PRACTICAL NO. 1 AND 2:

WATER QUALITY ANALYSIS

Water quality analysis is important for many purposes. The methods given in this booklet are sensitive methods to analyze constituents in minute concentrations in inland water bodies. Therefore follow all the instructions given very carefully to obtain the accurate results...

Following methods are given in this booklet:

(1) Determination of Dissolved Oxygen by Winkler's Method.
(2) Biological Oxygen demand
(3) Determination of rate of primary productivity
(4) Determination of Chloraphyll-a concentration in water
(5) Determination of alkalinity
(6)Determination of water Hardness
(7) Determination of Orthophosphate
(8) Determination of Nitrate
(9) Determination of Ammonia
(10) Determination of Dissolved sulphide

1. DETERMINATION OF DISSOLVED OXYGEN IN WATER
(Winkler's methods)

Reagents: **Manganese-II-sulphate solution:** **(1)**

*** 100 g $MnSO_4$ * $4H_2O$ are dissol_ved in 200 ml of distilled water.**

Winkler's reagent: **(2)**

*** 100 g NaOH + 50 g KJ are dissolved in 200 ml of distilled water.**

Phosphoric acid H_3PO_4 (85%, s.g.1.17). **(3)**

{Instead of H_3PO_4 one may use also H_2SO_4 (50%)}.

Sodiumthiosulphate (titrant): 0.01 N $Na_2S_2O_3$ **(4)**

Starch indicator: **(5)**

*** Dissolved 1g of starch in 100 ml of distilled water, heat gently.**

Procedure:

1. on the lake:

- Fill the water sample - carefully avoiding air bubbles- into a glass bottle of exactly known volume (between 100 and 150 ml), equipped with a ground-in stopper;
- Add below the surface 0.5 ml of manganese solution and 0.5 ml of Winkler's reagent;
- Close the bottle and shake it vigorously.

Chemical reactions:

OH^- - ions originating from Winkler's reagent react with the Mn^{2+}- ions, forming a white precipitate:

$$Mn^{2+} + 2\ OH^- \longrightarrow Mn(OH)_2$$

Oxygen dissolved in the water reacts with this precipitate, yielding brown manganese-IV-hydroxyde:

$$Mn(OH)_2 + 1/2O_2 \longrightarrow MnO(OH)_2$$

2. In the laboratory:

- Allow the brown $MnO(OH)_2$ to settle;
- decant a few ml of the supernatant;
- add 3 ml of H_3PO_4 (or1 ml of concentrated H_2SO_4 instead);
- shake the bottle to dissolve the precipitate;
- then titrate the solution **immediately** with thiosulphate.

Chemical reactions:

Mn^{4+}-ions are reduced to Mn^{2+}-ions in the presence of acid, hereby oxidizing the iodide from Winkler's reagent to iodine:

$$MnO(OH)_2 + 4H^+ + 2\ I^- \longrightarrow I_2 + 3H_2O + Mn^{2+}$$

The liberated molar amount of iodine corresponds to the amount of oxygen originally present. It is then determined by titration with thiosulphate:

$$I_2 + 2\ S_2O_3^{2-} \longrightarrow 2I^- + S_4O_6^{2-}$$

In this titration thiosulphate is oxidized to tetrathionate, whereas the iodine is reduced to iodide. As an indicator for Winkler titration, starch is used that forms a blue iodine-starch complex. The amylose of the starch consists of chains of molecules curled up to form channel-like hollow spaces in which chains of iodine are deposited, such that by interaction the blue colour appears. When all iodine molecules are reduced to iodide, the blue colour disappears

.

Titration:

* Place the whole content of the bottle into the an Erlenmeyer flask and titrate with 0.01 N $Na_2S_2O_3$ until it has a faint yellow colour.
* Then add 1 ml of starch indicator. The solution becomes blue.
* Titrate now until the blue colour has disappeared.

Calculation:

From the above equations it is obvious that

2 moles of $S_2O_3^{2-}$ react with $1/2O_2$.

Therefore 1 mole of $S_2O_3^{2-}$ reacts with $1/4O_2$. This corresponds to 8 g O_2 (molecular weight of O_2 being 32).

1 ml of thiosulphate solution contains 0.01 mmol of $S_2O_3^{2-}$, corresponding to 0.08 mg O_2. To find the amount of oxygen dissolved in 1 l of lake water, multiply by 1000 and divide by the bottle volume corrected for the ml of reagent added:

$$\frac{\text{ml 0.01 N } Na_2S_2O_{4\ \text{cunsumed}} * 0.08*1000}{ml_{\text{bottle volume}} - ml_{\text{reagent added}}}$$

Possible interferences:

Oxydizing and reducing substances (organic compounds; NO_2^-; Mn^{4+}; Mn^{7+}; Cl_2; S^-; SO_3^{2-}; Fe^{2+}; Fe^{3+}).

2. DETERMINATION OF BOD

The **B**iochemical **O**xygen **D**emand is a measure for the degradability of organic material in water by microorganisms.

$$O_2 + \text{organic carbon} \longrightarrow CO_2$$

The reaction is ferformed at 20^{0}C and in the dark (to exclude photosynthetic production of oxygen). The *demand*, the oxygen consumed by bacteria for the degration within a certain period of time, is obtained as the difference between the initial content of oxygen and the content measured after 2 or 5 days.

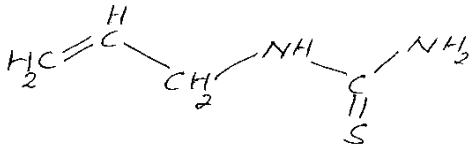
Determination:
The content of oxygen is measured according to Winkler's method (seep.7). Waters with low amount of organic material can be used undiluted, but should be enriched with oxygen. The O_2-content at measuring time should be at least 2 mg O_2 /l. If it is less, the water has to be diluted, either with clean river water or with an artificial medium. (The dilution water must contain nutrients for the bacteria.)
Composition of an artificial medium:

chemical	mg/l
K_2HPO_4	21.75
KH_2PO_4	8.5
$Na_2HPO_4 \times 2H_2O$	33.4

NH_4Cl	1.7
$MgSO_4$	22.5
$CaCl_2 \times 2\ H_2O$	36.5
$FeCl_3$	0.25

The artificial medium is saturated with oxygen (either by vigorously shaking or by bubbling with an aquarium pump).The oxygen content of the dilution water has to be determined always at the same times as that of the samples.

Sources of error	**Remedial measures**
* Degradation of organic material may also occur via denitrification ($NO_3 \longrightarrow NO_2 \longrightarrow N_2O \longrightarrow N_2$)	Addition of 5 drops of 5 % allylthiourea-solution / 100 ml of sample



➢ Low pH	Neutralization (pH of 7-8 should be adjusted)
➢ Presence of active chlorine	Equivalent addition of 0.01 M $Na_2S_2O_3$
➢ Low BOD	
1. Too few microorganisms present	Inoculation with 0.3 ml / l of sedimented domestic sewage or 2 ml / l of biologically purified sewage or 5 – 10 ml / l of river water
2. Little organic material present, 3. Or slow degradation process	

Simultaneous determination of COD yields the amount of organic material present. The relation COD / BOD_5 is a rough indication for the kind of process of biochemical degradation:

COD = 4 slow degradation (e.g.lignin)

BOD_5

COD =1, 5 quick degradation (e.g.domestic waste)
BOD_5

Calculation: the reaction must follow first order kinetics.

Calculation:

$$\frac{d[O_2]}{dt} = \frac{d[org.C]}{dt} = -k[org.C]$$

$$\int_{org.C_0}^{org.C_t} \frac{d[org.C]}{org.C} = -k\int_0^t dt$$

$$\ln\frac{[orgC]_t}{[orgC]_0} = -kt = 2,3\log\frac{[orgC]_t}{[orgC]_0}$$

$[org.C]_{0,t}$: organic carbon at time 0 or t respectivly.

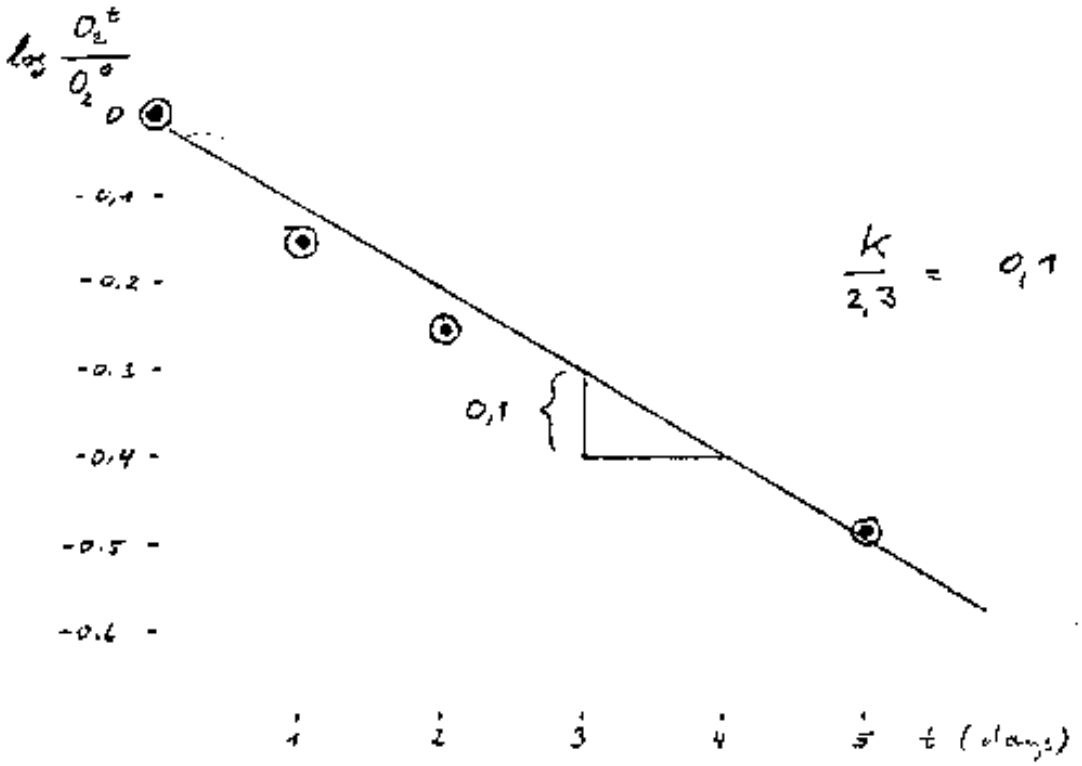
$[org.C]_{0,1}$: organic carbon at time 0 or t respectively.

A plot of 2, 3 $\log\frac{O_2{}^t\ mg/l}{O_2{}^o mg/l}$ Versus time allow to determine k.

The smaller the value for k the faster the degradation is performed.

Example:

time	**mg O_2/l**	**log $\frac{O_2{}^t\ mg/l}{O_2{}^o mg/l}$**
0	8.96	
1	6.32	-0.15
2	4.96	-0.256
5	2.93	-0.485



For a diluted sample the BOD is calculated according to the following formula:

$$\{(A_0 - A_1) - [(B_0 - B_1) - (B_0 - B_1)\ 1/X]\}\ X$$

X = dilution factor

A_0 = BOD at time 0

A_1 = BOD at time 1

B_0 = BOD of the dilution at time 0

B_1 = BOD of the dilution water at time 1

3 DETERMINATION OF RATE OF PRIMARY PRODUCTIVITY

Determination of rate of primary productivity in a column of water at different depths , using light and dark bottle method. This should be measured above the compensation point, in the euphotic region of the water body. (Use your Secchi Disk transparency values to calculate the euphotic limit.

Theory: Phytoplankton productivity can be summarized in the universal equation:

$6\ CO_2\ +\ 12\ H_2O$ Light pigment receptor $C_6H_{12}O_6\ + 6H_2O + 6O_2$

Procedure: light and dark bottle method:

The dark bottle should be covered in black plastic or cloth rather than painted black. The following should be estimated by Winkler method.

(a) The initial amount of O_2 in a sample at the desired depth.

(b) The amount of Oxygen in a sample entrapped in a transparent stoppered bottle and incubated in the same depth.

(c) The amount of Oxygen in another sample from the same depth incubated in the same manner but in a dark bottle into which no light can penetrate.

The same procedure is repeated at different depths ranging from the surface to the lower limit of the euphotic zone. After a given period of time (about 5 hrs) during day, the Oxygen content in both the light and dark bottles are estimated. This can be repeated for a few more period of in order to cover the whole day.

Calculations:

$$\mathbf{X = \frac{8.0\ X\ C_b\ V_b}{Va(V_{f\text{-}2.0)}V_f}}$$

X = Oxygen concentration in water mg/l

V_b= Volume of Sodium Thio Sulphate

V_a = Volume taken for titration (ml)

V_f = Bottle volume, with stopper in place (ml)

If the whole contents of the bottle are titrated, then

$$= \frac{8.0 \times C_b V_b}{(V_f - 2.0)} \text{ ml}$$

The amount of Oxygen used in respiration is the difference between the O_2 contents (Xi) in the initial bottle and the X_D in the dark bottle.

$$= (X_i - X_D) \text{ mg } O_2 \text{ } l^{-1} h^{-1}$$

Net primary production is the difference between the O_2 contents in the light bottle (X_L) & the initial bottle; $(X_L - X_i)$ mg O_2 L^{-1} h^{-1}

Gross primary production is the sum of net primary production and respiration which is equal to the difference between the O_2 content in the light bottle and dark bottle.

$$(X_L - X_d) \text{ mg } O_{2\,L}{}^{-1} h^{-1}$$

During photosynthesis six O_2 molecules are used to produce one molecule of $C_6 H_{12} O_6$, $6O_2$ & $C_6 H_{12} O_6$

Therefore 32 mg O_2 = 12 mg C

1mg O_2 = 12/32 mg C

Therefore primary production = 12/32 X $(X_i - X_D)$ mg C $l^{-1} h^{-1}$

Therefore primary production = 12/32 $^{(X}{}_L - X_D)$ mg Cl^{-1} h^{-1}

Assumptions made:

The rate of respiration in the dark bottle is equal to that in the light bottle (respiration as measured in this technique represent community respiration).

Q: What precautions should you take in the above estimation?

4. DETERMINATION OF CHLOROPHYLL-A

Pigment concentration:

Measure of the concentration of photosynthetic pigments can be used to estimate the biomass of phytoplanktonic populations. The methodology for measuring pigments is relatively direct and accurate and can be performed both on algae separated from the water as well as in vivo.

Degradation products of the chlorophyll phaeophytins are structurally similar to chlorophyll except that the Mg is lost from the ring structure. Pheophytins also absorb the light at some wave lengths. However pheophytin concentrations can be estimated separately on the same sample for which Chlorophyll is determined. Thus pigment analysis can yield sensitive approximation of algal biomass.

Sample preparation:

Water samples should be filtered through either membrane or glass fiber filters. Pore sizes of the filter must be sufficiently small to retain all algae of the smallest dimensions. If membrane filters are used a pore size of 0.8m (e.g. Millipore AA) is recommended. If glass fiber filters are employed, a pore size of 0.5 to 0.7m should be used. The pressure differential during filtration should not exceed 0.5 atm, to minimize damage to delicate organisms. The amount of sample required for filtration depends on the phytoplankton concentration. Filtration should be done as rapidly as possible avoiding exposure to bright light or temperature. After filtration, filters should be used immediately in extraction or stored in small dark desicator & immediately frozen.

Procedure:

(1) Filter 250ml sample through glass fibre course (G F C) filter and place the filter in 3ml absolute methanol in tubes covered with Aluminium foil. Keep in the dark.
(2) After an hour of extraction, crush against the sides of the glass vessel using a glass roller.

(3) Then rinse twice in methanol and filter the final solution through a GFC filters to obtain a colour solution.

(4) Make final extraction into 10ml with methanol.

(5) Record the extinction at length of 665nm and 750 nm in the spectrophotometer.

(6) Acidify with 5 drops of 0.1m HCl

(7) Neutralize with $MgCO_3$ powder, filter & read the extinction at 665 nm and 750nm.
Calculations:

Acid factor for Chlorophyll a in methanol ® = 1.5

αc = absorption coefficient for Chl a in methanol = 74.51 $g^{-1}cm^{-1}$

$$\textbf{Chl a mg/l} = (A - B) - (C - D) \times \frac{R}{R-1} \times \frac{V}{1 \times v} \times \frac{1}{\alpha c}$$

A= reading of Ch a, b & c at 665nm before acidifying.

B= reading of Ch c and phaeophytin at 750nm before acidifying.

C= reading of Chc and phaeophytin at 665nm after acidifying

D= reading of Ch a and phaeophytin at 750 nm

V= Volume of filtered sample (250 ml)

v =Volume of standardised methanol (10 cm^3)
1= Path length (1 cm)

Lorenzen, C J (1967): Determination of Chlorophyll a phaopigments Spectrophotometric equations. Limnol. Oceanogr. 12, 343 - 346

5 DETERMINATION OF ALKALINITY

Determine following in the given water samples, E and F

a. pH, conductivity

a. Total Alkalinity

b. Phenolphthalein alkalinity

c. Carbonate alkalinity

d. Total CO_2 by

i. Calculations based on pH and Conductivity of the original sample.

ii. Using acidimetric indicator end point.

e. Acidity

Alkalinity of water refers to the quantity and kinds of compounds present which collectively shift the pH to alkaline side of neutrality. The property of alkalinity is imparted by presence of HCO^-_3, CO_3^{2-} and less frequently by borate, silica and phosphates.

Students are advised to read IBP Handbook No .8 Methods for physical and chemical analysis of fresh water. H.l.Golterman et al 1978. Section 9.7 and 9.8 for further details.

Principle

The amount of $CO_3^{2-} + OH^-$ is determined by titration with acid to pH 8.3 , the end point being detected with phenolphthalein.

The amount of HCO_3^- is determined by further titration with acid to an end point pH between 4.2 and 5.4 with methyl orange or mixed indicator as end point indicator.

There are at least 4 qualities commonly reported.

Quantity point	**Symbol**	**Compound**	**End pH**
1. Phenolphthalein Alkalinity	P A	$OH^- + CO_3^{2-}$	8.3
2. Total Alkalinity	TA	$OH^- + CO_3^{2-} + HCO_3^-$	4.2 to 5.4
3. Carbonate Alkalinity	CA	$CO_3^{2-} + HCO_3^-$	
4. Total Alkalinity	TC	$CO_3^{2-} + HCO_3^- + CO_2$	

Reagents

A. Hcl 0.100 M(100 mmol l^{-1}) or 0.050 M

B. Phenolphthalein indicator

Dissolve 0.5g of Phenolphthalein in 50 ml of 95% ethanol, and add 50 ml of water. Add dilute (e.g. 0.05M) CO_2 free NaOH solution drop wise. Until the solution turns faintly pink.

C. Mixed indicator

Dissolve 0.02 of Methyl red and 0.08g of Bromocresol green in about 100ml. of 85% ethanol. This indicator is suitable over the pH range 4.6-5.2.

D. Methyl orange indicator 0.05%

Dissolved 0.05g of methyl orange in about 100ml of water this indicator is suitable for equivalence points below pH 4.6.

Procedure

Mix 100ml of sample / samples with 2 drops of Phenolphthalein indicator(B) in a conical titration beaker. If the solution remains colourless PA =0 and the total alkalinity is determined as described below. If the solution turn red , determine the PA by titrating with standard acid colour practically disappears. (The very faint colour at the end point is best estimated by comparing with a standard end point in pH=8.3 Buffer(E).

Add 3 drops per 100 ml of sample either the mixed indicator or Methyl orange and determine the TA by continuing the titration to the second equivalence point. Read the burette when the first tendency to change colour appears. With this approximate value of TA, and with the initial pH with conductivity of the water, the approximate value of total CO_2 may be found from table 3.4 and 3.5 (provided).

Add 3 drops of mixed indicator or Methyl orange to 100 ml buffer with the same pH as the calculated end point and continue the titration until the same colour is observed in the sample as the buffer. Loss of CO_2 minimized.

Calculations

A. Phenolphthalein alkalinity = $(OH) + (1/2\ CO_3^{2-})$
Let PA = Phenolphthalein alkalinity (Concentration) (m mol l^{-1})

Then PA;

$$\mathbf{PA = \frac{C_a * V_{8.3}}{V_s}}$$

Where C_a = Concentration of acid (m mol l^{-1})
$V_{8.3}$ = Volume of acid used to titrate to pH 8.3 (ml)
V_s = Volume of sample(ml)

B. Total Alkalinity = $(OH^-) + (1/2\ CO_3^{2-}) + (HCO_3^-)$
Let TA = Total Alkalinity (concentration) (m mol l^{-1})

Then TA ;

$$\mathbf{TA = \frac{C_a * V_{4\text{-}6}}{V_s}}$$

Where C_a = Concentration of acid (m mol l^{-1})
$V_{4\text{-}6}$ = Total volume of acid used to titrate the second end point (pH 4-6) (ml)
V_s = Volume of sample (ml)

C. Bicarbonate = (HCO_3^-)
Let *X* = concentration of HCO_3^- (m mol l^{-1})

Then X;

$$\mathbf{X = (TA- PA) = \frac{V_{8.3} - V_{4\text{-}6}}{V_S}}$$

D. Carbonate Alkalinity
(Use values for B from table 3.4 provided).

Carbonate Alkalinity = (½ CO_3^{2-}) + (HCO_3^-)

Let CA = Carbonate alkalinity (concentration) (m mol l^{-1})
Then CA;

$$\mathbf{CA = TA- 0.01\ B}$$

Where B is a factor with the dimensions of concentration, depend primarily on (OH^-) and to smaller extent on ionic strength. (Estimated from conductivity).

E. Total CO_2
Total CO_2= (1/2 CO_3^{2-}) + (HCO_3^-) + (CO_2)

Let TC = Total CO_2 concentration (m mol l^-)

Then TC;

$$\mathbf{TC = \alpha\ CA.}$$

(The factor α is obtained from table 3.5 provided)

ii.Determination of total CO_2 by acidimetric indicator

Procedure
Add several drops of phenolphthalein to 100 ml of the sample. Then titrate with 0.05 M NaOH until the solution has a faint pink colour if the solution turns pink when the indicator is added, titrate with 0.05M HCl instead. Then add the mixed indicator and titrate with 0.05M HCl solution.

Reagents

A. HCl 0.1 M or 0.05M
B. NaOH 0.1 M or 0.05M
C. Mixed indicator: Dissolve 0.02g of of methyl red and 0.08g of Bromocrisol green in about 100 ml of 95% ethanol. This indicator i8s suitable over the pH range 4.6- 5.2

Calculation:

Let X = Concentration of CO_2 in the water sample (m mol l^{-1})
Then X

$$\mathbf{X = C_a \frac{(V_2 - V_1)}{V_s}}$$

Where C_a = Concentration of Hcl(m mol l^{-1})
$V_2 - V_1$ = difference in volume of Hcl at the second and first equivalence points(ml)
V_s = Volume of sample (ml)

(f). **Determination of acidity in sample " G "**

Principle: The sample is titrated with dilute $Ba(OH)_2$ to pH 8.6(many water samples contain no titratable acid).

Reagents:

A. 1/2$Ba(OH)_2$, o.o1M = 10 m mol l^{-1}.Dissolve 3.2 g of $Ba(OH)_2$. 8 H_2O in 2 litres of boiled H_2O. Store the solution in a container which is connected directly to a buret. Allow any $BaCO_3$ precipitate to be settle before transferring the solution to the burette. Standardize with HCl.
B. Mixed indicator.
Mix 10ml of 0.1% thymol blue(in 50% alcohol) with 30 ml of 0.1 phenolphthalein(in 50% alcohol) At pH 8.6 the colour changes from yellow(acid) to red violet(alkaline).

Procedure: Put a suitable accurately measured 50 ml of sample in a titration flask, add one drop of indicator, and titrate with Ba $(OH)_2$ solution A to the end point.

Calculation:

Let X = acidity of the water sample (m mol l^{-1})
Then X;

$$\mathbf{X = \frac{C_b * V_b}{V_s}}$$

Where C_b = Concentration of Ba $(OH)_2$ (m mol l^{-1})
V_b = Volume of Ba $(OH)_2$ (ml)
V_s = Volume of sample (ml)

Given your results for:

I. Quantitative analysis of Ca. Mg (from previous practical)
Cl^- , SO_4^{2-}, CO_3^{2-}, HCO_3^{2-} and total CO_2

II. pH and Conductivity if the samples in a table.

A- +NaCl B

C- + Na_2SO_4 D water samples from the pond.

6. DETERMINATION OF TOTAL HARDNESS

Principle:

Disodium salt of Ethylene Diamine Terra Acetic acid(EDTA) forms stable unionized complexex with Ca^{2+} and Mg^{2+}. When Eriochrome black T is added to solutions containing Calcium and Mg ions, a complex is formed which is pink. By adding of standard EDTA the dye may change back to its original blue form. EDTA solution removes Ca^{2+} and Mg^{2+} from the dye complex to form the corresponding EDTA complex. The end points indicated by the change of colour of the dye.

Reagents:

1) ½ Na_2 EDTA 0.020 M- 20 m mol^{-1}(Na_2 EDTA 0.01M) Dry Na_2 EDTA(A.R) at 80^oC, Dissolve 3.722g of Na_2 EDTA in 500 ml H_2O and dilute to 1000ml(1ml is equivalent to 0.4 mg Ca^{2+}

2) Borax buffer for Ca^{2+} + Mg^{2+} , pH 12
Dissolve 8g of $Na_2B_4O_7.10\ H_2O$ in 160 ml of H_2O add to this solution 2g of NaOH + 1g of Na_2S- 9 H_2O previously dissolved together in 20ml of H_2O and dilute the mixed solution to 20ml **Dilute 10 times** with H_2O before use.

3) Eriochrome Black T indicator
Grind together 0.4g of Eriochrome Black T. and 100g of NaCl(AR) and keep stopper bottle.

Procedure:
Mix 25ml sample with 1ml of the diluted buffer in a 100ml flask. Add about 100mg of indicator powder and heat at 70^oC. titrate with EDTA solution when the wine red colour changes to blue.

Calculation:

$$X = \frac{Ca * V_t \text{ m mol}^{-1}}{V_s}$$

X = Concentration of Ca^{2+} + Mg^{2+} in the water sample (m mol^{-1})
Ca = Concentration of ½ Na_2 EDTA (m mol^{-1})
V_s= Volume of Sample (ml)
V_t= Volume of EDTA to the end point
Reference: Water analysis: Mackereth et al

7. DETERMINATION OF ORTHOPHOSPHATE
(Ammonium molybdate method)

For phosphate determinations all the glass ware used (bottles, pipettes, measuring cylinders etc.) have to be washed with 10% H_2SO_4 and then rinsed with distilled water, because phosphate is ubiquitous!

Reagents: **Ammonium molibdate solution**

* Dissolve 3 g of $(NH_4)_6Mo_7O_{24} * 4\ H_2O$ p.a 100 ml of distilled water.

(Stored in a plastic bottle out of direct sunlight, the solution is stable for a long time).

Sulphuric acid solution

* Place 225ml of distilled water into a bottle,
* add 35 ml of concentrated H_2SO_4 p.a (sp.gr. 1.82). After cooling the Solution is stored in a glass bottle.

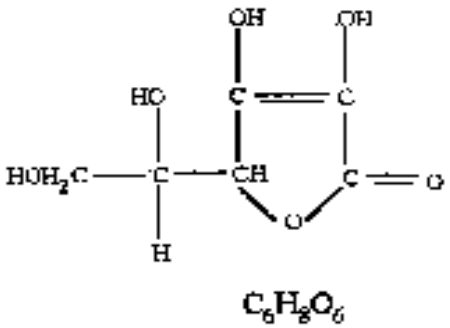
Potassium antimonyl-titrate solution

* Dissolve 0.136 g of $K[C_4H_2O_6Sb(OH)_2]* 1/2H_2O$ in 100 ml of distilled water. Heat, if necessary. The solution is stable for some months.

Mixed reagent

***** Mix together: 100 ml of reagent (1)
250 ml of reagent (2),
100 ml of reagent (3),
50 ml of reagent (4).

The mixed reagent cannot be stored for more than 6 hours. Prepare therefore only the required quantity.



Ascorbic acid

Principle of chemical reaction:

Ammonium molybdate forms with phosphoric acid at very low pH-value a yellow substance:

$$(NH_4)_6Mo_7O_{24} + PO_4^{3-} \xrightarrow{H_2SO_4} (NH_4)_3[P(Mo_3O_{10})_4] =$$

$$\text{“}(NH_4)_3PO_4 * 12\ MoO_3\text{”}$$

Under the influence of reducing agents this yellow substance is transformed into a mixture of molybdanous oxydes, that have a deep blue colour.

$$(NH_4)_3PO_4 * 12\ MoO_3 \xrightarrow{\text{Ascorbate}} (NH_4)_3PO_4 * 12\ MoO_{3-x}(OH)_x$$

(x can be e.g. 0.5 or 1).

Procedure:

A) For the **standard curve:**

Stock solution: 1 g of PO_4-P/l

* Dissolve 4.393 g of KH_2PO_4 (MW: 136.09 g /mol) in less than 1 l of distilled water.
* Add 1 ml of concentrated sulphiuric acid.
* Make up to 1 l with distilled water.

Working solution: 1 mg PO_4-P/l

* Dilute the stock solution 1: 1000.
* Prepare 50 ml of each of the following concentrations:

0	20	40	100	µg PO_4-P/l
0	1.0	2.0	5.0	ml of *WS*

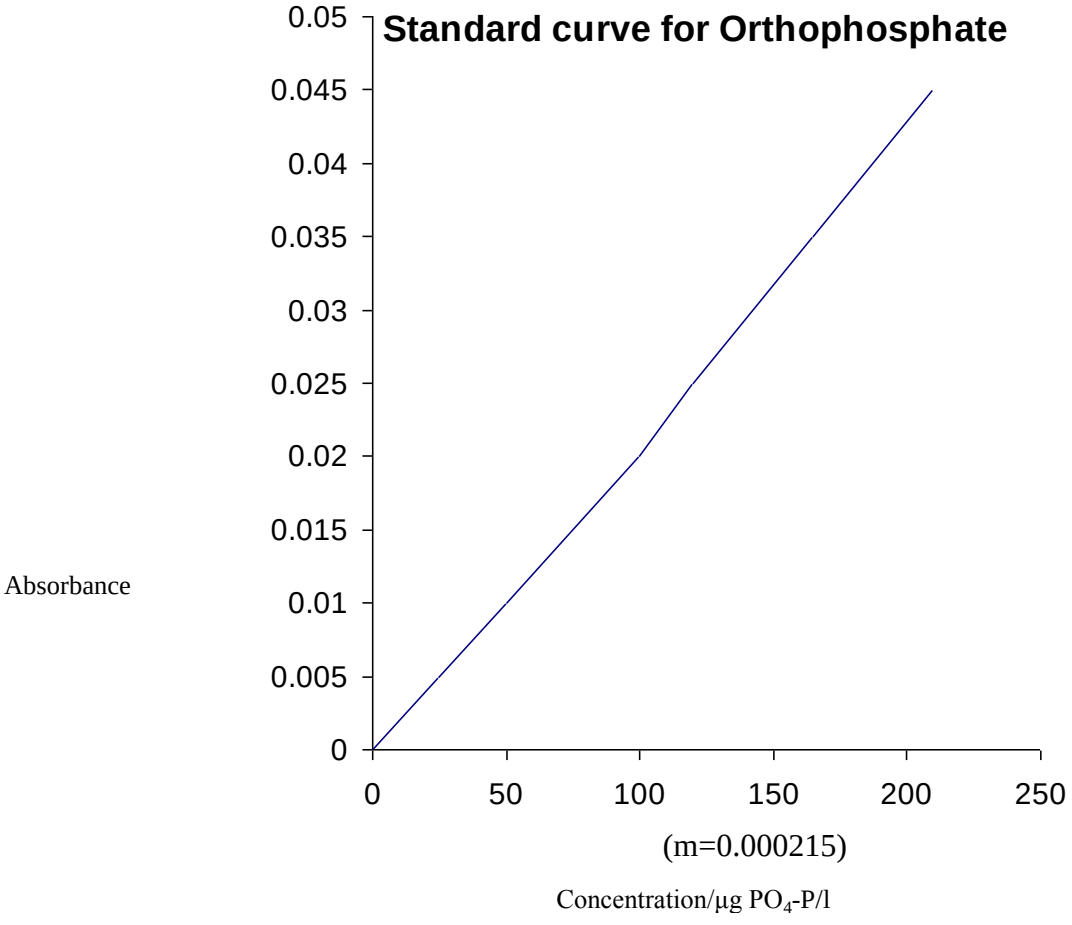
B) For the measurement:

* Place 50 ml of the filtered lake sample in an Erlenmeyer flask,
* Add 5 ml of mixed reagent to Lake Sample and standard series
* Mix
* After 5 minutes (or within the next 2 - 3 hours) measure the extinctions at a wave length Of 720 nm or 885 nm.

Calculation:

Plot the extinctions against the standard concentrations and read the concentration of your sample from the graph.

STANDARD CURVE FOR ORTHOPHOSPHATE



8. DETERMINATION OF NITRATE

(Sodium-salycilate method)

Reagent: **Na-salycilate solution** (1)

* Dissolve 0.5g Na-salycilate p.a in 100 ml of distilled water

(Prepare freshly, when needed).

Sulphuric acid (2)

concentrated sulphuric acid p.a. (sp.gr. 1.82)

NaOH-tartrate solution (3)

*Dissolve 80 g NaOH p.a. in 180 ml of distilled water,

* Add immediately (as long as the solution is still hot)

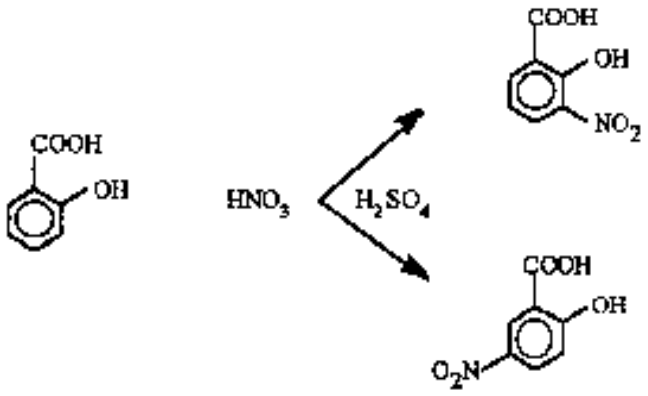
10g K-Na-tartrate p.a. and dissolve it.

* make the solution up to 200 ml.

Chemical reactions:

Nitrate ions (NO_3^-) form with Na-salycilate nitro-salycilic acid. (Salycilic acid is a basis for the production of dyes). This reaction is performed in water-free medium in the presence of sulphuric acid. Therefore the samples have to be evaporated to dryness after addition of the Na-salycilate. The residues, a mixture of ortho- and para-nitro-salycilic acid, are dissolved in concentrated H_2SO_4. In alkaline solution they develop a yellow colour.

Mechanism of the reaction:



Ortho form

Para form

Procedure:

A) For the **standard curve:**

Stock solution: 0.1 g NO_3-N/l (MW: 101.11 g/mol).

* Dissolve 0.722 g of potassium nitrate (KNO_3p.a) in 1 l of distilled water.

Working solution: 10 mg NO_3-N/l

* Dilute the stock solution 1:10.

concentrations:

0	0.5	1.0	2.0	$MgNO_3$-N/l
0	0.5	1.0	2.0	Ml of *WS*

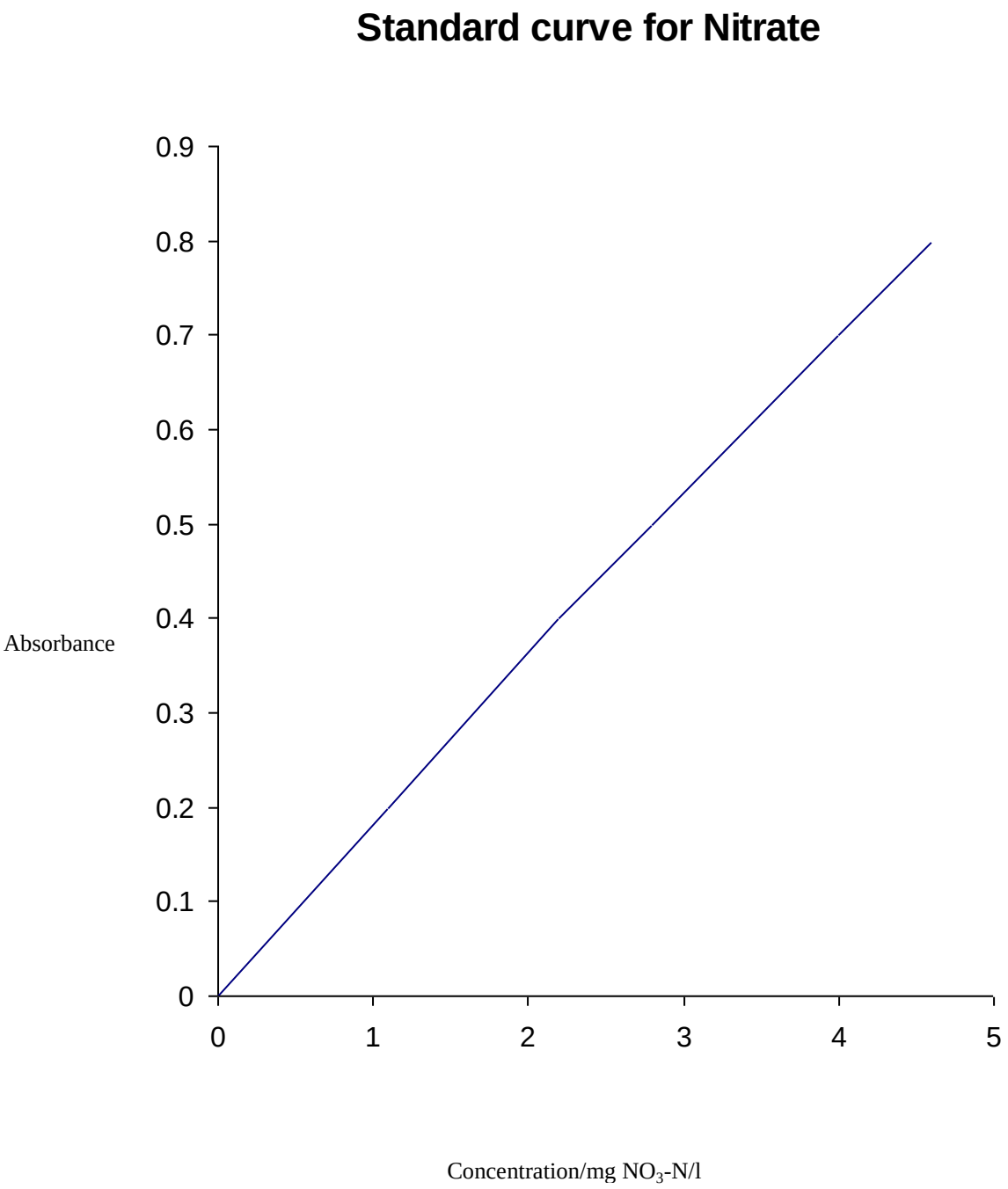
* The above mentioned ml of *WS* are pipetted in evaporation bottles.
  NO WATER IS ADDED!

B) Evaporation and measurement

* Place 10 ml of the filtered lake sample in an evaporation bottle,
* Add to Lake Sample and standard series 1 ml of (**1**);
* Put the bottles into the oven and evaporate to dryness at a temperature of 95^0 C.
* Dissolve the residue quantitatively in 1 ml of H_2SO_4 (**2**), swirling the bottle carefully While it is still warm.
* Add 50 ml distilled water and mix.
* Add 7 ml of (**3**) and make up with distilled water to a final volume of 100 ml.
* Mix again and measure the extinction immediately at a wave length of 420 nm.

STANDARD CURVE FOR NITRATE

m=0.193



9. DETERMINATION OF AMMONIA
(Indophenol blue method)

Reagents**:** **Ammonia free de-inonized water (AFW)** (1)

* Deionized water is passed over an acid cation exchange resin (e.g. hydogenated form of Dowex 50WX8, 20 - 50 mesh).

NaOH-EDTA solution (2)

* Dissolve 1.4 g NaOH p.a. in 100 ml of 0.1 M EDTA (Titriplex III).

Phenolic solution (3)

* Dissolve 12 g Na-salycilate and 100 mg Na-nitroprusside in 100 ml AFW (must be prepared freshly when needed).

Hypochlorite solution (4)

* 120 mg Di-chlor-sodium-cyanurate are dissolved in 100 ml of (2).

Chemical reactions: see p. 26

(Literature: M. D.Krom (1980) The Analyst: vol.105, no 1249, p.305)

Procedure:

All the glass ware used for this determination has to be washed with 10% HCl and carefully rinsed with AFW!

A) For the **standard curve:**

Stock solution: 1 g NH_4-N/l

* 4.717 g ammonium sulphate $(NH_4)_2SO_4$ p.a. dried at 100^0C are dissolved in about 200 ml of AFW;
* Add 2 ml of concentrated H_2SO_4;
* Make up to 1 l with AFW.

Working solution: 1 mg NH_4-N/l

* Dilute the stock solution 1: 1000 with AFW (for higher accuracy do it in two steps,

e.g.

* 1. Step make up 10 ml of *stock solution* to 1 l with AFW: ***WS 1***.

* 2. Step make up 50 ml of ***WS 1*** to 500 ml with AFW: ***WS 2.***

0	40	100	200	300	mg/l
0	2.0	5.0	10.0	15.0	ml *WS*

B) For the measurement

* Place 50 ml of the filtered lake sample in an Erlenmeyer bottle;

* Add to lake Sample and standard series 2 ml of (**3**) and mix.

* Add 2 ml of (**4**), mix again.

* Put the samples for 90 ml minutes in a dark place to develop the colour.

* Then measure the extinction within the next 48 hours at a wave length of 685 nm.

10. DETERMINATION OF THE QUANTITY OF TOTAL AND DISSOLVED SULPHIDE

Principle:

The S^{2-} is precipitated with $CdCl_2$ in similar bottles used in **Winkler** method. When the precipitate is settled, supernatant is removed and the Cd^{2+} is dissolvec in an acid iodine solution. The excess iodine is titrated with S_2O_3.

$$H_2S + I_2 \longrightarrow S + 2H^+ + 2\ I^-$$

Reagents :

A. Hcl 4M – 1 litre 12m Hcl diluted to 3 litre

B. $Na_2S_2O_3$ solution, (0.025M= 25 m mol l^-)

Dissolve 6.2 g 0f $Na_2S_2O_3$ .5 H_2O

1g of Na_2CO_3 and dilute to 1 litre. Store in a brown bottle.

C. $CdCl_2$ 2%

Dissolve 20g $CdCl_2$ in 1 litre of H_2O.

D. Iodine Solution: ½ I_2 0.025M = 25 M mol l^{-1}

Dissolve 20 g of KI in 50 ml of H_2O and add 3.17 g of I_2. After the Iodine has dissolved, dilute to 1 litre and standardize against standardized $Na_2S_2O_3$ with starch as indicator.

E. Starch indicator, 1 %

Disperse 1 g of of starch in 100 ml of water and warm to 80^{o}C- 90^{o}C stir well, allow to cool and 0.1g of salicylic acid.

Procedure:

Fill the glass stopper bottle with given water sample (or in the field). The volume of the bottle should be about 110ml and should be known. Add 1 ml CdCl2 solution, replace the stopper carefully to avoid inclusion of air bubbles and thoroughly mix the content by inverting and rotating the bottle several times for about 10 seconds. When the precipitate has settled to the lower third of the bottle, repeat the mixing and then allow the precipitate to settle completely leaving a clear supernatant. Dissolve the precipitate in an exactly known small volume of Iodine solution (D) and 5ml of Hcl (a). Titrate the excess iodine with standardized S_2O_3 using 2ml of starch as an indicator added only towards the end of the titration.

Interference's

If much organic matter is present. The precipitate should be collected on a membrane filter and washed with water.