Reagents

$FeCl_3$ solution (0.2%) was prepared by adding few drops of conc. HCl to 0.2 g of $FeCl_3$, and the mixture was diluted with water to the mark in 100 mL volumetric flask. $K_3[Fe(CN)_6]$ solution (0.2%) was prepared by dissolving 0.2 g of $K_3[Fe(CN)_6]$ salt in 100 mL of water in a volumetric flask. Sulfide stock solution of concentration 1000 ppm was prepared by dissolving 0.748 g of $Na_2S{\cdot}9H_2O$ in 100 mL water, and the mixture was stored in a refrigerator. Working standards were prepared from stock solution by appropriate dilution on the day of use. By adding an appropriate volume of acid into water, 6 M HCl was prepared. Cetylpyridinium chloride solution (0.05%) was prepared in water. Absorbing solution was prepared by dissolving 0.01 g of zinc acetate and 0.02 g of sodium citrate in 100 mL and adjusting the pH to 12 using 0.1 M KOH solution.

Aqueous procedure

Aliquots (1 – 5 mL) of 2 μg mL^{-1} sulfide standard solutions were transferred into a series of 25 mL calibrated flasks containing 2 mL of $FeCl_3$ solution and 2 mL of potassium ferricyanide solution. The contents were mixed well and allowed to stand for 10 min. Two milliliters of 6 M HCl were added and the mixture was then diluted to the mark with distilled water. The absorbance values were measured at 760 nm using 1 cm quartz cuvettes.

Extraction procedure

Aliquots (1 – 5 mL) of 0.5 μg mL^{-1} sulfide standard solutions were transferred into a series of 25 mL calibrated flasks containing 2 mL of $FeCl_3$ solution and 2 mL of potassium ferricyanide solution. The contents were mixed well and left to stand for 10 min. Two milliliters of HCl were added and then the mixture was diluted to the mark with distilled water. The solutions were then transferred into 60 mL separating funnels for the extraction. Two and a half milliliters of cetylpyridinium chloride and 3 mL of isoamyl acetate were added; the mixture was equilibrated for about a minute and allowed to stand for 3 min for phase separation. After the aqueous phase was removed, 2 mL of ethanol was added into the separating funnel. The micellar phase dissolves in the organic phase and gives a homogeneous solution. The organic phase was collected into a 5-mL volumetric flask made up to the mark with ethanol before its absorbance measurement at 760 nm, as shown in Fig. 1.

Determination of dissolved sulfide in sewage water samples

Water samples from different sewer lines were collected on different days. The collected water samples were filtered using Whatman filter paper to remove any suspended particulate matter. Ten milliliters of the filtered water sample were treated with 1 mL of 1 M sodium hydroxide and centrifuged to remove the metal ions precipitated in the form of their respective hydroxides. The residue was washed with 5 mL portions of water to collect the sulfide in the form of sodium sulfide and each mixture was centrifuged again. The centrifugate was taken in a 25-mL volumetric flask; to that 1 mL of formaldehyde (1000 μg) was added to mask the sulfite ions and 2 mL of sulfamic acid (5%) solution was added to convert nitrite ions into nitrogen. Analysis was carried out as explained in the above aqueous procedure by taking 1 mL of pretreated water sample.

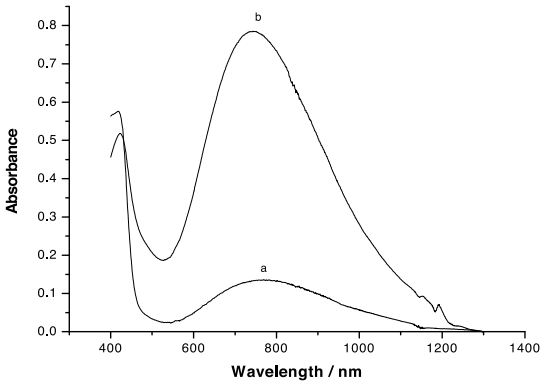

Fig. 1 Absorption spectra (sulfide = 2 μg). (a) Prussian Blue complex in aqueous phase, 25 mL. (b) Prussian Blue complex in organic phase, 5 mL.

Determination of hydrogen sulfide from ambient air

Air samples were drawn through 30 mL of alkaline zinc acetate absorber solution using an impinger. The sampled solution was made up to 50 mL with absorber solution. Ten milliliters of the made up solution were added to a 25-mL calibrated flask containing 2 mL of $FeCl_3$ solution and 2 mL of potassium ferricyanide solution; then two drops of acid was added to make the pH of the solution acidic. The contents were mixed well and allowed to stand for 30 min. Then 2 mL of acid was added and the solution was diluted to 25 mL with distilled water. The absorbance values were measured at 760 nm.

Results and Discussion

This method is based on the reaction of iron(III) with sulfide and the subsequent interaction of reduced iron(II) with ferricyanide to form Prussian Blue complex and its color intensity measurement. The color produced is directly proportional to the amount of hydrogen sulfide/sulfide. Preliminary studies have been carried out by using 1 mM $FeCl_3$, 5 mM potassium ferricyanide solution, 6 M HCl and 10 μg of sulfide in 25 mL of aqueous phase. All the reaction parameters have been optimized to get the maximum absorbance.

Effect of iron(III) ion concentration

The effect of iron concentration was examined in order to establish the optimum quantity of iron required for maximum absorbance by varying the volume of $FeCl_3$ solution. Different volumes of $FeCl_3$ solutions were taken in a series of 25 mL volumetric flasks. Then 10 μg of sulfide and 2 mL of ferricyanide solution were added to each flask followed by 1 mL of acid. After 10 min the solutions were made up to the mark with distilled water and the absorbance values were measured at 760 nm. These studies revealed that 2 mL of ferric

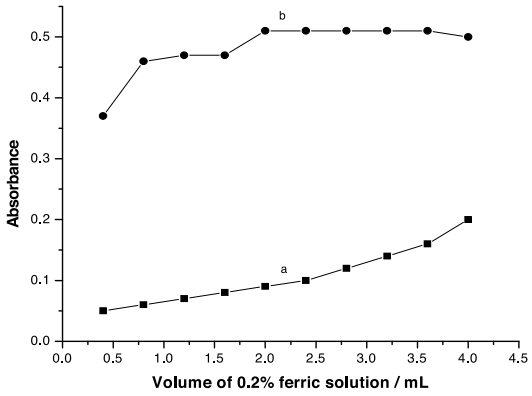

Fig. 2 Effect of ferric iron concentration. (a) Reagent blank absorbance *vs*. water, (b) sample absorbance *vs*. water.

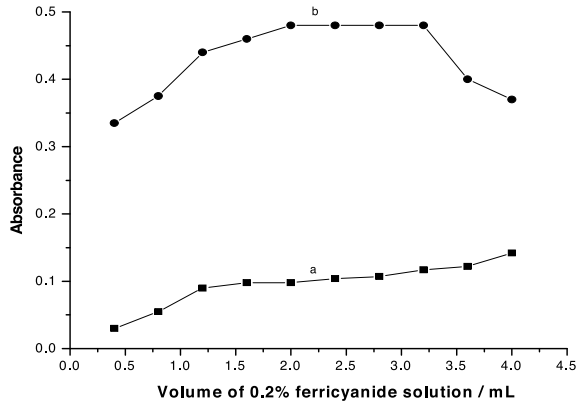

Fig. 3 Effect of ferricyanide concentration. (a) Reagent blank absorbance *vs*. water, (b) sample absorbance *vs*. water.

iron solution is sufficient to give maximum absorbance. Higher concentrations of iron did not enhance the sample absorbance value, but the blank value increased with the increase in the volume of iron(III) solution as shown in Fig. 2. Hence, 2 mL of iron(III) solution has been fixed as an optimum concentration and was used in all further studies.

Effect of ferricyanide ion concentration

The effect of ferricyanide concentration was carried out by varying the volume of 0.2% ferricyanide solution. These studies revealed that 2.0 mL of ferricyanide solution is sufficient to give maximum absorbance to the sample. Absorbance values of the sample remained constant up to 3.2 mL. Hence, 2 mL of 0.2% ferricyanide solution has been fixed as the optimum concentration. In all further studies, 2 mL of 0.2% ferricyanide solution was used, as shown in Fig. 3.

Order of addition of reagents

After other parameters were fixed, the different orders I, II, III, IV as summarized in Table 1 were checked by taking 6 μg of sulfide to ascertain the influence of the order in which reagents were added. Among them the following order: iron(III), ferricyanide, sulfide followed by the addition of hydrochloric acid after full color development, gave maximum absorbance. Hence this order of addition of reagents was followed throughout the experiment.

Table 1 Order of addition of reagents (sulfide = 6 μg)

Sl. No.	Order of addition	Blank absorbance	Sample absorbance
I	Iron(III) + sulfide + ferricyanide + acid	0.020	0.232
II	Iron(III) + acid + sulfide + ferricyanide	0.009	0.082
III	Iron(III) + ferricyanide + acid + sulfide	0.019	0.131
IV	Iron(III) + ferricyanide + sulfide + acid	0.036	0.614

Table 2 Interference study

Interfering ion	Tolerance limit/μg	Interfering ion	Tolerance limit/μg
HCHO	>1000	PO_4^{3-}	>400
SO_3^{2-}	>3	Ca^{2+}	>250
SO_3^{2-a}	250	CO_3^{2-}	>200
NO_2^-	1	Zn^{2+}	>100
NO_2^{-b}	25	Cu^{2+}	>20
NO_3^-	>500	Pb^{2+}	50
Cl^-	>1000	Tartarate,	50
F^-	>750	oxalate	

a. Sample was treated with 1 mL of formaldehyde (1000 μg mL^{-1}).
b. Sample was treated with 5 mL of 1% sulfamic acid.

Table 3 Effect of surfactants

Serial No.	Surfactant used	Nature of extraction
1	None	Partial
2	Tween 80 (neutral)	Partial
3	Sod. lauryl sulfate (anionic)	Partial
2	Cetylpyridinium chloride (cationic)	Complete
3	Cetrimide (cationic)	Complete

Sulfide = 2 μg.
The complex was extracted into 3 mL of organic solvent in presence of surfactant from 25 mL of aqueous phase and made up to 5 mL using ethyl alcohol.

Effect of interfering ions

The effects of common air and water pollutants on the determination of sulfide were studied by introducing the species under examination in the form of the respective anions and cations along with sulfide. Among the different ions studied, sulfite and nitrite gave positive interference at levels of 3 and 1 μg, respectively, in aqueous medium. The interference from sulfite and nitrite was overcome up to 250 and 25 μg respectively by masking them with 1 mL of 1000 μg mL^{-1} HCHO and 5 mL of 5% sulfamic acid. Tolerance limits of different anions and cations are listed in Table 2.

Extraction study

If one wants to lower the detection limit, the Prussian Blue complex can be extracted into an organic solvent; thereby, the limit of detection can be substantially lowered. Initially, several organic solvents were tried to extract the color complex into organic phase. There was no quantitative extraction in any of these solvents, hence we have made an attempt to extract the color complex quantitatively in the presence of a surfactant. Surfactants were known to solubilize, concentrate and organize the analyte species according to their hydrophilic, hydrophobic, electrostatic and specific interactions. Generally they alter the

effective microenvironment *i.e*. polarity, viscosity, acidity as well as the spectral parameters of solubilized chemical species. The use of organized surfactant molecular assemblies increases the sensitivity, selectivity and precision of the methods.[21]

A variety of surfactants including cationic, anionic and neutral surfactants were tried for the extraction of the Prussian Blue complex quantitatively into organic phase. It could be extracted quantitatively into an organic solvent only in the presence of cationic surfactants, as shown in Table 3. Hence, cationic surfactants like cetrimide and cetylpyridinium chloride were tried to extract the complex into the organic phase. Both of these surfactants facilitated the quantitative extraction of the color. However, cetylpyridinium chloride has been used in all further studies for the extraction of color complex into organic solvent. The following mechanism has been proposed for the interaction of surfactant with Prussian Blue complex which makes the phase separation occur very easily with enhanced sensitivity. The cationic surfactant entraps the color complex by forming unorganized micellar assemblies in the aqueous phase based on the interaction of the highly polar end group *i.e*. cyano of Prussian Blue and the hydrophilic end of the surfactant molecule. However, in the presence of organic solvent, the surfactant molecules undergo organization and form well-organized assemblies after entrapping the color complex, as shown in Fig. 4. The entrapped complex gives a heterogeneous phase due to the solubility of the micelles and the insolubility of Prussian Blue in the organic solvent. Addition of ethanol to this phase gives a clear homogeneous phase due to the solubility of both analyte species as well as micelles in ethanol. Hence, ethanol was added to the reaction mixture after extracting the color complex with isoamyl acetate before the measurement of absorbance values.

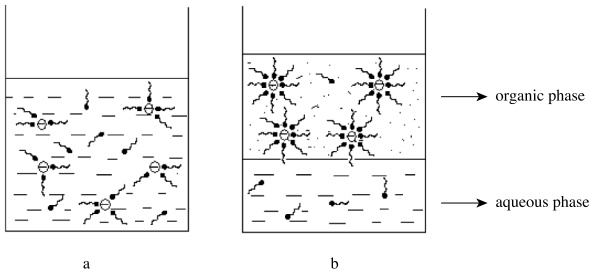

Fig. 4 Schematic pathway of phase separation in the presence of surfactant molecules. Prussian Blue complex entrapped in (a) unorganized micellar assemblies in aqueous phase, (b) organized micellar assemblies in organic phase.

Application study

The proposed method has been applied to the determination of residual hydrogen sulfide levels in a laboratory fume hood and ambient atmospheric air in the vicinity of open sewer lines after fixing it in a suitable trapping medium. It has been applied to estimate the dissolved sulfide in the sewage water samples also. Quantification of hydrogen sulfide/sulfide in the natural samples has been done using above recommended procedures. To check the validity of the method, we spiked the samples with a known quantity of sulfide and the recovery of total sulfide was carried out by the proposed method as well as by the standard methylene blue method. The results obtained by the proposed method are in good agreement with those of the standard method,[22] as shown in Tables 4 – 6.

Conclusion

The proposed method based on the micellar-mediated extraction of Prussian Blue complex into organic solvent formed by the reaction of ferric iron with sulfide and its subsequent reaction with ferricyanide provides a very useful and simple approach to quantify hydrogen sulfide or sulfide from a variety of environmental matrices. This method is less prone to

Table 4 Determination of sulfide in sewage water

Sample[a] No.	Sulfide found/μg		Sulfide added/ μg	Total sulfide found/μg		Recovery of added sulfide, %	
	Proposed method	Standard method		Proposed method	Standard method	Proposed method	Standard method
1	4.13	4.17	2	6.06	6.17	98.85	100.00
2	4.77	4.74	3	7.68	7.70	98.84	99.48
3	Not found	Not found	4	3.86	3.95	96.50	98.75

Filtered water samples were used for analysis. a. Water samples were collected from different locations.

Table 5 Determination of residual hydrogen sulfide in the ambient air in the laboratory fume hood

Sample No.	Volume of air sampled[a]/L	Hydrogen sulfide found				Sulfide added/μg	Total sulfide found/μg		Recovery of added sulfide, %	
		Proposed method		Standard method						
		μg	ppb[b]	μg	ppb[b]		Proposed method	Standard method	Proposed method	Standard method
1	80	14.50	130.3	15.4	138.40	3	17.03	18.30	97.79	99.50
2	72	5.95	59.41	5.90	58.91	3	8.71	8.87	97.37	99.76

Trapping solution, 30 mL of alkaline zinc acetate. The solution was made up to 50 mL after sampling hydrogen sulfide from ambient air. a. Air samples were collected on different days. b. Concentration of H_2S (ppb) = sulfide (μg) × 719/V, here V is the volume of air sampled in liters and 719 is the conversion factor to convert μg L^{-1} of H_2S to ppb of H_2S at 298 K and at 101.3 kPa.

Table 6 Derermination of hydrogen sulfide in the ambient air near the open sewer line

Sample No.	Volume of air sampled[a]/L	Hydrogen sulfide found				Sulfide added/μg	Total sulfide found/μg		Recovety of added sulfide, %	
		Proposed method		Standard method						
		μg	ppb[b]	μg	ppb[b]		Proposed method	Standard method	Proposed method	Standard method
1	82	8.01	70.23	8.00	70.14	3	11.02	11.00	100.1	100
2	85	5.1	43.14	4.90	41.14	—	—	—	—	—

Trapping solution, 30 mL of alkaline zinc acetate. The solution was made up to 50 mL after sampling hydrogen sulfide from ambient air. a. Air samples were collected on different days. b. Concentration of H_2S (ppb) = sulfide (μg) × 719/V, here V is the volume of air sampled in liters and 719 is conversion factor to convert μg L^{-1} of H_2S to ppb of H_2S at 298 K and at 101.3 kPa.

interference from common anions and cations. The proposed method has been applied to determine hydrogen sulfide in ambient air after fixing it as zinc sulfide using an alkaline zinc acetate absorbing solution. The proposed method has also been applied to determine sulfide dissolved in sewage water samples. The results obtained by the proposed method have been compared with those from the standard method and the values are in good agreement. The proposed method can be used as an alternative method to the existing methods.

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