Manual for pH Determination Version 1.0:

Determination of the pH of sea water using the indicator dye *m*-cresol purple

This SOP was adapted from Dickson et al (2007, SOP 6b) for use with the GOA-ON in a Box equipment (list is available here on GOA-ON website). More information about GOA-ON in a Box efforts and related SOPs referenced below are available on the GOA-ON website under Resources.

"GOA-ON in a Box" is the name used to describe equipment being provided through an international scientific capacity building effort being conducted to support the Global Ocean Acidification Observing Network.

1. Overview

This procedure describes a method for the spectrophotometric determination of the pH of sea water on the total hydrogen ion concentration pH scale. The total hydrogen ion concentration, $[H^+]$, is expressed as moles per kilogram of sea water.

This SOP was adapted from Dickson et al (2007, SOP 6b).

Sections 2 and 3 give a summary of the principles of spectrophotometric measurement of pH

Sections 4 to 9 give details of the method and the calculations

Section 10 provides information on quality assurance

Appendix 1 describes the Excel sheet template used for calculating pH from the absorbance data

Appendix 2 contains instructions on using the AquaMate TM700, the spectrophotometer provided as part of "GOA-ON in a Box"

Appendix 3 is a Quick Guide for the determination of pH using a spectrophotometric method

This pH SOP also includes a spreadsheet for the calculation of pH from the measured absorbance values.

2. Definition

The total hydrogen ion concentration of sea water includes the contribution of the medium ion sulfate and is defined as

$$[H^+] = [H^+]_F(1+S_T/K_S)$$

$$\backsimeq [H^+]_F + [HSO_4^-] \quad (1)$$

where $[H^+]_F$ is the *free* concentration of hydrogen ion in sea water, S_T is the total sulfate concentration ($[HSO_4^-] + [SO_4^{2-}]$) and K_S is the acid dissociation constant for HSO_4^-. The pH is then defined as the negative of the base 10 logarithm of the hydrogen ion concentration:

$$pH = -\log_{10}\left(\frac{[H^+]}{\text{mol kg}-\text{soln}^{-1}}\right). \quad (2)$$

3. Principle

A full description of the method is given in Dickson et al (2007). This section gives a brief overview of the principle of the spectrophotometric method of determining pH in seawater.

The values of pH are determined by adding an indicator dye to sea water. For the

sulfonephthalein indicators such as *m*-cresol purple, the reaction of interest at seawater pH is the second dissociation

$$HI^-(aq) = H^+(aq) + I^{2-}(aq) \quad (3)$$

where I represents the indicator dye, which is present at a low level when measuring a sea water sample. The total hydrogen ion concentration of the sample can then be determined:

$$pH = pK(HI^-) + \log_{10}\frac{[I^{2-}]}{[HI^-]}. \quad (4)$$

The principle of this approach uses the fact that the different forms of the indicator have substantially different absorption spectra. Thus, the information contained in the composite spectrum can be used to estimate $[I^{2-}]/[HI^-]$.

At an individual wavelength, λ, the measured absorbance in a cell with a path length, l, is given by the Beer–Lambert law as

$$\frac{A_\lambda}{l} = \varepsilon_\lambda(HI^-)[HI^-] + \varepsilon_\lambda(I^{2-})[I^{2-}] + B_\lambda + e \quad (5)$$

where B_λ corresponds to the background absorbance of the sample and e is an error term due to instrumental noise. Provided that the values of the extinction coefficients: $\varepsilon_\lambda(HI^-)$ and $\varepsilon_\lambda(HI^{2-})$ have been measured as a function of wavelength, absorbance measurements made at two or more wavelengths can be used to estimate the ratio $[I^{2-}]/[HI^-]$.

In the case that only two wavelengths are used, and provided that the background can be eliminated effectively by a subtractive procedure, (5) can be rearranged to give (assuming no instrumental error)

$$\frac{[I^{2-}]}{[HI^{-}]} = \frac{A_1/A_2 - \varepsilon_1(HI^{-})/\varepsilon_2(HI^{-})}{\varepsilon_1(I^{2-})/\varepsilon_2(HI^{-}) - (A_1/A_2)\varepsilon_2(I^{2-})/\varepsilon_2(HI^{-})} \quad (6)$$

where the numbers 1 and 2 refer to the wavelengths chosen. For the best sensitivity, the wavelengths corresponding to the absorbance maxima of the base (I^{2-}) and acid (HI^{-}) forms, respectively, are used. The various terms ε are the extinction coefficients of the specified species at wavelengths 1 and 2, respectively

4. Apparatus

4.1 Glass Pasteur pipette

A glass Pasteur pipette is used to carefully and quickly transfer the sample from the newly opened sample bottle into the spectrophotometric cuvette.

4.2 Spectrophotometric cuvettes

The spectrophotometer provided in the GOA-ON kit uses a 1 cm wide cuvette (sometimes called a cell), with a volume of either 2.5 mL or 3.5 mL. Other cuvettes can also be used (refer to Table 1 below). Good quality quartz cuvettes are preferable, but plastic cuvettes are acceptable. These should be handled carefully, and discarded when scratched or discoloured.

4.3 Micropipette

A Gelman micropipette is used to add the dye to the cell. It should be of ~ 0.1 mL capacity with a plastic tip.

4.4 High-quality spectrophotometer

For work of the highest sensitivity and precision, a double-beam spectrophotometer is desirable. However, good results can be obtained with a high quality single-beam instrument. A Thermo Scientific™ Orion™ AquaMate 7000 Vis spectrophotometer is provided with the GOA-ON Kit.

4.5 Temperature-control system for spectrophotometer

The spectrophotometer temperature should be regulated to within 0.1°C. If this is not possible, then the temperature should be stable, and measured to within 0.1°C. Refer to Section 7.1

4.6 System to warm/cool samples to measurement temperature

The sample temperature should be regulated to within 0.1°C. If this is not possible, then the temperature should be stable, and measured to within 0.1°C. Refer to Section 7.1

5. Reagents

5.1 Solution of *m*-cresol purple (see pH Dye SOP)

A 2 mmol L^{-1} solution of m-cresol purple dye adjusted to be in the range 7.9 ± 0.1 pH units, which is chosen to be similar to the sample pH, is required. The dye solution is made in a sodium chloride matrix so that the ionic strength is similar that of seawater. The procedure for making the dye is given in the pH Dye SOP.

6. Sampling and sample preservation

Measurements of pH can be performed within 6 hours of sampling, or pH samples can be preserved with mercuric chloride and brought back to the lab for analysis. Take the water sample using the Water Sampling SOP, ensuring that there is minimal gas exchange while sampling.

7. Procedure for measuring pH

The pH measurement should be made first, before measuring alkalinity. Open the sample bottle immediately before making the measurement.

7.1 Warm/Cool temperature of sample to 25.0°C (± 0.1°C), or to lab temperature

This is done by placing the sample bottles in a thermostated compartment or waterbath for a few hours. If this is not practical, then leave the samples, the cuvettes and the dye at lab temperature for a few hours. Record the temperature of the sample at the time of measurement.

7.2 Measure absorbances for the cell + sea water

Fill the cuvette by quickly and carefully transferring sample from the sample bottle to the cuvette using a Pasteur pipette.

Clean and dry the exterior of the cuvette, being careful not to smudge the optical surface; place the cuvette in the sample compartment of the spectrophotometer. Be sure to align the cuvette so that the optical surfaces are in the light path. If using the AquaMate™ 7000 spectrophotometer provided with the GOA-ON Kit, then ensure that the sample holder is aligned so that the sample is in the light path.

Measure and record the absorbances at three wavelengths: 730 nm, 578 nm, and 434 nm. These correspond to a non-absorbing wavelength (730 nm for *m*-cresol purple) and the wavelengths corresponding to the absorption maxima of the base (I^{2-}) and acid (HI^{-}) forms of the dye respectively (578 and 434 nm).

Programming the AquaMate™ 7000

Refer to Appendix 2.

7.3 Inject dye into cell

Remove the cuvette from the spectrophotometer. Add the appropriate volume of of dye solution (~2 mmol L^{-1}) to the sample using the micropipette, check Table 1 for the volume of dye to add for the cuvette that you are using. Replace the cap (if used) and gently shake the cell to mix the sea water and dye. This amount of dye should produce absorbance values of between 0.4 and 1.0 at each of the two absorbance peaks. Adjust the amount of dye if necessary.

Table 1.

Cuvette volume / mL	Path length of cuvette	Volume of dye solution to add / µL
1.5	1 cm	24
2.5	1 cm	40
3.5	1 cm	56
4.5	1 cm	72

7.4 Measure absorbances of cell + sea water + dye

Return the cuvette to the spectrophotometer and again measure the absorbances at the three wavelengths: 730 nm, 578 nm, and 434 nm. Be sure to position the cuvette in the same way as for the baseline measurement (Section 7.2).

Note: The difference between the baseline absorbance (sea water sample only) and the absorbance of the sample + dye at 730 nm should be no greater than ± 0.001 to ±0.003; if this value is exceeded, the cell should be removed and the optical windows cleaned before the absorbances are measured again.

7.5 Measure the temperature of the sample

Measure the temperature of the sample to ± 0.1°C.

7.6 Dispose of the sample waste

The spent sample in the cuvette must be disposed of appropriately as it contains mercuric chloride. Refer to the Hg Processing SOP for details.

The remaining sample can be used for alkalinity analysis. Seal the bottle until needed.

8. Calculation and expression of results

8.1 Correction of measured absorbances

The measured sample absorbances need to be corrected for the background absorbance (without dye), and for any baseline shift (using the absorbance at 730 nm). This can be done

using the spreadsheet calculation (pH SOP spreadsheet and Appendix 1), or using the function on the AquaMate™ 7000 (Section 7.2).

The method is described here, for completeness:

At each of the three wavelengths, subtract the absorbances measured for the background measurement (without dye) from the corresponding absorbances measured for the system containing dye. In addition, the absorbance measured at a non-absorbing wavelength is used to monitor and correct for any baseline shift due to error in repositioning the cell, instrumental shifts, *etc*. This assumes that the magnitude of any observed baseline shift is identical across the visible spectrum. To do this, subtract the measured shift from the background-corrected absorbances at wavelengths 1 and 2 to obtain the final corrected absorbance value at each wavelength.

These final absorbance values, corrected for background absorbances and any observed baseline shifts, are used to calculate A_1/A_2, the absorbance ratio which describes the extent of protonation of the dye.

To summarise:

A_{1b} = absorbance at 578 nm, seawater only
A_{1s} = absorbance at 578 nm, seawater + dye
$A_{1c} = A_{1s} - A_{1b}$

A_{2b} = absorbance at 434 nm, seawater only
A_{2s} = absorbance at 434 nm, seawater + dye
$A_{2c} = A_{2s} – A_{2b}$

A_{3b} = absorbance at 730 nm, seawater only
A_{3s} = absorbance at 730 nm, seawater + dye
$A_{3c} = A_{3s} – A_{3b}$

$A_1 = A_{1c} – A_{3c}$
$A_2 = A_{2c} – A_{3c}$

8.2 Calculation of the pH of the sea water + dye

This calculation is included in the Spreadsheet Appendix 1 and pH SOP spreadsheet. A description of the formula used is given here for completeness.

The pH of the sea water and dye in the cell is computed from

$$\mathrm{pH} = \mathrm{p}K_2 + \log_{10} \frac{A_1/A_2 - \varepsilon_1(\mathrm{HI}^-)/\varepsilon_2(\mathrm{HI}^-)}{\varepsilon_1(\mathrm{I}^{2-})/\varepsilon_2(\mathrm{HI}^-) - (A_1/A_2)\varepsilon_2(\mathrm{I}^{2-})/\varepsilon_2(\mathrm{HI}^-)} \qquad (7)$$

where pK_2 is the acid dissociation constant for the species HI^- (expressed on the total hydrogen ion concentration scale in mol kg-soln^{-1}), and A_1 and A_2 are the corrected absorbances measured at the wavelengths corresponding to the absorbance maxima of the base and acid forms, respectively. The various extinction coefficient terms ε correspond to values measured for the specified species at wavelengths 1 and 2, respectively (Table 2).

Table 2 Extinction coefficient ratios for *m*-cresol purple.

$\varepsilon_1(HI^-)/\varepsilon_2(HI^-)$	0.00691
$\varepsilon_1(I^{2-})/\varepsilon_2(HI^-)$	2.2220
$\varepsilon_2(I^{2-})/\varepsilon_2(HI^-)$	0.1331

λ_1 = 578 nm; λ_2 = 434 nm.

The equilibrium constant K_2 is a function of salinity and temperature and has been determined by careful laboratory measurements[3]. For *m*-cresol purple,

$$pK_2 = \frac{1245.69}{(T/\mathrm{K})} + 3.8275 + 0.00211(35 - S) \quad (8)$$

where $293 \le T/\mathrm{K} \le 303$ and $30 \le S \le 37$.

Note: Although DelValls and Dickson (1998) have suggested that this pK_2 may be in error because of an error in calibrating TRIS buffer, it seems that there may be a compensating error that largely mitigates the proposed correction. The pK_2 given here is that from Clayton and Byrne (1993).

8.3 Correction for pH change resulting from addition of the dye

The addition of indicator dye to the sea water sample will perturb the pH (another acid–base system has been added!). Although care is taken to minimize this (by adjusting the dye solution pH), it is desirable to correct for the addition of dye to obtain the best pH measurements. The perturbation of the pH by the dye addition is about 0.003 pH units.

The procedure is not included here, however details are available in SOP6b in Dickson et al (2007).

8.4 Correction for dye impurities

For the best measurements, purified dye is required, as commercially available dye contains impurities. The effect of this, and corrections for some brands of dye are given in Douglas and Byrne (2017). The effect of dye impurity is not included here.

9. Example calculation for pH from spectrophotometer absorbance

t = 25°C,

S = 35,

pK_2 = 8.0056,

Measured absorbances:

Sea water (*sw*): A_{434} = 0.02433 ; A_{578} = 0.01936 ; A_{730} = 0.08365

Dye + sea water (*swd*): A_{434} = 0.45123 ; A_{578} = 0.84574 ; A_{730} = 0.08298

After addition of dye,

$$A_1/A_2 = \frac{(A_{587swd} - A_{587sw}) - (A_{730swd} - A_{730sw})}{(A_{434swd} - A_{434sw}) - (A_{730swd} - A_{730sw})}$$

$$A_1/A_2 = \frac{(0.84574 - 0.01936) - (0.08298 - 0.08365)}{(0.45123 - 0.02433) - (0.08298 - 0.08365)} = 1.93430.$$

and thus

$$\text{pH} = 8.0056 + \log_{10}\left(\frac{1.93430 - 0.00691}{2.2220 - 1.94705 \times 0.1331}\right) = 7.997.$$

Note: if the dye correction is calculated the resulting pH is 8.001

10. Quality assurance

It is good practice to regularly and frequently assess the quality of the pH measurement system. The measurement uncertainty can be determined by analyzing Tris buffer (refer to Buffer SOP) and by collecting and analyzing duplicate samples (refer to Data QC SOP).

The use of property and range control charts is fully described in SOP 22 of Dickson et al (2007), and the statistical techniques used in the quality assessment are described in SOP 23 of Dickson et al (2007).

The spectrophotometric performance of the instrument used can be confirmed using reference materials that are available from the U.S. National Institute for Standards and Technology (NIST). SRM 2034 is a holmium oxide solution in a sealed cuvette that allows the wavelength accuracy of the spectrophotometer to be determined; SRM 930d is a set of absorbance filters that allows the absorbance measurement accuracy to be verified. Property control charts of these measurements should be maintained, and the spectrophotometer adjusted if it goes out of tolerance. (Nevertheless, the procedure detailed here is fairly insensitive to minor changes in spectrophotometer performance.)

A more important concern is that the spectrometer must have a high stability.
This can be confirmed by making a series of repeated measurements on a system of constant absorbance (e.g., SRM 930d or a thermostated buffer solution containing indicator dye) and computing the standard deviation at the wavelengths of interest.

11. Bibliography

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Appendix 1

Excel sheet for calculation of pH, for use with the Spreadsheet for pH Determination

=273.15+C2

=(1245.69/K2)+3.8275+0.002111*(35-B2)

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
				Absorbance of seawater only			Absorbance of seawater + dye			Calculated Values						
Sample	**Salinity**	**Temp / °C**	**Dye vol / mL**	**$A_{730, sw}$**	**$A_{578, sw}$**	**$A_{434, sw}$**	**$A_{730, sw+dye}$**	**$A_{578, sw+dye}$**	**$A_{434, sw+dye}$**	**Temp / K**	**$\varepsilon_{1\,(HI-)}/\varepsilon_{2\,(HI-)}$**	**$\varepsilon_{1\,(I2-)}/\varepsilon_{2\,(HI-)}$**	**$\varepsilon_{2\,(I2-)}/\varepsilon_{2\,(HI-)}$**	**pK_2**	**A_1/A_2**	**pH**
Example	35	25.0	0.08	0.08365	0.01936	0.02433	0.08298	0.84574	0.45123	298.15	0.00691	2.222	0.1331	8.0056	1.934303	7.997

=(I2-F2)-(H2-E2))/(J2-G2)-(H2-E2))

=O2+LOG((P2-L2)/(M2-(P2*N2)))

Measured values	
Salinity	salinity of sample
Temp / oC	temperature of the seawater sample at time of measurement [°C]
Dye vol / mL	volume of dye added to the seawater in the cuvette [mL]
$A_{730, sw}$	Absorbance of the seawater at 730 nm
$A_{578, sw}$	Absorbance of the seawater at 578 nm
$A_{434, sw}$	Absorbance of the seawater at 434 nm
$A_{730, sw+dye}$	Absorbance of the seawater + dye at 730 nm
$A_{578, sw+dye}$	Absorbance of the seawater + dye at 578 nm
$A_{434, sw+dye}$	Absorbance of the seawater + dye at 434 nm
Calculated values	
Temp / K	temperature of the seawater sample at time of measurement [K]
$\varepsilon_{1\,(HI-)}/\varepsilon_{2\,(HI-)}$	Extinction coefficient ratios for m-cresol purple
$\varepsilon_{1\,(I2-)}/\varepsilon_{2\,(HI-)}$	Extinction coefficient ratios for m-cresol purple
$\varepsilon_{2\,(I2-)}/\varepsilon_{2\,(HI-)}$	Extinction coefficient ratios for m-cresol purple
pK_2	acid dissociation constatn for m-cresol purple
A_1/A_2	absorbance ratio
pH	pH on the total scale, at the measurement temperature

Appendix 2

Programming the AquaMate™ 7000

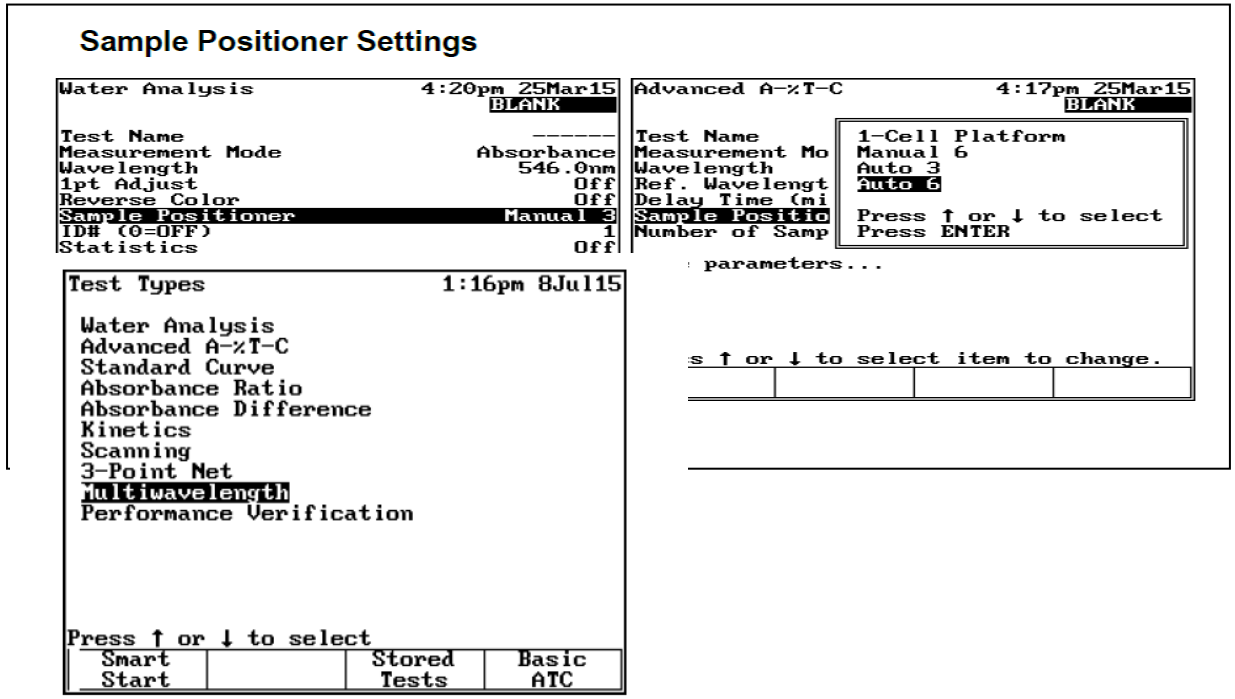
These instructions will help to set up the AquaMate™ 7000 spectrophotometer to measure the absorbance at the three different wavelengths required for the measurement of pH in seawater using m-crsesol purple. For a full description of the operation of the AquaMate™ 7000, refer to the manual, available at

https://www.thermofisher.com/order/catalog/product/AQ7000

Select Cuvette Sample Position 2

Use carousel Position 2, The three-position carousel has one adjustable 13 to 25 mm round vial holder in position B, one 10 mm square vial holder in position 2 and one 20 to 50 mm (long path) rectangular vial holder in position 4.

Use the Sample Positioner command on the menu to select Sample position 2.



Set up a Method for measuring the absorbance at three wavelengths

1. Press the **test** key to access the Test Types menu.
2. Press the ▲or ▼ key to highlight Multiwavelength and press the **enter** key

3. Highlight and change the displayed test parameters, including Test Name, Measurement Mode and Sample Positioner.

Test Name – give your method a name, for example sw pH
Measurement Mode – Absorbance
Sample Positioner – Auto2

4. Press the Set nms function key to add the wavelengths
Press the Add nm function key, use the numeric keypad to enter the wavelength value and press the enter key.
For m-cresol purple, use the three wavelengths 434nm, 578 nm and 730 nm

5. Press the **Run Test** function key.

Recalling an Existing Multiwavelength Method

1. Press the **test** key to access the Test Types menu.
2. Press the ▲or ▼ key to highlight Multiwavelength and press the **enter** key.
3. To recall an existing multiwavelength method, press the **Stored Tests** function key.
4. Press the ▲or ▼ key to highlight Load internal test or Load test from USB drive and press the **enter** key.
5. Press the ▲or ▼ key to highlight the desired test and press the **enter** key.

Taking Measurements with Multiwavelength Method

Once the parameters have been set for the multiwavelength test:

1. Press the **Run Test** function key.
2. Prepare the sample, first the seawater only (blank), secondly the seawater+dye.
3. Measure the absorbances:
   - Place the cuvette containing the seawater into position 2 on the vial holder carousel and press the **Measure Blank** function key. Once the blank has been measured, remove it from the carousel.
   - Add the dye then place the cuvette containing the seawater + dye into position 2 on the vial holder carousel and press the **Measure Sample** function key.
   - Repeat for each sample.

5. When all of the samples have been measured, press the **Save Data** function key and follow the onscreen prompts to save the sample measurements to the USB memory stick.

It is a good idea to also record the absorbance values in your notebook.

Using the pH SOP Spreadsheet

Note that the pH SOP Spreadsheet is set up for separate entry of the absorbance of the seawater only, and seawater +dye. The spreadsheet will need to be edited if using this method.

Appendix 3
Quick Guide for Determination of seawater pH using m-cresol purple

1. Materials:

Gather all of your materials from the GOA-ON kit and read through the entire set of instructions before you begin preparing your solutions and building your setup.

- Your water samples
- Spectrophotometer
- Cuvettes
- Pasteur Pipette
- Micropipette
- Thermometer
- 2 mmol L^{-1} m-cresol purple dye solution
    - 0.202 g of M-cresol purple sodium salt
    - 10.2 g NaCl
    - 250 mL of deionized (DI) water
    - 0.1 mol L^{-1} NaOH
    - 0.1 mol L^{-1} HCl
    - One 250 mL volumetric flask
- Kimwipes
- Container for chemical waste water
- Paper and pen or titration Excel sheet

2. Preparation and Setup:

a. Prepare your dye

1. Weigh out 0.202 g of m-cresol purple sodium salt and 10.2 g of NaCl salt
2. Add dionised water to the 250 mL volumetric flask until half full.
3. Add the salts via a funnel, rinse the funnel and weighing vessels into the flask.

4. Dissolve the salts completely.
5. Carefully fill the flask to the fill line with DI water to make 250 mL of dye.
6. Measure the pH of the dye with an electrode. Adjust until the pH is between 7.8 and 8.0
    a. If the pH of the dye solution is less than 7.8, add 0.1 mol L^{-1} NaOH solution dropwise until the pH increases to 7.9±0.1
    b. If the pH of the dye solution is greater than 8.0, add 0.1 mol L^{-1} HCl solution dropwise until the pH increases to 7.9±0.1
7. Transfer to a screw cap glass bottle and seal. This dye solution should last for several months, but check the pH before each use, and adjust if necessary.

b. Bring the samples to the lab temperature

Leave the samples, the cuvettes and the dye at lab temperature for a few hours.

c. Set up spectrophotometer

1. Turn the power onto the spectrophotometer
2. Load the multi -wavelength method if using
3. Orientate the turret so that the square cuvette holder is in the light path

3. Perform the Measurement

Remember that the sample contains mercuric chloride. Wear gloves

1. Fill the cuvette with sample using a Pasteur pipette. Ensure that there are no bubbles in the cuvette, no drops on the outside of the cuvette and no smudges or scratches
2. Place in the sample holder in the Spectrophotometer
3. Measure the absorbance at 730 nm, 578 nm, and 434 nm and record the values
4. Remove the cuvette from the Spec. then add 40 µL of dye solution (for 2.5 mL cuvette, check Table 1 for the dye volume needed for different sized cuvettes. Mix carefully. Wipe off any drops on the outside of the cuvette, avoiding smudges on the optical surface.
5. Place in the sample holder in the Spectrophotometer, in the same orientation as in Step 2.
6. Measure the absorbance at 730 nm, 578 nm, and 434 nm and record the values
7. Measure the temperature of the solution.

8. Discard the solution

4. Calculate the pH

Enter the data into the pH SOP Spreadsheet, in the green cells. The spreadsheet is set up assuming that the absorbance of the seawater, and the absorbance of the seawater+dye are recorded separately. If the blank correction is done automatically then the spreadsheet will need to be adjusted accordingly.

The yellow and blue cells will calculate, giving the pH value in the blue column.