Environmental Analysis I

Protocol

Experiments
1-6

21st of April 1999
28th of April 1999
5th of May 1999
12th of May 1999
19th of May 1999

Headed by: Mag. **Wolfgang Rottler**

Handed in by:

Pierre Madl (9521584)

Dieter Soukup (9520822)

Maricela Yip (9424495)

Salzburg, June 16th 1999

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Appendix

Note: Rinse all equipment thoroughly with deionized water before use.

Exp. 1: Anion detection with Capillary Electrophoresis (Day 1, 21st of April 1999)

Capillary electrophoresis is a process in which charged species (ions or colloidal particles) are separated based upon differential migration rates in an electrical field.
Their mobilities are affected by the size, shape, viscosity, ion strength, pH, and dielectric constant of the solute.
Separation depend upon differences in electrical properties among analytes. A buffer filled capillary is placed in two buffered containers. A few *nL* of sample is injected into the end of the capillary (opposing the detector). The components migrate under the influence of the external electric field towards the other electrode passing through a detector on the way.

material: Waterproof marker
10mL beaker
100mL beaker
250mL volumetric flask w/ stopper
4 x 100mL volumetric flasks -"-
spatula
50µL glass syringe
Capillary Electrophoresis (P/ACE) w/ 4 vials + 1 plastic bullet
High-precision single-pan balance
Paper towels

chemicals: Deionized water
≈ 0.25g Potassium-Chromate (K_2CrO_4)
≈ 50mg Tetradecyl-Trimethyl-Ammonium-Bromide (TdTAB)
≈ 20mg Sodium-Chloride (NaCl)
≈ Tri-Potassium-Phosphate ($K_3PO_4 \cdot 7H_20$)
≈ 20mg Sodium-Nitrate ($NaNO_3$)
≈ 20mg Sodium-Sulfate (Na_2SO_4)
sample 100mL sample obtained from lecturer

1.1 Buffer: The positive charges of the cationic buffer are attracted to the fixed negative charges on the capillary surface (silanol), resulting in a typical double layered structure. The negative charges of the electrolytic buffer (Br^-), are responsible for the *reverse electro-stacking* phenomenon. It does not take place in the sample zone (site of electrokinetic injection), but in the section of the electrolyte b/w the electrode and the injection end of the capillary. This relatively concentrated Br^--zone causes the electro-osmotic flow (EOF) to reverse once an external high DC voltage is applied. Like a plunger, these neutralized positive ions ringing the inner surface of the tubing along with the negatively charged bromide ions are attracted to the positive electrode (anode), carrying solvent molecules with them (anions, cations and neutral species). A unique feature of EOF is that the flow profile is nearly flat; i.e. EOF does not significantly contribute to band broadening.

Purpose: Neutralizing the negatively charged inner fused-silica tubing of the silanol groups. This is achieved by covering the inner capillary lining with a cationic tenside. A relatively high buffer concentration is used in order to keep the adsorption effects on the inner wall small;

Procedure: Potassium-Chromate-buffer with TdTAB;

- In order to determine the exact mass amount of Potassium-Chromate, and TdTAB required, use formula 1.1 and 1.2, to obtain a concentration of 5mMol/L and 0.5mMol/L respectively;
- weigh the calculated mass of each compound separately in a 10mL beaker, transfer them (flush w/ deionized water) into one 250mL flask and fill up to the mark with deionized water; seal and mark flask properly (group, content and concentration).

Formula 1.1 ($n = c \cdot V$)

$n_{Salt} = c_L \cdot V_L$ [mol]	c_L, desired concentration [mol/L] V_L, desired volume [L]

Formula 1.2 (conversion from mol to gram)

$m_{Salt} = \frac{n_{Salt}}{M_{Salt}}$ [g]	n_{Salt}, molar amount [mol] M_{Salt}, molar mass [g/mol]

Preliminary results (indicated in gray):

	c_L [mol/L]	V_L [L]	n_{Salt} [mol]	$M_{Salt.}$ [mg/mol]	m_{Salt} [mg]
Potassium-Chromate (K_2CrO_4)	$5E^{-3}$	0.25	$1.25E^{-3}$	194.22	242.8
TdTAB	$0.5E^{-3}$	0.25	$125E^{-6}$	336.42	42.08

1.2 Reference Solution (Standard Solution):

Purpose: Reference solutions are required to identify any unknown sample provided by the lecturer. Both quality (type of anion present in the sample) as well as quantity (amount contained in the sample) can be identified.

Procedure: The mass concentration required can be converted into 10mg/100mL, which is still manageable with a *high precision* flat pan balance.

- Determine the exact mass concentration of anionic reference solutions (chloride, sulfide, nitrate, phosphate - see formula 1.3 and 1.4) to achieve a mass concentration of 100mg/L = 10mg/0.1L.
- Weigh the calculated mass separately in a 10mL beaker, transfer them into individual 100mL flask and fill up to the mark with deionized water; mark each flask properly.

Note: Rinse all equipment thoroughly with deionized water before use.

Formula 1.3 ($n = c \cdot V$)

$c_{Anion} = \frac{\beta_{Anion}}{M_{Anion}}$ [mol/L]	β_{Anion}, mass concentration of anion	[g/L]
	M_{Anion}, molar mass of anion	[g/mol]

Formula 1.4 (1mol of salt dissociates into 1mol of anion and 1mol of cation)

$m_{Salt} = c_{Anion} \cdot M_{Salt} \cdot V$ [g]	c_{Aniom}, molar anion concentration	[mol/L]
	M_{Salt}, molar mass of salt	[g/mol]
	V, volume of final solution	[L]

Preliminary results of mass weighed per 0.1L volumetric flask (indicated in gray):

Salt	Anion	β_{Anion} [g/L]	M_{Anion} [g/mol]	c_{Anion} [g/L]	M_{Salt} [g/mol]	β_{Salt} [mg/L]	m_{Salt}* [mg]
Sodium Chloride (NaCl)	Cl^-	0.1	35.45	$2.821E^{-3}$	58.44	164.9	16.49
Sodium Nitrate ($NaNO_3$)	NO_3^-	0.1	62.07	$1.613E^{-3}$	84.99	137.1	13.71
Sodium Sulfate (Na_2SO_4)	SO_4^{2-}	0.1	96.08	$1.041E^{-3}$	142.0	147.8	14.78
Tri-Pot. Phosphate ($K_3PO_4 \cdot 7H_2O$)	PO_4^{3-}	0.1	94.97	$1.053E^{-3}$	338.4	356.3	35.63

(*) The 0.1L volumetric flask was used to keep consumption of chemicals as low as possible;

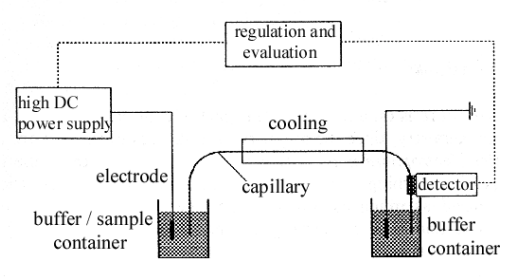

Fig 1.1 Capillary electrophoresis (CE) system for the analysis of anions
Left electrode: Cathode (-)
Right electrode: Anode (+)

Fig 1.2 Capillary Electrophoresis with peripheral computing device

1.3 Execution of Capillary Electrophoresis (Standard Solution):

Purpose: Reference solutions are required to identify any unknown probe provided by the lecturer. Both quality (type of anion present in the sample) as well as quantity (amount contained in the sample) can be identified;

Procedure: After a 3min warm-up phase of the P/ACE-Capillary Chromatographer, turn on the Computer and run the PACE software package;

- Verify the following program parameters (in the *General Settings* menu)

Initial Conditions: Inlet Sample Tray: 48 Vials Outlet Sample Tray: 48 Vials Cartridge Temperature: 25°C Sample Temperature: 25°C

- Verify the following sample-settings (in the *TIME PROGRAM* menu)

	Sampling settings for the *scanning* procedure: Sample Current: 21µA						
#	Time	Event	Value	Duration	Inlet Vial	Outlet Vial	Summary
1		rinse with pressure	1.5 bar	2.00min	buffer in: A1	buffer out: B1	forward
2		inject with pressure	10mbar	10sec	sample inject: A1	buffer out: A1	override; if o.k. forward
3	0.0min	separate w/ pressure	15kV	10min	buffer in: A1	buffer out: A1	1.00min ramp - rev. Polarity
4	6.0min	end					

<u>*P/ACE* software package - Follow the steps as suggested</u>:

I. Press the *LOAD*-icon (in the *CONTROL* menu) and place the two buffer filled vails into the appropriate slots (position A1 of both inlet and outlet cartridge).

II. Place the sample (from lecturer) into the cartridge holder (position A1),

i) Initialize preparative steps prior to scan by clicking the ***HOME*** button (*CONTROL* menu).

i) Once positioned, prepare scan by activating the ***SINGLE RUN*** button in the *CONTROL* menu.
Save settings; e.g.: c:\pacemdq\methods\ua99.met
and the scan itself in: c:\pacemdq\data\ua_99\gruppe2\ as 001
(software switches automatically to the *METHOD*-menu)

i) Run the program by clicking onto the ***START*** button in the *METHOD*-menu.
Double click of the left mouse button into the scan-image, selects the appropriate zoom factor; zooming is possible by using the left mouse button (click, drag, and release) over the site of interest; undo a zoom by holding the **SHIFT**-key and double-clicking the left mouse button; Progress of scan and actual status of the PACE can be observed by swapping between the two display modes using the **ALT+TAB** keys;

i) ***STOP*** aborts a running scan (in the *METHOD* menu);

i) The ***ANALYZE*** button provides mathematical support when using the right mouse button to mark certain sections of a displayed peak.

III. Qualitative Analysis:

- Add 20µL of nitrate into the sample-vail of the sampling cartridge (A1) with the 50µL syringe, and repeat entire procedure (starting from position II).
- Add 20µL of sulfate into the sample-vail (sample + nitrate) of the sampling cartridge (A1) with the 50µL syringe, and repeat entire procedure (starting from position II).
- Add 20µL of chloride into the sample-vail (sample + nitrate + sulfate) of the sampling cartridge (A1) with the 50µL syringe, and repeat entire procedure (starting from position II).
- Finally, add 20µL of phosphate into the sample-vail (sample + nitrate + sulfate + chloride) of the sampling cartridge, and repeat entire procedure (starting from position II).

Note: Flush syringe first with deionized water and with the sample a couple of times before introducing another shot into the sample-vail (plastic bullet), shake well or use vortex if available.

IV. Quantitative Analysis: This procedure is executed w/ type of reference solution identified during the qualitative analysis.

- Prepare 4 vails - one blank and 3 sample vails containing distinct volumetric amounts of 10, 20, 30µL (β_{anion}=0.1mg/L) and fill up w/ deionized water;
- Execute step I and II of the above routine for each vail individually.

1.4 Final results of Electrophoretic Analysis: After initial difficulties, the results shown were obtained only once the capillary tube has been replaced by a new one (5th of May 99).

Qualitative Analysis: Nitrate injection into the sample vail was done with 10µL rather than 20µL in order to observe the response without triggering an overshooting reaction; following injections were done with 20µL because nitrate response showed to be very moderate. Phosphate injection was cancelled since the sample has been found to contain traces of sulfate and chloride.

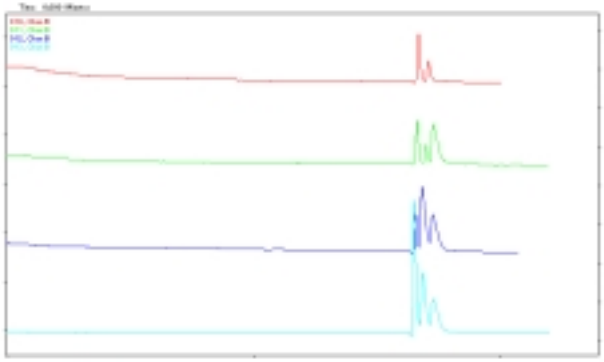

Fig 1.3 Sample Identification (see chart in appendix for further details)
unknown sample

Sample vail after injecting 20µL NO_3^-

Sample vail after injecting 20µL SO_4^{2-}

Sample vail after injecting 20µL Cl^-

Quantitative Analysis (indicated in gray): Since the sample vail was already scanned and memorized during the qualitative analysis only four extra vails are needed in the quantitative analysis. In accordance with the lecturer only the amount of chloride was determined.

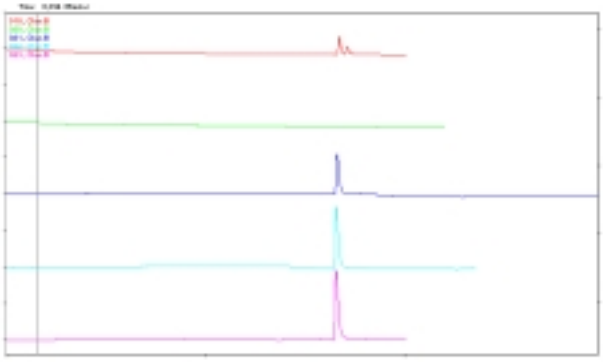

Fig 1.4 Quantitative determination (Cl^- only):
Vail#S w/ sample (peak: left Cl^-, right SO_4^{2-})
$A_{Cl\text{-}Probe}$ = 18972; $A_{SO4\text{-}Probe}$ = 10747
Vail#1: 100mL H_2O (blank sample)

Vail#2: 10µL Cl^- and 90mL H_2O

Vail#3: 20µL Cl^- and 80mL H_2O

Vail#4: 30µL Cl^- and 70mL H_2O

Formula 1.5 Conversion of the mass concentration from the reference solution to vail:

$\beta_{Vail} = \frac{\beta_{Cl\text{-}ref.} \cdot V_{Cl\text{-}injected}}{V_{Vail}}$ [g/L]	β_{Vail}, mass concentration [g/L] V_X, volume [L]

Final results after converting via the diagram obtained from the P/ACE

Vail [-]	$\beta_{Cl\ ofreference\ sltn}$ [mg/L]	$V_{Cl\ injected}$ [µL]	V_{Vail} [µL]	A.U. [-]	β_{Vail} [mg/L]
1	0.1	0	100	0	0
2	0.1	10	100	56802	10
3	0.1	20	100	92303	20
4	0.1	30	100	112473	30
Sample	-	-	100	18972	3.34

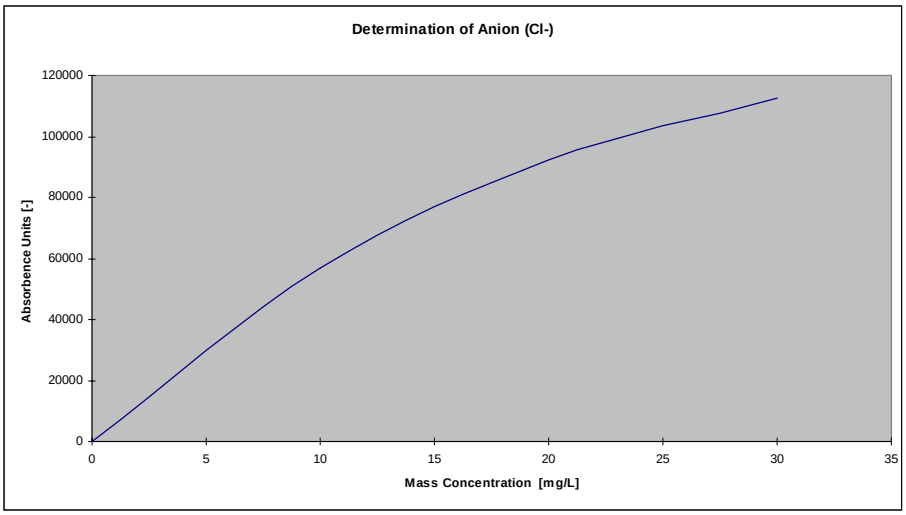

Fig. 1.5 Chart of Chloride absorbance versus mass concentration (for a more detailed display, see appendix)

1.5 Discussion and Evaluation:

Qualitative Analysis: Examination of the sample with the CE produced a two-peak spectrum, suggesting the presence of two different anionic species; the identification of these species was done by adding small amounts of the known anionic reference solution (NO_4^-, SO_4^{2-}, Cl^-, V=20µL / β=0.1g/L) in order to observe the scanning results; any match of the added reference solution and the unknown species would result in an increase of that particular peak. As can be seen in Fig. 1.3, the analytical procedure identified the unknown peaks as belonging to chloride (left peak of sample) and sulfate (right peak of sample).

Quantitative Analysis: Due to the limited time available, only the chloride peak has been quantitatively evaluated. To obtain the quantity of the chloride ions present in the sample, a dilution series was made of the reference chloride solution. According to the mass concentrations used (as determined by Formula 1.5, ranging from β=10 to 30mg/L), a graph of *Mass Concentration* vs. *Absorbance Unit* was established (Fig. 1.5). According to the memorized data of the qualitative analysis, the absorbance of the chloride peak of the sample can be considered to be still within the linear section of the overall plot (18972 AU) which corresponds to a mass concentration of **3.34mg/L** of Cl^-.

Exp. 2: Phosphate determination w/ Spectro-Photometer (Day 2, 28th of April 1999)

Ammonium-Molybdate $(NH_3)_6Mo_7O_{24}$ in an acidic medium (pH = 0.5) converts into a yellowish acid $H_3P(Mo_3O_{10})_4$. By adding 4-Methyl-Amino-Phenol-Sulfate (Photo-Rex) and Sodium-Bisulfite, the acid will be reduced to a bluish Molybdenum-Blue $(MoO_{3-x}(OH)_x$. The extent of the color reaction can be altered by adding traces of phosphate into the solution; the amount of phosphate determines the intensity of the bluish hue which can be detected photometrically by using a double-beam spectro-photometer.

Material: Peleus rubber-bulb
5mL measuring pipette AS-class
50mL volumetric pipette AS-class
1000mL volumetric flask w/ stopper
4 x 100mL volumetric flask w/ -"-
8 x 50mL volumetric flask w/ -"-
9 x 100mL beaker
Hot-plates
Spectrophotometer + 10 cuvets
Digital single-pan balance
Protection glasses
Pair of latex gloves
Paper towels
Marker pen

chemicals: approx. 2L Deionized water
10g Ammonium- Molybdate $(NH_3)_6Mo$
10g Citric Acid powder
2g 4-Methyl-Amino-Phenol-Sulfate "Photorex" $C_{14}H_{20}N_2O_6S$
17.5g Sodium-Bisulfite Na_2SO_4
50mL Sulfuric Acid H_2SO_4 (w = 96%)
≈150mg Potassium Dihydrogen-Phosphate KH_2PO_4

sample: 50mL water sample (f/ lecturer)

2.1 Reagent Solutions and **Dilution Series**:

Purpose: To makes photo-detection possible, both the calibration solution and the sample containing traces of phosphate have to be enriched with chemicals to render the solution acidic.

Procedure: 5 different solutions are required:

Solution-1: Ammonium-Molybdate;
- add 10g of Ammonium-Molybdate in a 100mL volumetric flask and fill up till the mark with deionized water; mark properly and shake well.

Solution-2: Citric Acid;
- add 10g of Citric Acid powder in a 100mL volumetric flask and fill up till the mark with deionized water; mark properly.

Solution-3: Sulfuric Acid;
- pipet 50mL of Sulfuric Acid into a 100mL vol. flask and fill up till the mark with deionized water;

Note: Use gloves and glasses; execute with great care - *add acid to water not the other way around*! Reaction is highly exothermic (place flask in a cold water filled container to provide extra cooling); mark properly and shake well.

Solution-4: Active Reagent;
- mix 2g of Photorex and 17.5g of Sodium-Bi-sulfite into a 100mL volumetric flask and fill up with deionized water; warm up the flask (≈50°C) onto a hot-plate to facilitate the dissolving process; mark the flask properly and shake well.

Solution-5: Phosphate Solution in variable concentrations;
- add 143.3mg of Potassium Dihydrogen-Phosphate into a 1000mL volumetric flask and fill up with deionized water; equivalent of 100mg of phosphate per 1L of water (according to formula 2.1); converted = 0.1mg of PO_4^{3-}/mL of water;
- for the dilution series, pipet the appropriate amount of phosphate solution into separate 50mL volumetric flasks to obtain a gradually increasing concentration per flask (according to results below) and fill up till the mark with deionized water; mark the flask properly and shake well.

Formula 2.1: 1mol of KH_2PO_4 = 1mol of PO_4^{3-}

$$m_{PO4} = \frac{m_{KH2PO4}}{M_{KH2PO4}} \cdot M_{PO4} \quad [g]$$

m, mass [g]
M, molar mass [g/mol]

Preliminary results of calibration series - indicated in gray (in parenthesis proper mass concentration):

m_{KH2PO4} [mg]	M_{KH2PO4} [g/mol]	M_{PO4} [g/mol]	m_{PO4} [mg]	Flask #1 [mL]	Flask #2 [mL]	Flask #3 [mL]	Flask #4 [mL]	Flask #5 [mL]	Flask #6 [mL]	Flask #7 [mL]
143.3	136.1	94.97	100	0.1 (0.1mg/L)	0.2 (0.2mg/L)	0.5 (0.5mg/L)	1.0 (1.0mg/L)	1.5 (1.5mg/L)	2.0 (2.0mg/L)	2.5 (2.5mg/L)

2.2 Spectrometric Analysis:

Purpose: Light-absorbing characteristics of each sample correlates with the nitrate content in each sample. Therefore, the Spectro-Photometer needs to be reset with the blank sample (0mg/L); the measurements can then be executed in an increasing order of nitrate-content (starting with calibration samples $0_{Calibr.}$ Up to $8_{Calibr.}$ Including the unknown test-sample).

Procedure: Mix the reagents and fill the photometric cuvets;

- transfer the content of each 50mL flask into 100mL beaker (7 reference concentrations + 1 unknown sample + 1 blank sample - in total 9 flasks); volumetric losses are neglectable;
- add the following solutions into each beaker:
  2.5mL of diluted Sulfuric Acid;
  0.5mL of Citric Acid;
  3mL of active reagent (Photorex-solution);
  4mL of Ammonium Molybdate solution

Calibrating photometer to zero:

- fill two cuvets with the blank sample ($0_{Calibr.}$) and place them into the scan compartment; upper = reference holder, lower = sample holder and execute autozero-function;
- remove blank sample from sample holder and repeat measurement with reference solutions $1_{Calibr.}$ To $7_{Calibr.}$ Plus unknown probe ($8_{Calibr.}$) and record absorbance from display;

Note: Do not touch cuvet at scan-window;

- plot chart from the read-outs obtained and determine PO_4^{3-} concentration of the unknown sample.

2.3 Final results of Spectrometric analysis at λ = 730nm - (indicated in gray):

	Dilution Series - Calibration samples								Probe
Flask-#	$0_{Calibr.}$	$1_{Calibr.}$	$2_{Calibr.}$	$3_{Calibr.}$	$4_{Calibr.}$	$5_{Calibr.}$	$6_{Calibr.}$	$7_{Calibr.}$	8_{Sample}
$S\text{-}Meter_{Reading}$	0.000	0.039	0.074	0.178	0.360	0.521	0.690	0.863	0.068
PO_4^{3-} [mg/L]	0.00	0.10	0.20	0.50	1.00	1.50	2.00	2.50	0.17

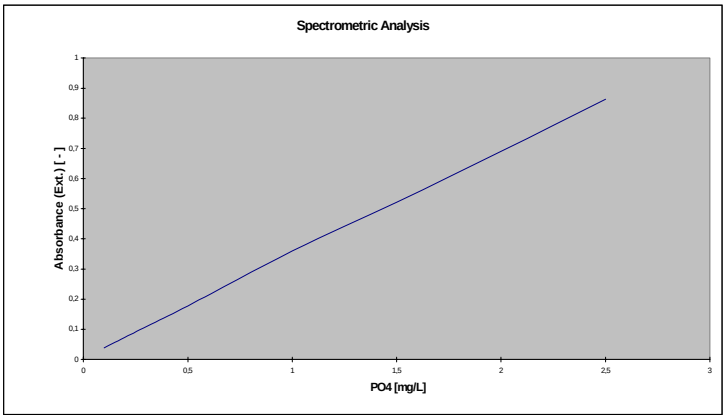

Fig 2.1 Chart of photo-spectrometric absorbance of phosphate with increasing concentrations (dilution series)
(see also appendix-tables for a more detailed chart)

Fig 2.2 Dilution series of reference solutions for the spectro-photometer; from left to right: increasing PO_4^{3-} concentrations.

2.4 Discussion and Evaluation: After establishing the dilution series of the calibration solution PO_4^{3-} (β=0.1g/L) - including a blank value to make sure that there is no offset in the ordinate - the absorbance of each cuvette was determined with the spectro-photometer. Already at this stage, the colorimetric analysis indicates the approximate value of the unknown sample (Fig. 2.2), which suggests that the number of dilutive steps of the dilution series could have been cut by half, i.e. excluding calibration samples 5 to 7. The absorbance data obtained were used to plot a chart showing the *Mass Concentrations* vs. *Absorbance*. The linear progression obtained (Fig. 2.1) was used to determine the unknown mass concentration of the sample provided by the lecturer, which has been found to contain 0.17mg/L PO_4^{3-}.

Exp.3: Heavy Metal Detection with TLC (Thin Layer Chromatography - Day 3, 5th of May 1999)

TLC is a less sensitive method than the previous, (which requires relatively high doses). It relies on the different abilities of substances to stick to surfaces (stationary phase) while the carrier medium (mobile phase) pushes the components along. In TLC, the stationary phase consists of a silicagel-coated glass plate which slightly dips into the carrier medium (vertical position) of a sealable container. According to the adherence of the components, certain elements will migrate faster upwards the plate than the others. The R_F-value obtained from the final separation pattern is a immediate indicator of the relationship between the reference substances and an unknown sample.

material: Peleus rubber-bulb and
- 1mL Volumetric pipet AS-class
- 2mL Volumetric pipet AS-class
- 10mLVvolumetric pipet AS-class
- 50mL Volumetric pipette AS-class
- 10x 2µL volume capillary
- 50mL Vol. flask w/ sprayer + pump
- 50mL Volumetric flask w/ stopper
- 5x 100mL Vol. flask w/ stopper
- 1000mL Volumetric flask w/ -"-
- 10mL Beaker
- 100mL Beaker
- 50mL Separator flask, stopper + tripod
- Spatula
- Precision digital flat-pan balance
- Chromatographic chamber (TLC)
- TLC plate (20 x 20 cm) coated w/ silicagel $60F_{254}$
- Pencil and waterproof marker
- UV-Lamp (254nm)

chemicals:
- ≈20mL Toulene C_7H_8
- ≈100mL Acetone CH_3COCH_3
- ≈50mL Chloroform $CHCl_3$ (w = 99%)
- ≈20mL Acetic Acid CH_3CO_2H (w = 90%)
- ≈50mL Sodium hydroxide NaOH-1M
- ≈1g Sodium diethyl-dithio-carbamate $C_5H_{10}NNaS_2 \cdot 3H_2O$
- ≈20mg Dithizone $C_{13}H_{12}N_4S$
- Ammonia solution 25% NH_3

few grams of:
- Copper-II-nitrate $Cu(NO_3)_2 \cdot 3H_2O$
- Lead-II-acetate $(CH_3COO)_2Pb \cdot 3H_2O$
- Cobalt-II-nitrate $Co(NO_3)_2 \cdot 6H_2O$
- Nickel-II-nitrate $Ni(NO_3)_2 \cdot 6H_2O$

sample: 50mL sample (f/ lecturer)

3.1 Preparation of chemical reagents

Purpose: Metal chelation is strongly pH-dependant; an acetic acid buffer is used to make sure that the pH does not change during the reaction. The spraying reagent is used in the final procedure to make the heavy metal spots on the TLC visible.

Procedure: Buffered medium at a distinct pH:

- According to formula 3.1 and 3.2, pipet the 5.7mL of concentrated HAc and 50mL of 1M NaOH into a 1L volumetric flask; fill up w/ deionized water, mark properly and shake well.

NaDDTc, a Chelate used to trap metal ions:

- To obtain a solution containing 1% of active reagent, dissolve 1g in a 100mL volumetric flask (or 0.5g in a 50mL flask) and fill up w/ deionized water; mark properly and shake well.

Spraying reagent:

- Dissolve 20mg of Dithizon w/ Acetone in a 100mL vol. flask; mark properly and shake well; store spraying reagent in a dark and cool place.

Formula 3.1: Concentration of HAc

$$c_{HAc} = \frac{w_{solution} \cdot \rho_{solution}}{100 \cdot M_{substance}} \quad [mol/L]$$

w, mass percentage	[%]
ρ, density of HAc	[g/L]
M, molar mass of HAc	[g/mol]

Formula 3.2: (to obtain a 1M HAc)

$$V_{concentr.} = \frac{c_{diluted} \cdot V_{diluted}}{c_{concentrated}} \quad [L]$$

c, concentration	[mol/L]
V, volume	[L]

Preliminary results (indicated in gray; (*) density value from label on bottle of manufacturer):

reference solution	concentrated HAc 90%				diluted HAc		conc. HAc
	w [%]	ρ* [g/L]	M_{HAc} [g/mol]	$c_{conc.}$ [mol/L]	$c_{diluted}$ [mol/L]	$V_{diluted}$ [mL]	$V_{conc.}$ [mL]
HCl	99	1060	60.05	17.48	1.00	100	5.7

3.2 Heavy metal reference solutions

Purpose: Identification of a heavy metal is possible by using several test-solutions containing a known quantity of heavy metal salts which have to be prepared separately.

Procedure: Preparation of heavy metal samples

- Table 1. lists the mass concentration of each mineral ion required. Determine the mass concentration of each salt (using formula 3.3), weigh each mass of metal salt into 5 separate 100mL volumetric flasks and mark properly;
- fill up to the mark w/ deionized water and shake well;

Table 1.

metal	mass concent. [mg/mL]
Co	**1** (= 100mg/0.1L)
Ni_{1x}	**1** (= 100mg/0.1L)
Ni_{5x}	**5** (= 500mg/0.1L)
Cu	**1** (= 100mg/0.1L)
Pb	**5** (= 500mg/0.1L)

Formula 3.3: 1mol of dissociated salt = 1mol of metal-ion

$$m_{Salt} = \frac{M_{Salt}}{M_{Metal}} \cdot m_{Metal} \quad [g]$$

m, mass [g]
M, molar mass [g/mol]

Preliminary results of salts to be weighed to obtain the desired mass concentrations (in gray):

metal [salt]		β_{Me} [mg/0.1L]	m_{Metal} [mg]	M_{Salt} [g/mol]	M_{Metal} [g/mol]	m_{Salt}* [mg]
Co:	$[Co(NO_3)_2 \cdot 6H_2O]$	100	100	291.04	58.93	493.9
Ni_{1x}:	$[Ni(NO_3)_2 \cdot 6H_2O]$	100	100	290.81	58.70	495.4
Ni_{1x}:	$[Ni(NO_3)_2 \cdot 6H_2O]$	500	500	290.81	58.70	2477
Cu:	$[Cu(NO_3)_2 \cdot 3H_2O]$	100	100	241.60	63.55	380.2
Pb:	$[(CH_3COO)_2Pb \cdot 3H_2O]$	500	500	379.34	207.2	183.1

(*) mass for 0.1L flask; (**) only 1mL was extracted from the original metal-solution

3.3 Extraction of heavy metal ions from the aqueous phase

Purpose: Most metals form complex ions. Using a complex forming reagent at a distinct pH (a chelating ligand like NaDDTc), metal-ions can be extracted from a watery solution. Any chelate-complex formed (captured metal-ion) changes from hydrophilic to lipophilic status, whereas unloaded complex reagents remain polar. The so formed non-polar complexes can be easily extracted and examined, using an unpolar solvent (e.g. chloroform) and a separation flask.

Procedure: Use soft pencil to define start positions on TLC-plate before proceeding with metal extraction. Each metal reference solution and unknown sample is subject to the following treatment;

1. Extract 1mL of heavy metal solution (from 0.1L flask), pipet into separation flask (make sure stopcock is in closed position), and add the following chemicals into the separation flask:
   8mL of NaOH-HAc-buffer,
   2mL of chelating agent (NaDDTc - usually accompanied by an intense color reaction)
   10mL of chloroform
2. close with stopper and shake well for a minute or so;
   Note: Make sure to ventilate repeatedly by holding the flask up side down and opening the stopcock - vapor pressure of chloroform exerts a considerable pressure;
3. extract the lower phase (containing the chelated heavy metal complex) in a tiny beaker and
4. pipet 2µL from this extract onto the predefined position of the TLC-plate; (w/ 2µL vol. capillary).
5. repeat procedure for all of the metal probes prepared (steps 1 to 4);

- place charged plate into the developing chamber using toulene as the mobile phase (make sure that starting line does not dip into the toulene) - allow approx. 50mins for a clear separation to occur, or >13cm b/w upper limit and line of origin (see Fig. 3.3).
- remove plate once separation is satisfactory, outline upper limit w/ soft pencil, degas and spray w/ dithizon solution in a zick-zack pattern (plate turns reddish) under aspirator (dithizon is toxic!);
- hold plate over an open bottle of ammonia solution (ammonia vapor enforce visible reaction - plate turns yellowish) and check separation under UV-lamp;

Note: Dispose off any unused organic phase containing the heavy metals properly - recycle!

3.4 Final results of TLC-procedure:

Fig. 3.1 Heavy metal extractions

....before phase separation

....after phase separation

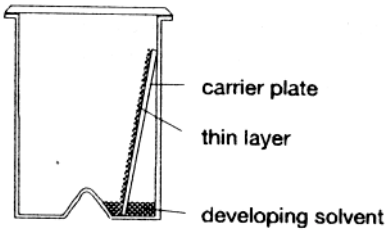

Fig 3.2 Development chamber for TLC

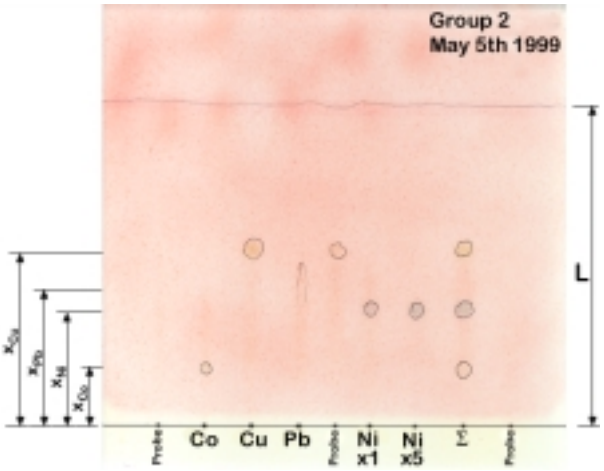

Fig. 3.3 Scan of the chromatogram obtained after spraying it with Dithizon solution and exposing the plate to ammonium (open bottle of 25% ammonium solution is sufficient to obtain a visible reaction); **Note**: Both substances are toxic - execute both procedures under aspirator.

Retardation Factor: $R_F = \frac{x_{Ion}}{L}$ x_{Ion}, distance from line of origin to center of spot
L, upper limit of solvent front

3.5 Discussion and Evaluation: Already after the final steps of procedure 3.2 (phase separation), the color of the extract provided some clues of the kind of heavy metals contained in the sample (horizontally positioned flask on the right hand side of Figure 3.1).

All samples (reference solutions and unknown sample) have been placed onto the TLC-plate, using the 2µL volume capillary. To make sure that the unknown sample will provide a detectable readout, it has been placed on three different separate sites of the plate (Fig. 3.3). Each of the reference solutions has been placed once and also onto an overall spot (∑ - excluding the unknown sample) to observe the separation capabilities of the mobile phase used. The plate was given a total of 50mins separation time in the chamber before outlining the upper limit of the mobile phase, and finally was treated with reagents to make heavy metal detection more evident. Since the substances were clearly visible and were limited in number, any extra confirmation, by using the Rf-value, was not executed. It can be said with certainty that the unknown sample definitively contained **copper** and probably traces of **nickel**. The poor showing of the left and right chromatographic traces probably are either due to an insufficient buffering capacity used in this experiment; instead of the calculated 5.7mL of acetic acid, an erroneous 1.8mL was added instead in order to prepare the buffer or to an non-proper application of the samples onto the plate.

Exp.4: Titrimetric **COD Determination** (Chemical Oxygen Demand - Day 4, 12th of May 1999)

Permanganate is considered a strong oxidant able to oxidize many in/organic substances (carbohydrates, phenoles, certain proteins, Fe^{2+}, Cl^-, SO_4^{2-}, etc.). This property is used to determine the degree of contamination of weakly polluted waters. An acidic water sample containing a definite amount of permanganate kept at near boiling conditions oxidizes those pollutants and itself reduces Mn(VII) into Mn(II). To determine the extent of contaminating agents, an equivalent amount of oxalic acid added destroys any remaining permanganate molecules in the solution. Therefore, a reverse titration with permanganate solution yields the amount of contaminants originally present in the sample.

Unbalanced REDOX equation in *acidic* solution (K^+, H^+ considered spectator ions; ON = oxidation number):
Half Reactions: **R** = Reduction; **O** = Oxidation;

$\overset{ON:+7}{MnO_4^-} \leftrightarrow \overset{ON:+4}{Mn^{2+}} + 4H_2O$
$= MnO_4^- + 8H^+ \rightarrow \overset{ON:+4}{Mn^{2+}} + 4H_2O$
O: $\overset{ON:+3}{C_2O_4^{2-}} \rightarrow \overset{ON:+4}{2CO_2}$

Electron equalization:
R: $MnO_4^- + 8H^+ + 5e^- \leftrightarrow Mn^{2+} + 4H_2O$
$= 2MnO_4^- + 16H^+ + 10e^- \leftrightarrow 2Mn^{2+} + 8H_2O$
O: $C_2O_4^{2-} \rightarrow 2CO_2 + 2e^-$
$= 5C_2O_4^{2-} \rightarrow 10CO_2 + 10e^-$
Adding the two half-reactions:
$2MnO_4^- + 16H^+ + 10e^- + 5C_2O_4^{2-} \rightarrow 2Mn^{2+} + 8H_2O + 10CO_2\uparrow + 10e^-$
Final REDOX-EQ:
$2MnO_4^- + 16H^+ + 5C_2O_4^{2-} \rightarrow 2Mn^{2+} + 8H_2O + 10CO_2\uparrow$

material: Peleus rubber-bulb and
5mL Volumetric pipet AS-class
10mL Volumetric pipet AS-class
15mLVvolumetric pipet AS-class
100mL Volumetric pipette AS-class
50mL Volumetric flask w/ stopper
2x 100mL Volumetric flask w/ stopper
500mL Volumetric flask w/ stopper
2x 1000mL Vol. flask w/ stopper
15mL Beaker
250mL Erlenmeyer flask
Watch glass (lid f/ Erlenmeyer)
25mL Burette w/ stand and funnel
Spatula
Hot plate w/ int. magnetic stirrer and magnetic rod (30mm)
3 Granules of boiling chips
Digital flat-pan balance
Pestle and mortar
Protection glasses
Pair of latex gloves
Waterproof marker
Conductivity Meter w/ int. T.-Meter

chemicals:
≈7g Oxalic acid $C_2H_2O_4 \cdot 2H_2O$
≈4g Potassium Permanganate $KMnO_4$
≈10mL Sulfuric Acid H_2SO_4 (w = 96%)

samples:
1L tap water sample
1L river water (Hellbrunner Bach)
1L pond water (Uniteich)

4.1 Preparation of chemical reagents

Purpose: Three solutions (acidic, oxidizing and reducing agents) are required; according to the final Redox-EQ, 2mol of $KMnO_4$ are needed for a complete reaction with 5mol of $C_2H_2O_4$. For practical purposes, the figures should be changed to 0.2mol of $KMnO_4$ and 0.5mol of $C_2H_2O_4$.

Procedure: <u>Solution 1 - concentrated $KMnO_4$ solution</u>:

- Using a 0.1L instead of 1L flask, add the 3.16g (formula 4.1) of grinded $KMnO_4$ powder (pestle and mortar) into a 100mL vol. flask; fill up w/ deionized water, mark properly and shake well until all $KMnO_4$ is dissolved completely;
- to make procedure more sensitive to tiny fractions of pollutants, dilute the $KMnO_4$ solution by a factor of 100; i.e. pipet 10mL of the solution into a 1L vol. flask, fill up w/ deionized water, mark properly and shake well;

<u>Solution 2 - Oxalic Acid</u>:

- Using a 0.1L instead of a 1L vol. flask, weigh 6.3g (formula 4.1) of oxalic acid into a 100mL vol. flask; fill up w/ deionized water, mark properly and shake well;
- similarly, to maintain molar relations while reducing with the oxidizing agent, dilute the $C_2H_2O_4$ solution by a factor of 100; i.e.: pipet 10mL of the solution into a 1L vol. flask, fill up w/ deionized water, mark properly and shake well;

<u>Solution 3 - Sulfuric Acid (Redox Reaction requires a H^+ donor)</u>:

- pipet 10mL of Sulfuric Acid into a 50mL volumetric flask containing about 30mL of deionized water. **Note**: Use protective gloves and glasses; execute with great care - *add acid to water not the other way around*! Reaction is highly exothermic (place flask in a cold water filled container to provide extra cooling); mark properly and shake well.

Formula 4.1: mass of solid substances required

$m_{Substance} = n_{solution} \cdot M_{substance}$ [g]	n, molar amount	[mol]
	M, molar mass of Substance	[g/mol]

Preliminary results (indicated in gray):

	concentrated Solutions				Informative only	
solution	$n_{solution}$ [mol]	$M_{Substance}$ [g/mol]	$m_{Substance}$ [g]	$m_{Sub\ in\ 0.1L\ flask}$ [g]	$c_{in\ 0.1L\ flask}$ [mol/L]	$c_{1/100}$ [mol/L]
$KMnO_4$	0.2	158.04	31.61	3.161	0.2	0.002
$C_2H_2O_4$	0.5	126.07	63.04	6.304	0.5	0.005

4.2 Boiling and Titration procedure

Purpose: Prepare hot-plate, Erlenmeyer flask and buret with stand for both boiling and titration. Since permanganate solution is a strong oxidant, execute a preliminary rinse to get rid of any residual contaminants of the equipment used (it is highly suggested to rinse all the equipment with chemicals to be used later on);

1. pipet 100mL of deionized water and 5mL of diluted sulfuric acid solution into the Erlenmeyer flask;
2. add 3 boiling chips, magnetic rod into the flask and turn on the hot plate;
3. fill up burette completely with diluted $KMnO_4$ solution (c=5mmol/L);
4. once the acidic mixture of water and diluted acid boils, add 15mL of $KMnO_4$ (titrant) from the burette, cover Erlenmeyer flask with watch-glass and *keep* simmering for 10mins more to obtain a complete reaction with any contaminants (analyte turns deeply violet);
5. afterwards, pipet 15mL of diluted oxalic acid (c = 0.005mol/L) into the mixture - violet hue of analyte vanishes completely;
6. after a minute or so, add $KMnO_4$-titrant to the analyte until a persistent light pinky shade reappears (lasting about 30secs) at that point titration should be terminated;

Note: to confirm concentrations of analyte and titrant, add 15mL of diluted oxalic acid (c = 0.005mol/L) into the mixture and titrate w/ diluted $KMnO_4$ solution (c = 0.005mol/L); the volumetric consumption should match exactly 15mL; if this is not the case, (especially when not freshly made), titrant can still be used but requires modification by f = $15/(V_{Titrant\ used})$, otherwise f = 1;

7. repeat procedure (steps 1 to 6) 3 times by using tab water, river water and pond water samples instead of deionized water;

- determine the amount of mass consumed by in/organic compounds of the samples examined using formula 4.2;
- determine conductivity of any water sample used.

Fig 4.1 Titration and boiling procedure with $KMnO_4$

Formula 4.2: formula obtained from *Schneppl-Schwedt* Analyt. Chem. Umwelt Praktikum

$m_{Titrant} = \frac{[(15+V_{Titrant}) \cdot f - 15] \cdot 316}{V_{Water\ Sample}}$	$V_{Titrant}$, volume of $KMnO_4$	[mL]
	$V_{Water\ Sample}$, volume of sample	[mL]
	f, correction factor (see step 7)	[-]

4.3 Final Results (indicated in gray):

	Tap water	River water	Pond water
$V_{Titrant}$ [mL]	0.9	2.4	3.3
$m_{Titrant}$ [mg]	2.84	7.58	10.43
Conductivity [µS/cm]	338	533	484
Temperature [°C]	16.9	21.3	20.4

4.4 Discussion and Evaluation:

Potassium-Permanganate was used for the entire procedure in order to examine water samples that are low (tab water) to moderately contaminated (pond water) with organic pollutants. Indeed as expected, the tab water analysis revealed a very low extent of contamination; whereas, the pond water, with its various kinds of aquatic fauna, showed an elevated level of organic contaminants (feces of ducks and fish).
According to the table 4.1, the tap water sample (**2.84mg** $KMnO_4$) and the river water sample (**7.58mg** $KMnO_4$) do fit into the first category; whereas, the pond water sample (**10.43mg** $KMnO_4$) can be considered to fit into the "clean surface water" category.
To increase the overall credibility of this procedure it would be better to use distilled water for any dilutive steps and Potassium-Chromate as the oxidative reagent (stronger oxidant than $KMnO_4$).

Table 4.1: Level of organic strain according to $KMnO_4$ consumption

Class	**used amount of $KMnO_4$ [mg]**
Pure spring and groundwater bodies	3-8
Drinking water	<12
Clear surface waters	8-12
Moderately polluted rivers	20-35

Exp.5: Titrimetric Determination of Ca^{2+} and Mg^{2+} (Day 5: 19th of May 1999)

Individual determination of Mg^{2+} and Ca^{2+} ions can be achieved by fixing Mg^{2+} at a high pH value to $Mg(OH)_2$ while Ca^{2+} can be titrimetically quantified with EDTA; a consecutive lowering of the pH protonizes the Mg-complex and makes it available for another titrimetic analysis with EDTA; together (Ca^{2+} and Mg^{2+}) represent the overall (total) hardness of an unknown water sample.

material: Peleus rubber-bulb and
1mL Volumetric pipet AS-class
100mL Volumetric pipette AS-class
4 x 10mL beaker
250mL Erlenmeyer flask
10mL Burette w/ stand and funnel
Spatula
Hot plate w/ integrated magnetic stirrer and magnetic rod (30mm)
Waterproof marker

chemicals:
≈5mL Sodium-hydroxide
NaOH (w = 32%) in 10mL beaker
≈5mL Hydrochloric Acid,
HCl (w = 32%) in 10mL beaker
≈5mL Hydrogen-Peroxide
H_2O_2 (w = 30%) in 10mL beaker
≈5mL concentrated Ammonia
NH_3 in 10mL beaker
≈50mL Titriplex-III-solution
(0.1mol/L) $Na_2EDTA \cdot 2\ H_2O$
3 Indicator buffer tablets (H_2O hardness determination w/ titriplex solutions)
3 tips of Calcon-Carbon Acid (CCA)
$C_{21}H_{14}N_2O_7S$ (1:99 of CCA:NaCl)

samples: lL tap water sample
1L river water (Hellbrunner Bach)
1L pond water (Uniteich)

5.1 Titration of Ca^{2+}-ions

Purpose: To avoid any interference with Mg^{2+}-ions, while titrating calcium, the pH of the sample has to be artificially risen beyond pH10, to force the formation of an insoluble magnesium-hydroxide precipitate.

Mg-elimination: $Mg^{2+} + 2NaOH \rightarrow Mg(OH)_2\downarrow + 2Na^+$

CCA-indicator: $CaIn^-_{red} \leftrightarrow Ca^{2+} + In^{3-}_{blue}$

Chelation: $Ca^{2+} + H_2Y^{2-} \leftrightarrow CaY^{2-} + 2H^+_{(neutralized\ by\ OH-)}$

Calcon carbonic acid is used as a standard indicator at this particular pH. Once all the Ca-ions are chelated by the titrant-EDTA, the remaining indicator molecules are destroyed by EDTA, causing a change of color from red to blue.

Procedure: Prepare titrating apparatus and fill burette with titriplex III solution;

- pipet 1mL of NaOH and a tip full of CCA-indicator to the 250mL Erlenmeyer containing a 100mL water sample; dissolve completely (sample turns reddish);
- titrate analyte with titriplex-III-solution (titrant) until (ligand forms Ca-complex) bluish replaces reddish hue of solution.

Note: Check stopcock while titrating - color change occurs quite abruptly.

5.2 Titration of Mg^{2+}-ions

Purpose: Liberation of magnesium ions from magnesium hydroxide is achieved by lowering the pH value with HCl. A tiny amount of H_2O_2 destroys the CCA-Indicator used earlier. Adding some ammonia rises the pH value again to previous levels (≈ pH 10) while creating a buffering system as well.

$NH_3 + HCl \leftrightarrow NH_4Cl$ (a persistent NH3 smell indicates the presence of the buffer)

Placing the flask onto a hotplate eliminates any residual traces of H_2O_2 left after CCA-decomposition at which stage the solution turns colorless again.

Titration is then executed in this buffered solution of NH_3 and Eriochrome Black-T (Indicator tablets). The indicator complexes the free metal ions causing a wine-red solution. Once EDTA chelates all the free Mg-ions during titration and becomes present in slight excess, the solution turns green as a consequence of the reaction:

ErioBT indicator: $H_2In^- \leftrightarrow In^{3-} + H^+_{(neutralized\ by\ OH-)}$

$In^{3-} + Mg^{2+} \leftrightarrow MgIn^-_{(red)}$

Chelation: $MgIn^- + H_2Y^- \leftrightarrow MgY^{2-} + In^{3-}_{(green)} + 2H^+_{(neutralized\ by\ OH-)}$

The former Ca-EDTA complex is stable enough to withstand this treatment; therefore, Ca^{2+} does not interfere with this titration at all.

Procedure: Prepare titrating apparatus and fill burette with titriplex III solution;

- pipet 1mL of HCl and 1mL of H_2O_2 into the flask to destroy previously used indicator and place flask on hotplate until bluish color is completely gone; cool off the flask before executing next step - (place in cold water bath);
- pipet 1mL of NH_3 and one indicator buffer-tablet to the Erlenmeyer flask (sample turns reddish once tablet has dissolved completely);
- titrate analyte with titriplex-III-solution (titrant) until greenish replaces reddish hue;

Note: Check stopcock while titrating - color change occurs quite abruptly.

- Repeat both procedures outlined in 5.1 and 5.2 with at least two more other water samples.

Fig 5.1 Titrimetric determination of Ca^{2+}-ions

5.3 Final results of hardness determination: Each of the titrations performed yield the individual cation content of the water sample; using formula 5.1 and 5.2, the molar concentrations of consumed titrant can be directly converted into dH (German hardness).

Formula 5.1: 1mol titrant chelates 1mol metal ions

$c_{Metal} = \frac{V_{Titrant} \cdot c_{Titrant}}{V_{Water\ Sample}}$	[mol]	V_X, volume solution X $c_{Titrant}$, concentration of -"-	[L] [mol/L]

Formula 5.2: 1°dH = 1mgMeO

$dH_{Analyte} = V_{Titrant} \cdot c_{Titrant} \cdot M_{MeO}$	[°]	$V_{Titrant}$, volume of titriplex-III $c_{Titrant}$, concentration of -"- M_{MeO}, molar mass of MgO/CaO	[mL] [mol/L] [g/mol]

Results (indicated in gray) $V_{Water\ Sample}$ = 0.1L

water sample	$Ca^{2+} \cdot M_{CaO}$ = 56.08 [g/mol]				Mg^{2+} M_{MgO} = 40.31 [g/mol]				Total hardness	
	$V_{Titrant}$ [mL]	$c_{Titrant}$ [mol/L]	c_{Metal} [mmol/L]	dH_{Ca} [°]	$V_{Titrant}$ [mL]	$c_{Titrant}$ [mol/L]	c_{Metal} [mmol/L]	dH_{Mg} [°]	c_{Mg+Ca} [mmol/L]	$\sum_{dH}$ [°]
tap	1.61	0.1	1.61	9.03	0.41	0.1	0.41	1.65	2.02	10.7
river	2.46	0.1	2.46	13.8	0.94	0.1	0.94	3.79	3.40	17.6
pond	2.45	0.1	2.45	13.7	1.01	0.1	1.01	4.07	3.46	17.8

5.4 Discussion and Evaluation: Determination of the total hardness was done with three different water samples. Tab water with a total of 2.02mmol/L Ca-Mg has been classified - according to the table 5.1 - as being in the transition class of soft to hard (as expected by the fact of Salzburg City's geographic location). Whereas, the samples taken from the river and the pond - both around 17.7mmol/L Ca-Mg - can be considered as belonging to hard water calss. This gap between the samples are probably due to the poor working water treatment facility installed at the campus which provides the laboratories with tab water.
The results obtained have been found to be the same with those observed by the other two groups who took part in this years *Environmental Analysis* project.

Table 5.1: Classification of total hardness of water bodies

Total hardness [mmol/L]	**Corresponding class in** [°dH]	**Classification** [-]
0 - 1	0 - 5.6	very soft
1 - 2	5.6 - 11.2	soft
2 - 3	11.2 - 16.8	Soft to hard
3 - 4	16.8 - 22.4	hard
> 4	>22.4	very hard

In comparison with experimentation 6 (quicktests), the results obtained do not exactly match with those of experiment 5; the mismatch is most likely related to the fact that the sites from which the samples were taken were not identical. One has to keep in mind, especially in water chemistry, that the site of sampling as well as the method of sampling does have a significant impact upon the final results.

Exp.6: Quicktests used in Water Sampling (Day 5: 19th of May 1999)

6.1 Sampling and On-site Analysis:

Purpose: On site sampling and analysis provides a quick estimate of the chemo-physical constitution of a water sample.

If it is suspected that waste water is being discharged, a minimum of 3 samples should always be taken:

- from the suspected source of discharge;
- from the polluted water downstream of the suspected site;
- from a non-polluted section of water, as far as possible upstream from the conjectured point of discharge.

material: squeeze-flask of deionized water
Conductivity Meter
Oxical-Meter
Aquaquant Quicktest (Merck)
f. BOD, Ca, Hardness, pH, NH_4^+, NO_2^-, NO_3^-,
Merckoquant Teststrips (Hardness)
Riedel deHaen Aquanol (PO_4^{3-})

Procedure: Execute test according to the instructions given in the instructions manual of each quicktest box.

Ammonium - colorimetric determination of NH_4^+;
Ammonia reacts with a chlorinating agent to form monochloramine, which reacts with thymol resulting in 2,2-isopropyl-5.5-methyl-indophenol;

Nitrite - colorimetric determination of NO_2^-;
The determination of the nitrite takes place after diazotization of sulfanilic acid and coupling with N-[naphtyl-(1)]-ethyline diamin hydrochloride which forms a violet azo dye;

Nitrate - colorimetric determination of NO_3^-;
Nitrate reduced to nitrite reacts with sulfanilic acid; the diazonium salt which results from this is coupled with 2.5-dihydroxy-benzoic acid to the corresponding azo dye;

Colorimetric determination of pH;
Colorimetric determination of the pH with a mixed indicator;

Total Hardness - titrimetric determination;
Calcium and magnesium ions form a colored chelate comples with the indicator; through titration with Titriplex-III (disodium salt) metal ions are bound to these chelate formers and the indicator's chelate complex is destroyed with a change in color;

Residual Hardness (Carbonate Hardness) - acid binding capacity;
Titration of a sample water with hydrochloric acid using a mixed indicator;

Phosphate - colorimetric determination of PO_4^{3-};
Reaction w/ molybdate ion to form a phospho-molybdate which is selectively reduces to intensly colored molybdenum blue.

O_2 Test - titrimetric determination of Oxygen O_2, or electronically with Oxical-Meter;
In an alkaline milieu oxygen oxidizes bivalent (dibasic) manganese into higher-valence manganese hydroxides (precipitates). After acidifying, manganese hydroxides dissolve into Mn(III) ions. Mn(III) is reduced to Mn(II) with iodine. The iodine which is released during this process is equivalent to the oxygen concentration of the sample and is determined iodometrically.

Conductivity is determined in the laboratory with a conductivity meter.

6.2 Final results of physical and chemical measurements:

	Pond	**Influx**	**Outflux**
Temperature [°C]	19.8*	16.5*	20.6
Conductivity [µS/cm]	596*	594	597*
pH [-]	7.8	n.e.	n.e.
Nitrite [mg/L]	≈ 0.02	n.e.	≈ 0.03
Nitrate [mg/L]	≈ 5	n.e.	≈ 10
Ammonium [mg/L]	≈ 0.3	n.e.	n.e.
Phosphate [mg/L]	≈ 0	n.e.	n.e.
Carbonate Hardn. [°dH]	≈ 16.5	n.e.	n.e.
Total Hardn. [°dH]	≈ 21.5	n.e.	n.e.
O_2 - Oxygen [mg/L]	≈ 12* (9.5)**	≈ 15.7*	≈ 8.8*
O_2 - Oxygen [%]	65*	173*	n.e.

(*) determined electrically; (**) determined chemically, (n.e.) not executed

Fig 6.1 Assemblage of Quicktests

6.3 Discussion and Evaluation:

The values listed in the table of results have been executed according to the sector assigned to the groups taking part in this years course. Due to lack of coordination on behalf of the students, some crucial parameters of both influx and outflux have not been recorded. Therefore, final results of the eutrophic effects by aquatic fauna could not be deducted.

Several water samples have been taken from different depths. The location (not the depth) has been recorded on the sampling report (see results 6.2), along with the weather conditions and other specific observations (sunny day with mild temperatures).

Although quicktests do not exactly quantify the extent of a contaminated water body, they often provide some clues whether further laboratory analysis are required or not. Better results can be achieved by using quicktests which rely on titrimetric methods rather than on colorimetric comparison.

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