Chapter 4

Micelle Mediated Trace Level Sulfide Quantification through Cloud Point Extraction

4.1 Introduction

Sulfide is one of the most essential parameter to monitor in water matrices due to its high toxicity for aquatic life. Dissolved sulfide is toxic to some fresh water fish even at the concentration level less than 1 ppm [1]. The threshold limit value of sulfide concentration in water is in the range 0.05–0.1 ppm according to world health organization (WHO) [2]. Sulfide anion is an important constituent of aqueous systems wherever microbial colonies flourish. Because of its toxicity and removal ability of dissolved oxygen, there are limits on the total level of sulfide permitted in waste discharges [3]. Hence its concentration needs to be controlled especially in water and waste water discharges. Monitoring of aqueous sulfide is one of the important parameter in industrial effluents as the discharges containing sulfide cause the contamination. The treatment of such water generally involves aeration, chlorination or flocculation and other processes [4]. The treated effluent must meet regulatory specifications before it can be discharged into environment. Thus it is mandatory to determine sulfide levels during various stages of treatment [5]. Although sulfide is currently regarded as a core parameter for assessing environmental quality of a given system, clean matrices such as mineral water or anaerobically formed biogenic sulfide in natural waters [1]. Determination of sulfide in the waste water or the first stage effluent is a challenging problem due to complex nature of the matrix. Both petroleum and kraft process based paper and pulp industries generate sufficient quantities of sulfide bearing waste water. Sodium sulfide is one of the most widely used reagents for the removal of hair from animal hides with processing liquors possessing sulfide ion concentration up to 2000 ppm [6]. Sulfide gets released into aquatic environments substantially through bacterial mobilization of sulfur containing minerals.

Several methods have been reported for the quantification of dissolved aqueous sulfide. These are mainly based on titrimetric, chromatographic and spectroscopic techniques. Among these, spectroscopic methods have been proved to be important due to its simplicity, selectivity and sensitivity. Methylene blue method is considered as a standard method for the quantification of sulfide due to its sensitivity and easy adaptability. But the method suffers from the interference of thiols and acidity plays a critical role in the production of phenothiazinium
dye. Number of methods have been reported by adapting the same reaction using different analytical techniques like flow injection analysis, solid phase extraction, solid phase reflectometry [7, 8]. In addition to the spectrophotometry, various other techniques like atomic fluorescence spectroscopy (AFS), atomic absorption spectroscopy (AAS), flow injection analysis (FIA), inductively coupled plasma atomic emission spectroscopy (ICPAES) [9–13] have been successfully utilized for the quantification of sulfide. These techniques require expensive instrumentation and skilled technical personnel to operate the systems. Hence spectrophotometric methods find widespread use due to their simplicity and easy operation. Organic solvents have been extensively used for the extraction of the metal-ligand complexes in spectrophotometric analysis to lower the detection limit and to improve the sensitivity. However the use of organic solvents is not preferred in recent years due to green protocols as they are toxic in nature. Hence surfactants have been used as alternative reagents to enhance the sensitivity of the reaction as well as analyte preconcentration in cloud point based extraction procedures.

The combination of pronounced hydrophobic and hydrophilic properties within a molecule gives surfactant molecules unique properties on dissolution in water [14]. Surfactants on dissolution in water form organized molecular assemblies called micelles if the critical micelle concentration (CMC) exceeds. Organized molecular assemblies have potential utility in chemical analysis because micelles enhance the solubility of organic compounds in water by providing local non polar environment [15]. Watanabe and coworkers have introduced cloud point extraction (CPE) as a promising new separation and extraction technique as an alternative in place of organic solvents [16]. CPE based on the clouding phenomenon of surfactants has become attractive because it offers many advantages over traditional liquid-liquid extraction methods. Recently cloud point extraction of ethylene blue for the estimation of sulfide in water samples has been reported [17].

Neutral surfactants have been extensively used in sensitizing the reactions in various analytical applications [15, 16]. However the use of cationic surfactant as a sensitizing agent has not been fully explored in recent years [18–20]. The use of cationic surfactant i.e. cetylpyridinium chloride (CPC) as a sensitizing agent to quantify trace level sulfide by its reaction with ferric iron and its subsequent complexation of reduced ferrous iron with nitroso-R salt to form a green colored complex has been explored in this study. The complex has been quantitatively extracted into surfactant rich phase in presence of potassium iodide. The
method has been applied for the quantification of dissolved sulfide in the leachate, bore well and pond water samples and the results obtained by the proposed method are in good agreement with standard methylene blue method [21].

4.2 Experiment
4.2.1 Apparatus
Absorbance measurements were made using Shimadzu Scanning Spectrophotometer (model UV-3101PC) using 1 cm quartz cuvettes. All pH measurements were carried out using a Control Dynamics digital pH meter (Model APX 175). All reagents were analytical grade and used without further purification. Distilled water was used throughout the experiment.

4.2.2 Reagents
Iron (III) solution (100 μg/mL): Prepared by dissolving 0.086 g of ammonium ferric sulphate dodecahydrate in few drops of concentrated sulfuric acid and then diluted up to the mark in 100 mL volumetric flask with distilled water.
Sulfide stock solution (1000 ppm): Prepared by dissolving 0.748 g of Na₃S·9H₂O in 100 mL of water and the solution was stored in a refrigerator. Working standards were prepared from stock solution by appropriate dilution on the day of use.
Nitroso-R salt (0.1%): 100 mg of Nitroso-R salt was dissolved in 100 mL of water.
Di-sodium hydrogen phophospatre (0.2M): Prepared by dissolving 2.82 g of anhydrous Na₂HPO₄ in 100 mL of water.
Sodium dihydrogen phosphate (0.2M): Prepared by dissolving 2.61 g anhydrous KH₂PO₄ in 100 mL of water.
EDTA (0.01 M): Prepared by dissolving of 0.372g in 100 mL of water.
Cetylpyridinium chloride monohydrate (1.39 mM): Prepared by dissolving 0.05 g of cetylpyridinium chloride in 100 mL of water.
Potassium iodide (10%): Prepared by dissolving 10 g in 100 mL of water.

4.2.3 Aqueous procedure
Aliquots (0.2–1 mL) of 10 μg/mL sulfide solutions were transferred into a series of 10 mL volumetric flasks containing 1.5 mL of 100 μg/mL of Fe (III) solution. Then 0.5 mL of 0.1% nitroso-R salt solution followed by 3 mL of phosphate buffer (pH 7) was added. The solutions were diluted to the mark with distilled water and the absorbance values were measured at 710 nm (Fig.4.1).
**Fig.4.1** Absorption spectra. a, b - reagent blank and sample in aqueous phase. a', b' - reagent blank and sample in micellar phase (Sulfide = 2 µg)

### 4.2.4 Extraction procedure

Aliquots (0.2–1.8 mL) of 1 µg/mL sulfide solutions were transferred into a series of 10 mL volumetric flasks containing 1.5 mL of 100 µ g/mL of Fe (III) solution. Then 0.5 mL of 0.1% nitroso-R salt solution, 3 mL of phosphate buffer of pH 7 followed by 1.5 mL of EDTA were added and the contents were mixed well. Then 1 mL of cetylpyridinium chloride followed by 1.5 mL of potassium iodide was added. The solutions were diluted to the mark with distilled water and the contents were mixed well. The reaction mixture has been centrifuged for 6 min. at 4000 rpm. The aqueous phase was separated by decantation process and the micellar phase was homogenized by the addition of ethanol and its absorbance was measured at 710 nm (Fig.4.1).

### 4.3 Results and Discussion

The proposed method is based on the reaction of sulfide with ferric iron solution under neutral condition and the subsequent complexation of reduced ferrous iron with nitroso-R-salt
(Scheme 4.1). The initial studies were carried out using 100 μg/mL of iron (III), 1 mL each of 0.1% nitroso-R salt, phosphate buffer (pH 7). Absorbance values of the resulting green color was measured at 710 nm and correlated to the concentration of sulfide.

Nitroso-R salt is a sulfonated compound of 1-nitroso-2-naphthol and its metal complexes are easily soluble in water. Because of high water solubility, the Fe (II)-nitroso-R complex cannot be extracted into any organic solvent. To improve the sensitivity and working range of the method, the cloud point extraction has been explored using cationic surfactant as a sensitizer. Cloud point extraction can be used when the target species are hydrophobic in nature. Though the Fe (II)-nitroso-R salt complex is water soluble, it has been successfully extracted into surfactant rich phase and it can be explained through the following mechanism. When the concentration of surfactant is lower than the CMC, only slightly soluble ion associates can form between anionic ligand and surfactant monomers causing turbidity [14]. Electrostatic interaction between the metal–ligand complex and the cationic surfactant takes place through the negatively charged SO₃⁻ group of the ligand and the positively charged head group of the surfactant molecule. The solubilizing effect of the surfactant begins at critical micellar concentration (CMC) and above, hence the neutral complex gets trapped into the micelles (Fig.4.2). Once the complex gets incorporated into the micellar core it becomes easy to separate it from the aqueous phase. Addition of salts to ionic micelle solutions reduces the mutual electrostatic repulsions of charged head groups. This leads to an increased aggregation number and micellar diameter. High concentrations of salt cause cationic surfactant solutions to separate into immiscible surfactant rich and surfactant poor phases [22].
Scheme 4.1 Species responsible for color.
Fig 4.2 Schematic representation of metal-ligand complex at different stages in presence of cationic surfactant. (a) neutralized complex in bulk solution (b) symbolic representation of electrostatically neutralized complex (c) incorporation of metal-ligand complex into the hydrophobic core of micelle (d) agglomeration of the complex entrapped micelles to form the tubular or rod like structures.
4.3.1 Optimization study
All the reaction parameters have been optimized to get the maximum sample absorbance and minimum reagent blank.

4.3.1.1 Effect of iron (III) ion concentration
The effect of metal ion concentration on the absorbance of the complex was studied in the concentration range 25–500 µg/mL. The absorbance of the complex, Fe (II)-Nitroso-R salt-cetylpyridinium chloride against reagent blank was measured at 710 nm. The absorbance value increases steadily in the concentration range 25–100 µg/mL of iron, there after it remained almost constant as shown in the Fig.4.3. The maximum absorbance was obtained at a concentration of 150 µg/mL of iron (III) and it has been fixed as an optimum concentration and used in all further studies.

![Graph of absorbance vs iron (III) ion concentration](image)

Fig.4.3  Effect of Ferric (III) concentration

4.3.1.2 Effect of nitroso-R salt
The effect of nitroso-R salt concentration on the absorbance of the sample was investigated by varying its concentration in the range 0.2–0.8 mL of 2.6 mM ligand solution. The sample absorbance increased with increase in the volume of ligand as shown in the Fig.4.4. At higher concentration, there exists a competition between
ligand and its iron (II) complex to extract into the surfactant rich phase so the concentration of ligand in surfactant rich phase increased. It resulted in the increase of blank value by suppressing the sample absorbance. Hence 0.5 mL of ligand concentration has been optimized to get the maximum sample absorbance with minimum blank value.

### 4.3.1.3 Effect of pH

The effect of pH on the formation of metal–ligand complex was carried out in the pH range 7–12 as the complexation takes place only in alkaline condition. The variation of pH was carried out by using phosphate buffer solutions of different pH values. The sample absorbance remains almost constant throughout the pH range studied but the blank absorbance value increased with the increase in the medium pH. In order to maintain the low blank absorbance value, the medium pH was maintained in the range 7–8 (Fig. 4.5). It was achieved by the addition of 3 mL of phosphate buffer to the reaction mixture and it was optimized and used in all further studies.
Fig. 4.5  Effect of pH.

4.3.1.4 Effect of surfactant
Surfactants have been used to extract the metal–ligand complexes efficiently without using any organic solvents [23]. Hence an attempt has been made to extract the Fe (II)–nitroso-R salt complex from the aqueous solution using different types of surfactants. These surfactants are known to form aggregates which are called micelles which entrap the complexes very efficiently facilitating phase separation. Several surfactants have been tried to separate the metal-ligand complex from aqueous phase. After adding surfactant, the solutions were heated to different temperatures to cause cloud point formation. Once clouding takes place the phase separation can be efficiently carried out by simple centrifugation procedure. Among the surfactants used, Triton X 100 a neutral surfactant could not cause any quantitative separation even after heating up to 80°C. Since the complex is anionic in nature the use of cationic surfactant may facilitate the quantitative extraction of the metal-ligand complex. Therefore several cationic surfactants were used in order to extract the metal-ligand complex quantitatively from aqueous phase. The list of the surfactants used for the extraction of the complex has been listed in Table 4.1. Among these, only cationic surfactants could quantitatively extract the Fe(II)-nitroso-R-salt complex from aqueous phase. The clouding results at room temperature itself, hence the cationic surfactants have been used in the present
investigation. The monomer molecules of the cationic surfactant initially neutralize the anionic complex

**Table 4.1** Effect of surfactants

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Sample v/s blank</th>
<th>Temperature(°C)</th>
<th>Absorbance after CPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.08</td>
<td>RT</td>
<td></td>
</tr>
<tr>
<td>Triton X100</td>
<td>No extraction</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>CTAB</td>
<td>0.50</td>
<td>RT</td>
<td></td>
</tr>
<tr>
<td>CPC</td>
<td>0.56</td>
<td>RT</td>
<td></td>
</tr>
<tr>
<td>Cetrime</td>
<td>0.51</td>
<td>RT</td>
<td></td>
</tr>
</tbody>
</table>

RT - Room temperature (27 ± 2°C)

CTAB - Cetyltrimethyl ammonium bromide

CPC - Cetylpyridinium chloride

![Graph showing the effect of cetylpyridinium chloride](image)

**Fig.4.6** Effect of cetylpyridinium chloride.

The micelles entrap the complex into its core and undergo clouding at room temperature. Among the three cationic surfactants, CPC gave a higher absorbance value to the sample when compared to the other surfactants, hence CPC has been used as a surfactant in all further
studies. The effect of CPC concentration on the extraction of the complex was investigated by varying its concentration by adding of 0.6–1.0 mL of 1.39 mM solution. Extraction has not been observed below 0.6 mL of the surfactant. Extraction of the complex increased with increase in concentration up to 1 mL, there after sample absorbance value was almost remain constant (Fig.4.6). Hence 1 mL of 1.39 mM concentration of the surfactant has been fixed as the optimum value and incorporated in all further studies.

4.3.1.5 Effect of electrolyte
Separation of charged micelles from the aqueous phase can be improved by the addition of ionic salts like NaCl, KI, etc. High concentration of salt causes cationic surfactant solutions to separate into immiscible surfactant-rich and surfactant-poor phases. Different volumes of 1–2.5 mL of potassium iodide (10%) solutions were used to study the effect of concentration of the salt on the extraction of the complex in presence of cationic surfactant, cetylpyridinium chloride. No quantitative extraction was observed in the absence of KI. The complete extraction was caused with the increase in the concentration of the KI (Fig.4.7). There is a decrease in the absorbance

![Graph showing effect of KI in phase separation](image)

**Fig.4.7** Effect of KI in phase separation
values of both blank and sample beyond 1.5 mL of potassium iodide, hence an optimum volume of 1.5 mL of 10% KI has been used in all further studies to get the low blank and high sample absorbance values.

### 4.3.1.6 Effect of EDTA concentration

Addition of EDTA suppresses the blank absorbance value by masking the excess of unreacted iron (III). Therefore EDTA was used to lower the blank absorbance value. The EDTA concentration was varied in the concentration range 0.5–3.0 mL. In the absence of EDTA, the blank value was very high and it is decreased with the rise in the concentration of the masking agent (Fig.4.8). Hence 1.5 mL of 0.02 M EDTA has been used as an optimum concentration in all further studies to obtain the maximum sample absorbance with low blank value.

![Graph showing the effect of EDTA concentration on absorbance](image)

**Fig.4.8** Effect of EDTA

### 4.3.1.7 Effect of centrifugation time

The effect of centrifugation time for the efficient phase separation was examined by centrifuging the resulting cloud phase at 4000 rpm at different time intervals of 2–8 minutes. Beyond five minutes the absorbance values of the complex has remained constant, hence six minutes time has been fixed as the optimum centrifugation time.
4.3.1.8 Phase diagram
The influence of potassium iodide as an electrolyte on the extraction of Fe (II)-nitroso-R complex was examined at various cetylpyridinium chloride (0.05%) concentrations in the range from 0.6–1.4 mL of 1.39 mM. It is evident from the Fig.4.9 that the extent of extraction has increased in presence of higher concentrations.

\[\text{Absorbance vs. volume of cetylpyridinium chloride (1.39 mM) (mL)}\]

Fig.4.9 Phase diagram of the CPC-water-KI ternary system.

of potassium iodide. The addition of potassium iodide was varied between 0–1 mL of 0.05 % concentration. The sample absorbance values increased with increase in KI concentration. Hence 1 mL of potassium iodide (0.05%) was used as an optimum concentration. This behavior can be explained in terms of faster dehydration of surfactant molecules due to the presence of inorganic ions i.e. K$^+$ and I$^-$ competing for interaction with water molecules, i.e., salting out effect.

4.3.2 Interference study
The effect of foreign ions has been studied in order to apply the developed method to determine sulfide in real samples. The tolerance limits of different cations and anions are shown in Table 4.2. No strong interference has been observed from most of the ions except
sulfite and cobalt. Sulfite interfered even at 10μg/mL due to its redox behaviour. It was overcome upto 100 μg/mL by adding 1000 μg of formaldehyde which forms the hydroxymethane sulfonic acid adduct which prevents the sulfite interference in the reaction.

Table 4.2  Interference study

<table>
<thead>
<tr>
<th>Interfering ion</th>
<th>Tolerance limit (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>SO₃²⁻</td>
<td>10</td>
</tr>
<tr>
<td>SO₃²⁻ᵃ</td>
<td>100</td>
</tr>
<tr>
<td>NO₂⁻</td>
<td>20</td>
</tr>
<tr>
<td>NO₂⁻ᵇ</td>
<td>100</td>
</tr>
<tr>
<td>SO₄²⁻, CO₃²⁻</td>
<td>&gt;200</td>
</tr>
<tr>
<td>I⁻, PO₄³⁻</td>
<td>400</td>
</tr>
<tr>
<td>Zn²⁺, Ca²⁺</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Cu²⁺, Ni²⁺</td>
<td>25</td>
</tr>
<tr>
<td>Pb²⁺</td>
<td>200</td>
</tr>
<tr>
<td>Cd²⁺</td>
<td>50</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>600</td>
</tr>
<tr>
<td>Co²⁺</td>
<td>05</td>
</tr>
<tr>
<td>HCHO</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

ᵃSample was treated with 1 mL of formaldehyde (1000 μg/mL).
ᵇ Sample was treated with 5 mL sulfamic acid (0.5%).

Formaldehyde did not interfere even at 1000 μg level, hence it has been used as a masking agent for sulfite. Nitroso-R salt is a well known ligand for metal ions like Fe, Co, Ni, Cu etc. Cobalt forms an orange colored complex with nitroso-R salt solution under the optimized conditions and interfered as low as 5 μg/mL. Other metal ions like mercury, lead, copper did not interfere. Nitrite interfered at 20 μg/mL and has been overcome by adding 1 mL of 0.5% sulfamic acid up to 100 μg/mL. The addition of sulfamic acid to the reaction mixture minimizes nitrite interference by reducing the nitrite into nitrogen. Other anions like sulphate, phosphate, iodide, carbonate, chloride and nitrate did not interfere significantly in the present method.
4.3.3 Application study

Water passing through the landfill which collects the dissolved and suspended matter from it is called leachate. Generally leachate may contain nitrate, phosphates, organic matter and sulfide etc in significant quantities [24]. Solid wastes placed in a sanitary landfill may undergo a number of biological, chemical and physical changes. Aerobic and anaerobic decomposition of the organic matter results in both gaseous and liquid products. Sanitary landfills are one of the important sources of groundwater contamination. Other sources include septic tanks, mining and agricultural activities and leaking underground storage tanks. In all cases the threat of contamination to ground water depends on the specific geological and hydrological conditions of the site. Leaking chemicals pass through the soil to the ground water system.

The developed method has been applied to determine trace level sulfide concentrations from the leachate samples as well as water samples collected from the ponds and the bore wells located near the landfills. Known quantities of sulfide were added and the recovery studies have been carried out to validate the proposed method.

The leachate and water samples collected were filtered using Whatman filter paper to remove any suspended and colloidal particulate matter. 20 mL of the filtered sample was taken in 25 mL volumetric flask and 1 mL of formaldehyde (1000 µg/mL) was added followed by 1 mL of sulfamic acid (0.5%). The sample was diluted to the mark and the analysis was carried out by taking 3 mL aliquot of sample following the procedure described above. The samples were spiked with known amounts of sulfide and the recovery of sulfide added was studied by the proposed method as well as standard method [25]. The results obtained by the proposed method are in good agreement with the standard method (Table 4.3 and 4.4).
<table>
<thead>
<tr>
<th>Sample</th>
<th>Sulfide found (ppm)</th>
<th>Sulfide added (ppm)</th>
<th>Total sulfide found (ppm)</th>
<th>Recovery of added sulfide (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A*</td>
<td>2.81±0.37</td>
<td>2.88±0.12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B*</td>
<td>1.94±0.28</td>
<td>1.9±0.15</td>
<td>1.0</td>
<td>2.86±0.22</td>
</tr>
<tr>
<td>C*</td>
<td>1.03±0.21</td>
<td>1.05±0.12</td>
<td>2.0</td>
<td>3.00±0.18</td>
</tr>
<tr>
<td>D*</td>
<td>ND</td>
<td>ND</td>
<td>1.0</td>
<td>1.03±0.20</td>
</tr>
<tr>
<td>D**</td>
<td>ND</td>
<td>ND</td>
<td>1.0</td>
<td>1.09±0.18</td>
</tr>
</tbody>
</table>

Values are the average of three measurements ±RSD.

ND – not detected

* Sulfide determined by aqueous procedure

** Sulfide determined by cloud point extraction procedure
<table>
<thead>
<tr>
<th>Sample</th>
<th>Sulfide found (ppm)</th>
<th>Sulfide added (ppm)</th>
<th>Total sulfide found (ppm)</th>
<th>Recovery of added sulfide (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.297±0.37</td>
<td>0.5</td>
<td>0.810±0.033</td>
<td>101.6</td>
</tr>
<tr>
<td>B&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.475±0.28</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.139±0.21</td>
<td>0.5</td>
<td>0.661±0.036</td>
<td>103.4</td>
</tr>
<tr>
<td>B&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.636±0.030</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are the average of three measurements ±RSD

<sup>a</sup> water samples were collected from bore wells located near dump sites.

<sup>b</sup> water samples were collected from ponds located near dump sites.
4.4 Conclusions
The proposed micelle mediated cloud point extraction procedure for the determination of trace quantities of dissolved aqueous sulfide is simple, sensitive and carried out at ambient temperature using cetylpyridinium chloride as a cationic surfactant. No major interferences from the common cations and anions have been found. The proposed method doesn’t require any organic solvent for the extraction purpose and it has been applied to determine trace level sulfide in leachate samples and water samples. The results obtained by the proposed method are in good agreement with the standard method. It can serve as an alternative to the existing methods.

4.5 References
1. S. Gorji, M. Bahram, Analytical Methods, 2, 948 (2010).