North East Regional Heat
Schools Analyst Competition 2013

'Coral in Distress'

LABORATORY HANDBOOK
Royal Society of Chemistry
Analytical Division

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hydrogen 1 H 1.0079																		helium 2 He 4.0026
lithium 3 Li 6.941	beryllium 4 Be 9.0122											boron 5 B 10.811	carbon 6 C 12.011	nitrogen 7 N 14.007	oxygen 8 O 15.999	fluorine 9 F 18.998	neon 10 Ne 20.180
sodium 11 Na 22.990	magnesium 12 Mg 24.305											aluminium 13 Al 26.982	silicon 14 Si 28.086	phosphorus 15 P 30.974	sulfur 16 S 32.065	chlorine 17 Cl 35.453	argon 18 Ar 39.948
potassium 19 K 39.098	calcium 20 Ca 40.078	scandium 21 Sc 44.966	titanium 22 Ti 47.867	vanadium 23 V 50.942	chromium 24 Cr 51.996	manganese 25 Mn 54.938	iron 26 Fe 55.845	cobalt 27 Co 58.933	nickel 28 Ni 58.693	copper 29 Cu 63.546	zinc 30 Zn 65.39	gallium 31 Ga 69.723	germanium 32 Ge 72.61	arsenic 33 As 74.922	selenium 34 Se 78.96	bromine 35 Br 79.904	krypton 36 Kr 83.80
rubidium 37 Rb 85.468	strontium 38 Sr 87.62	yttrium 39 Y 88.906	zirconium 40 Zr 91.224	niobium 41 Nb 92.906	molybdenum 42 Mo 95.94	technetium 43 Tc [98]	ruthenium 44 Ru 101.07	rhodium 45 Rh 102.91	palladium 46 Pd 106.42	silver 47 Ag 107.87	cadmium 48 Cd 112.41	indium 49 In 114.82	tin 50 Sn 118.71	antimony 51 Sb 121.76	tellurium 52 Te 127.60	iodine 53 I 126.90	xenon 54 Xe 131.29
caesium 55 Cs 132.91	barium 56 Ba 137.33	lutetium 71 Lu 174.97	hafnium 72 Hf 178.49	tantalum 73 Ta 180.95	tungsten 74 W 183.84	rhenium 75 Re 186.21	osmium 76 Os 190.23	iridium 77 Ir 192.22	platinum 78 Pt 195.08	gold 79 Au 196.97	mercury 80 Hg 200.59	thallium 81 Tl 204.38	lead 82 Pb 207.2	bismuth 83 Bi 208.98	polonium 84 Po [209]	astatine 85 At [210]	radon 86 Rn [222]
francium 87 Fr [223]	radium 88 Ra [226]	lawrencium 103 Lr [262]	rutherfordium 104 Rf [261]	dubnium 105 Db [262]	seaborgium 106 Sg [266]	bohrium 107 Bh [264]	hassium 108 Hs [269]	meitnerium 109 Mt [268]	ununnilium 110 Uun [271]	unununium 111 Uuu [272]	ununbium 112 Uub [277]		ununquadium 114 Uuq [289]				

	57-70	lanthanum 57 La 138.91	cerium 58 Ce 140.12	praseodymium 59 Pr 140.91	neodymium 60 Nd 144.24	promethium 61 Pm [145]	samarium 62 Sm 150.36	europium 63 Eu 151.96	gadolinium 64 Gd 157.25	terbium 65 Tb 158.93	dysprosium 66 Dy 162.50	holmium 67 Ho 164.93	erbium 68 Er 167.26	thulium 69 Tm 168.93	ytterbium 70 Yb 173.04			
	89-102	actinium 89 Ac [227]	thorium 90 Th 232.04	protactinium 91 Pa 231.04	uranium 92 U 238.03	neptunium 93 Np [237]	plutonium 94 Pu [244]	americium 95 Am [243]	curium 96 Cm [247]	berkelium 97 Bk [247]	californium 98 Cf [251]	einsteinium 99 Es [252]	fermium 100 Fm [257]	mendelevium 101 Md [258]	nobelium 102 No [259]			

* Lanthanide series

** Actinide series

Introduction

Corals are marine animals. They typically live in colonies of many identical individual 'polyps'. The group includes the important reef builders that inhabit tropical oceans and secrete calcium carbonate to form a hard skeleton. Reefs are extremely diverse marine ecosystems which host over 4,000 species of fish and many other marine animals. Coral reefs are under stress around the world. Localized threats to coral ecosystems include coral mining, agricultural and urban runoff, pollution, overfishing, disease and the digging of canals and access into islands and bays. Broader threats are sea temperature rise, sea level rise and pH changes from ocean acidification, all associated with greenhouse gas emissions. In an attempt to educate the public about these problems and also to protect endangered types of corals and associated fish species 'Ocean World' has established large coral ecosystem tanks at their aquaria to facilitate breeding programmes and research. However, some of the tanks are exhibiting signs of 'coral distress'. Your task is to analyse the water from the affected tanks and to try to deduce the root cause.

Background

In this section, the theory of each analytical method is explained. Enough detail is provided to allow you to understand what you are doing and why you are doing it. You will also be provided with information of the typical concentrations necessary for healthy aquaria.

Units and ppm

In this booklet you will encounter several ways of describing concentration. The first is "molarity". This should be familiar to you and describes the number of moles per cubic decimetre (mol dm^{-3}) of each chemical. This is also sometimes referred to as mol L^{-1} or M. The second is "parts per thousand" or "ppt". This unit may not be familiar to you, but it simply describes the number of g of a particular analyte per litre of aqueous solution, for example 1 g of sodium in 1 litre of water would be 1 ppt sodium. The third is "parts per million" or "ppm". This again describes the number of mg of a particular analyte per litre of aqueous solution, for example 1 mg of copper in 1 litre of water would be 1 ppm copper. It is frequently encountered for elements rather than molecules, i.e. copper rather than copper sulphate. Other common units for concentration are mg L^{-1} & g L^{-1}.

Titrimetric analysis of calcium

Calcium can be determined using many analytical techniques, but a straightforward method is to titrate the calcium solution with ethylenediaminetetraacetic acid (EDTA) using a coloured indicator to determine the 'end point'. The calcium solution is pipetted into a conical flask, the pH buffered and a total hardness tablet added as an indicator. At this stage, the solution is red. The burette is filled with EDTA solution, and then it is slowly delivered into the conical flask. The EDTA reacts with any available calcium to form a complex ion. At the end point a blue solution is produced.

Calcium is the fifth most abundant element in the Earth's crust. It is essential for living organisms, particularly in cell physiology, and is the most common metal in many animals. Corals need sufficient calcium and magnesium amongst other trace elements to flourish. For healthy coral growth a typical concentration of between 350-400 ppm is needed. This is much higher than the level of calcium needed for other sea life such as snails and shrimps where 70-140 ppm is sufficient.

Spectrophotometric determination of copper

Many molecular species absorb radiation in the UV or visible regions of the electromagnetic spectrum. Solutions containing such molecules may be colourless or coloured, and the amount of light absorbed depends on the concentration of the molecules. It is slightly more complex than that, indeed the absorbance (A) of any solution is related to three parameters; the molar absorptivity coefficient (ε) of the molecules at the wavelength used, the concentration of the absorbing species (c) and the path length (l) through the solution. This is expressed as Beer's law (equation 1) and describes the relationship between the measured absorbance and the concentration of the absorbing species.

$$A = \varepsilon c l \qquad \text{(equation 1)}$$

In experiments like the one here, the pathlength is usually fixed at 1 cm. This is simply achieved by using a glass, plastic, or quartz cuvette that has holds a column of liquid exactly 1 cm in thickness. The molar absorptivity coefficient is usually different for each absorbing species present, and has the units of $mol^{-1}dm^3cm^{-1}$. The molar absorptivity coefficient can readily be determined by producing a calibration graph of absorbance versus concentration (measure the absorbance of a series of solutions of known concentration). Assuming a 1 cm cuvette was used, then the gradient of the line is ε. Once the calibration graph has been produced, it is relatively simple to determine the concentration of the analyte in a solution where the concentration of the analyte is not known. The absorbance of the solution can be read from the graph and by rearranging equation 1, the concentration can easily be calculated.

The only problem with this method is that many species do not absorb light at a convenient wavelength or there are interferences. An alternative approach is to measure the analyte indirectly, by derivatising it to a species that absorbs at a suitable wavelength. Ideally this procedure should eliminate any interference since the derivatising reagent should only react with the analyte of interest. Copper is a good example where direct analysis by spectrophotometry would be difficult. Instead, we react the copper with cuprizone and ammonia to form a blue complex. This gives a blue solution whose absorbance is conveniently measured at 600 nm.

Copper is an essential nutrient to all higher plants and animals but in sufficient amounts it can be poisonous or even fatal to all organisms. Copper is used in anti-fungal/anti-bacterial treatments and against 'Ich' (ichthyophthirius multifiliis) a parasitic disease which appears as random white spots on the surface of the skin or gills in fish so is very helpful in maintaining a healthy fish population. It is typically added as copper sulphate at a concentration of 0.1 mg L^{-1} copper. Corals are very sensitive to copper poisoning. In an aquarium tank it is imperative that calcium levels are maintained as these help to prevent copper absorption by the corals leading to 'coral bleaching' which is often fatal.

Atomic emission spectrometric determination of sodium

When ions are heated in a flame, some of them become electronically excited. In order to return to the electronic ground state, they have to emit light at a characteristic wavelength. The amount of light is proportional to the temperature of the excitation source and the concentration of ions present. Thus if a flame in used to excite the ions, and the flame temperature is constant, then the amount of light emitted will be proportional to the concentration.

While all elements can be made to emit light, the temperatures required can be extreme. For some elements, e.g. lithium, sodium, and potassium, emission can be obtained at much lower temperatures. Flame photometry uses a cool flame to excite the ions, and thus only a few elements emit. This means that the emission spectrum is very simple, and unwanted emission wavelengths can be readily removed using a filter. Here, we use a flame photometer to measure the sodium emission from a suspect sample. Aqueous samples of sodium ions are aspirated into the methane/air flame and a percentage become electronically excited. As they return to the ground state, they emit light; this gives rise to the characteristic yellow colour in the flame. A filter is used to block out the other wavelengths so that only that due to the sodium emission reaches the detector. The amount of sodium in the sample can easily be determined from a calibration graph. This is produced by first analysing a set of calibration standards and plotting a graph of sodium concentration vs. instrument reading. The unknown concentrations can then be determined from the graph.

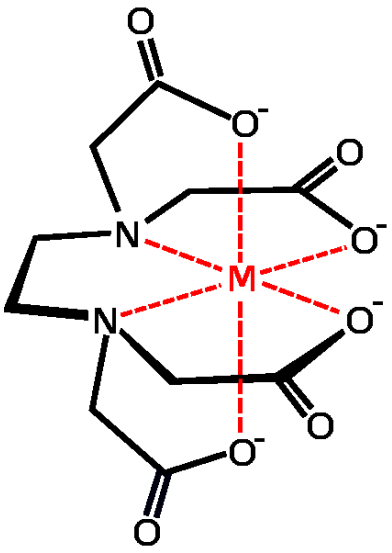
Seawater has an average salinity of about 3.5% or 35 ppt ie 35 g of dissolved salts, predominantly sodium chloride in 1000 g water. Equivalent to 14 ppt sodium.

What is required?

In your team of three decide who is going to do each analysis; then read the experiment you are going to do. Each experiment has a section explaining the hazards of the chemicals you will be using; you should read this section carefully. Once you are happy with what you are going to do, you should begin the practical work. Pool the results of the three experiments in your 'neat copy' of the answer booklet and hand this in together with the graphs. Make sure your names are correctly and clearly spelled as there is nothing worse than a participation certificate with your name spelled incorrectly! Good luck and enjoy the challenge. There are marking guidelines alongside the tasks to guide you in the depth of answer required. Good communication is very important so make sure your calculations are laid out well.

Experiment 1: Titrimetric analysis of calcium

The ions involved in water hardness i.e. calcium ($Ca^{2+)}$ and magnesium (Mg^{2+}) ions can be determined by titration with a chelating agent, ethylenediaminetetraacetic acid (EDTA). It is these ions which allow the coral to build up its structural integrity. In this practical, tablets for detecting Ca^{2+} ions will be utilised as the end point indicator in the titration as they change from yellow to red. One EDTA molecule reacts with one metal ion to form a complex ion. (Image from Wikimedia commons)



Safety:
PPE must be worn. Safety spectacles and lab coat.
Spillages must be mopped up immediately.
If you get any on your skin wash off with water.

Reagents:
0.005 mol dm^{-3} EDTA solution
aquarium water
NH_3-NH_4Cl buffer solution
'total hardness tablets' – indicator (5 provided)
distilled water

Apparatus:
50 mL burette
25 mL bulb or graduated pipette
5 mL calibrated pipette (2 mL buffer)
pipette filler
funnel
250 mL conical flask
glass rod/stirrer
white tile/filter paper for under titration flask
clamp, boss and stand
permanent marker or labels

Results Table:

	rough	Run 1	Run 2	Run 3
Final reading (mL)				
Initial reading (mL)				
Titre (mL)				
Identify results used in average titre				
			Average titre	

Experimental Procedure:
Take all precautions to ensure an accurate titration

1. Fill burette with EDTA
2. Pipette 25 mL aquarium water into the conical flask
3. Add 2 mL buffer solution and one calcium tablet
4. Crush tablet with the glass rod and swirl to dissolve
5. Carry out a rough titration with 0.005 mol dm^{-3} EDTA until the solution turns from red to blue

6. Complete accurate titrations until concordant results (within 0.10 mL) are achieved
7. Calculate average titre value
8. Calculate the number of moles of EDTA in the titre

9. From the reaction equation determine the number of moles of calcium ions in the 25 mL aquarium water sample

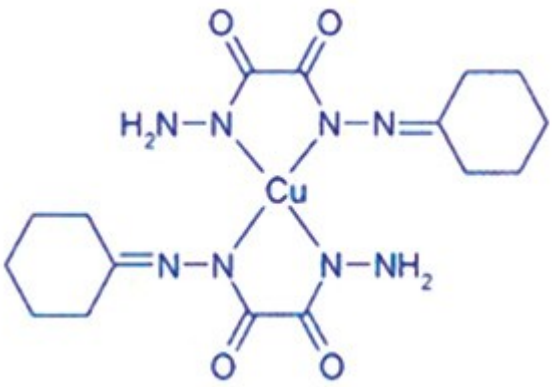
10. Calculate a value for the molarity of Ca^{2+} ions in mol dm^{-3} in the aquarium water

11. Determine the concentration of Ca^{2+} ions in mg dm^{-3} and ppm (Periodic table on inside front cover of booklet)

12. Comment on your findings

Experiment 2: Spectrophotometric determination of copper

This analytical test is based on the quantitative formation of a blue complex when Cu^{2+} ions react with cuprizone (oxalic acid bis cyclohexylidine hydrazide) in an ammoniac solution. The absorbance of the complex can be measured photometrically at 600 nm.



(image from labint-online.com)

Safety:

PPE must be worn. Safety spectacles and lab coat.
Spillages must be mopped up immediately. If you get any on your skin wash off with water.
copper solutions are accumulatively poisonous but at this strength no significant adverse health effects
cuprizone at this strength no significant adverse health effects
2 mol dm^{-3} ammonia irritant at this strength

Reagents:

2 mol dm^{-3} ammonia solution
cuprizone solution
copper (II) stock solution (100 mg L^{-1})
aquarium water
distilled water

Apparatus:

7 x 100 mL volumetric flasks
1 mL graduated pipette for ammonia solution
5 or 10 mL graduated pipette for copper (II) solution
20 mL bulb pipette for cuprizone solution
cuvette(s)
spectrophotometer set at 600 nm
permanent marker or labels

Preparation of a 10 mg L^{-1} intermediate copper (II) solution:

Transfer 10 mL copper stock solution to a 100 mL volumetric flask and fill to the mark with distilled water. Invert several times to ensure thorough mixing.
Below is an explanation of how to use the dilution equation.

$$c_1v_1 = c_2v_2$$

Where:
c_1 is concentration of initial solution (100 mg L^{-1})
v_1 is volume of initial solution (10 mL)
c_2 is final concentration
v_2 is final volume (100 mL)

rearranging $v_2 = c_1 v_1 \div c_2$
$= 100 \times 10 \div 100$
$= 10$ mg L^{-1}

Preparation of calibration solutions:
Transfer the required amounts (calculate using the dilution equation) of copper (II) intermediate (10 mg L^{-1}) solution, cuprizone solution and ammonia solution as laid out in the table below to a 100 mL volumetric flask and fill to the mark with distilled water. Invert the flask several times.

Preparation of test solution from aquarium water:
Pipette 20 mL cuprizone and 1 mL ammonia solution into a 100 mL volumetric. Fill to the mark with aquarium water. Invert the flask several times.

ALLOW THE VOLUMETRIC FLASKS TO STAND FOR THE BLUE COLOUR TO DEVELOP – 20 MINUTES

Consult a demonstrator about the operation of the spectrophotometer.

Check the wavelength on the spectrophotometer is set to 600 nm then measure the absorbance of your calibration standards and the aquarium standard.

1. Complete the results table. You should obtain two readings for each solution.

2. Calculate the average absorbance for each solution.

copper (II) strength (mg L^{-1})	Volume of intermediate copper (II) solution (mL)	Cuprizone solution (mL)	ammonia solution (mL)	absorbance run 1	absorbance run 2	average
0.0		20	1			
0.1		20	1			
0.2		20	1			
0.3		20	1			
0.5		20	1			
aquarium water		20	1			

3. Plot a graph of concentration vs average absorbance for the cuprizone complex.

4. Use your graph to determine the concentration of copper (II) ions in the aquarium water.

   Answer: _____ mg L^{-1}

   _____ ppm

5. Is the copper ion content appropriate for the prevention of Ich?

Experiment 3: Atomic emission spectrometric determination of sodium

Safety:

PPE must be worn. Safety spectacles and lab coat.
Spillages must be mopped up immediately.

Reagents:

oven dried sodium chloride
aquarium water
distilled water

Apparatus:

1 L volumetric flask
6 x 100 mL volumetric flask
10 mL graduated pipette
pipette filler
permanent marker or labels

Procedure:

1. Prepare a 100 ppm stock solution of sodium (from sodium chloride) by weighing 0.255 g of sodium chloride, and dissolving it in deionised water. Make up to 1 L.
2. By dilution of the 100 ppm stock solution, prepare five calibration solutions in the range 0-40 ppm sodium. (see page 7 for dilution equation)
3. Prepare a diluted sample of aquarium water as follows:
   Take 1 mL aquarium water and dilute to 100 mL – call this the intermediate aquarium water
   Take 10 mL of this intermediate aquarium water and dilute to 100 mL – call this the diluted sample
4. Consult a demonstrator about the operation of the flame photometer.
5. Check the filter on the flame photometer is set to sodium, and then aspirate your 40 ppm calibration solution into the flame photometer to check that the response is on the scale.
6. Once you are happy that the top calibration standard is on the scale, aspirate each of your standards in increasing concentration order and then the aquarium diluted sample. Note down the readings in the table provided. It is best to take two readings for each solution.

ppm sodium	volume of 100 ppm used	reading run 1	reading run 2	average reading
0				
10				
20				
30				
40				
diluted aquarium water				

7. Plot a graph of concentration vs. average reading for the sodium emission.

8. Use your graph to determine the concentration of sodium in the diluted aquarium sample.

   Answer:_____

9. Now work back to estimate the concentration of sodium in the neat aquarium water.