Fisheries and Oceans Canada | Pêches et Océans Canada
Ecosystems and Oceans Science | Sciences des écosystèmes et des océans

Canadian Science Advisory Secretariat (CSAS)

Research Document 2025/015

National Capital Region

Ion Selective Electrode (ISE) Method Overview and Variations in Implementation Descriptions for the Determination of Sulfide in Marine Sediment Samples

D.K.H. Wong and F.H. Page

Fisheries and Oceans Canada
St. Andrews Biological Station
125 Marine Science Drive,
St. Andrews, New Brunswick E5B 0E4

December 2025

Canada

Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

Published by:

Fisheries and Oceans Canada
Canadian Science Advisory Secretariat
200 Kent Street
Ottawa ON K1A 0E6

http://www.dfo-mpo.gc.ca/csas-sccs/
DFO.CSAS-SCAS.MPO@dfo-mpo.gc.ca

ISSN 1919-5044
ISBN 978-0-660-75874-9 Cat. No. Fs70-5/2025-015E-PDF

Correct citation for this publication:

Wong, D. and Page, F.H. 2025. Ion Selective Electrode (ISE) Method Overview and Variations in Implementation Descriptions for the Determination of Sulfide in Marine Sediment Samples. DFO Can. Sci. Advis. Sec. Res. Doc. 2025/015. v + 54 p.

Aussi disponible en français :

Wong, D. et Page, F.H. 2025. Aperçu de la méthode de mesure des sulfures dans les échantillons de sédiments marins par électrode à ions spécifiques (EIS) et variantes de sa mise en œuvre. Secr. can. des avis sci. du MPO. Doc. de rech. 2025/015. v + 62 p.

TABLE OF CONTENTS

ABSTRACT

This document is part of a Canadian Science Advisory Secretariat (CSAS) process to give Fisheries and Oceans Canada (DFO) science advice pertaining to the sulfide ion selective electrode (ISE) method. This method is currently the only approved regulatory tool within Canada used to determine the oxic state of the benthos as a result of organic loading from finfish aquaculture activities. ISE is an easily operated and highly cost-effective technique compared to other high level instrumental methods. However, due to its manual nature, methodological steps can be prone to subjective interpretation by the analyst resulting in inconsistent execution by different individuals and/or laboratories. In terms of sulfide monitoring, this can potentially lead to the generation of variable, and inaccurate data with ramifications to regulatory decision making. The sulfide ISE method was initially evaluated by Wildish et al. (1999) and subsequently adopted for use by New Brunswick, Nova Scotia, and nationally as part of the Aquaculture Activities Regulations (DFO 2018a), which British Columbia follows as part of Annex 8 of the Aquaculture Activities Regulations guidance document. In their environmental monitoring standard operating procedures (SOPs), New Brunswick (NB DELG 2018), Nova Scotia (NS DFA 2021), and British Columbia (DFO 2018b) all refer to Wildish et al. (1999) for measurement of sulfide in collected sediment samples. However, they deviate in certain aspects from Wildish et al. (1999), and also amongst themselves, e.g., the number of standards used to calibrate the ISE, the time from sample collection to analysis, etc. In this paper, we list the necessary steps required for the determination of sulfide in sediment samples, and also document deviations from Wildish et al. (1999) by the aforementioned regulators, along with procedural differences between one another. A proposed methodological procedure for determination of sulfide in sediment samples is also presented, which could lead the way for development of a revised and more prescriptive SOP that can be adopted nationally.

INTRODUCTION

Fisheries and Oceans Canada (DFO) is responsible for the federal regulation and management of the aquaculture industry throughout Canada under the Aquaculture Activities Regulations (AAR; DFO 2018a). These regulations define the conditions under which an aquaculture operator may deposit organic material and are regulations under section 36 and section 35 of the *Fisheries Act*.

DFO's regulatory mandate recognises that there are interactions between aquaculture operations and the natural environment. The risks associated with these interactions are considered and addressed through a suite of regulatory tools.

Under the AAR (DFO 2018a), the finfish aquaculture industry is required to conduct monitoring of the oxic state of the seafloor impacted by their operations. The protocols for conducting the sampling and for measurement of total free sulfide are outlined in the regulations and associated monitoring standards documents of British Columbia (DFO 2018a, DFO 2018b), New Brunswick (NB DELG 2018), and Nova Scotia (NS DFA 2021). Newfoundland and Labrador typically do not monitor for sulfide due to the hard bottom nature of the substrate found around aquaculture sites there. In the Gulf Region, where the main aquaculture activity is shellfish farming, monitoring of sulfide is typically not required.

The method required by management for determining sulfide in sediments associated with aquaculture activities is often referred to as the Ion Selective Electrode (ISE) method for determining total 'free' dissolved sulfide. The method was initially suggested by Hargrave et al. (1997) as a more cost-effective proxy for biodiversity sampling. The method was described more fully and tailored for use for management of the organic waste related environmental impact of the in-situ aquaculture industry by Wildish et al. (1999) and subsequently refined and clarified by Wildish et al. (2004). Wildish et al. (2001) also proposed the use of sulfide measurement as one of two indicators which could be used as a cost-effective method for monitoring salmon aquaculture.

The method is referred to in the regulatory documents of the Government of Canada (DFO 2018a) and the provinces of British Columbia (DFO 2018b), New Brunswick (NB DELG 2018), and Nova Scotia (NS DFA 2021). The method has been used in many scientific studies related to aquaculture activities occurring within, and outside of, Canada but it has not been incorporated into the management regimes of other countries.

A review of the various federal and provincial government documents relevant to the management and regulation of aquaculture environmental impacts shows numerous inconsistencies between implementations and several nuances that are not associated with references to scientific publications. The currently applied AAR (DFO 2018a) monitoring protocol used at marine finfish sites was adopted by DFO, and it incorporates some of the regional differences that existed in provincial monitoring programs at that time.

The Aquaculture Management Directorate within DFO has requested science advice specific to several questions pertaining to the ISE method due to management's recognition of inconsistencies with execution of the method, and some practical implementation concerns raised by the private sector. This review is also as a result of a routine effort by DFO to review, update, improve, and work towards the development of a nationally harmonised and robust approach for the analysis of sediment sulfide for its use as a proxy for sediment oxic state.

This paper focuses on the ISE analytical method employed by Canadian regulators and the differences in some of the steps observed between each region. It does not discuss the collection and/or storage of sediment samples prior to their analysis.

Questions from this CSAS meeting's Terms of Reference:

1. What are the effects of sediment sample storage time and conditions (i.e. temperature, vacuum-sealed, etc.) on the measurement of total free sulfide as compared to total free sulfide measured immediately upon sample collection?
2. Are these relationships consistent across sediment types and/or total free sulfide concentrations?
3. Is there a combination of storage conditions and storage time post collection that would result in expected total free sulfide measurements within +/- 5%, 10% and 15% of the value obtained from measuring total free sulfide immediately following sediment sample collection?
4. Are there steps in the ion-selective electrode (ISE) total free sulfide measurement protocol that are open to interpretation by the analyst and to which differences will result in different measured concentrations of total free sulfides?
5. Review ISE total free sulfide measurement methodologies and develop standard procedures for sample storage time, storage conditions, and analyses.
6. Characterize the natural spatial variability of sediment sulfide levels.

This paper addresses questions 4 and 5 of the above questions.

BACKGROUND TO ION SELECTIVE ELECTRODES

The ISE method requires the use of an ion selective electrode selective to the target ion (e.g. S^{2-}) and temperature probe attached to a pH/ORP (oxidation-reduction potential)/Ion meter. ISE is one of the most technologically simple, and least cost prohibitive techniques available for chemical analysis. There are many types of ISEs available on the market which allow for the selective and quantitative determination of a vast spectrum of substances, e.g. glass membrane for pH measurement; solid-state electrode for, for example, sulfide (S^{2-}), fluoride (F^{-}), silver (Ag^{+}); liquid based electrode for calcium (Ca^{2+}), and compound electrode for CO_2. They allow for rapid and simple analysis of samples without sophisticated and time consuming sample preparation techniques, are unaffected by sample colour or turbidity, and are suited for use in the field or laboratory (Nico2000 2011).

ISEs are essentially electrochemical half-cells in which a potential difference, which is dependent on the concentration (activity) of a particular ion in solution arises across the electrode/electrolyte interface. The relation between ionic concentration and electrode potential is given by the Nernst equation:

$$E = E^{0} + \left(\frac{2.303RT}{nF}\right) \times \log(A)$$

Where:

E is the total potential (mV) developed between the sensing and reference electrodes,

E^{o} is a constant which is characteristic of the particular ISE/reference pair,

2.303 is the conversion factor from natural to base 10 logarithm,

R is the Gas Constant (8.314 joules/degree/mole),

T is the absolute temperature (273.15 K + °C),

n is the charge of the ion (with sign),

F is the Faraday Constant (96 500 coulombs),

log (A) is the logarithm of the activity of the measured ion either 2.303 log base 10, or the natural logarithm ln.

Figure 1a shows a simplified schematic for an ISE set up with separate indicator and reference electrodes. For current applications, a combination solid state electrode can be used which has the reference electrode built into the electrode housing with an inorganic compressed sensing pellet membrane fixed to a sensing module. The electrode (silver/sulfide) used for sulfide determination is of this type, Figure 1b.

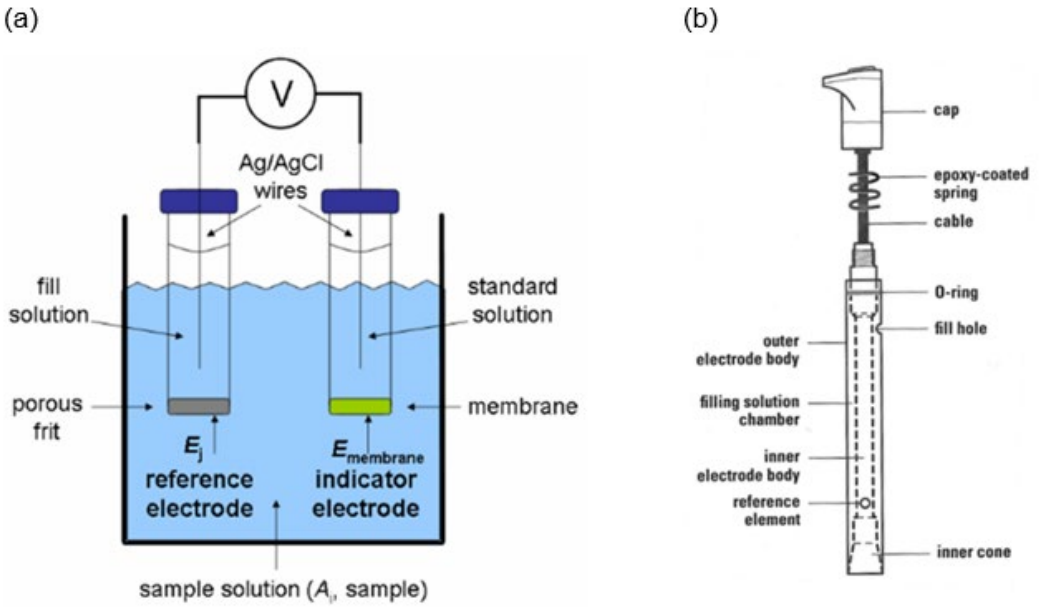

Figure 1. Schematics of (a) an ion selective electrode with external reference electrode and (b) a silver/sulfide combination solid state electrode (Thermo Scientific 2009).

As the name suggests, ion selective electrodes are not ion specific, they are sensitive to other ions to a certain extent, though this interference may be insignificant unless there is a higher proportion of interfering ions to primary ions. For example, the silver/sulfide ISE is prone to interference from mercury although most significantly when using it to determine the silver anion (Ag+) rather than the sulfide (S2-) ion since mercury sulfide (HgS) and mercuriosulfanylmercury (Hg2S) salts are extremely insoluble (Thermo Scientific 2009).

Ion selective electrodes have their limitations but with careful use, frequent calibration, and an awareness of the limitations, ISEs can achieve accuracy and precision levels of ±2% or ±3% for some ions and thus compare favourably with analytical techniques which require far more complex and expensive instrumentation (Nico2000 2011). Thermo Scientific (2009) states that their ISE can achieve ±4% reproducibility with hourly calibrations.

METHOD VARIABILITY

Before reviewing the analytical method, it is worthwhile to briefly discuss the variability which can arise from analysis. This factor is also discussed more in-depth in Page and Wong (In press).

All measurements are prone to error, i.e. the difference between the observed value and true value of the sample being analysed. These errors can originate from many areas associated with the sample analysis process. For example, in the case of the ISE method, inconsistent sample handling procedures (samples analysed immediately versus being stored for up to 36 or 72 hours), and poorly defined methodological procedures resulting in individual interpretation of the analysis steps make this an inherently noisy (i.e., highly variable) method which can compromise the accuracy and precision of the results. The best approaches to minimising errors are through the use of comprehensive SOPs, training, and possibly to have one analyst conducting the analyses (which limits analyst variability), however the latter is not viable since sulfide analysis is undertaken by multiple regions and laboratories.

The best methods are those which are reproducible by multiple laboratories and analysts. Ideally, an analytical method should undergo an evaluation and validation process to ensure it is suitable for its intended use, especially if derived data are used for regulatory decision making. Methods should be validated for, but not limited to:

Parameter	Definition*
Linearity	The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.
Accuracy	The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.
Precision	The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.
Robustness	The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.
Reproducibility	Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

* = ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology (1995)

Research has been conducted to investigate various aspects of the sulfide ISE method (Wildish et al. 1999; Wildish et al. 2004; Chang et al. 2014; Wong et al. unpublished), however a formal evaluation and validation process resulting in the production of a standardised method suitable for determination of dissolved sulfide in sediment samples has not been conducted. The fact that the method has not undergone a formal validation process is of concern, especially since the derived data are used for regulatory decision making. A lack of standardisation has also led to the method, often referred to as “Wildish et al (1999)”, to be utilised differently resulting in the adoption of differing procedures which could affect the level of accuracy towards the determined results.

As indicated above, the sulfide ISE method is an inherently noisy method with variability being driven by many factors, some of which contribute a higher degree of variance than others. Figure 2 outlines the main areas that may introduce variability/error to the generated results. To minimise analytical variability, analysts should follow a comprehensive SOP that would set out each step in a clear and concise manner. An example of a proposed SOP is presented in Appendix 1.

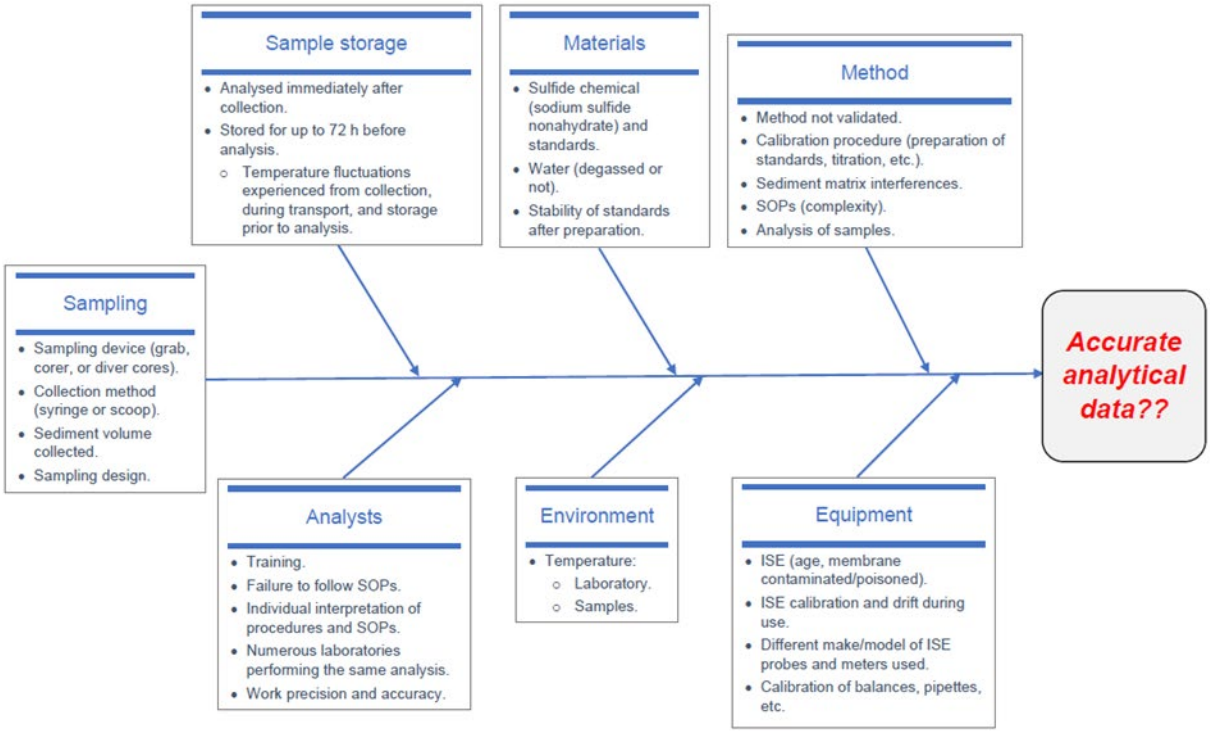

Figure 2. Sources of possible variability associated with the sulfide ISE method.

ANALYTICAL STEPS FOR DETERMINATION OF SULFIDE BY ISE

A flow chart representing the methodological steps considered essential for the accurate and precise determination of sulfide in sediment samples is presented in Figure 3. A comparison of the actions indicated by various science authors, and those outlined in federal and provincial regulations are detailed in Appendix 2.

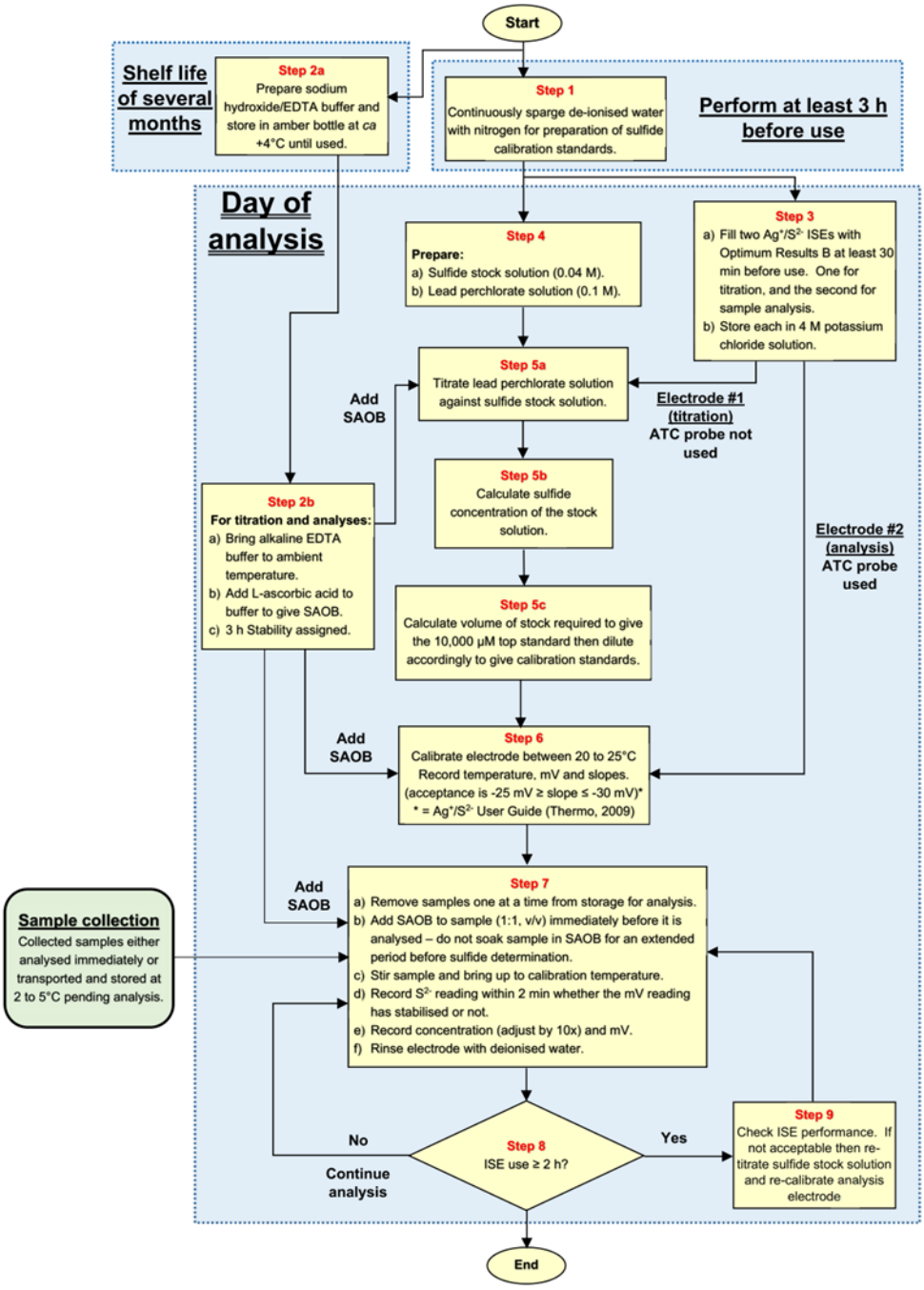

Figure 3. Flowchart of analytical steps required for the accurate determination of sulfide in sediment samples.

The following steps refer to the flowchart presented in Figure 3.

STEP 1: DEOXYGENATION OF WATER

PURPOSE OF OXYGEN REMOVAL

Sulfide is highly volatile and is readily oxidised in the presence of oxygen. To minimise the chemical oxidation process during preparation of sulfide calibration standards, it is essential to remove dissolved oxygen from the water used during this process, otherwise the loss of sulfide could impact accurate calibration of the ISE electrode-meter system and thus accurate quantification of sulfide in the samples. There are a few methods available to degas water, however the efficiency and time to achieve this are dependent on the technique used.

SPARGING WITH AN INERT GAS

Continuously sparging deionised water with a high purity inert gas, e.g. nitrogen, before, and during its use is one method. This process involves the use of a cylinder of inert gas with an attached regulator which is connected by a gas line to an air stone having consistently sized pores (e.g., 0.5 µM) which can deliver a fine, slow moving stream of uniform bubbles. The gas pressure required to produce such a stream is dependant on the type of air stone used, therefore no ‘one gas pressure’ is suitable for this procedure, the analyst should rely on the size of bubbles being produced. Coarse bubbles, generated from high porosity air stones and/or the use of high gas pressures, are not recommended since these are less efficient at degassing due to the smaller surface-area-to-volume ratios, and the speed at which the bubbles travel through the water column. Deoxygenating occurs when the stream of inert gas bubbles rises through the water column slowly, causing the inert gas to dissolve which in turn displaces the dissolved oxygen (DO) from the water.

In the following example using high purity nitrogen (>99.999%) as the sparging gas, the initial DO concentration in the water (1 L) was ca. 7 mg/L which decreased to around 20 µg/L by 2 h post start of sparging. DO levels were monitored using a PreSens oxygen microsensor connected to a Microx 4 Trace meter. The DO concentration stayed steady at this minimal level throughout the sparging process and only steadily increased after the nitrogen was turned off after the 24 h experimental period. In this experiment, a 1 L volume was used, if however a larger volume was to be prepared, the time to fully degas the water may vary compared to this example. Butler et al. (1994) determined purging with nitrogen to be the most efficient method for removal of oxygen from water when compared to boiling and sonication. A representative plot of this degassing process is presented in Figure 4.

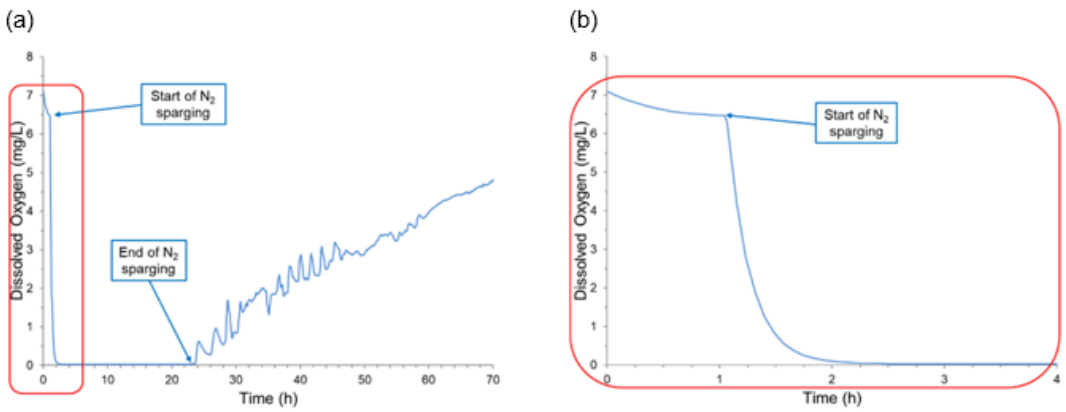

Figure 4. Plots of (a) deionised water sparged with nitrogen to remove dissolved oxygen and reoxygenation of the water after sparging was terminated, and (b) expanded portion showing the speed of deoxygenation after the sparging process was commenced. The experiment was conducted by D. Wong (unpublished).

CHEMICAL DEGASSING

Chemical deoxygenation of water is another method which can be utilised to remove DO from water. This method was tested using the oxygen scavenging agent sodium sulfite anhydrous. Figure 5 shows a plot of DO in deionised water after addition of sodium sulfite anhydrous at a concentration of 1% (w/v). Levels of DO were monitored using the same PreSens system setup mentioned previously. Initial DO in the water was ca. 9 mg/L which dropped within 5 min after addition of sodium sulfite anhydrous to ca. 260 µg/L (Replicate 1) and ca. 40 µg/L (Replicate 2). The minimum DO concentration attained was ca. 9 µg/L in both replicates which was comparable to degassing using nitrogen gas.

Replicate 2 started to re-oxygenate after approximately 2.5 days whereas Replicate 1 did so just before 7 days. This suggests that this process may not be consistent, however the de-oxygenated period is sufficient for analysing a batch of samples in one day. This method may be beneficial if access to nitrogen or other inert gas is not possible. It should be noted that the interaction of the sodium sulfite anhydrous with the reagents and sediments used for the ISE method has not been fully tested at this time, therefore further work would be required if this were to be adopted as a degassing method.

In the above experiment, a 1 L volume of water was used, if however a larger volume was to be prepared, the time to fully remove DO from the water and for it to re-oxygenate may vary compared to this example.

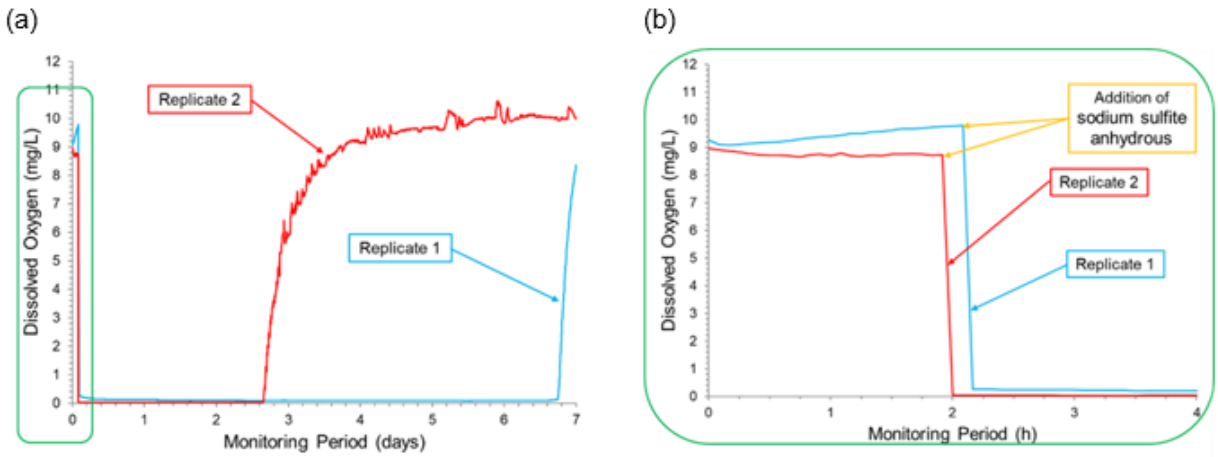

Figure 5. Plots of (a) deionised water chemically degassed using sodium sulfite anhydrous at a concentration of 1% (w/v) then monitored over a 7 day period and (b) expanded portion of the plot up to 4 h showing rapid deoxygenation of the water after addition of the sodium sulfite anhydrous chemical.

BOILING

A more simplistic method is to boil water which drives off any dissolved gases. An experiment conducted by Wong et al (unpublished) tested the effectiveness of this method compared to sparging and chemical degassing. Continuous monitoring of DO levels in the water during and after boiling was not possible due to the temperature tolerance of the PreSens fiber optic sensor and constraints of the experimental setup, therefore DO concentrations were only determined before and after boiling once the water had cooled to ambient temperature. The initial DO concentration of the water was determined to be 7.53 mg/L (using the same PreSens setup detailed previously). The water was then brought to a vigorous boil on a hotplate. After boiling for 1 h, the water was transferred to a media bottle and filled to the top (no head space) then capped tightly, sealed with electrical tape, and allowed to cool at ambient temperature on the bench. After 24 h, the DO was retested and found to contain 4.95 mg/L (i.e., 65% of initial). Assuming the capping prevented any influx of DO from the air into the boiled water, it may be concluded that boiling is an ineffective method to degas water. This conclusion is backed up by Butler et al. (1994) who suggested that a back reaction occurs where atmospheric oxygen is redissolved in the water and carried back into solution during the boiling process which resulted in the variability of their results and also the ineffectiveness of the method.

STABILITY OF SULFIDE IN DEGASSED WATER

The stability of sulfide prepared at 400, 1 200 and, 8 000 µM using nitrogen degassed water (continuously sparged until preparation of the sulfide samples) and sodium sulfite deoxygenated water (1% w/v) was assessed by Wong et al. (unpublished). Results (Figure 6, Table 1) showed that in all situations the sulfide concentration decreased with time with the rate of degradation being decreasing from low to high initial sulfide concentrations (i.e., the rate was greatest for the 400 µM and lowest for the 8 000 µM solutions (400 µM > 1 200 µM > 8 000 µM)) in both waters tested. However, sulfide degradation at 400 µM and 1 200 µM was lower in the chemically deoxygenated water. At 8 000 µM, loss of sulfide was equivalent in both waters tested. Sulfide loss was generally less in water deoxygenated by sodium sulfite than in the sparged water. This is contrary to the understanding that sulfide would not degrade in oxygen free media. In the nitrogen sparged water, degradation was likely due to reoxygenating immediately after

cessation of sparging to enable preparation of the sulfide solutions. With the chemically deoxygenated water, oxygen levels should have been very low (ca. 9 µg/L), at least until ca. 48 h. This oxygen content may still be high enough for oxidation reactions to occur. Another area of loss could be volatisation since the containers housing the standards still contained headspace.

It can be concluded that sulfide is not stable for an extended period of time even when prepared in degassed, or deoxygenated water. Therefore, standards should be prepared and used immediately to calibrate the ISE to mitigate any inaccuracies due to sulfide loss.

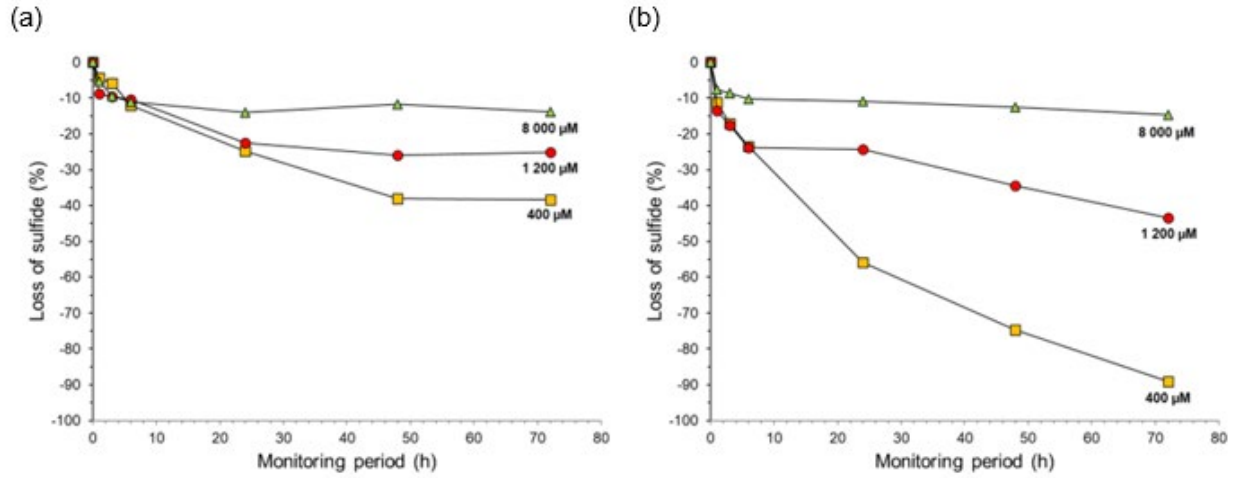

Figure 6. Loss of sulfide at concentrations of 400, 1 200, and 8 000 µM in (a) sodium sulfite anhydrous deoxygenated water and (b) nitrogen sparged water. Reoxygenation begins about 48 h after chemical degassing and immediately after cessation of nitrogen degassing.

Table 1. Loss of sulfide at concentrations of 400, 1 200, and 8 000 µM in sodium sulfite deoxygenated and nitrogen sparged deionised water.

Time-point (h)	Loss of sulfide (%)					
	Sodium sulfite deoxygenated water			Nitrogen degassed water		
	400 µM	1 200 µM	8 000 µM	400 µM	1 200 µM	8 000 µM
0	0.0	0.0	0.0	0.0	0.0	0.0
1	-4.3	-8.8	-5.3	-11.3	-13.6	-7.6
3	-5.8	-9.6	-9.4	-17.2	-17.6	-8.7
6	-12.1	-10.4	-11.1	-23.5	-23.8	-10.2
24	-24.7	-22.5	-14.0	-55.9	-24.3	-10.9
48	-38.1	-25.9	-11.8	-74.8	-34.5	-12.5
72	-38.4	-25.1	-13.9	-89.2	-43.4	-14.6

RESEARCH AND REGULATIONS

The use of degassed deionsed or distilled water is documented by all parties in Appendix 2, however the method of degassing is not mentioned by Wildish et al. (1999), DFO (2018b), NB DELG (2018), nor NS DFA (2021). Wildish et al. (2004) however did detail the use of a fine stream of nitrogen gas to degas the deionised water for 10 min. Based on Figure 4 however, 10 minutes may be insufficient to fully degas water, however this would depend on the volume that was being degassed.

STEP 2: PREPARATION OF SULFIDE ANTI-OXIDANT BUFFER (SAOB)

PURPOSE OF SAOB

Sulfide anti-oxidant buffer (SAOB) acts as an ionic strength adjuster (ISA), and also facilitates the formation of the divalent sulfide ion (S^{2-}) for sulfide ISE analysis. The buffer comprises 3 components, (1) sodium hydroxide, (2) ethylenediaminetetraacetic acid (EDTA) disodium salt, dihydrate, and (3) L-ascorbic acid (L-AA), each of which has it own function to play in the analysis of sulfide in sediment.

SODIUM HYDROXIDE

The pH of the marine environment is generally ca. 7 to 8, in these situations soluble sulfide exists as hydrogen sulfide (H_2S) and bisulfide (HS^-), Figure 7. The ISE technique however relies on detection of S^{2-}, therefore H_2S and HS^- must be deprotonated to this divalent form. To fully accomplish this, the sample pH must be increased to > 14 using a highly alkaline solution. The use of SAOB, which contains a high concentration of sodium hydroxide rapidly facilitates this

reaction in aqueous media, equations (a) and (b). However it is unclear if these reactions are instantaneous in sediment due to the complex nature of this matrix.

$$H_2S \rightleftharpoons HS^- + H^+ \quad \text{(a)}$$

$$HS^- \rightleftharpoons S^{2-} + H^+ \quad \text{(b)}$$

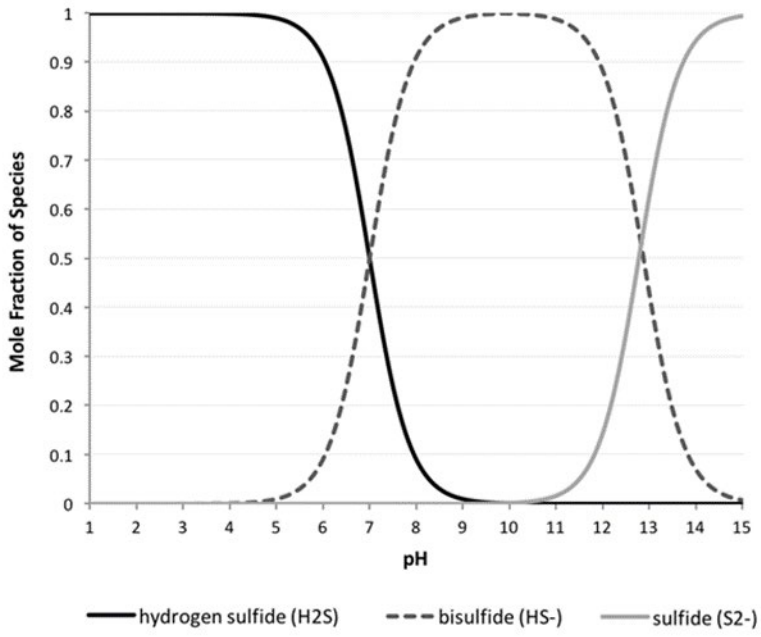

Figure 7. Proportions of sulfide species at varying pH.

Ion selective electrodes respond to the activity of an ion which is dependant on the concentration of the ion and the ionic strength of the sample. If concentration curves are used then the activity coefficient (eq. 1) of the ion in the standards and samples is assumed to be the same (eq. 2).

$$a_i = f_i[i] \quad \text{(eq. 1)}$$

Where a_i is the activity of the ion, f_i is the activity coefficient of the ion, and $[i]$ is the molar concentration of the ion. In very dilute solutions f_i approaches 1, therefore:

$$a_i = [i] \quad \text{(eq. 2)}$$

Therefore measurement of ion activity will yield the ion concentration. However, as concentrations increase, f_i decreases and a_i and $[i]$ deviates more resulting in loss of accuracy. To overcome this effect, an ion strength adjuster (ISA) is added to calibration solutions and samples to increase their respective ionic strengths to a relatively high, equivalent level. The presence of sodium hydroxide in the SAOB serves as an ionic strength adjuster.

EDTA (ETHYLENEDIAMINETETRAACETIC ACID DISODIUM SALT, DIHYRATE)

The second component of the SAOB is disodium EDTA dihydrate which is widely used as a chelating agent to bind metal ions as ligands forming water soluble complexes. It has many roles, e.g. in the health sector where chelation therapy is used for removal of heavy metals from blood to treat metal poisoning (Wu, 2016) and environmental remediation (Gluhar et al. 2020). The role of EDTA in the buffer in the total 'free sulfide' ISE method discussed here is the removal of potentially interfering metal ions in the sample to allow measurement of the free ions of interest.

L-AA (L-ASCORBIC ACID)

L-AA is the L-enantiomer of ascorbic acid (vitamin C), it is an antioxidant and for this application is used as an oxygen scavenger to inhibit the oxidation of sulfide during sample analysis. Therefore, the effect of exposure to oxygen during the measurement process (slurry stirring, etc.) should be minimized.

RESEARCH AND REGULATIONS

British Columbia (DFO 2018a), New Brunswick (NB DELG 2018), and Nova Scotia (NS DFA 2021) reference Wildish et al. (1999) for preparation of the alkaline EDTA portion of the SAOB which contains sodium hydroxide (2 M) and disodium EDTA dihydrate (0.2 M). Its preparation therefore appears standardised (Appendix 2), however, there is disparity between storage conditions of this buffer. Wildish et al. (1999) and Wildish et al. (2004) did not record the storage condition whereas Chang et al. (2014) and NB DELG (2018) stored it at approximately +4°C. The shelf life of this reagent has not been assessed, however the +4°C storage temperature may give the solution several months stability although this value is based on a sodium hydroxide solution containing 0.5 M EDTA solution at pH 8 (Beynon & Bond, 2001). NS DFA (2021) does not mention storage conditions, and DFO (2018b) states storage at 4°C after addition of the L-AA component.

After addition of L-AA to the alkaline EDTA solution to produce the SAOB, the solution slowly changes colour from a clear yellowish solution to clear brown due to oxidation of the L-AA. No stability data was afforded to the SAOB although all parties did assign it a 3 h shelf life.

Wong et al. (unpublished) conducted an experiment to determine if aging a solution of SAOB for 3 h would affect its ability to accurately quantify sulfide, Table 2. Results show that there was no effect on quantification of sulfide even though the L-AA had oxidised (solution had turned from clear yellowish to brown/red in colour). Thus, SAOB is deemed suitable for use up to 3 h after preparation and with oxidation of the L-AA component. After 3 h however, the SAOB's performance is uncertain and would require further work to determine it's efficacy.

Table 2. Comparison of sulfide quantification using freshly prepared SAOB versus aged (3 h) SAOB.

Nominal sulfide conc. (µM)	Determined sulfide conc. (µM)								Difference compared to fresh SAOB (%)
	Fresh SAOB				Aged SAOB*				
	Rep. 1	Rep. 2	Rep. 3	Mean	Rep. 1	Rep. 2	Rep. 3	Mean	
250	258	229	205#	231	255	248	207#	237	+2.6
750	727	784	597#	703	773	774	587#	711	+1.2
2500	2460	2570	1950#	2327	2460	2490	1930#	2293	-1.4
7500	7630	7510	6150#	7097	7570	7640	5830#	7013	-1.2

* = SAOB buffer aged for 3 h prior to use.

= Sulfide concentrations lower than nominal, but results accepted since it is the difference between fresh SAOB, and aged SAOB analyses that are being compared.

STEP 3: ELECTRODE CONDITIONING

PURPOSE OF FILLING SOLUTION

Ion selective electrodes are electrochemical half-cells which convert the activity (concentration) of a specific ion in solution into an electric potential across the electrolyte/electrode interface. The use of a filling solution allows for a bridge between the electrode sensing membrane and reference element of the silver/sulfide electrode which allows the generated signal to be passed to the meter. The silver/sulfide ISE (9616BNWP, Thermo Scientific) can use two types of filling solution for sulfide determination, Optimum Results A or B. Optimum Results B “can be used for most silver or sulfide measurements and titrations” whereas Optimum Results A “is recommended for precise silver measurements” (Thermo Scientific, 2009). The period from addition of filling solution to use of the ISE is not documented in the user guide (Thermo Scientific, 2009). However, solid state electrodes do not need to be conditioned and are ready for use immediately (Cammann and Schroeder, 1977). To prolong the life of the sensing membrane while the ISE is in use, the ISE should be stored in a solution of 4 M (saturated) potassium chloride (Thermo Scientific, 2009).

RESEARCH AND REGULATIONS

New Brunswick and Nova Scotia regulators both use the same make/model of electrode (9616BNWP, Thermo Scientific) and filling solution (Optimum Results B). British Columbia (DFO 2018b) on the other hand does not state the use of a specific electrode but they do give 9616BNWP as an example which would require Optimum Results A as the filling solution if used (Appendix 2). Chang et al. (2014) conducted a comparison of Optimum Results A and B filling solutions and found that they gave similar results with the Optimum Results A producing slightly more accurate and less variable results (lower standard errors with means closer to the nominal concentrations, Table 3).

Table 3. Interlaboratory comparison of using Optimum Results A and B filling solutions for the quantification of sulfide in solution, reproduced from (Chang, et al. 2014). Sulfide concentrations are given as means ± standard errors.

Lab	Filling solution	Sulfide concentration (µM)			
		250	750	2 500	7 500
Laboratory A	A	252 ± 3.1	746 ± 4.4	2 472 ± 22.7	7 385 ± 146.8
Laboratory A	B	240 ± 1.6	733 ± 10.5	2 472 ± 60.2	7 360 ± 216.1
Laboratory B	A	261 ± 4.8	756 ± 15.2	2 457 ± 57.9	7 457 ± 100.0
Laboratory B	B	253 ± 5.9	733 ± 22.4	2 342 ± 86.7	6 980 ± 273.2
Combined	A	257 ± 3.0	751 ± 7.7	2 464 ± 29.7	7 421 ± 85.4
Combined	B	246 ± 3.5	733 ± 11.8	2 407 ± 54.0	7 170 ± 175.7

As mentioned above, the period between addition of filling solution and use of the ISE is not stated by the manufacturer, however Wildish et al. (1999), NB DELG (2018) and NS DFA (2021) state that the ISE should be filled 24 h before use whereas Wildish et al. (2004) did not stipulate a time period. DFO (2018b) states 30 min which Chang et al. (2014) and Wong et al. (unpublished) also found to be a suitable time for the ISE to stabilise (constant mV) after the filling solution was added.

STEPS 4 AND 5: PREPARATION OF SOLUTIONS AND STANDARDS

PURPOSE OF SOLUTIONS

To obtain accurate results during analysis, the ISE must be calibrated using solutions containing known concentrations of the sulfide analyte. A sulfide stock solution is initially prepared then serially diluted to give a range of solutions containing sulfide over the concentration range, 100 to 10 000 µM. Sodium sulfide nonahydrate is the chemical used to prepare the calibration standards and can be purchased from numerous manufacturers and suppliers. Ideally ACS grade, which meets or exceeds the standards set out by the American Chemicals Society, or purer should be used. However, the higher the purity used, the higher the cost of the chemical. An indication of purity of the purchased batch of chemical can be confirmed by obtaining the certificate of analysis from the manufacturer.

Sodium sulfide nonahydrate is extremely hygroscopic, light sensitive, and volatile, meaning that over time its purity will be affected. Therefore, the amount weighed to prepare a stock solution will not necessarily contain the expected amount of sulfide due in part to the weight gain of moisture, therefore the expected concentration of the stock solution may be inaccurate. This will result in the prepared calibration standards containing inaccurate concentrations of sulfide which will impact accurate calibration of the ISE and subsequent quantification of samples. For

example, if the ISE is calibrated using solutions containing less sulfide than expected, the determined sample concentrations will be overestimated (analyst is assuming more sulfide than actually present) which could have regulatory implications if classification thresholds are crossed. Therefore, careful titration of the stock solution is considered a critical step.

PREPARATION OF SOLUTIONS

Sulfide stock solution – analyst prepared

The molarity of the prepared stock sulfide solution is calculated using equation 3 where Wt_a is the amount of sodium sulfide nonahydrate weighed, 240.18 is the molecular weight of sodium sulfide nonahydrate and the 100 (mL) is the volumetric flask volume used to prepare the stock solution. Typically weighing 0.96072 g (assuming 100% purity) into a 100 mL volumetric flask and making up to volume gives a 0.04 M solution.

$$\left[S^{2-}\right] = \frac{Wt_a}{240.18} \times \frac{1000}{100\ mL}\ (Molar) \qquad \text{(eq. 3)}$$

To obtain an accurate sulfide concentration, a titration of the prepared stock solution should be undertaken with lead perchlorate (see Preparation of titrant section). From the prepared stock solution, a series of dilution standards is prepared using degassed deionised water (see Step 1) to cover the calibration range of the ISE method, i.e. 100 to 10 000 µM.

Sulfide – Certified Reference Material (CRM)

It is recognised that premade sulfide certified reference materials (CRMs), with accompanying certificates of analysis, can be purchased from various chemical suppliers. These CRMs are manufactured with varying concentrations, and can be used as an alternative to analyst prepared stock solutions (above). Cranford el al. (2020) used such a reference material to calibrate their uv spectrophotometer when quantifying total free sulfide in sediment porewater samples. However, at the time of this CSAS process the effectiveness and accuracy of these materials had not been evaluated for ISE use, therefore these are not discussed further in this report.

Lead perchlorate trihydrate solution (titrant)

For this method, the titrant used is a solution of 0.1 M lead perchlorate trihydrate. The concentration of the prepared titrant is calculated using eq. 4, where $Wt._b$ is the amount of lead perchlorate trihydrate weighed, 460.15 is the molecular weight of lead perchlorate trihydrate and 25 mL is the volumetric flask volume used. Typically, weighing 1.150 g into a 25 mL volumetric flask and making up to volume with deionised water will give a 0.1 M solution. Unlike the sulfide stock solution, the titrant does not need to be prepared using degassed deionised water. However, since it is prepared at the same time as the sulfide stock solution, degassed deionised water can be used.

$$[Pb(ClO_4)_2 \cdot 3H_2O] = \frac{Wt._b}{460.15} \times \frac{1000}{25\ mL}\ (Molar) \qquad \text{(eq. 4)}$$

TITRATION PROCEDURE

Titration is a common laboratory method employed for the quantitative determination of an unknown concentration of identified analyte in solution (titrand). It involves the addition of a titrant of known concentration to an established volume of titrand until the endpoint of the

reaction is reached (i.e., the analyte in the titrand is used up). For the sulfide ISE method, a potentiometric precipitation titration is applied which involves a reaction with the titrant and the given analyte to form a precipitate. An indicator electrode (a silver/sulfide ISE in this case) is used to monitor the potential changes in the solution as the titrant is added. The potential change proceeds slowly at the start of the titration but increases rapidly as the end point is approached. The endpoint is considered to be the volume of titrant which elicits the largest change in potential (the maximum rate of change in potential), and when further addition of the titrant does not produce any precipitate, although the former is the primary indicator in this case. A titration curve and 1st derivative plot can then be generated from the potentiometric readings and titrant volumes dispensed, Figure 8.

The sulfide concentration of the stock solution can then be calculated (eq. 5), where $Vol._c$ is the volume of titrant required to reach the end-point (peak rate of change), d is the concentration of lead perchlorate trihydrate (eq. 4) and $25\ mL$ is the volume of sulfide stock solution used for the titration.

$$[S^{2-}] = \frac{Vol._c \times d}{25\ mL}\ (Molar) \qquad \text{(eq. 5)}$$

The current titration method uses 100 µL of titrant after the fast titration stage, i.e. addition of large volumes of titrant (Appendix 1). To improve the resolution of measurement and thus the accuracy of the sulfide determination, this volume could be lowered to, for example, 50 µL then 25 µL as the end point is neared. Titration can be a very accurate procedure capable of ± 0.1% of the total sulfide ion concentration of the sample (Thermo Scientific, 2009), however this is dependent on several factors, Table 4.

Table 4. Factors affecting accuracy of titration experiments.

Concentrations	Preparing and using the wrong concentrations of titrant and/or titrand.
	Sample degradation, contamination.
Misreading volume	More relevant when a burette is used. The method described here uses displacement pipettes at set volumes – however if the accuracy of the pipette is compromised then volume inaccuracies will occur, therefore calibration of the pipette is vital.
End point error	More likely with acid-base titrations where colour changes occur – individual perception.
	For potentiometric titrations, this is less likely to occur since it is based on the mV response
Using equipment incorrectly	For example, incorrect volume dispensed due to presence of air bubbles when aspirating the titrant.
	Analyst's pipetting skills.
Other errors	Human error (e.g. pipetting skills, if using pipettes to dispense titrant).

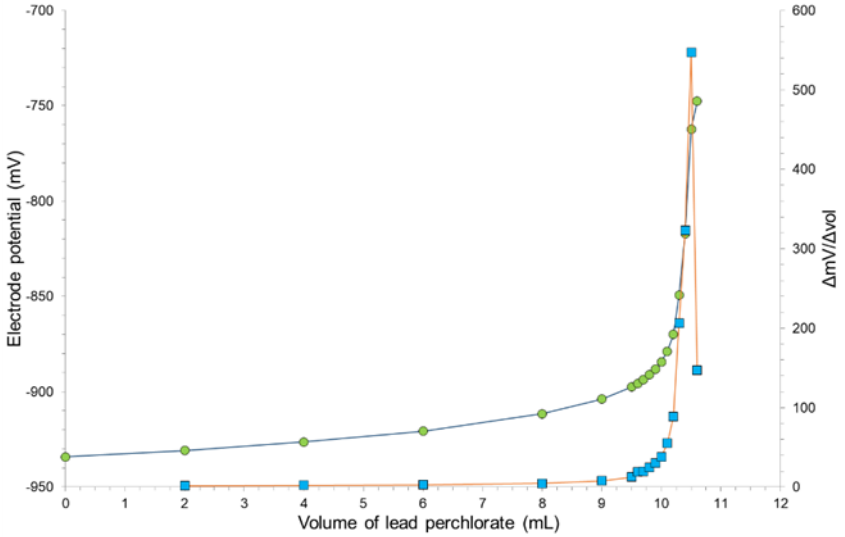

Figure 8. Representative titration curve (-●-) and 1st derivative plot (-■-) obtained from the titration of 0.1 M lead perchlorate against 0.04 M sodium sulfide nonahydrate.

DETERMINATION OF CHEMICAL PURITY

The purity of the sodium sulfide nonahydrate is important to know, whether it is a newly purchased or aged batch, for preparation of accurate calibration standards. The purity of the sodium sulfide nonahydrate chemical can be calculated using eq. 6 where, $[S^{2-}]_{titrated}$ is the determined sulfide concentration (Molar) by titration (eq. 5), and $[S^{2-}]_{weighed}$ is the calculated concentration (Molar) of the stock solution by weight (eq. 3). The purity is essential for compensating for the amount of chemical required to give an accurate stock concentration, e.g. to prepare a 100 mL solution of ca. 0.04 M stock from a chemical of 80% purity, you would need to weigh 1.2009 g instead of 0.96072 g (100 % purity). From the authors experience (Wong), it has been noted that the purity of the sodium sulfide nonahydrate chemical can fall below 80% if used over a prolonged period of time.

$$Purity\ (\%) = \frac{[S^{2^-}]_{titrated}}{[S^{2-}]_{weighed}} \times 100 \qquad \text{(eq. 6)}$$

RESEARCH AND REGULATIONS

Regulations by DFO (2018b), NB DELG (2018), and NS DFA (2021) follow the procedure documented in Wildish et al. (1999) for the preparation of the sulfide stock solution (Appendix 2). Based on that method, the prepared stock (also the top calibration standard) contained 10 000 µM of sulfide (0.2402 g sodium sulfide nonahydrate, MW = 240.18, into 100 mL) assuming 100% chemical purity, although realistically this is never the case since even a newly purchased chemical is <100% pure. Once opened, the chemical begins to degrade, however purging the container and storing the chemical with a nitrogen headspace at ca. +4°C can slow down this process since moisture and oxygen are driven out. Storing the chemical in a desiccator also inhibits any degradation. Titration of this stock was not mentioned nor performed according to the respective SOPs. The same is true for Wildish et al. (1999) and Wildish et al. (2004). As mentioned above, if the sodium sulfide nonahydrate is not 100% pure the calibration standards

will contain less sulfide than expected resulting in the inaccurate calibration of the ISE and hence quantification of samples.

Wildish et al. (1999) and the regulators all detail preparation of the top calibration standard (10 000 µM) by weighing the nominal amount (0.2402 g) of sodium sulfide nonahydrate. Since this amount would be difficult to attain accurately, any deviation from the nominal weight would be reflected in the actual sulfide concentration of the prepared calibration solution. This deviation would not include any purity issues noted above, therefore the magnitude of the deviation could potentially be much higher. Since the meter's calibration points are set at nominal values, i.e. 10, 50, 100, 500, and 1 000 (equivalent to 100, 500, 1 000, 5 000, and 10 000 µM), calibration standards containing less (or more) sulfide than expected would impact accurate calibration of the ISE and therefore accurate quantification of samples.

The approach used by Chang et al. (2014) and the one routinely used by one of us (Wong) follows a different stock standard preparation method. In this approach a ~0.04 M stock solution is titrated with lead perchlorate to determine the actual sulfide concentration present (section 3.4.3). The volume of stock required to produce a 10 000 µM standard is then calculated (eq. 7), where $[S^{2-}]$ is the sulfide concentration by titration, 10 mL is the flask volume used, and 1×10^6 is the conversion to micromolar (µM).

$$Vol.req'd\ for\ 10\ 000\ \mu M\ STD = \frac{10\ 000\ \mu M}{[S^{2-}]} \times \frac{10}{1\times10^6} \qquad \text{(eq. 7)}$$

For example, if the titrated stock was determined to be 0.039575 M (from eq. 5), then the volume required to produce a 10 000 µM standard would be:

$$= \frac{10\ 000}{0.039575} \times \frac{10}{1\times10^6} = 2.526\ ml$$

The dilution scheme (Table 5) was then followed to prepare the required calibration standards.

Table 5. Dilution scheme used by Chang et al. (2014) and Wong to prepare sulfide standards for ISE calibration.

Standard	Standard used	Vol. used (mL)	Final vol. (mL)	Nominal sulfide conc. (µM)
Stock	-	-	-	By titration
A	Stock	X	10	10 000
B	A	5	10	5 000
C	B	2	10	1 000
D	C	5	10	500
E	D	2	10	100

X = Volume calculated from equation 7.

There is no mention of the dilution scheme used to prepare standards from the prepared stock (10 000 µM) by NB DELG (2018) nor NS DFA (2021), however DFO (2018b) does mention how diluted standards should be prepared. Methodology by Wildish et al. (1999), who used 2 calibration standards (100, and 1 000 µM), was referenced by DFO (2018), NB DELG (2018), and NS DFA (2021) for preparation of their calibration standards. However, NB DELG (2018) and NS DFA (2021) use 5 standards (100, 500, 1 000, 5 000, and 10 000 µM) whereas DFO (2018b) uses only 3 (100, 1 000, and 10 000 µM), although they state that 10 µM can also be used if required. The 10 µM calibration level is considered irrelevant since the lowest environmental rating for monitoring is Oxic A which corresponds to a sulfide concentration of <750 µM, therefore quantifying ≤10 µM is not required.

STEP 6: CALIBRATION OF ISE

PURPOSE

Calibration of any analytical instrument is essential for quantification of unknown analyte concentrations in test samples. This procedure ensures that the instrument is functioning within acceptable specifications and that the generated data are both accurate and precise within the defined method parameters. It involves the use of solutions containing known concentrations of the test analyte and measuring the response of the instrument to these standards to a derived relationship.

CALIBRATION OF ISE

For chromatographic and spectroscopic analytical techniques such as HPLC, GC, UV/Vis, etc., calibration is performed using a least squares linear regression in which the response of the analyte is plotted against known concentrations of the analyte over a given linear range. This produces a calibration curve with the relationship:

$$y = mx + c$$

where y is the analyte response, m is the slope of the calibration curve, x is the concentration of the analyte, and c is the intercept of the calibration curve on the y-axis (see Figure 9a). In contrast, if a calibration curve was plotted for an ISE calibration, a logarithmic relationship would be obtained, Figure 9b. However in practice, due to the Nernstian response of ISEs (see Background to Ion Selective Electrodes section), the potential difference is directly proportional to the logarithm of the ion concentration which allows construction of a linear calibration line.

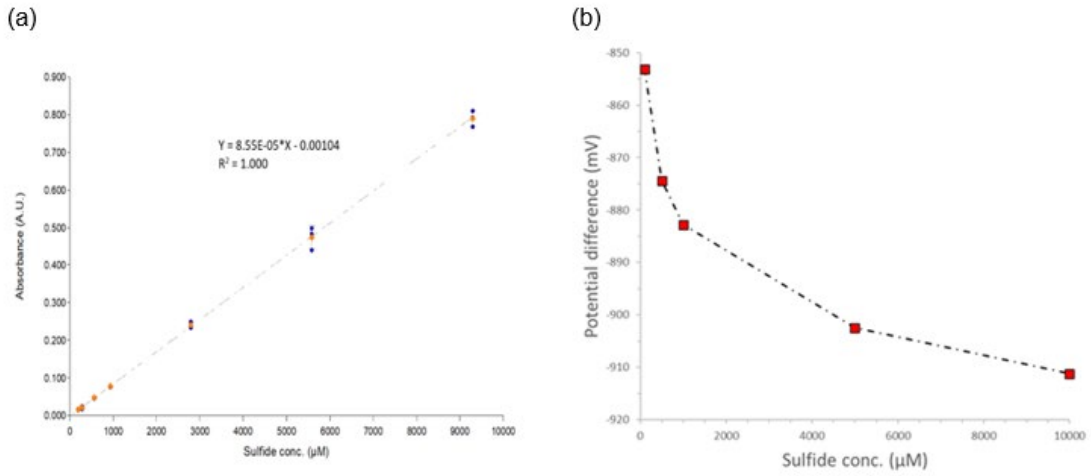

Figure 9. Comparison of calibration curves derived from instrument response against sulfide concentration by (a) colorimetric absorbance, and (b) ion selective electrode techniques. The colorimetric technique generates a linear least squares regression relationship whereas the ISE method derives a logarithmic relationship.

For the silver/sulfide ISE, calibration is performed by direct calibration (Thermo Scientific, 2009), which is used by all parties in Appendix 2. This method is recommended for samples above 0.32 ppm (1×10^{-5} M ≡ 10 µM) sulfide with a 2 point calibration being sufficient as long as these bracket the expected sample range, although more points are required in non-linear regions (Thermo Scientific, 2009). For the silver/sulfide ISE, calibration should be performed between 20 to 25°C, which will give acceptable slopes between -25 to -30 mV (Thermo Scientific, 2009). The calibration procedure entails preparation of sulfide standards of known concentration which correspond to preset order of magnitude range options in the meter, e.g. meter setting 10 → 100 µM (standard); 50 → 500 µM; 100 → 1 000 µM; 500 → 5 000 µM; and 1000 → 10 000 µM. The meter settings are an order of magnitude (10 times) less than the calibration standards used, therefore test results must be multiplied by this factor to obtain accurate sulfide concentrations when analysing sediment samples. Rather than using a least squares linear regression approach which derives a single relationship from a multi-point calibration, the meter utilises a segmented (or point-to-point) approach whereby a series of calibration curves is produced with one curve or calibration segment between successive calibration points. For example, if a 5-point calibration was performed, as is the case for New Brunswick (NB DELG, 2018) and Nova Scotia (NS DFA, 2020), four calibration curves would be constructed in the meter's memory, i.e. from 100 to 500 µM, 500 to 1 000 µM, 1 000 to 5 000 µM, and 5 000 to 10 000 µM, see Figure 10 for a representative plotted example using derived experimental data. The slope of the calibration curve is the mV response per decade of concentration change which is theoretically -29 mV/decade for the divalent form of sulfide (S^{2-}), although the slope is temperature dependent (Nico2000 2011). During sample analysis, the potentiometric reading (mV) of the test sample determines which calibration curve (segment) is used to calculate the sulfide concentration. With this point-to-point calibration approach, if even one of the calibration points is outwith the acceptance criteria (-25 to -30 mV, Thermo Scientific, 2009), the calibration would have to be repeated from the beginning. This is unlike the least squares linear regression approach which allows outliers to be removed if they fall outside the acceptance range. Also unlike the regression approach, the segmented approach does not generate an uncertainty or error estimate associated with the calibration and hence measurements are not associated with uncertainties.

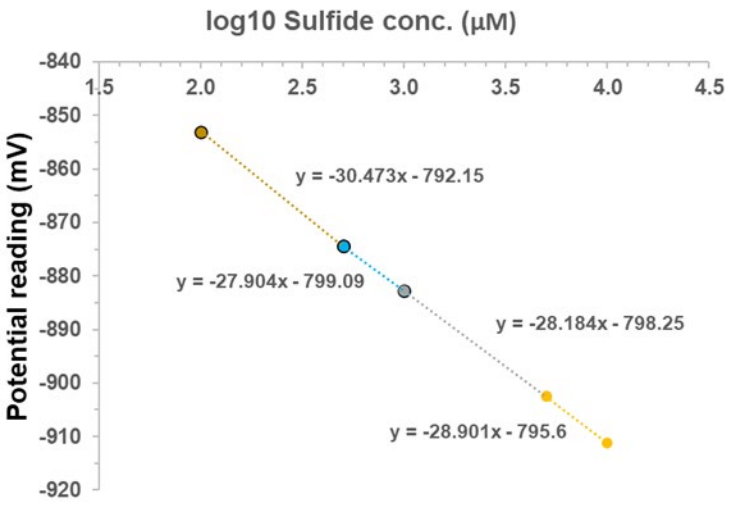

Figure 10. Representative 5-point segmented calibration (4 x 2-point calibration curves, 100 to 10 000 µM) reproduced from empirical data.

RESEARCH AND REGULATIONS

The calibration procedure used by New Brunswick (NB DELG, 2018) and Nova Scotia (NS DFA 2021) is fairly well documented (Appendix 2), they use five calibration points (100, 500, 1 000, 5 000 and 10 000 µM), as did Chang et al. (2014) and Wong (in practice). British Columbia (DFO 2018b) on the other hand uses three calibration points (100, 1 000, and 10 000 µM), although if low sulfide is expected then this is modified to 10, 100 and 1 000 µM. Wildish et al. (1999) however only used 2 calibration points (100 and 1 000 µM). Wildish et al. (2004) recommend three points (10, 1 000, and 10 000 µM). Calibration of the ISE by each of the regulatory authorities covers the environmental classification range for sulfide in sediment detailed in Table 6.

Table 6. Environmental impacts related to varying sulfide concentrations in sediment (Chang et al. 2014).

Environmental rating	Sediment sulfide conc. (µM)	Effects on marine sediments
Oxic A	< 750	Low effects
Oxic B	750 to 1 500	
Hypoxic A	1 500 to 3 000	May be causing adverse effects
Hypoxic B	3 000 to 4 500	Likely causing adverse effects
Hypoxic C	4 500 to 6 000	Causing adverse effects
Anoxic	> 6 000	Causing severe damage

Thermo Scientific (2009) states that calibration should be conducted between 20 to 25°C, with an acceptable slope for a 10-fold concentration difference of ca. -28 mV (acceptance range -25 to -30 mV). Only New Brunswick (NB DELG 2018), and Nova Scotia (NS DFA 2021), document this range along with the use of an automatic temperature compensation (ATC) probe to measure the sample temperature during analysis. All the regulators detail an acceptance criteria of -27 to -33 mV for slope values but no mention of this range can be found in Thermo Scientific (2009) so it is unclear how this acceptance range is derived.

Chang et al. (2014) conducted an interlaboratory comparison to test the accuracy of quantification when using either a 3-point or 5-point calibration curve, Table 7. Results showed that 3-point calibration curves afforded more accurate quantification compared to 5-point calibrations but there was no significant difference with the Laboratory A data. Laboratory B produced better results with the 3-point calibration due to outliers with their 5-point trials. The improved quantification using a 3-point calibration may be due to the 100 to 500 µM and 1 000 to 5 000 µM standards used in the 5-point calibration having less than a decade change in sulfide concentration between them.

Table 7. Interlaboratory comparison of using 3-point and 5-point calibration curves for the quantification of sulfide in solution, reproduced from (Chang et al. 2014).

Lab	No. of calibration points	Test solution sulfide concentration (µM)			
		250	750	2 500	7 500
Laboratory A	5	241 ± 3.5	740 ± 8.3	2 484 ± 19.6	7 543 ± 86.0
Laboratory A	3	254 ± 5.2	765 ± 11.7	2 513 ± 34.2	7 550 ± 151.0
Laboratory B	5	229 ± 11.2	672 ± 21.0	2 146 ± 71.0	6 478 ± 245.3
Laboratory B	3	246 ± 6.5	736 ± 20.2	2 439 ± 86.9	7 158 ± 203.8
Combined	5	235 ± 5.8	706 ± 13.2	2 315 ± 50.4	7 011 ± 168.8
Combined	3	250 ± 4.2	751 ± 11.8	2 476 ± 46.3	7 354 ± 130.6

STEP 7: SAMPLE ANALYSIS

BACKGROUND

Sediment analysis is performed by taking the collected sediment sample and adding a volume of SAOB to give a ratio of 1:1. This converts H_2S and HS^- in the sample to S^{2-} for detection by the ISE and also increases the ionic strength of the sample to a high and constant level to enable determination of ion concentration instead of activity. The sediment and SAOB mixture is stirred to obtain a homogenous sample then the ISE and ATC probe are inserted into the slurry. The sample is then brought up to the calibration temperature, an effective method being to use the warmth of one's hand, and then allowing the meter reading to stabilise. Analysis of samples

must be performed at the same temperature at which the calibration was established to eliminate any temperature effects which could result in a ca. 4% difference for a 1°C deviation, hence the use of the ATC probe to monitor the sample temperature in real time.

An important factor to consider is the time that SAOB is in contact with the sediment before a sulfide reading is taken. Too short a time may not allow the SAOB to fully react with the sediment to produce the divalent sulfide ion and may also not allow the sample to reach the calibration temperature. Too long a period would allow the aforementioned reaction and temperature variables to be reached but accuracy could be compromised since sulfide levels could have changed during that time. Accurate and consistent data are dependent on a standardised read time window. Brown et al. (2011) concluded that a residence time of 10 min for sediment in a pH 14 environment (equivalent to exposure to SAOB) results in potential for bound sulfides to be solubilised resulting in artificially high sulfide concentrations. Wong et al. (unpublished) conducted a soak time experiment using sediment collected from Nova Scotia and Southwest New Brunswick to test if leaving SAOB in contact with sediment would affect quantification of sulfide. Results (Figure 11) showed that sulfide increased in the Nova Scotia sediment but decreased in the New Brunswick sediment. It is unclear whether the rise in concentration in the Nova Scotia sediment was due to solubilisation of bound sulfides or whether the ISE was taking a long time to stabilise due to potential sediment matrix effects. This phenomenon of continual increasing sulfide past the 2 min analysis window stipulated in the ISE method in low concentration samples has been observed by one of us (Wong) during study specific analysis of sediment samples containing very low sulfide concentrations. The New Brunswick sediment demonstrated stable sulfide early on but then started to degrade steadily. It is unclear why this degradation was taking place since the Nova Scotia sediment maintained constant levels without any apparent degradation once concentrations had plateaued. Therefore other factors may be driving this loss in the New Brunswick sediment. This was a limited test using sediment derived from two locations, therefore it is unclear how reproducible these results are or how other sediment types would respond, e.g. spatially different or high sulfide sediments.

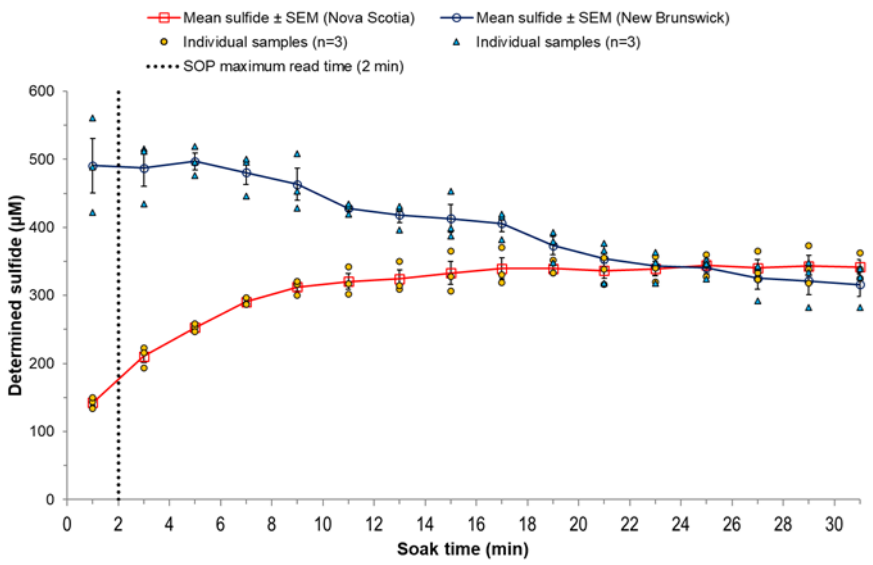

Figure 11. Soak time experiment to determine effect of prolonged exposure of SAOB to sulfide in sediment.

Analytically, the most accurate time for sulfide determination would be immediately after SAOB addition which would attain the most representative in-situ results with little to no time for effects. However this timeframe would not allow for the sample to reach the calibration temperature or for the electrode to stabilise (ca. 1 min), therefore this is not a practical possibility. Hence a 2 min window has been adopted for the ISE method by regulators (NB DELG 2018, NS DFA 2021, and DFO 2018b) which is sufficient for the sample to come up to calibration temperature, and for the electrode to stabilise but not so long as to markedly affect quantification.

RESEARCH AND REGULATIONS

Due to the chemical properties of sulfide (i.e. volatile, easily oxidised, etc.) it is desirable to analyse sediment samples immediately after collection to minimise any sulfide loss. However, New Brunswick (NB DELG 2018) and Nova Scotia (NS DFA 2021) regulators allow for storage of samples between 2 to 5°C for up to 72 h prior to analysis (Appendix 2). In contrast, British Columbia sites are so remote that transportation of samples is not feasible, therefore analyses are conducted onsite enabling immediate analysis (within 5 min) after collection (DFO 2018). The question of whether there are any effects on sulfide concentrations as a result of storing sediment samples prior to analysis is the focus of the second Wong and Page paper in this CSAS process.

Wildish et al. (1999) used a 5 mL sediment volume for their research along with a sediment to SAOB ratio of 1:1. All regulators follow the same ratio, however, the sediment volumes analysed differ between regions, 10 mL for British Columbia (DFO 2018b), 5 mL for New Brunswick (NB DELG 2018), and 3 mL for Nova Scotia (NS DFA 2021).

It is known that prolonged exposure to the high alkaline environment after SAOB addition has the potential to solubilise bound sulfide in the sediment. Therefore a maximum analysis time window of 2 minutes is followed by all regulators, which is also an adequate time period to enable the sediment sample to be brought up to the calibration temperature.

It is unclear what matrix effects different sediment types (i.e., muddy, sandy), in addition to its composition (i.e., feed, faeces, high sulfides) have on quantification of sulfide in porewater during this time due to grain size, and organic content differences. It is also unclear what the implications are for using a calibration relationship established using deionised fresh water for measuring sulfide in salty marine sediment pore water.

Hargrave et al. (2008) mentioned that sulfide concentrations can be expressed as $\mu M \cdot mL^{-1}$ if the sediment water content is measured in sediments of differing porosities, *e.g.* coarse to fine grain sediments. However, most analysts have not made this correction and little evidence exists to determine the empirical support for the correction. This issue is discussed further in Page and Wong (this CSAS).

STEPS 8 AND 9: ISE CALIBRATION CHECK

PURPOSE

Analysing samples immediately after ISE calibration garners the most accurate and precise results. However during use, the ISE will be subject to drift due to the electrode response gradually changing over time resulting in loss of accuracy during a sequence of measurements. To check its performance, the ISE should be checked at least once every 2 h using a freshly prepared solution of the lowest sulfide standard (100 µM). If the measurement has changed by > 4% the ISE should be recalibrated (Thermo Scientific, 2009).

RESEARCH AND REGULATIONS

The manufacturer's recommendation of checking the ISE every 2 h is not implemented by any of the regulators. New Brunswick (NB DELG, 2018) indicates that the ISE must only be used for a maximum of 3 h then it should be recalibrated. Nova Scotia (NS DFA, 2021) states the ISE is stable for 3 h and British Columbia (DFO 2018b) does not specify a time limit for ISE calibration performance although it does state that an "additional sulfide reading from a sample should be taken once every 20 samples" (Appendix 2). However, a solution containing a known concentration of sulfide, as indicated by Thermo Scientific (2009), is a more appropriate indicator of ISE performance than taking an additional sample reading. It is also unclear whether the first sample of the batch of 20 is reread, if so, then sulfide could have changed significantly in that time thus providing an inaccurate comparison.

Wildish et al. (1999) and Wildish et al. (2004) did not mention a time limit for ISE use whereas Chang et al. (2014) and Wong et al. (unpublished) did not use electrodes for more than 2 h during their experimental work, therefore ISE performance was not rechecked.

Chang et al. (2014) did perform an interlaboratory calibration stability test whereby an electrode was calibrated and its performance tested over a 96 h period using freshly prepared test solutions containing known concentrations of sulfide, Table 8. Data showed that sulfide values changed > 4% within 1 h and 2 h after calibration based on the 100 µM test solution (also the lowest calibration level). ISE performance dropped by ca. -20% by 96 h post calibration at all concentration levels investigated.

Analysis of sediment samples by contract laboratories as part of environmental monitoring has shown that the presence of feed and/or faeces in the sediment can deposit a film across the membrane's surface resulting in electrode drift of as much as 10% to 15% within 1.5 h of calibration (personal correspondence). Therefore the 2 h limit as set out by the manufacturer may not be appropriate for samples containing high organic content which means that electrode performance may have to be checked sooner based on the sediment type and composition.

Table 8. Interlaboratory assessment of calibrated ISE performance. The ISE was calibrated then tested over 96 h using freshly prepared solutions containing known concentrations of sulfide at each timepoint, reproduced from Chang et al. (2014). Results were compared against a freshly calibrated ISE at each time point which acted as a control (data not presented).

Lab	Time (h)	100 µM	Rel. diff. from T_0 (%)	500 µM	Rel. diff. from T_0 (%)	1 000 µM	Rel. diff. from T_0 (%)	5 000 µM	Rel. diff. from T_0 (%)	10 000 µM	Rel. diff. from T_0 (%)
Laboratory A	0	103	0.0	504	0.0	991	0.0	4957	0.0	9908	0.0
	1	101	-1.9	494	-2.0	975	-1.6	4843	-2.3	9922	0.1
	2	98	-4.9	493	-2.2	964	-2.7	4852	-2.1	9803	-1.1
	3	93	-9.7	479	-5.0	943	-4.8	4717	-4.8	9590	-3.2
	4	97	-5.8	487	-3.4	958	-3.3	4823	-2.7	9625	-2.9
	12	93	-9.7	466	-7.5	909	-8.3	4488	-9.5	8882	-10.4
	24	87	-15.5	447	-11.3	882	-11.0	4508	-9.1	8993	-9.2
	48	89	-13.6	443	-12.1	862	-13.0	4278	-13.7	8653	-12.7
	72	81	-21.4	413	-18.1	802	-19.1	3995	-19.4	8308	-16.1
	96	81	-21.4	408	-19.0	807	-18.6	4035	-18.6	7977	-19.5
Laboratory B	0	104	0.0	500	0.0	992	0.0	4950	0.0	9994	0.0
	1	99	-4.8	485	-3.0	971	-2.1	4830	-2.4	9938	-0.6
	2	98	-5.8	491	-1.8	963	-2.9	4784	-3.4	9731	-2.6
	3	95	-8.7	477	-4.6	944	-4.8	4649	-6.1	9526	-4.7

Lab	Time (h)	100 µM	Rel. diff. from T_0 (%)	500 µM	Rel. diff. from T_0 (%)	1 000 µM	Rel. diff. from T_0 (%)	5 000 µM	Rel. diff. from T_0 (%)	10 000 µM	Rel. diff. from T_0 (%)
	4	94	-9.6	474	-5.2	933	-5.9	4672	-5.6	9388	-6.1
	12	92	-11.5	460	-8.0	893	-10.0	4431	-10.5	8930	-10.6
	24	89	-14.4	446	-10.8	878	-11.5	4380	-11.5	8712	-12.8
	48	87	-16.3	435	-13.0	851	-14.2	4142	-16.3	8163	-18.3
	72	83	-20.2	417	-16.6	809	-18.4	3970	-19.8	8012	-19.8
	96	82	-21.2	410	-18.0	810	-18.3	3963	-19.9	7867	-21.3

Rel. diff. = Relative difference

SUMMARY AND CONCLUSIONS

- The sulfide ISE method, even though it is currently used for regulatory monitoring and decision making, has not been formally validated to demonstrate its suitability for its intended purpose.
- Regulators refer to Wildish et al. (1999) and Wildish et al. (2004) for sulfide analysis but they have also adopted their own nuances to the method.
- Analytical procedures detailed in the SOPs by different jurisdictions are not comprehensive, thus processes are open to individual interpretation which can increase the error and variability of generated results.
- The ISE method is prone to variation due in large part to execution by different analysts and laboratories nationwide and individual interpretation of each analytical step.
- Titration of the prepared stock sulfide solution is not documented in any of the regulator's SOPs, therefore it is assumed that this procedure is not performed. This is a critical step due to the chemical properties of the sodium sulfide nonahydrate chemical. The lack of this step would result in inaccurate ISE calibration and thus impact accurate sample quantification.
- Titration is essentially a laboratory based technique due to safety issues (equipment and chemicals used) and the required accuracy of determination. If collected samples are to be analysed immediately after collection (as per recommendation in paper 2 of this CSAS, Wong and Page (In press)), the practicality of titrating in the field whether in a land based mobile lab or on a boat should be investigated. This has the potential to impact the accuracy of the determined sulfide concentration, and thus accurate calibration of the ISE.
- Execution of the sulfide ISE method is inconsistent between regulators. This includes:
    - Number of calibration points used.
    - Filling solution used for the make/model of ISE.
    - Volume of sediment sample analysed.
    - Time from sample collection to analysis (within 5 min versus up to 72 h).
- Time limit for ISE calibration check, 3 h for New Brunswick (NB DELG, 2017) and Nova Scotia (DFA, 2020) is longer than that recommended by the manufacturer (2 h) which will affect the accuracy of results.
- It is unclear what matrix effects, if any, the sediment fraction of the sample has on quantification of dissolved sulfide in the porewater.
- Calibration of the ISE is performed using deionised water standards (freshwater) whereas the analysed samples are in seawater (porewater) and sediment. Therefore it is unknown what effect (if any) this salinity difference and sediment matrix effect have on accurate quantification.

RECOMMENDATIONS

1. Titration of prepared stock solutions should be performed to confirm actual sulfide concentration prior to preparation of calibration solutions.
2. Evaluate the possible use of sulfide certified reference material (CRM) as an alternative to analyst prepared stock sulfide solutions.

3. Water (distilled/deionsed) should be degassed by sparging with an inert gas for at least 3 h prior to use rather than boiled.
4. Further research to investigate deoxygenating water with sodium sulfite anhydrous as an alternative to degassing with an inert gas.
5. Calibration standards should be used as soon as possible after preparation to avoid loss of sulfide and to afford accurate calibration of the ISE.
6. Use of a 3 point calibration (100, 1 000 and 10 000 µM) rather than 5 points to operate within a one order of magnitude difference for the calibration standards, or use a regression approach.
7. Assess what effect freshwater versus seawater standards has on ISE calibration and quantification.
8. The ISE should be recalibrated after 2 h of use as a matter of course to minimise loss of accuracy.
9. The performance of the ISE should be checked at the end to determine the loss in accuracy over the analysis period.
10. Further research to investigate if solubilisation of bound sulfide is an issue with spatially different sediments containing varying concentrations of sulfide (oxic to anoxic).
11. Even though extensive research work has been conducted to evaluate the ISE method, questions have been raised about the method by practitioners and regulators, indicating it should be formally validated to demonstrate that it is suitable for its intended purpose. This will also give credibility to the method for regulatory decision making.
12. Reproducibility between laboratories and multiple analysts must be demonstrated to be very high when the method is validated.
13. Once the method is validated, an SOP should be developed and distributed for use by third party laboratories to standardise the execution of the method. A suggested improved SOP has been provided in Appendix 1.
14. Even if the ISE method produces zero bias, there is an in situ variability associated with sulfide distribution in the benthic environment which can influence regulatory decision making. Therefore, this variability should be thoroughly explored to generate a better understanding of sulfide's spatial distribution which could potentially have consequences for sampling design.

REFERENCES CITED

Beynon, R., & Bond, J. (Eds.). 2001. Proteolytic Enzymes: A Practical Approach, 2nd ed. (2nd ed.). Oxford University Press.

Brown, K. A., McGreer, E. R., & Taekma, B. 2011. Determination of Total Free Sulphides in Sediment Porewater and Artefacts Related to the Mobility of Mineral Sulphides. *Aquat Geochem*(17), 821-839.

Butler, I. B., Schoonen, M. A., & Rickard, D. T. 1994. Removal of Dissolved Oxygen from Water: A Comparison of Four Common Techniques. *Talanta, 41*(2), 211-215.

Cammann, K., & Schroeder, A. H. 1977. Working with Ion Selective Electrodes. Springer-Verlag.

Chang, B., Lewis-Mcrea, L., Wong, D., MacKeigan, K., Page, F., Cameron, S., . . . Smith, A. 2014. Interlaboratory research on the factors affecting the calibration of electrodes used for sediment sulfide analyses at fish farms. Can. Tech. Rep. Fish. Aquat. Sci. 3090: v + 66 p.

Cranford, P., Brager, L., Elvines, D., Wong, D., & Law, B. 2020. A revised classification system describing the ecological quality status of organically enriched marine sediments based on total dissolved sulfides. Marine pollution Bulletin, 154, 111088.

DFO. 2018a. Aquaculture Activities Regulations guidance document.

DFO. 2018b. Aquaculture Activities Regulations guidance document. Retrieved 2019, from Annex 8: Program Protocols for Marine Finfish Environmental Monitoring in British Columbia.

Gluhar, S., Kaurin, A., & Lestan, D. 2020. Soil washing with biodegradable chelating agents and EDTA: Technological feasibility, remediation efficiency and environmental sustainability. Chemopshere, 257, 127226.

Hargrave, B., Holmer, M., & Newcombe, C. 2008. Towards a classification of organic enrichment in marine sediments based on biogeochemical indicators. Marine Pollution Bulletin, *56*, 810-824.

Hargrave, B., Phillips, G., Doucette, L., White, M., Milligan, T., Wildish, D., & Cranston, R. 1997. Assessing benthic impacts of organic enrichment from marine aquaculture. Water, Air and Soil Pollution(99), 641-650.

NB DELG. 2018. Standard Operating Practices for the Environmental Monitoring of the Marine Finfish Aquaculture Industry in New Brunswick.

Nico2000. 2011. Beginners Guide to ISE Measurements.

NS DFA. 2021. Standard Operating Procedures for the Environmental Monitoring of Marine Aquaculture Sites in Nova Scotia.

Page, F.H., and Wong, D.K.H. In press. Considerations of Bias and Precision Associated with ISE Based Measurements of Sediment ‘Free’ Sulfide. DFO Can. Sci. Advis. Sec. Res. Doc. 2025/017.

Thermo Scientific. 2009. User Guide - Silver/Sulfide Ion Selective Electrode.

Wildish, D. J., Akagi, H. M., Hamilton, N., & Hargrave, B. T. (1999). A Recommended Method for Monitoring Sediments to Detect Organic Enrichment from Mariculture in the Bay of Fundy. Can. Tech. Rept. Fish. Aquat. Sci. 2286: iii + 31 p.

Wildish, D. J., Akagi, H. M., Hargrave, B. T., & Strain, P. M. 2004. Inter-laboratory Calibration of Redox Potential and Total Sulfide Measurements in Interfacial Marine Sediments and the Implications for Organic Enrichment Assessment. Can. Tech. Rep. Fish. Aquat. Sci. 2546: iii+25 p.

Wildish, D. J., Hargrave, B. T., & Pohle, G. 2001. Cost-effective monitoring of organic enrichment resulting from salmon mariculture. Journal of Marine Science, 58, 469-476.

Wong, D., and Page, F. 2025. Storage Stability: Effect of Storage Time, Temperature and Preservation Method on Total Free Sulfide Measurements in Marine Benthic Sediment. DFO Can. Sci. Advis. Sec. Res. Doc. 2025/016. iv + 43 p.

Wu, H. 2016. Heavy Metals and Chelation Therapy. J Heavy Met Toxicity Dis., 1:1.

APPENDIX 1. PROPOSED STANDARD OPERATING PROCEDURE FOR THE ISE DETERMINATION OF SOLUBLE SULFIDE IN SEDIMENT SAMPLES

STANDARD OPERATING PROCEDURE FOR THE DETERMINATION OF SOLUBLE SULFIDE IN SEDIMENT SAMPLES FOR ENVIRONMENTAL MONITORING OF AQUACULTURE ACTIVITIES

1. Scope

This standard operating procedure (SOP) details standardised methodology for the quantitative determination of soluble sulfide in sediment samples using silver/sulfide ion selective electrodes. Samples are collected from in and around aquaculture lease sites using appropriate samplers as part of environmental monitoring of benthic habitats which could be affected due to excessive organic loading resulting from feed and faecal deposition from aquaculture activities.

2. Materials

The following lists the equipment and chemicals required to conduct analysis for the quantification of sulfide in sediment samples. Alternative manufacturers and suppliers of equipment and/or chemicals can be used as long as comparable performance is achieved. The only exceptions are the silver/sulfide ISE, Accumet™ pH/ORP/Ion meter and ATC probe, the listed manufacturer and models must be used unless they have been made obsolete – in which case, the newer model will be used.

2.1 Equipment

1. Combination silver/sulfide (Ag^{+}/S^{2-}) ion selective electrode (ISE) 9616BNWP (Fisher Scientific, P/N 13642266).
2. Accumet™ AP125 portable pH/ORP/Ion meter (Fisher Scientific, P/N 13636AP15A).
3. Accumet™ automatic temperature compensation probe (Fisher Scientific, P/N 13620AP53).
4. 5-Figure analytical balance.
5. Magnetic stir bar and plate.
6. Volumetric glass pipettes.
7. Volumetric flasks (amber).
8. Displacement pipettes (e.g. Gilson P5000, P1000, P200).
9. Glass liquid scintillation (LSC) vials (Fisher Scientific, 03340022, 20 mL).
10. Glass stir rods.
11. Glass filter for degassing water (Agilent Technologies, 01090-60009).
12. Glass weigh boats (Fisher Scientific, 14353D).
13. Disposable transfer pipettes (Fisher Scientific, 137119AM).
14. Culture tubes (Fisher Scientific, 149331C).
15. 100 mL Pyrex beaker.
16. 25 mL Graduated measuring cylinder.

17. Volumetric pipettes (5 mL and 2 mL).
18. Timer.

2.2 Chemicals

1. Sodium sulfide nonahydrate (Fisher Scientific, Cat. No. S425, Certified ACS grade, crystalline).
2. Lead (II) perchlorate trihydrate (Fisher Scientific, Cat. No. AC254592500, 99%).
3. Sodium hydroxide (MilliporeSigma, Cat. No. 221465, ACS reagent grade).
4. EDTA disodium salt dihydrate (MilliporeSigma, Cat. No. E4884, ACS reagent grade).
5. L-Ascorbic acid (MilliporeSigma, Cat. No. A0278, reagent grade).
6. Orion™ Optimum Results B filling solution (Fisher Scientific, Cat. No. 13642633).
7. 4 M (Approximate) potassium chloride, saturated (Fisher Scientific, Cat. No. 5900-32)
8. Nitrogen gas (Air Liquide, Alpha Gaz™ 2 grade).
9. Deionised water (MilliporeSigma, Milli-Q® 18.2 MΩ·cm @ 25°C, produced in-house).
10. Gel pack or ice (in-house).

2.3 Reagents

1. Sodium sulfide nonahydrate solution; 0.04 M.
2. Lead perchlorate trihydrate solution; 0.01 M.
3. Alkaline EDTA solution; 2 M sodium hydroxide/0.2 M EDTA disodium dihydrate.
4. SAOB; 0.2 M L-ascorbic acid in alkaline EDTA disodium dihydrate solution.

3. Analytical Procedure

A flowchart denoting the sequential order of steps for the determination of sulfide in sediment samples is presented in Figure A.1 of this SOP.

3.1 Degassing of deionised water

The volume of water required to be degassed will depend on the number of samples to be analysed, typically 2 L is sufficient for several batches.

The best option is to use a 2 L media bottle, drill a hole through the cap and feed the nitrogen line through and attach the glass filter. Fill the bottle with deionised water to the required level, cap and open the gas line until a fine steady stream of nitrogen comes through the glass filter. Degassing should be initiated about 3 h before the start of analysis and performed continuously to ensure the water is deoxygenated sufficiently during the analytical phase.

3.2 Preparation of solutions and reagents

3.2.1 Preparation of SAOB

Prepare the alkaline EDTA disodium dihydrate component of the SAOB by weighing sodium hydroxide (80 g) into a 1 L volumetric flask followed by EDTA disodium salt dihydrate (75 g). Add deionised water (ca. 600 mL, deoxygenation not required) and dissolve the two solutes by swirling the flask. CAUTION – the flask contents will get very hot due to the exothermic reaction of sodium hydroxide with the water. Once dissolved, allow the contents to come down to ambient temperature then make to volume with further deionised water. Transfer the buffer to a

1 L media bottle, cap and store at ca. +4°C in a fridge until use. A shelf life of 2 months can be assigned to this buffer based on its performance during its routine use, although the actual stability has not been fully tested. The volume of buffer prepared can be scaled up (or down) accordingly, depending on the expected number of samples to be analysed.

Before analysis, replicate amounts of L-AA (0.875 g, molecular weight 176.12 g/mol) can be weighed into 50 mL glass culture tubes which can be capped and stored in the dark until required. On the day of analysis, the alkaline EDTA solution should be removed from ca. +4°C to allow it to come up to ambient temperature before preparation of the SAOB.

The SAOB is prepared immediately before sample analysis. Use a 25 mL serological pipette to add 25 mL of alkaline EDTA solution into a culture tube containing the pre-weighed L-AA, cap and invert several times to aid dissolution. 25 mL is sufficient to perform the stock titration (Section 3.4), an ISE calibration (Section 3.6), or analysis of 5 samples (Section 3.7). This means that a fresh solution needs to be prepared after every 5 samples. However, a larger quantity can be prepared if desired using scaled up volumes and weights.

If left for a certain amount of time, the solution colour will darken to red/brown indicating that the L-AA is oxidizing. However, the solution is still viable up to 3 h after preparation, therefore the colour change does not affect its performance.

3.2.2 Preparation of 0.04 M sulfide stock solution

On each analytical occasion, a stock solution of sodium sulfide nonahydrate is prepared, titrated to determine the actual sulfide concentration, then serially diluted to give the standards needed to calibrate the ISE.

NOTE: if a new (or previously opened) container of sodium sulfide nonahydrate is to be used, it is advisable to perform a titration to determine its purity before it's use. This value can then be used to compensate for the actual weight of chemical required to give a 0.04 M stock standard.

The molecular weight of sodium sulfide nonahydrate is 240.18 g/mol, therefore for a 0.04 M solution, weigh ca. 0.961 g into a glass weigh boat (assuming 100 % purity). Transfer the contents into a 100 mL amber volumetric flask using degassed deionised water with a transfer pipette then add further degassed deionised water (ca. 500 mL) and gently swirl the flask to aid dissolution. Once fully dissolved, make to volume with further degassed deionised water, stopper the flask then invert about 4 times to obtain a homogenous solution. The actual concentration of the solution based on the amount weighed is calculated using the following equation:

$$\text{Sulfide conc. (M)} = \frac{\text{Amount weighed}}{240.18} \times \frac{1000}{100}$$

3.2.3 Preparation of 0.1 M Lead (II) perchlorate trihydrate

The molecular weight of lead (II) perchlorate trihydrate is 460.15 g/mol. Therefore for a 0.1 M solution, weigh ca. 1.150 g into a glass weigh boat. Transfer the contents into a 25 mL amber volumetric flask using degassed deionised water with a transfer pipette then add further degassed deionised water (ca. 10 mL) and gently swirl the flask to aid dissolution. Once fully dissolved, make to volume with further degassed deionised water, stopper the flask then invert about 4 times to obtain a homogenous solution. The actual concentration of the solution based on the amount weighed is calculated using the following equation:

$$\text{Titrant conc. (M)} = \frac{\text{Amount weighed}}{460.15} \times \frac{1000}{25}$$

3.3 Preparation of ISEs for analysis

Approximately 30 min prior to titration and sample analysis, fill two ISEs with Optimum Results B as per the manufacturer's instructions and store them in the 4 M potassium chloride storage solution. The ISE used for titration purposes does not require an ATC probe connected to the AP125 meter, however one is required for the ISE used for sample analysis. It is a good idea to tape the ATC probe against the ISE for ease of handling. The bottom of the ATC probe should be in line with the ISE sensing membrane with the fill hole not obstructed by the probe.

3.4 Titration of stock sulfide solution

Sulfide is highly volatile, hygroscopic, and readily oxidizable therefore it is conceivable that the amount weighed will not produce the expected concentration. Therefore, titration of the prepared stock is considered essential to generate accurate solutions for accurate calibration of the ISE.

Safety Appropriate PPE should be worn when using the lead (II) perchlorate trihydrate - refer to the appropriate SDS. The titration procedure should be conducted under a fume hood to eliminate exposure to lead fumes.

1. Prepare solutions of 0.04 M sulfide stock solution (Section 3.2.2), 0.1 M lead (II) perchlorate trihydrate (Section 3.2.3) and 25 mL of SAOB (Section 3.2.1).\
2. Put a magnetic stir bar into a 100 mL glass beaker then place onto the magnetic stir plate.
3. Pour the 25 mL of prepared SAOB into the beaker.
4. Measure out 25 mL of the prepared sulfide stock solution using the graduated cylinder and add to the beaker too.
5. Start the magnetic stir plate to gently mix the two solutions.
6. Rinse the ISE with deionised water and insert into the sample. Ensure that the AP125 meter is on the mV setting.
7. Titrate the sample with the 0.1 M lead (II) perchlorate trihydrate solution using the volumes presented on the titration template sheet in Figure A.2 of this SOP. Allow the mV readings to stabilize after each addition of titrant then record the value. Note: the time to reach stabilisation of the mV readings takes longer as the end-point of the reaction is reached. The endpoint is the volume of titrant that gives the largest difference in mV reading. Once this has been reached, proceed with a couple more additions of titrant to take the reaction past it to confirm the endpoint.
8. Perform the calculations presented in the template, Figure A.2 of this SOP. Calculate the actual sulfide concentration using:

$$\text{Titrated sulfide conc. (M)} = \frac{\text{Vol.}_{Pb(ClO_4)_2\cdot 3H_2O} \times \text{Conc.}_{Pb(ClO_4)_2\cdot 3H_2O}}{\text{Vol.}_{Na_2S\cdot 9H_2O}}$$

Where:

$\text{Vol.}_{Pb(ClO_4)_2\cdot 3H_2O}$ is the volume of lead (II) perchlorate trihydrate required to reach the reaction endpoint.

$\text{Vol.}_{Na_2S\cdot 9H_2O}$ is the volume of sulfide stock used (25 mL).

$\text{Conc.}_{Pb(ClO_4)_2\cdot 3H_2O}$ is the concentration of the prepared titrant (lead (II) perchlorate trihydrate).

The calculated purity value (Figure A.2 of this SOP) can be used on the next analytical occasion to compensate for the weight of sodium sulfide nonahydrate required to obtain a stock solution concentration of 0.04 M (≡ 40 000 µM).

3.5 Preparation of calibration standards

From the calculated sulfide concentration, work out the volume required to give a top calibration standard containing 10 000 µM using the following equation:

$$\text{Vol. required (mL)} = \frac{10\,000\ \mu\text{M}}{\text{Titrated sulfide conc. (M)}} \times \frac{\text{Flask vol. (mL)}}{1\times10^{6}}$$

Use an appropriate displacement pipette to dispense, into a 10 mL amber volumetric flask, the required volume of stock sulfide solution required to give the 10 000 µM standard. Make to volume using degassed deionised water, stopper the flask and gently invert 4 times to mix. Serially dilute the 10 000 µM standard with further degassed deionised water using the following scheme to obtain a range of calibration standards for calibration of the ISE prior to sample analysis.

Standard	Standard used	Volume used (mL)	Final vol. (mL)	Nominal sulfide conc. (µM)
Stock	n/a	n/a	n/a	40 000
A	Stock	x	20	10 000
B	A	10	20	5 000
C	B	4	20	1 000
D	C	10	20	500
E	D	4	20	100

x = from volume required calculated above.

3.6 Calibration of ISE

A representative template for recording ISE calibration is presented in Figure A.3 of this SOP.

1. Ensure the AP125 meter is cleared of previous calibration settings:
    1.1. Press the [mode] button to enter the [ion] screen
    1.2. Press [setup/meas] until the [Clear ion - std] page is displayed then press [std/enter] to delete the previous calibration.
2. Prepare an ice bath using either a gel pack or ice. This will be used to cool samples down if they exceed the calibration temperature during sample analysis.
3. Prepare a fresh 25 mL volume of SAOB solution, or prepare a bulk solution by scaling up the weights and volumes (Section 3.2.1).
4. Use a displacement pipette and transfer 5 mL aliquots of standard (A to E) into separate LSC vials.

5. Add 5 mL of SAOB to the lowest standard (100 µM) to give a 1:1 ratio and swirl gently to mix.
6. Calibration is performed from lowest to highest concentrations.
7. Remove the ISE with attached ATC probe from the storage solution, rinse with deionised water and blot dry.
8. Insert the ISE and ATC probe into the standard and select the mV display by pressing the [mode] button.
9. Gently swirl the sample periodically (warm it gently with your hand if required) and observe the mV reading and temperature.

    9.1 Once the temperature is between 20 to 25°C (cool the sample using the ice bath in Step 2 if the temperature exceeds the upper limit) and the mV reading has stabilised, record the mV reading on the calibration sheet (Figure A.3 of this SOP) then proceed to the [ion] screen by pressing [mode].

    9.2 Press [std/enter] then [setup/meas] repeatedly until [10] is displayed on the bottom row of the display (this is equivalent to the 100 µM standard).

    9.3 Press [std/enter] which will accept that standard.

    9.4 Record the temperature that's displayed. This will be the temperature at which the other standards will be calibrated.

    9.5 Rinse the ISE and ATC probe with deionised water and blot dry.

    9.6 Repeat Steps 8 to 9.5 for the 500 µM standard, [50] on the meter display. After calibration using this standard, press [setup/meas] 2 times, this will display the slope value for the 100 and 500 µM standards – press [enter] to accept this value. Record this value on the calibration sheet (Figure A.3 of this SOP). Rinse the ISE and ATC probe with deionised water.

    9.7 Repeat Steps 8 to 9.5 for the 1 000 µM standard, [100] on the display. Pressing [setup/meas] 2 times after this standard will give the slope value for the 500 and 1 000 standards – press [enter] to accept this value. Record this value on the calibration sheet (Figure A.3 of this SOP). Rinse the ISE and ATC probe with deionised water.

    9.8 Repeat Steps 8 to 9.5 for the 5 000 µM standard, [500] on the display. Pressing [setup/meas] 2 times after this standard will give the slope value for the 1 000 and 5 000 standards – press [enter] to accept this value. Record this value on the calibration sheet (Figure A.3 of this SOP). Rinse the ISE and ATC probe with deionised water.

    9.9 Repeat Steps 8 to 9.5 for the 10 000 µM standard, [1 000] on the display. Pressing [setup/meas] 2 times after this standard will give the slope value for the 5 000 and 1 0000 standards – press [enter] to accept this value. Record this value on the calibration sheet (Figure A.3 of this SOP). Rinse the ISE and ATC probe with deionised water and place back into the storage solution.

    9.10 Four slope values are produced from the calibration, 100 to 500 µM, 500 to 1 000 µM, 1 000 to 5 000 µM and 5 000 to 10 000 µM. The acceptance range for the obtained slopes is -25 to -30 mV when the standards are within 20 to 25°C (Thermo Scientific, 2009). If any slope is outside this range then the ISE calibration must be repeated.

3.7 Analysis of samples

Once the ISE has been calibrated, analysis of collected sediment samples will be performed at the same temperature that the calibration was performed at. If samples are analysed immediately after collection (BC), then storage is not an issue. However, if samples are collected and stored for up to 72 h after collection (NB and NS), they should remain in refrigerated storage (2 to 5°C) and removed for analysis on a one-by-one basis so as to not have them sitting at ambient temperature for a prolonged period of time which could promote degradation of sulfide.

1 Prepare a fresh 25 mL (or larger) solution of L-ascorbic acid in SAOB which will be sufficient for 5 samples or prepare a bulk solution by scaling up the weight and volume of L-ascorbic acid and SAOB (Section 3.2.4).

2 Pipette 5 mL of L-ascorbic acid in SAOB into a LSC vial – adding the buffer first seems to prevent/limit adhesion of the sediment to the vial wall when it's dispensed.

3 Remove a sediment sample from storage and record the sample number on the analysis sheet (Figure A.4 of this SOP).

4 Dispense the 5 mL sample into the vial for a 1:1 ratio and stir using a glass rod for ca. 5 sec to obtain a homogeneous sample.

Note: If the sediment sample volume is not 5 mL, the volume of SAOB should be adjusted so that it corresponds to the sediment volume being analysed, i.e, a 1:1 ratio is maintained. For example, 3 mL of sediment requires 3 mL of SAOB.

5 Remove the ISE from the storage solution, rinse with deionised water and blot dry.

6 Insert the ISE and ATC probe midway into the sample and start the timer (set to 2 min).

Note: for muddy sediments, it is hard to see if any particles have settled after stirring hence the placement of the ISE midway through the sample. However for sandy sediments, the ISE sensing membrane must stay in the aqueous phase and not come into contact with the settled particles since it has been observed that sulfide readings increase when this occurs.

7 Slowly bring the temperature of the sample up to the calibration temperature using the heat from your hand whilst monitoring the mV and ion values.

8 If the sample temperature exceeds the calibration temperature during analysis, immerse the vial briefly in the ice bath to bring it slightly below that of the calibration temperature then gently raise it back up using the warmth of your hand.

- If a stable reading is obtained within the 2 min window and the sample is at the calibration temperature, record the displayed concentration on the ion screen of the meter followed by the mV reading onto the analysis sheet.
- If however a stable reading is not possible within 2 min (e.g. due to the mV reading continuously dropping), but the sample is at the calibration temperature then record the determined sample concentration and mV reading and flag the sample appropriately.
- If the calibration temperature cannot be reached within 2 min, record the concentration and mV readings, and flag the result to report to regulators that the sample took greater than 2 minutes to stabilise and to reach the calibration temperature.

9 Remove the ISE and ATC probe from the sample, rinse both with deionised water then place the ISE into the storage solution.

10 Repeat Steps 2 to 9 for the remainder of the samples.

11 Multiply all concentrations by 10x to obtain the correct sulfide concentrations.

12 If all samples are analysed with 2 h of the ISE having been calibrated then the analysis can be ended. However, if analysis is expected to extend past 2 h, then the performance of the ISE must be checked using fresh solution of the lowest calibration standard (100 μM) (Thermo Scientific, 2009). If the change is >4% then the ISE will have to be recalibrated otherwise sample analysis can continue.

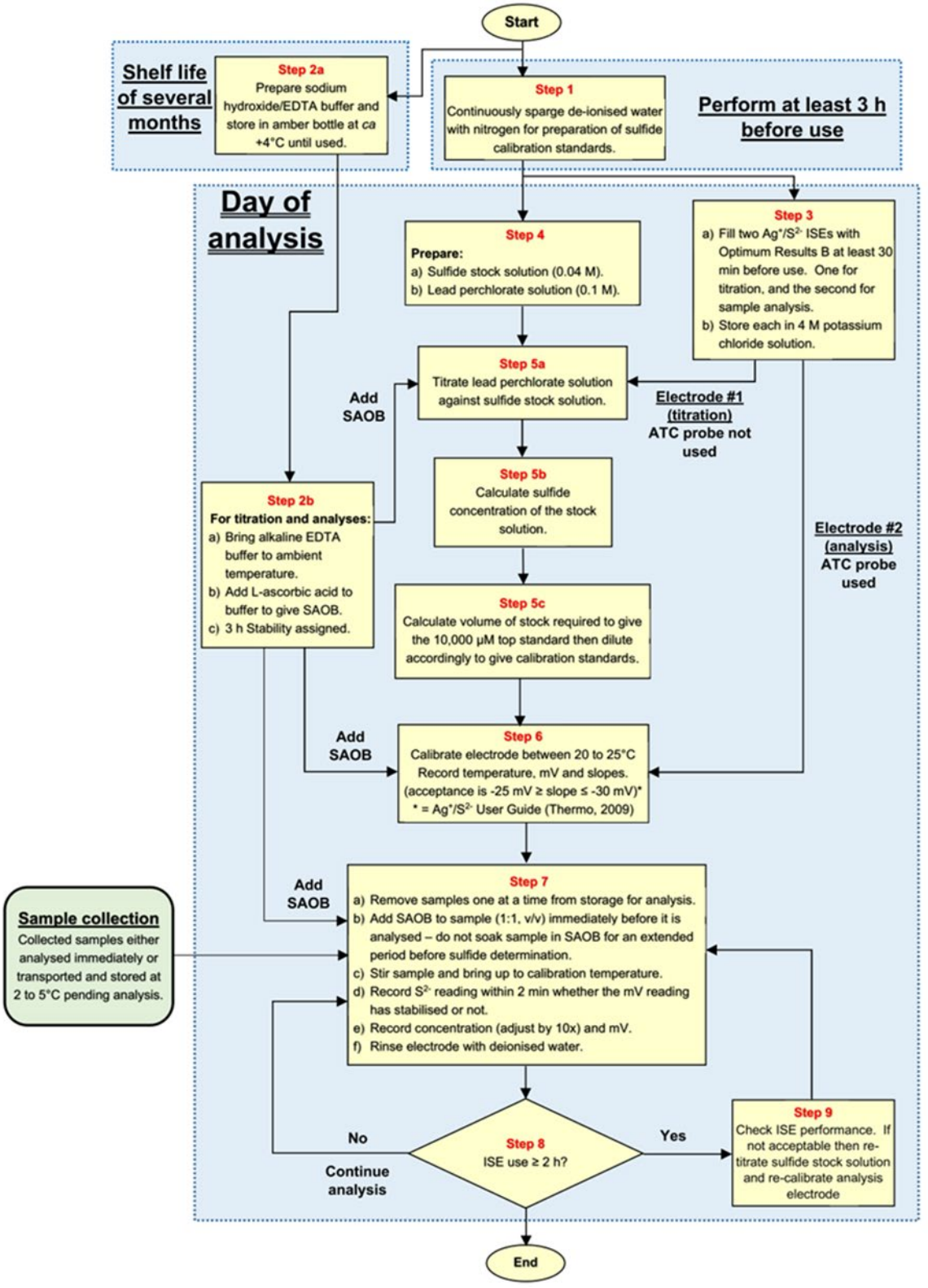

Figure A.1. Flowchart depicting analysis of sediment samples for total free sulfide.

Stock sulfide solution titration sheet.

(0.04 M = 0.96072 g into 100 mL or equivalents)

Sodium sulfide nonahydrate (g) :
(MW = 240.18)

(0.1M = 1.15038 g into 25 mL or equivalents)

Lead (II) perchlorate trihydrate (g) :
(MW = 460.15)

Titration					
Volume of lead perchlorate (mL)	Cumulative vol. (mL)	mV	Volume of lead perchlorate (mL)	Cumulative vol. (mL)	mV
0	0		0.1	10.6	
2	2		0.1	10.7	
2	4		0.1	10.8	
2	6		0.1	10.9	
2	8		0.1	11.0	
1	9		0.1	11.1	
0.5	9.5		0.1	11.2	
0.1	9.6		0.1	11.3	
0.1	9.7		0.1	11.4	
0.1	9.8		0.1	11.5	
0.1	9.9		0.1	11.6	
0.1	10.0		0.1	11.7	
0.1	10.1		0.1	11.8	
0.1	10.2		0.1	11.9	
0.1	10.3		0.1	12.0	
0.1	10.4		0.1	12.1	
0.1	10.5		0.1	12.2	

Stock concentration by weight:

$$\text{Conc. (M)} = \frac{\text{Wt.}_{Na_2S\cdot 9H_2O}}{240.18} \times \frac{1000}{\text{Flask vol. (mL)}}$$

Lead perchlorate concentration:

$$\text{Conc. (M)} = \frac{\text{Wt.}_{Pb(ClO_4)_2\cdot 3H_2O}}{460.15} \times \frac{1000}{\text{Flask vol. (mL)}}$$

Stock concentration by titration:

$$\text{Conc. (M)} = \frac{\text{Vol.}_{Pb(ClO_4)_2\cdot 3H_2O} \times \text{Conc.}_{Pb(ClO_4)_2\cdot 3H_2O}}{\text{Vol.}_{Na_2S\cdot 9H_2O}}$$

Calc. chemical purity:

$$\text{Purity (\%)} = \frac{\text{Conc. by titration}}{\text{Conc. by weight}} \times 100$$

Figure A.2. Representative template for titration of the prepared stock sulfide solution.

	ISE calibration			
Calibration temperature (°C)	Sulfide conc. (µM)	Calibrated on meter as:	mV	Point to point slope* (mV)
	100	10		
	500	50		100 µM to 500 µM
	1000	100		500 µM to 1 000 µM
	5000	500		1 000 µM to 5 000 µM
	10000	1000		5 000 µM to 10 000 µM

* = Acceptance range is -25 mV to -30 mV

Figure A.3. Representative template for ISE calibration.

Determination of sulfide in sediment by ISE

Sampling site: _____

Sampling date: _____

Analysis date: _____ **Analyst name:** _____

Sample #	mV Reading	Sulfide conc. from meter (µM)	Corrected conc. (x10) (µM)

Figure A.4. Representative template for a sample analysis recording sheet.

APPENDIX 2. PROCEDURAL COMPARISONS BETWEEN RESEARCH AND REGULATORS FOR THE DETERMINATION OF SULFIDE IN SEDIMENT SAMPLES

Flowchart Process		Rationale for Process	Science			Regulators		
			Wildish et al. (1999)	Wildish et al. (2004)	Chang et al. (2014)	NB DELG SOP (2018)	NS DFA SOP (2021)	BC (AAR, Annex 8) (2018)
Step 1	Deoxygenation of water.	Prevents oxidation of sulfide during preparation of standards.	Grade of water used not mentioned. Deoxygenation of water not mentioned.	Deionised water used. Degassed with fine stream of nitrogen for 10 min.	Deionised water (18.2 MΩ·cm) used. Degassed continuously with nitrogen before (at least 3 h) and during use.	Deionised or distilled water used. Deoxygenation method not detailed in SOP.	Deionised or distilled water used. Deoxygenation method not detailed in SOP.	Deionised or distilled water used. Deoxygenation method not detailed in SOP.
Step 2	Preparation of sulfide anti-oxidant buffer (SAOB)	Once added to the sample: H_2S and HS^- form S^{2-} for detection by ISE. Retards oxidation of sulfide in samples during analysis. Acts as an ion strength adjuster	Either: Purchased – no other details. or: 20 g NaOH/17.9 g $Na_2EDTA{\cdot}2H_2O$ in 250 mL distilled water – no expiry info. Prior to use, add 8.75 g L-ascorbic acid to buffer. Shelf life of 3 h.	References Wildish et al. (1999) document for sulfide analysis.	Made in-house. 160 g NaOH/150 g EDTA in 2 L deionised water – possible stability for several months when stored at ca. +4°C (product info from Sigma-Aldrich). Prior to use, add 0.875 g L-ascorbic acid for	Made in-house. Refers to Wildish et al. (1999). If premade, SAOB must be stored in refrigerator. If made on day of analysis it must be cooled to room temperature before use.	Made in-house. Refers to Wildish et al. (1999) for preparation method. States that SAOB must be cooled to between 2 and 5 °C prior to use due to exothermic reaction	Made in-house. Same method as detailed for Wildish *et al* (1999). De-aerated deionised water used. Stored in dark at +4°C after addition of L-ascorbic acid. Mentions deionised water must be at similar temperature to

Flowchart Process		Rationale for Process	Science			Regulators		
			Wildish et al. (1999)	Wildish et al. (2004)	Chang et al. (2014)	NB DELG SOP (2018)	NS DFA SOP (2021)	BC (AAR, Annex 8) (2018)
		buffer (ISAB) to increase ionic strength of the sample so that ion concentration can be measured.			every 25 mL buffer. At least 3 h shelf life.	SAOB + L-ascorbic acid combined before analysis. Stable for 3 h or make fresh batch if colour changes.	when prepared. SOAB + L-ascorbic acid combined prior to calibration. Shelf life of 3 h. Or prepare fresh solution when colour changes.	the sediment being analysed. 3 h shelf life for SAOB. 7 Day shelf life for alkaline EDTA buffer component of SAOB (i.e. minus L-AA)
Step 3	Addition of electrolyte and conditioning of ion selective electrode (ISE).	Filling solution acts as a voltage potential conductor between the sensing membrane and reference element of the ISE.	Orion Ag+/S2- half-cell electrode model 9416 with Orion reference electrode 90-01. Orion 90-00-01 filling solution used. Filled 24 h before use. ISE storage solution not mentioned.	Orion #9616 (Ag+/S2-) combination electrode. Either Optimum Results A or B filling solution used. Period between filling and use not stated.	Orion Ag+/S2- Ionplus ® Sure-Flow ® Solid state combination ISE (9616BNWP). Orion Optimum Results B filling solution used. Filled from 30 min to 24 h before use. Note: manufacturer does not	Orion Ag+/S2- Ionplus ® Sure-Flow ® Solid state combination ISE (9616BNWP). Orion Optimum Results B filling solution used. Filled at least 24 h prior to use.	Orion Ag+/S2- Ionplus ® Sure-Flow ® Solid state combination ISE (9616BNWP) . Orion Optimum Results B filling solution used.	No specific details about make and model of ISE used. Filling solution used dependant on the type of ISE used (e.g. if Orion 9616BNWP then use Orion Optimum Results A). Filled at least 30 min before use.

Flowchart Process		Rationale for Process	Science			Regulators		
			Wildish et al. (1999)	Wildish et al. (2004)	Chang et al. (2014)	NB DELG SOP (2018)	NS DFA SOP (2021)	BC (AAR, Annex 8) (2018)
				ISE storage solution not mentioned.	mention stabilisation period after addition of filling solution. ISE stored in saturated KCl solution (4 M).	ISE storage solution not mentioned.	Filled at least 24 h before use. ISE storage solution not mentioned.	ISE storage solution not mentioned.
Step 4	Preparation of sulfide stock solution	Used to prepare calibration standards.	Either: Purchased 3% Na2S·9H2O solution. or: Prepared 0.01 M solution by weighing 0.2402 g Na2S·9H2O into 100 mL volumetric flask and making to volume with distilled water. Shelf life of 48 h when stored in the dark.	No details given. Refers to Wildish et al. (1999).	Prepared in-house. 0.04 M Na2S·9H2O stock solution - weight of required chemical is purity corrected. Can be stored at ambient temperature and used on day of analysis if re-titrated.	Prepared in-house. Refers to Wildish et al. (1999) 0.01 M sulfide stock standard prepared. Stored under nitrogen in the dark until used - no shelf life detailed.	No details given. Refers to Wildish et al. (1999).	0.01 M Na2S·9H2O. Pre-weighed (0.2402 g) in N2 or He filled vials and made to volume (100 mL) with deaerated deionised water. Shelf life of 48 h (cool, dark, air excluded). Amount weighed not purity corrected.

Flowchart Process		Rationale for Process	Science			Regulators		
			Wildish et al. (1999)	Wildish et al. (2004)	Chang et al. (2014)	NB DELG SOP (2018)	NS DFA SOP (2021)	BC (AAR, Annex 8) (2018)
	Preparation of titrant	Titrant for titrating prepared sulfide stock.	Not prepared.	Not prepared.	0.1 M lead (II) perchlorate trihydrate.	Not mentioned.	Not mentioned.	Not mentioned.
Step 5a	Titration of sulfide stock solution.	To determine the sulfide concentration of the prepared stock solution.	Not performed.	Sodium sulfide nonahydrate (0.03 M) standard titrated against purchased lead perchlorate hexahydrate standard (0.1 M, Orion 948206).	Sodium sulfide nonahydrate stock solution (0.04 M) titrated against lead (II) perchlorate (0.1 M).	Not mentioned.	Not mentioned.	Not mentioned.
Step 5b	Calculation of stock concentration.	Sulfide conc. determined to enable preparation of calibration standards	Standards prepared in distilled water. Three calibration standards 100, 1 000 and 10 000 µM. No shelf life information.	No mention of water used, but assume distilled since 1999 followed. 0.1 M standard (prepared	Standards prepared in de-aerated deionised (18.2 MΩ·cm) water. Five calibration standards 100, 500, 1 000, 5 000	Standards prepared using de-aerated water (distilled or deionised). Five calibration standards 100, 500, 1000,	Standards prepared in de-aerated water (distilled or deionised). Five calibration standards 100, 500,	Standards prepared in de-aerated deionised water. Three calibration standards 100, 1 000 and 10 000 µM.

Flowchart Process		Rationale for Process	Science			Regulators		
			Wildish et al. (1999)	Wildish et al. (2004)	Chang et al. (2014)	NB DELG SOP (2018)	NS DFA SOP (2021)	BC (AAR, Annex 8) (2018)
				stock) for a concentration range of 10 000, 1 000 and 100 µM (3 calibration standards). Calibrate 50 to 100 µM for samples <100 µM. No shelf life information.	and 10 000 µM. Used immediately after preparation.	5000 and 10 000 µM. No shelf life information.	1 000, 5 000 and 10 000 µM. No shelf life information.	10 µM prepared if required. Shelf life of 3 h for 100 and 1 000 µM standards. Dilutions, 10 mL + 90 mL degassed water.
	Preparation of calibration solutions.	Prepared solutions used to calibrate the electrode to ensure accurate quantification of sulfide in samples.	-	-	-	-	-	
Step 6	ISE calibration.	To ensure that the electrode performance is within the	Accumet AP25 pH/ORP/ion meter.	No details but paper refers to Wildish et al. (1999).	Accumet AP125 pH/ion/ORP meter. Accumet ATC (13-620-19)	Accumet AP63 or AP125 pH/ion/ORP meter.	Accumet 63 or AP125 pH/ion/ORP meter.	Type of meter used not documented. No mention of temperature

Flowchart Process		Rationale for Process	Science			Regulators		
			Wildish et al. (1999)	Wildish et al. (2004)	Chang et al. (2014)	NB DELG SOP (2018)	NS DFA SOP (2021)	BC (AAR, Annex 8) (2018)
		manufacturer's specifications for accurate quantification of sulfide.	No temperature compensation probe mentioned. No mention of temperature range for calibration. 100 and 1 000 µM calibration standards used. STD:SAOB (1:1, v/v). No mention of rechecking calibration.		temperature compensation probe. STD:SAOB (1:1, v/v). Calibration should be checked after 2 h (manufacturer recommendation). Swirl, don't shake samples. Calibrate between 20 to 25°C. Record mV and slopes. Calculate slopes for 10-fold change in calibration standards (500 and 5000 µM plus 1000 and 10000 µM). Acceptance is -25 to -30 mV).	Accumet ATC (13-620-19) temperature compensation probe. STD:SAOB (1:1, v/v). Used for 3 h before re-calibration. Swirl, don't shake samples. Calibrate between 20 to 25 °C. Record mV for each standard and slope for 10,000 µM standard (acceptance -27 to -33 mV). Calculate 10-fold slope change (acceptance -25 to -30 mV).	Accumet ATC (13-620-19) temperature compensation probe. STD:SAOB (1:1, v/v). Calibration stable for 3 h. Swirl or stir to mix sample. Calibrate between 20 to 25°C. Record both µM and mV readings once the target temperature is reached for each standard. Record the displayed slope value provided after	compensation probe. No mention of temperature range for calibration. STD:SAOB (1:1, v/v). Calibrated every 3 h and at new farm site. Slope acceptance -27 to -33 mV.

Flowchart Process		Rationale for Process	Science			Regulators		
			Wildish et al. (1999)	Wildish et al. (2004)	Chang et al. (2014)	NB DELG SOP (2018)	NS DFA SOP (2021)	BC (AAR, Annex 8) (2018)
						Multiply sulfide concentration1 0x since AP63 or AP125 meter used.	500 µM (must be between -27 to -33) Displayed µM need to be multiplied by 10 – depends on meter used. Calculate 10-fold mV change to check ISE operation (-25 to -30 mV acceptance). Slope of 10,000 µM standard within -27 to -	

Flowchart Process		Rationale for Process	Science			Regulators		
			Wildish et al. (1999)	Wildish et al. (2004)	Chang et al. (2014)	NB DELG SOP (2018)	NS DFA SOP (2021)	BC (AAR, Annex 8) (2018)
							33 mV acceptance.	
Step 7	Sample analysis.	To accurately determine the concentration of sulfide in collected samples for regulatory monitoring or scientific data collection.	Syringe samples (stored in dark on ice) analysed ≤ 3 h after collection. Or store whole core on ice if > 3 h storage required. Add 5 mL SAOB to 5 mL sediment. Shake sample. Place ISE in sample and mix. No mention of reading sample at the calibration temperature. About 1 min to stabilise. No mention of multiplying results x10 to get adjusted readings.	Inter-laboratory comparison of analytical method, no detailed procedures for actual sample analysis but references Wildish et al. (1999).	Samples in syringes (stored in cooler with ice packs) analysed on day of collection (≤ 6 h). Add 5 mL sample to 5 mL SAOB. Stir with glass rod to mix sample. Insert ISE into sample (ca. 1/3 height of total sample) and swirl sample. Bring sample to calibration temperature. Take sulfide reading within 2 min (timer used) of ISE insertion into sample.	Samples in syringes stored at 2 – 5°C in the dark and analysis completed within 72 h of collection. 5mL Sediment volume analysed. Add SAOB + L-ascorbic acid to sediment in 1:1 (v/v) ratio. Swirl or stir samples, don't shake. No mention of electrode insertion into sample. Bring samples to calibration temperature.	Samples in syringes stored at 2 – 5°C in the dark and analysis completed within 72 h of collection. 3 mL Sediment volume analysed. Add SAOB + L-ascorbic acid to sediment in 1:1 (v/v) ratio. Swirl or stir samples, don't shake. No mention of ISE insertion into sample.	No storage - analyse sediment samples within 5 min of collection. SAOB added to sediment in 1:1 (v/v) ratio - 10 mL SAOB into graduated container then add sediment to 20 mL mark. Stir the sample. Insert and the stir sample with the electrode. Ensure the electrode tip is covered by the sample slurry. No mention of taking sample reading at the calibration temperature.

Flowchart Process		Rationale for Process	Science			Regulators		
			Wildish et al. (1999)	Wildish et al. (2004)	Chang et al. (2014)	NB DELG SOP (2018)	NS DFA SOP (2021)	BC (AAR, Annex 8) (2018)
			Rinse electrode with distilled water and blot dry.		Record both mV and ion concentration (x10 for adjusted conc.) Rinse electrode between samples with deionised water and dry.	Sulfide readings taken when stabilised (within 2 min). Record both mV and ion concentration (x10 for adjusted conc.). Rinse electrode with distilled water between measurements and blotted dry.	Bring sample to calibration temperature. Take reading within 2 minutes whether or not reading has stabilised or up to analysis temperature. Record both mV and ion concentration (x10 for adjusted conc.). Rinse electrode between samples with distilled water and blot dry.	Take reading when stabilised (1 to 4 min). No mention of recording mV and concentration or adjusting the concentration x10. Wipe ISE clean then insert into next sample. Mentions typical electrode stabilisation within 1-4 min but then also mentions if electrode doesn't stabilise within 1 to 2 min, check ISE with sulfide standard. If >20% deviation then recalibrate.
Steps 8 & 9	Calibration check. (Analysis time ≥ 2 h?)	Manufacturer gives 2 h limit for ISE use before	No mention of analysis time limit before ISE check/recalibratio	No details given but references	Check and recalibrate ISE according to manufacturer's	Use for a maximum of 3 h (first to last sample	States that calibration is stable for a	No mention of analysis time limit before ISE

Flowchart Process		Rationale for Process	Science			Regulators		
			Wildish et al. (1999)	Wildish et al. (2004)	Chang et al. (2014)	NB DELG SOP (2018)	NS DFA SOP (2021)	BC (AAR, Annex 8) (2018)
		rechecking performance. Recalibrate if lowest sulfide STD has changed by 4%.	n. However it is stated that the meter should be recalibrated before each batch of samples are run.	Wildish et al. (1999).	instructions if required	measurement) then recalibrate.	maximum of 3 h.	check/recalibration. ISE to be calibrated before the start of each sampling event. Recalibrated a minimum of every 3 h during an analytical session. From a performance check, if deviation of dilute S2- standard > expected value, then recalibrate the ISE. QC check performed every 20 samples or once per batch (if samples < 20).