







Risk analysis

Chemical	Formula	Hazard statement(s)		Precautionary statement(s)		Environmental/Waste aspects
Ammonium chloride 	NH ₄ Cl	H302	Harmful if swallowed.	P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.	Toxic to aquatic life./ Offer surplus and non-recyclable solutions to a licensed disposal company.
		H319	Causes serious eye irritation.			
Ammonium hydroxide 	NH ₄ OH	H315	Causes skin irritation.	P280	Wear eye protection/ face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor/ physician.	Toxic to aquatic life./ Offer surplus and non-recyclable solutions to a licensed disposal company.
		H318	Causes serious eye damage.	P305 + P351 + P338 + P310		
		H412	Harmful to aquatic life with long lasting effects.			
Sulphanilamide	H ₂ NC ₆ H ₄ SO ₂ NH ₂	ND	ND	ND	ND	Offer surplus and non-recyclable solutions to a licensed disposal company.
Naphtylamine 	C ₁₀ H ₇ NHCH ₂ CH ₂ NH ₂ · 2HCl	H315	Causes skin irritation.	P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.	Offer surplus and non-recyclable solutions to a licensed disposal company.
		H319	Causes serious eye irritation.			




Risk analysis (continued)

Chemical	Formula	Hazard statement(s)		Precautionary statement(s)		Environmental/Waste aspects
Hydrochloric acid 	HCl	H290	May be corrosive to metals.	P260	Do not breathe dust/ fume/ gas/ mist/ vapours/ spray.	May be harmful to aquatic organisms due to the shift of the pH. Do not empty into drains./ Offer surplus and non-recyclable solutions to a licensed disposal company.
		H314	Causes severe skin burns and eye damage.	P280	Wear eye protection/ face protection.	
				P303 + P361 + P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.	
H335	May cause respiratory irritation.	P304 + P340 + P310	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor.			
		P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.			
Sulfuric acid 	H ₂ SO ₄	H290	May be corrosive to metals.	P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.	Toxic to aquatic life./ Offer surplus and non-recyclable solutions to a licensed disposal company.
		H314	Causes severe skin burns and eye damage.	P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.	
				P310	Immediately call a POISON CENTER or doctor/ physician.	





Risk analysis (continued)

Chemical	Formula	Hazard statement(s)		Precautionary statement(s)		Environmental/Waste aspects
Ascorbic acid	$C_6H_8O_6$	ND	ND	ND	ND	Offer surplus and non-recyclable solutions to a licensed disposal company.
Ammonium heptamolybdate	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	ND	ND	ND	ND	Harmful to aquatic life./ Offer surplus and non-recyclable solutions to a licensed disposal company.
Potassium antimonyl tartrate 	$C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$	H302 + H332	Harmful if swallowed or if inhaled	P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.	Toxic to aquatic life with long lasting effects./ Offer surplus and non-recyclable solutions to a licensed disposal company.
				P273	Avoid release to the environment.	
				P301 + P312 + P330	IF SWALLOWED: Call a POISON CENTER or doctor/ physician if you feel unwell. Rinse mouth.	
		P304 + P340 + P312	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER or doctor/ physician if you feel unwell.			
		P391	Collect spillage.			
		P501	Dispose of contents/ container to an approved waste disposal plant.			


Risk analysis (continued)

Chemical	Formula	Hazard statement(s)		Precautionary statement(s)		Environmental/Waste aspects
Oxalic acid 	(COOH) ₂ ·2H ₂ O	H302 + H312	Harmful if swallowed or in contact with skin	P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.	Toxic to aquatic life with long lasting effects./ Offer surplus and non-recyclable solutions to a licensed disposal company.
				P301 + P312 + P330	IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell. Rinse mouth.	
		H318	Causes serious eye damage.	P305 + P351 + P338 +	P310 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor.	
Trisodium citrate dihydrate	Na ₃ C ₆ H ₅ O ₇ ·2H ₂ O	ND	ND	ND	ND	Offer surplus and non-recyclable solutions to a licensed disposal company.
Boric acid 	H ₃ BO ₃	H360FD	May damage fertility. May damage the unborn child.	P201	Obtain special instructions before use. P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.	Toxic to aquatic life./ Offer surplus and non-recyclable solutions to a licensed disposal company.
				P308 + P313	IF exposed or concerned: Get medical advice/ attention.	
Citric acid dehydrate 	C ₆ H ₈ O ₇ ·2H ₂ O	H319	Causes serious eye irritation.	P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.	Offer surplus and non-recyclable solutions to a licensed disposal company.


Risk analysis (continued)

Chemical	Formula	Hazard statement(s)		Precautionary statement(s)		Environmental/Waste aspects
Sodium hydroxide 	NaOH	H290	May be corrosive to metals.	P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.	Toxic to aquatic life./ Offer surplus and non-recyclable solutions to a licensed disposal company.
		H314	Causes severe skin burns and eye damage	P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.	
				P310	Immediately call a POISON CENTER or doctor/ physician.	
Sodium nitroprusside dihydrate 	Na ₂ Fe(CN) ₅ NO · 2H ₂ O	H301:	Toxic if swallowed.	P308 + P310:	IF exposed or concerned: immediately call a POISON CENTER or doctor/ physician.	Discharge into the environment must be avoided / Offer surplus and non-recyclable solutions to a licensed disposal company.
Sodium salt of dichloroisocyanuric acid  	C ₃ Cl ₂ N ₃ NaO ₃ · 2H ₂ O	H302	Harmful if swallowed.	P273	Avoid release to the environment.	Very toxic to aquatic life./ Offer surplus and non-recyclable solutions to a licensed disposal company.
		H319	Causes serious eye irritation.	P301 + P312 + P330	IF SWALLOWED: Call a POISON CENTER or doctor/ physician if you feel unwell. Rinse mouth.	
		H335	May cause respiratory irritation.	P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.	
		H410	Very toxic to aquatic life with long lasting effects.	P391	Collect spillage.	
P501	Dispose of contents/ container to an approved waste disposal plant.					


Risk analysis (continued)

Chemical	Formula	Hazard statement(s)		Precautionary statement(s)		Environmental/Waste aspects
Phenol 	C ₆ H ₅ OH	H301 + H311 + H331	Toxic if swallowed, in contact with skin or if inhaled	P260	Do not breathe dust/ fume/ gas/ mist/ vapours/ spray.	Toxic to aquatic life with long lasting effects./ Offer surplus and non-recyclable solutions to a licensed disposal company.
		H314	Causes severe skin burns and eye damage.	P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.	
		H341	Suspected of causing genetic defects.	P301 + P330 + P331 + P310	IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. Immediately call a POISON CENTER or doctor/ physician.	
		H373	May cause damage to organs through prolonged or repeated exposure.	P303 + P361 + P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.	
				P304 + P340 + P310	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER or doctor/ physician.	
H411	Toxic to aquatic life with long lasting effects.	P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.			

Risk analysis (continued)

Chemical	Formula	Hazard statement(s)		Precautionary statement(s)		Environmental/Waste aspects
Potassium peroxodisulfate 	K ₂ S ₂ O ₈	H272	May intensify fire; oxidiser.	P220	Keep/Store away from clothing/combustible materials.	Harmful to aquatic life./ Offer surplus and non-recyclable solutions to a licensed disposal company.
		H302	Harmful if swallowed.	P261	Avoid breathing dust.	
		H315	Causes skin irritation.	P280	Wear protective gloves.	
		H317	May cause an allergic skin reaction.	P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.	
		H319	Causes serious eye irritation.			
		H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.	P342 + P311	If experiencing respiratory symptoms: Call a POISON CENTER or doctor/ physician.	
H335	May cause respiratory irritation.					
Glycine	H ₂ NCH ₂ COOH	ND	ND	ND	ND	Offer surplus and non-recyclable solutions to a licensed disposal company.
Potassium dihydrogen phosphate	(KH ₂ PO ₄)	ND	ND	ND	ND	Offer surplus and non-recyclable solutions to a licensed disposal company.

Risk analysis (continued)

Chemical	Formula	Hazard statement(s)		Precautionary statement(s)		Environmental/Waste aspects
Mercury(II) chloride 	HgCl ₂	H300	Fatal if swallowed.	P260	Do not breathe dust/ fume/ gas/ mist/ vapours/ spray.	Very toxic to aquatic life with long lasting effects./ Offer surplus and non-recyclable solutions to a licensed disposal company.
		H314	Causes severe skin burns and eye damage.	P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.	
		H341	Suspected of causing genetic defects.	P301 + P330 + P331 + P310	IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. Immediately call a POISON CENTER/doctor.	
		H361f	Suspected of damaging fertility.	P303 + P361 + P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.	
		H372	Causes damage to organs through prolonged or repeated exposure.	P304 + P340 + P310	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor.	
		H410	Very toxic to aquatic life with long lasting effects.	P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.	

Chemicals

Before starting preparing chemicals, you must read the risk analysis to be aware of the Hazard and Precautionary statements as well as the Environmental/Waste aspects

Nitrate

25% Stock Buffer:

250g ammonium chloride (NH_4Cl) is dissolved in ~800mL MQ-water and 25mL concentrated ammonium hydroxide (25%) is added. Dilute to 1000mL with MQ-water.

2.5% Work Buffer:

100 mL of **Stock Buffer** is diluted to 1000mL with MQ-water.

Wash Buffer:

20 mL of **2.5% Work Buffer** is diluted to 1000mL with MQ-water.

Reagent A, Sulphanilamide:

1.675g sulphanilamide ($\text{H}_2\text{NC}_6\text{H}_4\text{SO}_2\text{NH}_2$) is dissolved in a mix of 16.75mL HCl (36%) and 150 mL MQ-water. **After cooling** the solution is diluted to 250mL with MQ-water. Stable for several months if stored at room temperature.

Reagent B, Naphtylamine:

0.1675g (1-Naphthyl) ethylenediamine dihydrochloride ($\text{C}_{10}\text{H}_7\text{NHCH}_2\text{CH}_2\text{NH}_2 \cdot 2\text{HCl}$) is dissolved in MQ-water and diluted to 250mL with MQ-water. Stable for 1-2 months if stored in refrigerator or until it becomes brown.

Phosphate

3 M Sulfuric acid:

COOLING! 41.75 mL concentrated sulfuric acid (H_2SO_4) (96%) is added very slowly under constant mixing and cooling to ~175 mL MQ-water. **After cooling** the solution is diluted to 250mL with MQ-water.

Reagent A, Ascorbic acid:

16.5g ascorbic acid ($\text{C}_6\text{H}_8\text{O}_6$) is dissolved in 125 mL MQ-water and diluted to 250 mL with **3 M Sulfuric acid** (H_2SO_4). Store in refrigerator, stable as long as it is colorless.

Reagent B, Ammonium heptamolybdate:

4.175g ammonium heptamolybdate ($(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$) and 0.1675 g potassium antimonyl tartrate ($\text{C}_8\text{H}_4\text{K}_2\text{O}_{12}\text{Sb}_2 \cdot 3\text{H}_2\text{O}$) are dissolved in 75 mL MQ-water and diluted to 250mL with **3 M Sulfuric acid** (H_2SO_4). Stable for several months if stored at room temperature.

Chemicals (continued)

Before starting preparing chemicals, you must read the risk analysis to be aware of the Hazard and Precautionary statements as well as the Environmental/Waste aspects

Total Dissolved Nitrogen (TN) and Phosphate (TP)

In addition to all chemicals used in the nitrate and phosphate analysis you also have to prepare;

1 M Sodium hydroxide:

COOLING! 40g sodium hydroxide (NaOH) is dissolved in MQ-water and diluted to 1000 mL with MQ-water.

Oxidation reagent (OR):

It is important that the potassium peroxodisulfate ($K_2S_2O_8$) used has a low nitrogen content. Merck #5092 is suitable with a maximum content of 0.0001% N.

50 g potassium peroxodisulfate ($K_2S_2O_8$) and 30 g boric acid (H_3BO_3) are dissolved in **425 mL 1 M sodium hydroxide** (NaOH) and diluted to 1000 mL with MQ-water. Stable for several months if stored in dark at room temperature.

TP/TN standard stock solutions (TPN SSS)

Dry 2g of glycine (H_2NCH_2COOH) and 1g of potassium dihydrogen phosphate (KH_2PO_4) at 70°C until constant weight.

1.126g of glycine (H_2NCH_2COOH) and 0.34023g of potassium dihydrogen phosphate (KH_2PO_4) are dissolved in MQ-water and diluted to 250 mL with MQ-water. This gives a concentration of 10 mM P and 60 mM N in TPN SSS

Stored in tightly capped dark bottle in refrigerator with 2-3 drops of saturated mercury(II) chloride ($HgCl_2$). Since mercury(II) chloride ($HgCl_2$) is very toxic it is better to prepare the standard solutions fresh when you are performing the analysis.

Working standard solution for TPN (WSS)

1 mL of TPN SSS is added to 100 mL MQ-water → **D** (100 μ M P & 600 μ M N)

WSS	Dilution volumes		[Phosphate]	[Nitrogen]
I	10 mL D	190 mL MQ-water	5 μ M	30 μ M
II	6 mL D	194 mL MQ-water	3 μ M	18 μ M
III	2 mL D	198 mL MQ-water	1 μ M	6 μ M

Chemicals (continued)

Before starting preparing chemicals, you must read the risk analysis to be aware of the Hazard and Precautionary statements as well as the Environmental/Waste aspects

Silicate

All reagents should be prepared and stored in plastic bottles

Reagent A, Molybdate:

COOLING! 24.75g ammonium heptamolybdate ((NH₄)₆Mo₇O₂₄·4H₂O) is dissolved in 175 mL MQ-water. To this solution, 50 mL of concentrated sulfuric acid (H₂SO₄) (96%) is added very slowly under constant mixing and cooling. **After cooling** the solution is diluted to 250mL with MQ-water. Stable for several months if stored at room temperature.

Reagent B, Oxalic acid:

90g oxalic acid ((COOH)₂·2H₂O) is dissolved in MQ-water and diluted to 1000 mL with MQ-water. Stable indefinitely if stored at room temperature.

Reagent C, Ascorbic acid:

17g ascorbic acid (C₆H₈O₆) is dissolved in MQ-water and diluted to 1000 mL with MQ-water. Store in refrigerator, stable for a couple of weeks.

Ammonium

Citrate buffer solution:

67g trisodium citrate dihydrate (Na₃C₆H₅O₇·2H₂O), 34g Boric acid (H₃BO₃), 19g Citric acid dihydrate (C₆H₈O₇·2H₂O) and 30g Sodium hydroxide (NaOH) are dissolved in MQ-water and diluted to 1000 mL with MQ-water. Stable for several months if stored at room temperature in a tightly closed bottle.

Reagent A, Phenol- nitroprusside:

8.75g Phenol (C₆H₅OH) and 0.1g Sodium nitroprusside dihydrate (Na₂Fe(CN)₅NO·2H₂O) are dissolved in MQ-water and diluted to 250 mL with MQ-water. Store in refrigerator, stable for several months.

Reagent B, Hypochlorite:

1g sodium salt of dichloroisocyanuric acid (C₃Cl₂N₃NaO₃ · 2H₂O) and 3.75g Sodium hydroxide (NaOH) are dissolved in MQ-water and diluted to 250 mL with MQ-water. Store in refrigerator, stable for several weeks.

Nitrate Analysis

Reagent A and B react with nitrite NO_2^- and forms a red coloured complex. The intensity of the colour can be measured with a spectrophotometer. To analyze nitrate NO_3^- we first have to reduce all NO_3^- to NO_2^- . This is done by passing the sample through a cadmium reduction column.

- Mark all sample-cups (Do not forget number of funnel)
Blank, standard and samples for each funnel

The **Wash buffer** should be poured into the funnels before use. Flush at least **2 whole funnels**.

Let it run through the funnels. **Never let the funnels go dry.**

- Fill 3 sample-cups with **30 mL** of Blank (MQ-water)
- Fill 3 sample-cups with **30 mL** of standard (1:1000)
- Fill 3 sample-cups with **30 mL** in each from each sample

- Add **1 mL** of **Work buffer** to all sample-cups. **VERY IMPORTANT!**
- Pour the sample in the funnel, discard the first **15 mL** into the waste-cup and collect the remaining **15 mL** into the sample-cup.
- Add **0.5 mL** of **Reagent A** to all sample-cups and mix.
- Wait **2-8 minutes**
- Add **0.5 mL** of **Reagent B** to all sample-cups and mix.
- Measure at **543 nm** after at least **8 minutes** stable for quite long

NOTICE: When you are done with the funnels. Pour some Wash buffer into the funnels to rinse them, at least 1 whole funnel, and let some of it stay in the funnel.

Calculate the concentration

Standard concentration [Std]= 1.613 μM NO_3^-

$$K = [\text{Std}] / (\text{Abs}_{\text{STD}} - \text{Abs}_{\text{BLANK}})$$

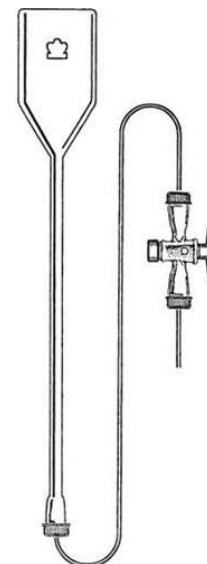
$$\text{Concentration} = K \times \text{Abs}_{\text{SAMPLE}}$$

Reagent A: Sulfanilamide

Reagent B: Naphtylamine

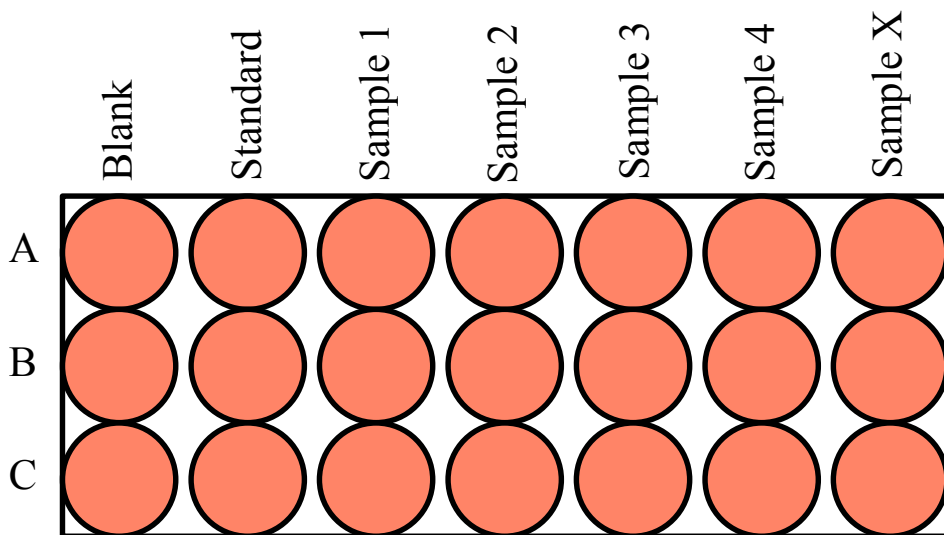
Work buffer: 2.5 % NH_4Cl

Wash Buffer: 0.05 % NH_4Cl



Phosphate Analysis

- Mark all test-tubes, 3 Blank, 3 standard and 3 for each sample
- Fill 3 test-tubes with **15 mL** of Blank (MQ-water)
- Fill 3 test-tubes with **15 mL** of standard (1:1000)
- Fill 3 test-tubes with **15 mL** in each from each sample
- Add **0.5 mL** of **Reagent A** to all tubes (**A,B,C**), put on corks and mix
- Add **0.5 mL** of **Reagent B** to **2 out of 3 tubes (B,C)**
- Measure at **882 nm** after **5 minutes** (Do not wait much longer)



Calculate the concentration

Standard Concentration [Std] = $1.052 \mu\text{M PO}_4^{3-}$

$$K = [\text{Std}] / (\text{Abs}_{\text{STD}} - \text{Abs}_{\text{BLANK}})$$

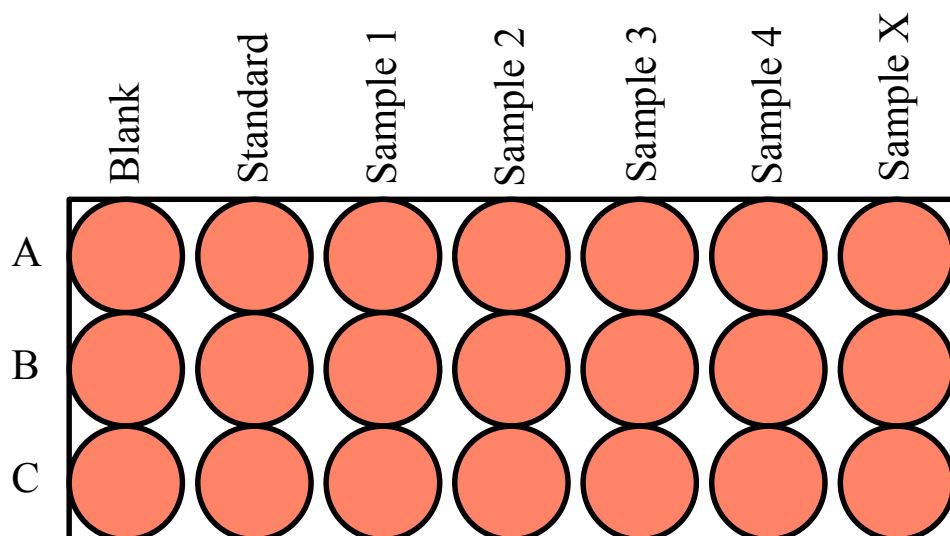
$$[\text{PO}_4^{3-}] = K \times (\text{Abs}_{\text{SAMPLE}} - \text{Abs}_{\text{SAMPLE BLANK}})$$

Reagent A: Ascorbic Acid

Reagent B: Molybdate

Silicate Analysis

- Mark the test-tubes, 3 Blank, 3 Standard and 3 for each sample
- Fill 3 test-tubes with **25 mL** of Blank (MQ-water)
- Fill 3 test-tubes with **25 mL** of standard (1:1000)
- Fill 3 test-tubes with **25 mL** in each from each sample
- Add **0.75 mL** of **Reagent A** to all samples (**A,B,C**), put the corks on the tubes in the first row (**A**).
- Wait **20 minutes**
- Add **0.75 mL** of **Reagent B** to the rest of the tubes (**B,C**)
- Add **0.75 mL** of **Reagent C** to the rest of the tubes (**B,C**)
- Put on corks to all and mix
- Wait **30 minutes**
- Measure at **810 nm**



Calculate the concentration

Standard Concentration [Std] = 1.664 μ M

$$K = [\text{Std}] / (\text{Abs}_{\text{STD}} - \text{Abs}_{\text{BLANK}})$$

$$\text{Concentration} = K \times (\text{Abs}_{\text{SAMPLE}} - \text{Abs}_{\text{SAMPLE BLANK}})$$

Reagent A: Molybdate

Reagent B: Oxalic Acid

Reagent C: Ascorbic Acid

Ammonium Analysis

If possible, the ammonium content should be measured immediately after sampling.

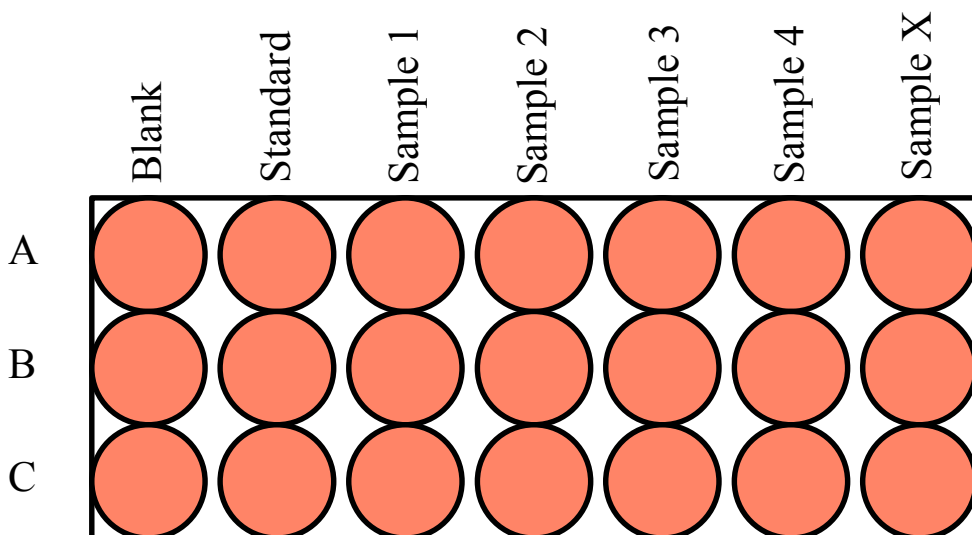
- Mark all tubes, 2 blank, 2 standard and 3 for each sample
- Fill 2 test-tubes with **12 mL** of Blank (MQ-water)
- Fill 2 test-tubes with **12 mL** of standard (1:1000)
- Fill 3 test-tubes with **12 mL** in each from each sample

Notice: Must work in hood/ventilated area, phenol is highly toxic.

- Add **1 mL** of **Citrate buffer** to all tubes (A,B,C)
- Add **0.5 mL** of **Reagent A** to all tubes (A,B,C), put the corks on the tubes in the first row (A).
- Add **0.5 mL** of **Reagent B** to the rest of the tubes (B,C)

Leave overnight in dark (minimum of 8 h in the dark)

Measure the next day at **630 nm**



Calculate the concentration

Standard Concentration [Std] = 7.139 μ M

$$K = [\text{Std}] / (\text{Abs}_{\text{STD}} - \text{Abs}_{\text{BLANK}})$$

$$\text{Concentration} = K \times (\text{Abs}_{\text{SAMPLE}} - \text{Abs}_{\text{SAMPLE BLANK}})$$

Citrate Buffer solution

Reagent A: Phenol-nitroprusside

Reagent B: Hypochlorite

Total Dissolved Nitrogen (TN) and Phosphate (TP)

Pre-analysis

The sample is filtered through precombusted A/E or GF/C glassfiber filters with acid cleaned filtration setup

- **3** oxidation bottles (red cap, 50 mL) is filled with **30 mL of sample**
- **3** oxidation bottles (red cap, 50 mL) is filled with **30 mL WSS, for each WSS**
- **3** oxidation bottles (red cap, 50 mL) is filled with **30 mL of MQ-water** to create **standard blanks**
- Add 4 mL oxidation reagent (OR) to each bottle (sample, standard solution and standard blank)
- 2 oxidation bottles (red cap, 50 mL) is filled with 4 mL OR to create **sample blanks**

- Close the caps tightly
- All bottles are autoclaved for **30 minutes** at 121°C (You must get the key for the autoclave to be able to change the time setting)
- Let the autoclave cool down to room temperature before opening
- Change the time setting back to 20 minutes when done with the autoclave

- After autoclaving, gently swirl the bottles to dissolve any eventual precipitate
- After this step the bottles can be stored for up to 3 months before analyses if the filtration is done at sampling

Total Dissolved Nitrogen (TN) and Phosphate (TP) (continued)

Analyses

Volume in oxidation bottles is adjusted to 40 mL with MQ-water

Total Dissolved Nitrogen (TN)

Follow protocol for nitrogen analysis

- Mark Erlenmeyer flasks the same way as oxidation bottles
- A subsample is taken from each oxidation bottle
- **10 ml subsample** is added to a graduated measuring cylinder
- **1 mL working buffer** is added to measuring cylinder
- **MQ-water** is added to reach **50 mL** total volume in measuring cylinder
- Content in measuring cylinder is poured into Erlenmeyer flasks
- Continue by following protocol for nitrogen analysis

Total Dissolved Phosphate (TP)

The remaining 30 mL of sample/standard/blanks in the oxidation bottles is used to determine total dissolved phosphate by follow the protocol for phosphate analysis with slight modifications

- Add 0.7 mL of each reagent A (Ascorbic Acid) and B (Molybdate)
- Reagent B (Molybdate) is added two minutes after reagent A (Ascorbic Acid)