

Optimization of Dissolved Fe(III) Determination in Coastal Water by Using Electrochemistry Approach and 2, 3-Dihydroxynaphthalene as the Binding Ligand

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An optimization procedure was developed in this study using Competitive Ligand Exchange-Adsorptive Cathodic Stripping Voltammetry (CLE-AdCSV) to determine the concentration of dissolved Fe(III) in coastal water collected at Pulau Redang, Terengganu. The method was optimized for the UV-irradiation period for dissolved organic digestion, while the pH of the samples prior to UV-irradiation was determined. Additionally, the types and concentrations of the artificial ligands, followed by the sample equilibration period for sample measurements, were confirmed and performed. A small standard deviation of 0.002 was obtained in this experiment, indicating that the data obtained were precise when the optimized method was applied. Those optimized method was applied to the samples at Station 3, and the data obtained were compared with the previous study. Our present data showed that the concentration of dissolved Fe(III) was low, approximately 6 - 82 times lower than previously reported data. As our study area was affected by the Northeast monsoon (NEM), the phenomenon of strong turbulence during the post-monsoon event could have caused the resuspension of bottom sediment to the upper layer of seawater. Hence, the total suspended solids (TSS) level could have increased in the water column, suggesting that Fe(III) was mainly attached to the particulate phase rather than during the dissolved phase.

Keywords: dissolved iron, coastal water, voltammetry, speciation, organic ligands

1. INTRODUCTION

Iron (Fe) is a key element in marine ecosystems and plays a vital role in the biochemistry and physiology of phytoplankton [1]–[3]. In natural water, dissolved Fe (dFe) exists in two oxidation states, which are the Fe(II) and Fe(III) forms [4]. These forms are involved in the formation of soluble organic, inorganic complexes, colloids, and particulate phases [5]. The availability of Fe in seawater is controlled by the solubility of Fe(III) [6], [7]. However, the Fe(III) concentration is low, ranging from 10^{-6} M in

river water, 10^{-6} - 10^{-9} M in coastal water, and 10^{-11} M in ocean water [8], [9] because of the tendency to form an oxyhydroxide precipitate [5] under natural pH and oxygenated conditions [10], [11]. By that, over 99% of the dFe(III) are found to form complexes with other organic ligands [12]–[16], in order to increase the Fe solubility in oxygenated water and to maintain the dFe in the euphotic zone [17], [18]. Subsequently, phytoplankton is able to uptake the soluble Fe for nutrients.

Several studies on metals found in marine systems have been published in Malaysia. However, most of these studies were conducted in biota and sediment settings. Thus, the applications were limited to dissolved and particulate metals (PM) [19] owing to their trace concentrations [20], [21], and the possible interference of the salt matrix during the analysis [22]. To counter these limitations, analytical methods such as spectrophotometry, atomic absorption spectrometry (AAS), inductively coupled plasma-mass spectrometry (ICP-MS), chemiluminescence, and fluorescence [9] have been developed.

In 2007, [21] provided baseline data for dissolved and particulate metals (Cadmium (Cd), Chromium (Cr), Manganese (Mn), and Lead (Pb)) at the southern coast of Terengganu using seawater concentrated on a Chelex-100 column (for the dissolved phase) and acid digestion (for the particulate phase), followed by metals' detection analysis using the ICP-MS. This method was applied by [23] to monitor the levels of dissolved and particulate Mn, Fe, and Copper (Cu) metals found in the surface water collected at Port Dickson, Negeri Sembilan over a year period (May 2014 to May 2015). In addition, this method was followed by [24] to determine the influence of Northeast monsoon on the distributions of dissolved Aluminium (Al), Cu, and Fe at Pulau Perhentian, Terengganu. However, this method requires complex and expensive analytical instruments, which limits their potential applications [9]. Moreover, preconcentration and separation techniques are not always possible as these approaches are time-consuming and prone to errors [25]. Furthermore, the data provided by [24] recorded a high standard deviation (SD), ranging from ± 0.71 to ± 21.87 $\mu\text{g/L}$ for dFe during October 2014. The findings suggested that the ICP-MS technique has a low precision in trace metals determination and might be due to the interference of seawater samples. The precision is defined by how similar the measurement is to another measurement and can be determined by the SD value. A high SD indicates low precision, whereas a low SD value indicates high precision [26].

To date, the electrochemical method has been found to be a very sensitive method for metal determination, with advantages such as simple equipment requirements, low cost, low detection limit, fast analysis speed, and easily automated detection [9]. According to [27], adsorptive cathodic stripping voltammetry (AdCSV) is a widely accepted technique used to measure the concentration of metal-binding ligands. [28] had applied this method to determine the distribution of dissolved and particulate Fe in the estuary system at Bagan Pasir, Perak, by using 2-(2-thiazolylazo)-p-cresol (TAC) as the competitive ligand. The saline water sample can be measured directly using this technique without pre-treatment. Moreover, in comparison to the SD, this technique recorded ± 0.02 to 0.67 mg/L of dFe [28], which is lower as compared to the ICP-MS technique [24].

Because our study focuses on coastal water, there is a need to perform a method optimization as the distributions (e.g., dissolved and particulate) of trace metals and their rates of reactivity may differ between the areas and are depending on environmental factors [23], such as salinity. For example, the salinity gradient was observed to change the nutrient limitation from phosphorus-limiting in freshwater to nitrogen-limiting in temperate coastal seas [29] due to the high content of sulfate in the sea salt. In

addition, a study conducted [30] at Pulau Perhentian, Terengganu, and Pulau Pangkor, Perak noted that more than 99.5% of dCu(II) bind to organic ligands with $\log K'_{CuL} > 12$, indicating the presence of strong organic ligands in coastal areas. Hence, the method developed by [28] might not be suitable for testing coastal seawater samples.

Pulau Redang, Terengganu is located on the east coast of Peninsular Malaysia and is therefore receiving the influence of Northeast monsoon (NEM) from November to Mac every year. The influence of weather tends to alter the physicochemical parameters (e.g., temperature, pH, salinity, DO, and nutrients) of seawater, thus affecting the trace metal speciation in a Malaysian coastal area [30]. According to [23], the distributions (e.g., dissolved and particulate) of trace metals and their reactivity rates differ between coastal areas and depend on environmental factors. This leads to the probability of optimization is capable of our study period (post-monsoon). Hence, this study aims to optimize the method conditions for determining the dFe(III) found in coastal seawater samples using the AdCSV method.

2. MATERIALS AND METHOD

2.1 Instrumentation and determination

The voltammetry apparatus consisted of a μ Autolab voltammeter with a three-electrode system (Metrohm Model VA797). The three electrodes included a) hanging mercury drop electrode (HMDE) as the working electrode, b) Ag/AgCl, saturated AgCl, 1M KCl as the reference electrode, and c) platinum wire as the counter/auxiliary electrode.

The samples were purged with nitrogen gas (purity 99.99%) for 300 seconds (s) to remove dissolved oxygen (DO) present inside the sample [37], followed by the deposition step. In this step, the Fe-AL complexes produced from the analysis were adsorbed onto the HMDE (the drop surface area of 0.45 mm^2) in -0.2 V for 60 s while stirring at 2000 rpm. An equilibration step was allowed to occur, and the scans produced during the ligand complexing determination steps were transferred to a Lenovo computer, and the peak height was evaluated using a software program (VA 797 Computrace). The details of voltammetric parameters, such as the deposition potential -0.2 V , deposition time 60s, pulse amplitude 0.05 V , pulse time 0.02 s, scan rate 90 mV/s , initial potential -0.2 V , final potential -0.8 V , were followed.

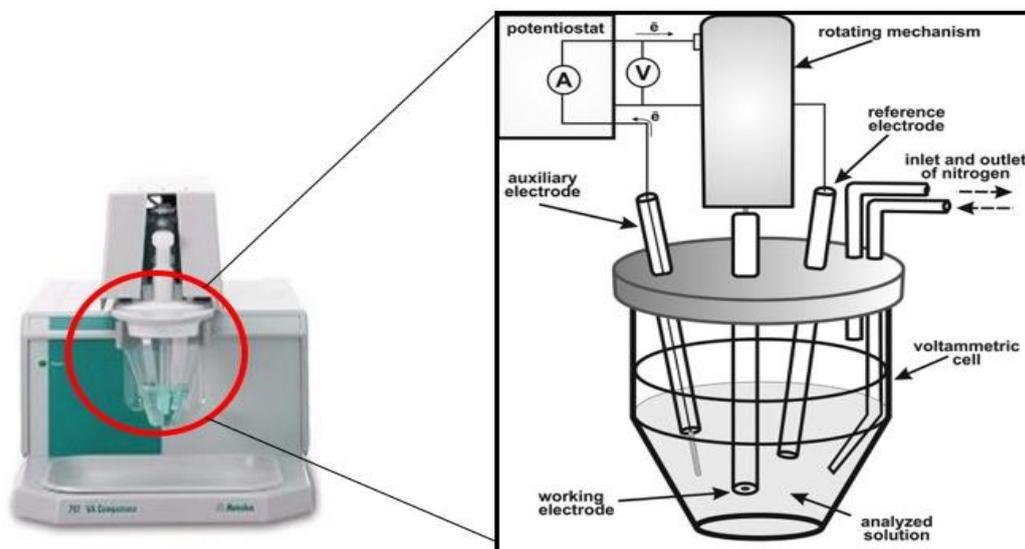


Figure 1. The figure of voltammetry with the three-electrode system (a) hanging mercury drop electrode (HMDE) as a working electrode, (b) Ag/AgCl, saturated AgCl, 1M KCl as the reference electrode, and (c) platinum wire as the auxiliary electrode

2.2 Samples and Reagents

This study aims to develop and optimizes the dFe(III) method using the electrochemistry approach. Therefore, the vertical seawater samples were first collected at Pulau Redang, Terengganu, in March 2019 (post-monsoon) by using a Van Dorm water sampler. Next, the samples were filtered through a cellulose nitrate filter membrane (pore size 0.20 μm , Whatman) as the substantial portion of dFe and Fe-binding ligands was present in that colloidal size [31]. The samples were kept in 250 mL low-density polyethylene (LDPE) bottles (Nalgene) and were immediately frozen to inhibit any biological activities that may alter the nature of the samples [32].

MilliQ-water (MQ, Millipore; $N\ 18.2\ \mu\Omega\ \text{cm}^{-1}$) was used for the reagents and sample preparation. The reagents needed for voltammetry analysis were the buffer solution to maintain the pH of the samples throughout the analysis and the artificial ligand (AL) solution used to produce the Fe complexes, and Fe standard solutions (10 ppm, 20 ppm, 30 ppm, and 50 ppm) used to calibrate the sample.

A 0.1 M borate buffer was prepared by dissolving boric acid (H_3BO_3 , MERCK) (final volume of 100 mL) in 0.3 M ammonia. Then, the pH of this mixture solution was adjusted to a pH of between 8.00 to 8.05 after the addition of 50 μL into 10 mL of seawater sample. A 0.01 M stock of 2-(2-thiazolylazo)-p-cresol (TAC, $\text{C}_{10}\text{H}_9\text{N}_3\text{OS}$, Sigma Aldrich) was prepared by dissolving TAC in methanol. The possible contaminating Fe in the mixture was cleaned by overnight equilibration with 20 μM of TAC and was filtered over a SepPak C18 column prior to the activation by 10 mL methanol (CH_3OH , MERCK), coupled with 10 mL 0.6 N hydrochloric acid (HCl, MERCK) and 20 mL mQ.

A 0.1 M stock buffer HEPES/bromate (Sigma Aldrich) (final volume of 50 mL) was prepared in ~ 0.05 M ammonia and 0.4 M potassium bromate, KBrO_3 (Sigma Aldrich) by using Milli-Q water. The pH of this mixture was adjusted to between 8.00 to 8.10 after the addition of 500 μL into 10 mL of

seawater sample. The possible contaminating Fe was removed by overnight equilibration with 100 μM manganese oxide (MnO_2 , MERCK) and was filtered by using a nylon syringe filter (pore size 0.20 μm , Whatman). All the pH adjustment for the buffer was made by using nitric acid (HNO_3 , MERCK) or ammonium hydroxide (NH_4OH , MERCK).

A 0.01 M TAC, salicylaldoxime (SA, $\text{C}_6\text{H}_4\text{CH}=\text{NOH}-2\text{-OH}$, Sigma Aldrich), and 2,3-dihydroxynaphthalene (DHN, $\text{C}_{10}\text{H}_8\text{O}_2$, Sigma Aldrich) (final volume of 30 mL) was dissolved in methanol. These ligands were to form a complex with Fe(III) ions that are present in the sample [33]. A series of Fe standards (between 10 ppm to 50 ppm) were diluted from a 1000 ppm standard stock (Sigma Aldrich) by using Milli-Q water. These standard solutions were prepared fresh weekly and kept refrigerated at 4°C.

2.4 Optimization analysis for determination of dFe(III)

Part 1: 10 mL of natural seawater sample (without modification) was poured into a polarography cell along with the addition of 50 μL of borate buffer (pH~8) and 40 μL of SA (final concentration 40 μM). Then, the sample was analyzed for dFe(III) as outline in 2.1. The step was repeated by using TAC (buffered by 50 μL of borate buffer) and DHN (buffered by 500 μL of HEPES/bromate), respectively. The CSV scan was produced at -0.40 V for SA and TAC, and at -0.60 V for DHN. The analytical performance of each AL in determining the dFe(III) was compared, and the best one is selected to be used in subsequent analysis. Next, the selected AL was further tested by increasing its concentration to 400 μM .

Part 2: UV irradiation step was performed on the natural seawater samples (30 ml) by using the UV digestion apparatus (Figure 2) for 15, 30, 60, 120, and 240 minutes in an acid-cleaned quartz tube to measure the optimum time in removing the organic complexes. The housing of the UV digester is made of heavy-duty PVC with a PTFE top with holes to hold the quartz sample tubes. The UV light is provided by a high-pressure mercury vapor lamp with a power output of 125 W, peaking at ~250 nm (Figure 2). The sample was prepared as mentioned in Part 1 by using the selected AL with its optimized concentration and was determined for dFe(III) as in 2.1. Then, the optimum UV period was applied to the next sample determination.

Part 3: The sample was further tested by adjusting the pH condition to pH ~2, and was UV irradiated for a period that was optimized in part 2. The pH of the sample was brought back to pH~8 by using 30% of NH_4OH (MERCK). During the determination, some samples were measured directly after the UV irradiation procedure, and some were leaving overnight after the addition of buffer and AL.

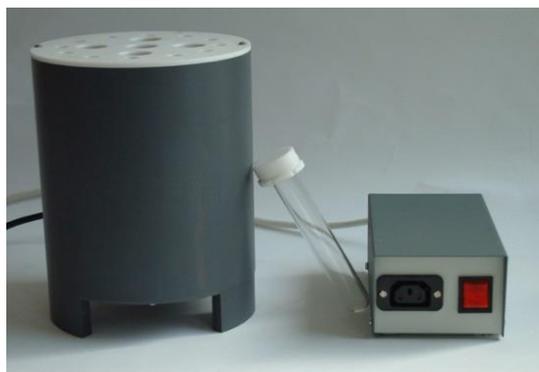


Figure 2. The figure of UV-digestion apparatus. The housing is made of heavy-duty PVC with a PTFE top with holes to hold quartz sample tubes. UV light is provided by a high-pressure mercury vapor lamp with a power output of 125 W, peaking at ~250 nm.

3. RESULTS AND DISCUSSION

Cathodic stripping voltammetry (CSV) is a technique used to determine trace and ultra-trace analytes from environmental, clinical, and industrial samples. Interestingly, this technique has an excellent sensitivity performance where it allows the determination of analytes in the highly diluted sample that is up to pM concentration. Moreover, the preconcentration step in CSV requires little or no reagent to be added, thus offering an advantage to minimize the risk of sample contamination. The basic instrumentation in CSV is potentiostat, three-electrode cells (i.e., working electrode, the reference electrode, and auxiliary electrode), and a computer for data collection. HMDE is the typical working electrode being used in CSV due to its reliability, where with the formation of a new drop, a new electrode surface is produced. Furthermore, this technique uses a small HMDE (i.e., a size of 0.45 mm^2) to minimize the health and environmental risk. [38].

3.1 Optimization of method for determination of $d\text{Fe(III)}$

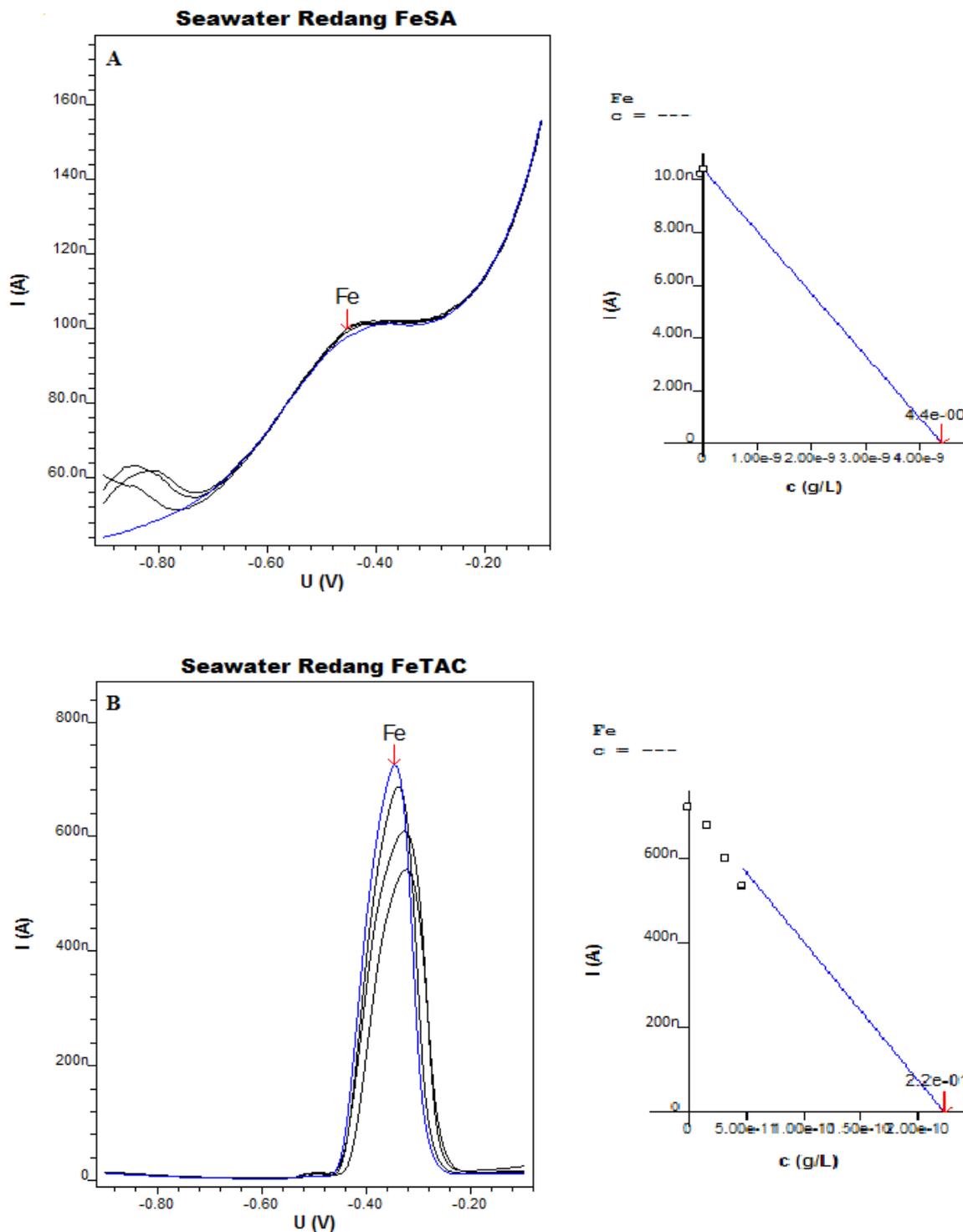
The method optimization for the determination of $d\text{Fe(III)}$ had carried out to improve the analytical response of CSV on the water off the Malaysian coast. By that, the seawater samples were sampled at Redang Island, Terengganu. The optimization procedure involved the choices of the best AL to be used for the rest of the experiment and its working concentration, followed by the period for the UV irradiation step and the sample's pH condition during UV irradiation. Lastly, the optimization procedure had done on time taken for the sample to reach equilibrium after UV irradiation. Hence, the data that was collected shown as in Figure 3-Figure 7:

3.1.1 Choice of the best artificial ligands

According to IUPAC, organic ligands are the molecules that can bind to the trace metals (in dissolved phase; $< 0.2 \text{ }\mu\text{m}$) to form stable complexes. The complexes had found to increase the Fe

solubility in oxygenated water and to maintain the dFe in the euphotic zone [17], [18]. Some examples of organic ligands are siderophores, humic substances (HS), exopolymeric substances, and transparent exopolymers [39].

For this optimization, the analytical response of CSV had evaluated on several AL named SA, TAC, and DHN to identify their analytical performance for the determination of dFe(III). The analysis had carried out under natural sample conditions with 40 μM of AL in each experiment. Hence, the titrations of the sample with the AL had illustrated in Figure 3.



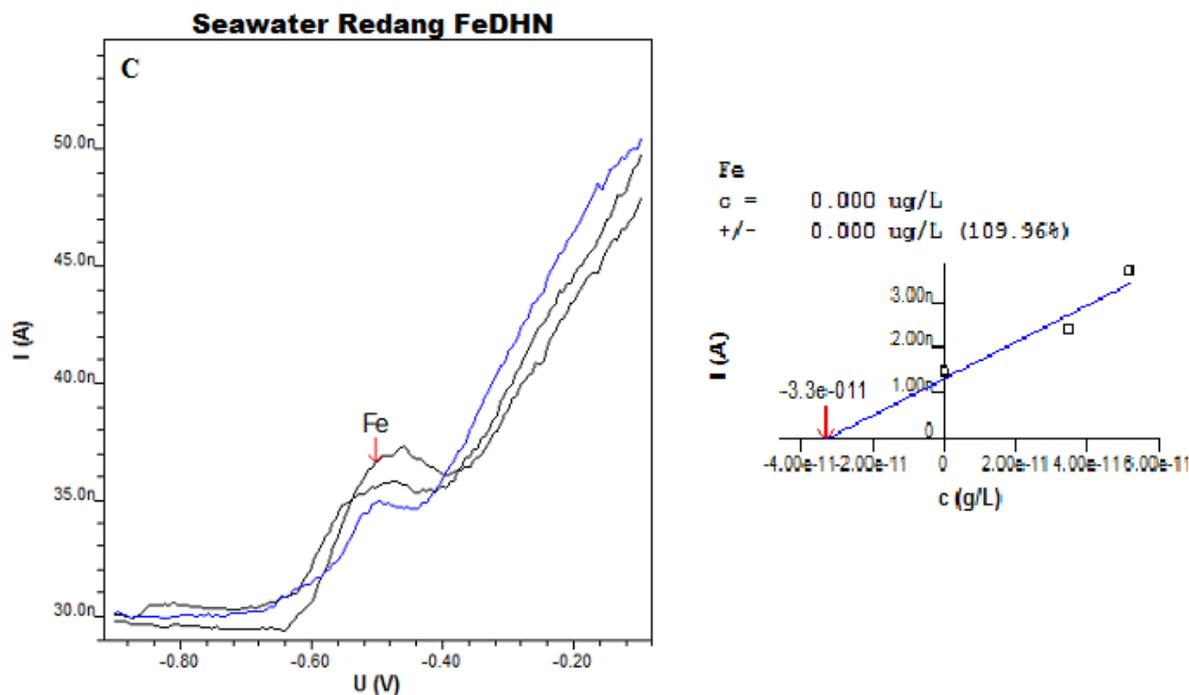


Figure 3. Voltammetric scans and calibration curves of dFe(III) obtained in seawater samples with different artificial ligands under the following conditions; (A) 0.5 mM of borate buffer, 40 μ M of SA, (B) 0.5 mM of borate buffer, 40 μ M of TAC and (C) 5 mM of HEPES/bromate buffer, 40 μ M of DHN. The sample was determined directly without modification, and the calibration curve was obtained by the standard addition method. The blue line has represented the scan of the sample, and the black line represents the scan of standard addition. The peak current produced at -0.40 V for SA and TAC and at -0.60 V for DHN.

Figure 3 shows the analytical response of CSV on dFe(III) after the voltammetry determination testing under different types of AL. There was no recorded data for dFe(III) due to a poor calibration curve obtained in all determination. In contrast to the calibration curve, only DHN gives an increased value in each addition of standard, while SA and TAC were the otherwise result.

In (A), the constant values of peak current for both sample and standard addition probably been explained by [33], which additional of SA as AL required a long adsorption time of 10 minutes and must achieve using the larger mercury drop electrode due to poor sensitivity. In this analysis, the deposition time applied was 60 s and the mercury size is 0.45 mm², indicating the inconvenient condition for determination with SA. Furthermore, a few numbers of complexes with slow dissociated rate are not fully outcompeted by SA in a short time [40]

Meanwhile, the addition of TAC in (B) seemed to suffer from the ligands' pre-peak effects, thus causing the limit of detection (LOD) issue [33]. However, the use of DHN in the analysis gave a better sensitivity for Fe detection, with recorded LOD of less than 10 pM and 60 s deposition time (10 times shorter than SA) [33].

Moreover, the presence of oxidants in the samples (such as peroxide, H₂O₂, and bromate, BrO₃⁻) could enhance the re-oxidation of Fe(II) formed during the stripping step, hence increasing the peak current [37]. In this analysis, the BrO₃⁻ solution was included in the preparation of the HEPES buffer

solution and was used along with the addition of DHN. Since the DHN shows better results than the TAC and SA, thus it was applied as the AL in subsequent dFe(III) determination testing for our samples. Hence, the determination of dFe(III) using TAC and SA as the AL was ignored.

3.1.2 Influence of concentration of DHN to the concentration of dFe(III)

The determination of dFe(III) using 40 μM of DHN in Figure 3(C) resulted in an unstable CSV signal, as the voltammogram produced was not smooth. The results much probably due to insufficient DHN to compete with natural ligands inside the samples. Thus, the concentration of DHN increased to tenfold to extend the strength of DHN over the natural ligands, and the results obtained was shown below:

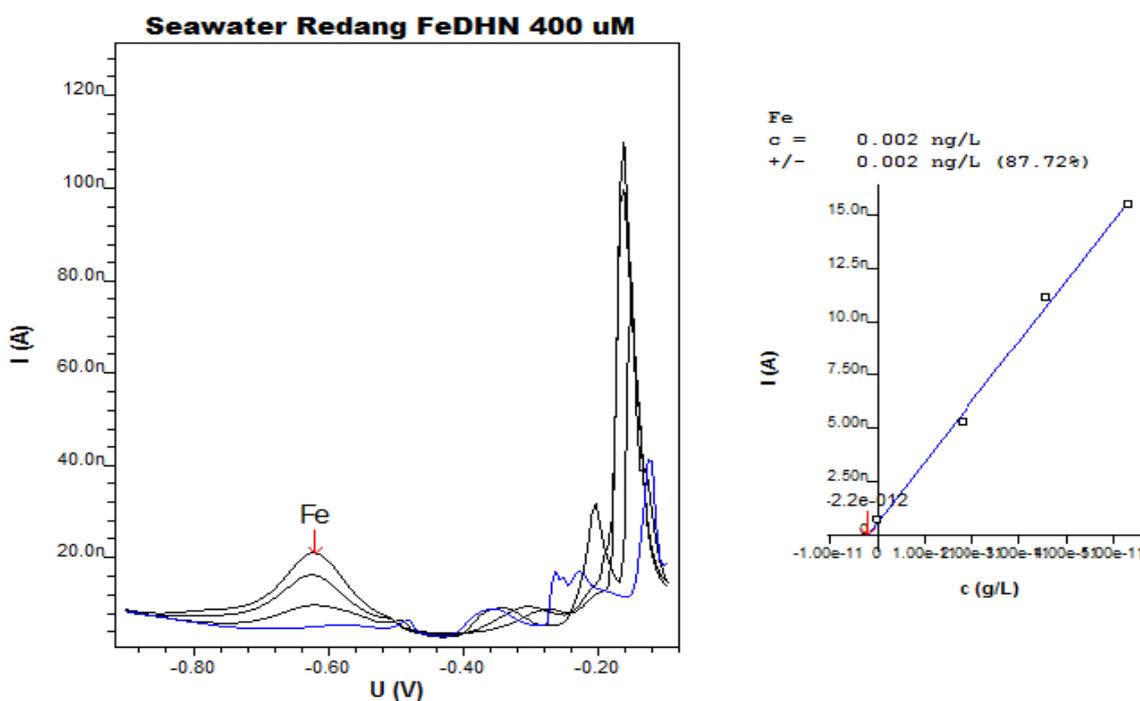


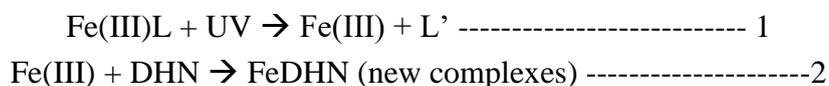
Figure 4. Voltammetric scan and calibration curve for dFe(III) obtained in seawater sample with 5 mM of HEPES/bromate buffer and 400 μM of DHN. The sample was determined directly without modification, and the calibration curve was obtained by the standard addition method. The blue line represented the scan of the sample, and the black line represents the scan of the standard addition. The peak current produced at -0.6 V

Figure 4 shows the peak current and calibration curve obtained in CSV after the determination of dFe(III) with high concentration of DHN. By comparing the result with Figure 3(C), a stable peak current was obtained when a high concentration of DHN was used (Figure 4), producing a smooth voltammogram in the determination. The previous study reported by [41] suggested that the overload titration at the concentration of a very high AL produces competition with the natural ligands and

subsequently increases the peak height that is linear with the added metals. Furthermore, the titration of the sample with a high level of DHN gives a better calibration for the sample, hence recorded 0.002 ng/L of dFe(III) (Figure 4). Thus the DHN concentration was set at 400 μM throughout the rest of the analysis for our coastal water samples.

3.1.3. Optimization of the UV irradiation period

Both analyses of samples in section 3.1.1 and section 3.1.2 were determined by using the natural seawater sample. However, under natural conditions, more than 99% of dFe(III) were complexed to organic ligands [42], and the presence of these complexes in the samples may reduce the analytical response in the voltammetry analysis [34]. UV-irradiation is the common techniques used to break down the dissolved organic matter (DOM) to prevent the formation of complexes with the trace metals in the water samples [43], gives the opportunity for AL (e.g., DHN) to form a new complex with the free Fe(III) during the determination. The photochemical reaction that occurs during the UV irradiation step was summarized as:



Where, L' represent the free organic ligands

In this analysis, the seawater sample was UV irradiated for 15, 30, 60, 120, and 240 minutes in a clean quartz tube under the natural seawater pH condition to determine the optimum time needed in breaking down the organic ligands in the samples for better determination. Then, the data collected were presented in Figure 5:

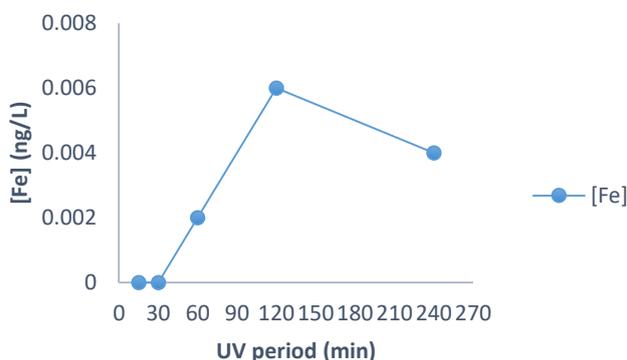


Figure 5. Influence of the UV irradiation period on the concentration of dFe(III). The line graph indicates the reading of dFe(III) on the samples that irradiated for 15, 30, 60, 120, and 240 minutes under the natural pH condition. The artificial ligands used was DHN at a concentration of 400 μM

Figure 5 shows the results obtained for the seawater samples treated with different UV irradiation periods. Based on the results, no dFe(III) was recorded in the samples that irradiated for 15 min and 30

min. However, it was recorded in the sample that irradiated for 60 min, 120 min, and 240 min, with the concentrations of 0.002 ± 0.003 ng/L, 0.006 ± 0.002 ng/L, and 0.004 ± 0.005 ng/L, respectively. This suggested that the concentration of dFe(III) was increased by the time when exposed to the UV light and was reach optimum in 120 min. The long period of 120 minutes is likely due to the presence of strong and highly concentrated organic substances in the study area (coastal water). As proven by [30], this statement showed that 99.5% of dCu(II) bind to the organic ligands in coastal areas located in Pulau Perhentian, Terengganu, and Pulau Pangkor, Perak. By comparing to the previous studies, the UV-irradiation period of 30 minutes was recorded for samples collected in coastal Dagujia River [9], 60 minutes for the samples collected in Mersey Estuary and Liverpool Bay [44], followed by 90 minutes for the samples collected in eastern North Atlantic Ocean [45]. The differences between the UV irradiation periods were probably due to the binding strengths of organic ligands in the samples. Hence, 120 minutes of UV irradiation was applied along with this analysis.

3.1.4. Influence of sample pH condition during UV-irradiation to the concentration of dFe(III)

In section 3.1.2, the UV irradiation step was applied on the seawater sample under its natural pH, and recorded the reading of 0.006 ± 0.002 ng/L for dFe(III) after 120 minutes of UV irradiation. By considering the suggestion by a previous study [9], [33], [35], which applies the acidify seawater sample during UV irradiation, hence an analysis was carried out to compare the efficiency of the UV system in breaking down the organic ligands in the sample under different pH condition (a) with natural seawater pH and (b) with acidified seawater to pH~2. Finally, the data collected was illustrated in Figure 6:

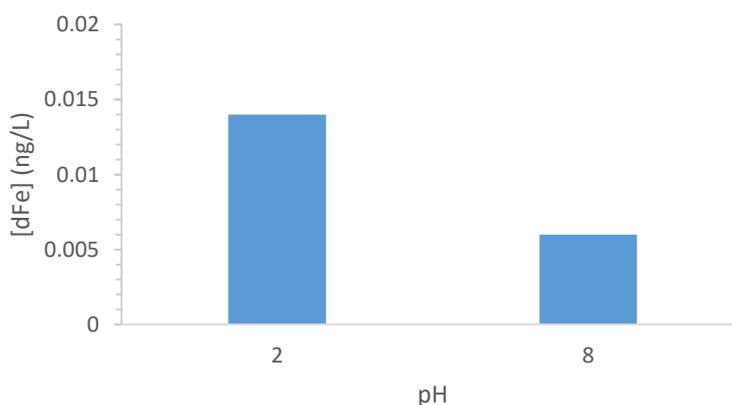


Figure 6. Effect of modifying the pH condition during UV irradiation step to the concentration of dFe(III). The bar graph represents the concentration of dFe(III) under different pH conditions as pH 2 indicates the acidic condition while pH 8 indicates the natural seawater condition. Samples were UV irradiated for 120 minutes and used DHN as the artificial ligand at 400 μ M.

Figure 6 shows the results of seawater samples tested with different pH conditions, such as natural seawater pH (pH 8) and acidify seawater pH (pH 2). Based on the results, the concentration of dFe(III) was higher at pH 2 than the concentration at pH 8, with the recorded concentrations of 0.014 ng/L ± 0.002 ng/L and 0.006 ng/L ± 0.002 ng/L, respectively. This shows that the results were about two

times higher when the sample was irradiated in acidic conditions, indicating the increase in efficiency of the UV system in breaking down the organic ligands in that sample. This is because the sample with acidic pH conditions could prevent the Fe precipitation and sample evaporation [46] and destroy all the Fe and organic matter complexes inside the sample, therefore releasing the unstable Fe complexes [9]. The new stable complex of Fe-DHN will be formed, hence increasing the peak current that produces in the determination. Therefore, all the sample was acidified to pH~2 prior to UV irradiation for the next optimization.

3.1.5. Influence of sample equilibration period to the concentration of $dFe(III)$

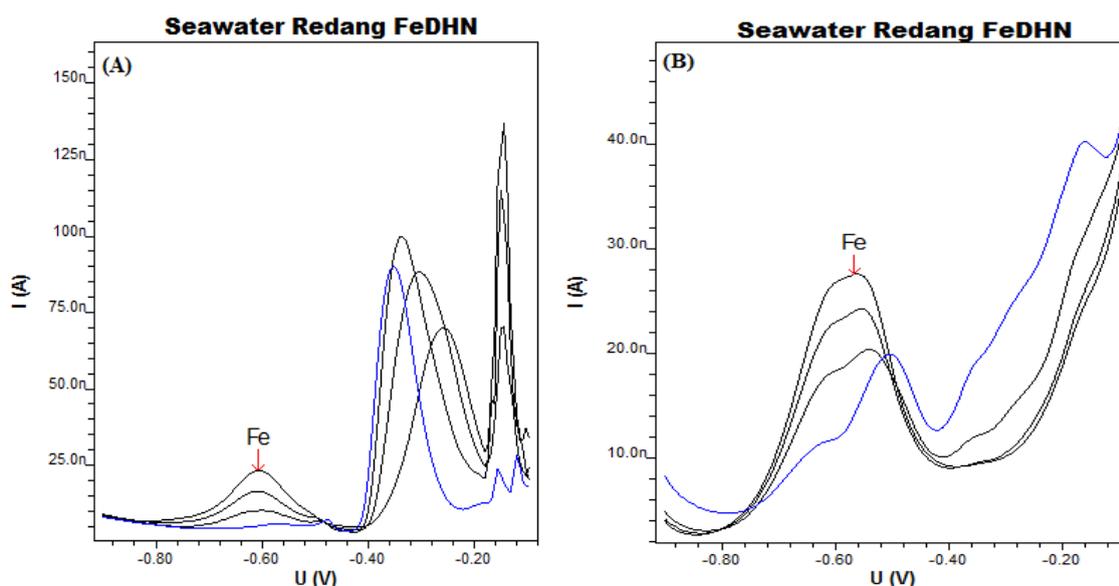


Figure 7. Voltammetry scans obtained from the seawater samples under two equilibration conditions; (A) sample was measure directly after UV irradiation, (B) sample was equilibrate overnight at pH 8 with 400 μ M of DHN. The blue line represented the scan of sample, and the black line represent the scan of standard addition.

Figure 7 shows the results of the seawater sample tested with two different equilibrium times, e.g. (A) the sample was measure directly (less equilibrium time) on the same day after the UV irradiation step, and (B) the sample was left overnight for equilibrium after the UV irradiation step, adjusting its pH to pH~8 followed by the addition of HEPES and DHN. The sample analyzed after overnight equilibrium was shown to have a peak that was shifted to the right (more positive) and was affected by the sample calibration, probably due to the pH change during the overnight reaction. The previous study by [47] found an anodic shift (shift to a more positive position) of the cathodic stripping peak for the cysteine-mercury complexes at a pH of lower than 8.5 (at pH 6.5 and pH 4.2). The previous finding could be used to describe our sample, as the stripping peak in (B) was produced at approximately -0.5 V, as compared to -0.6 V in the sample that was measured directly (Figure 7). Furthermore, [33] suggested that the use

of high DHN concentration could enhance the time taken for the AL to reach stability. Hence, the direct determination was the most suitable equilibrium method for our coastal water samples determination.

3.1.6. Precision analysis for method optimization

Table 1. Concentration, average, deviation, and relative deviation of dFe(III) after being tested three times

Sample	[dFe(III)], ng/L	Deviation, ng/L	Relative deviation, %	Standard Deviation
St.1 3m	0.024	0.002	4.55	0.002
	0.020	0.002		
	0.022	0.000		
Mean	0.022	0.001		

The optimized method was applied to the same sample several times in order to analyze the precision of the data. The data gained in this analysis were recorded in Table 1:

Based on Table 1, the concentrations of dFe(III) recorded were 0.024, 0.020, and 0.022 ng/L, respectively, with an average value of 0.022 ng/L recorded after the voltammetry determination test. By calculating the relative deviation (RD), our data were different from the mean value by 4.5%, with a standard deviation (SD) of 0.002. The low value of SD (as shown in Table 1) indicated that the high sample precision was obtained when this method was applied. Thus, this method was considered reliable to be used for the analysis of coastal seawater samples.

3.1.7 Application of optimization method on seawater samples

The optimization method was applied to our samples to verify their reliability. By that, the seawater samples from Station 3 were chosen for the analysis as the station is located near the island, hence may probably have a greater influence [24]. Figure 9 shows the sampling location at Pulau Redang, Terengganu, in March 2019 (post-monsoon):

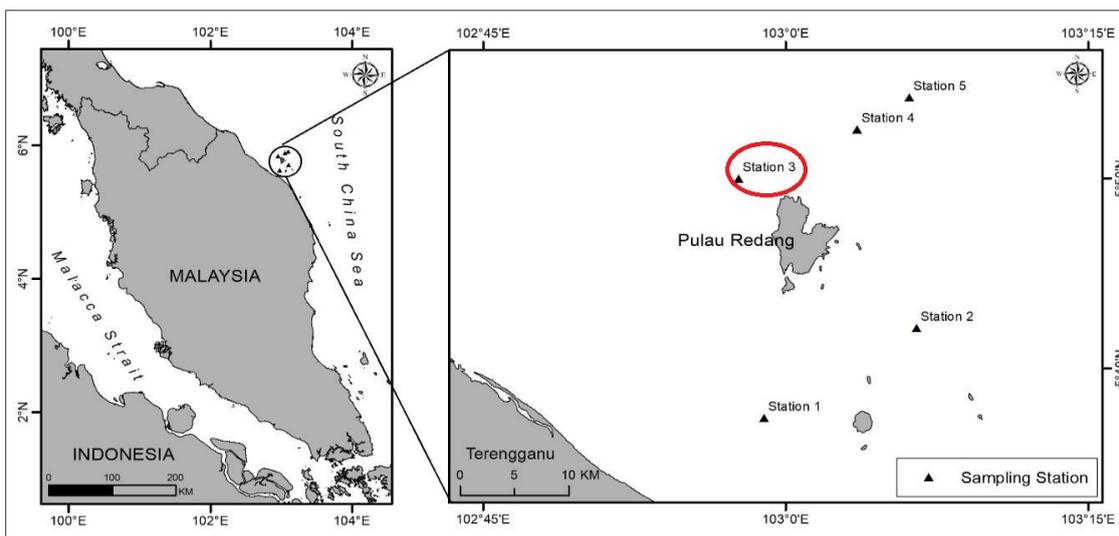


Figure 9. Sampling location at Pulau Redang, Terengganu in March 2019 (post-monsoon). Station 3 was chosen as the tested sample for the optimization method.

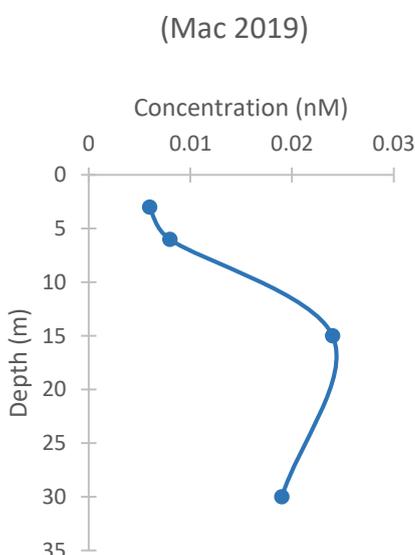


Figure 10. The vertical distribution of dFe concentration in Station 3 at Pulau Redang, Terengganu during post-monsoon season

Figure 10 shows the vertical distribution of dFe(III) concentration at St. 3, by using the CLE-AdCSV analysis coupled with the optimized method for the coastal water samples. The results showed a nutrient-like trace metals profile (as shown in Figure 10), as the concentration was low at the surface water and was enriched at the deeper water. This pattern may be due to the gravitational settlements and the decomposition of organic matter (OM) [21], [48]. Accordingly, the pattern was similar to the vertical distribution of dFe at Pulau Perhentian, Terengganu, in October 2016, reported by [24]. According to [49], the trace elements such as Cd, Cu, Fe, and Ni are examples of nutrient-type trace elements supplied from the demineralization of sinking bionic particles [24], [50].

In term of concentration, our data recorded the values from 0.006 ± 0.003 ng/L to 0.024 ± 0.005 ng/L (as shown in Figure 10), as compared to the values of 227.36 ± 1.72 $\mu\text{g/L}$ to 523.44 ± 4.43 $\mu\text{g/L}$ recorded at St. 1 from Pulau Perhentian, Terengganu [24]. A huge difference between the dFe concentrations was reported. This is because the data by [24] represented a total dissolved Fe concentration. Meanwhile, our data presented the concentration of dFe(III) species as the Fe bioavailability in our study area. Therefore, these findings highlighted the advantage of voltammetry testing over the ICP-MS analysis in species determination. According to [51], the voltammetric method is substance-specific and is able to differentiate between the different chemical forms of metal/metalloid ions.

Thus, the levels of dissolved trace metals (for speciation) in Malaysia coastal waters were compared with the dCu(II) concentrations at Pulau Perhentian, Terengganu, and Pulau Pangkor, Perak [30], as there was no data published for dFe(III) in the Malaysian coastal areas yet. The previous studies recorded 3.12 to 9.61 ng/L (PP1), 4.66 to 11.87 ng/L (PP2), and 2.09 to 9.11 ng/L (PG) of dCu(II) concentrations, respectively. Based on the data, the levels of dCu(II) concentration was in the unit of ng/L, similar to our data unit. According to [8], [9], the concentrations of dFe ranged from 10^{-6} to 10^{-9} M in coastal waters. However, the particle size used for dCu(II) was $0.45\ \mu\text{m}$ [30]. In contrast, our study used the $0.20\ \mu\text{m}$ particle size, suggesting the possible difference between the range of dissolved trace metals from both of the studies.

Pulau Redang is located on the southern coast of SCS, so the comparison with the previous data conducted at or near the SCS could be reliable. Table 1 shows the range of dFe(III) concentrations in Pulau Redang, Terengganu, as compared to the concentrations reported by previous studies:

Table 1. dFe concentration at Pulau Redang, Terengganu compared to the previous study

No	Study Area	Range of dFe (nM)	References
1.	Pulau Redang, Terengganu	0.006-0.024	This study
2.	Tropical South Eastern Pacific	0.080-1.200	[52]
3.	Sulu Sea	0.250-1.680	
	Philippine Sea	0.080-1.440	[53]
	Celebes Sea	0.280-1.830	
	South China Sea	0.250-1.980	
4.	Eastern North Atlantic	0.130-0.700	[54]
5.	Southern Ocean (Atlantic sector)	0.100-0.340	[55]
6.	Ross Sea	0.040-0.110	[56]

The previous studies recorded 0.04 to 1.98 ng/L of dFe(III) concentrations and were 6 to 82 times higher than our data, suggesting that the data collected were incomparable. The first understanding is that the trace Fe metals in our study area may present in the particulate Fe (pFe) (particle size $>0.45\ \mu\text{m}$) form during the post-monsoon event. A study by [57] (*unpublished work*) at the same area suggested that 95% of the trace zinc (Zn) metals existed in the particulate form, while the other 5% was present in the dissolved form during the post-monsoon season, with the concentrations of 82.26 to 289.46 mg/kg were recorded. The study highlighted the phenomena of high turbulence during the post-monsoon

season, thus causing the resuspension of bottom sediment to the upper layer of seawater and increasing the levels of total suspended solids (TSS) in the water column. Hence, the same phenomena might occur to our sample in describing the low dFe(III) concentration. This study recommended further investigation on the size fractionation of trace metals (either in the particulate, dissolved, or colloidal forms) in order to improve our understanding of the Fe biogeochemistry in the Malaysian coastal seawater.

4. CONCLUSION

In conclusion, the optimization method that used the AdCSV technique in this study could determine the dFe(III) concentration found inside the Malaysian coastal water. The method was applied to the seawater determination analysis and showed a reliable result. Some of the optimizations regarding the coastal sample determination procedures were:

- DHN was the best AL used to bind with free Fe(III) in the samples to form a new complex of FeDHN for the AdCSV method.
- A high concentration of DHN (400 μM) was needed in this analysis to compete with the natural ligands and to give a better sample calibration.
- Two hours of UV-irradiation were determined to be the optimum time needed to break down the binding of Fe-organic ligands in the samples.
- The samples must be acidified to pH~2 before undergoing the UV-irradiation process (UV-light is provided by a high-pressure mercury vapor lamp with a power output of 125 W, peaking at ~250 nm) to prevent the Fe precipitation and sample evaporation issues.
- Sample analysis must be done directly after the UV-irradiation step as the bindings reached stability in a shorter period with the high [DHN].

However, the data collected were far different from the previous studies, probably due to Fe trace metals that are mostly present in the particulate form rather than in the dissolved form, specifically in our study area. Thus, further investigation on the size fractionation of Fe is needed to describe the biogeochemistry of Fe(III) at the coastal water of Malaysia.

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