Travaux Pratiques-Pollutants analysis in the environment

(Day 1)

Purpose of the practical works:

In the practical work, the composition of 2 unknown solutions will be determined by potentiometric methods. The unknown solutions contain a ligand (i.e., weak diprotic acids) that are mainly used as a ligand in the complexation process of a specific metal ion.

In *Day 1*, the unknown solution (S_x) contains one ligand. The determination of the dissociation constants of the unknown ligand (pKa_s), which are obtained by potentiometric titration, will allow identifying it.

In *Day 2*, the unknown solution is a mixture of 2 ligands. Identity and concentration of the ligands contained will be obtained using the dissociation constants (pKa_s), and the concentration of titrant added.

Method: The potentiometric experiment is applied to determine the dissociation constants of the ligand. It consists of a titration where a strong base, i.e. NaOH, is used to titrate the unknown ligand until the two equivalence points are met monitoring the potential of the protons in solution.

Devices and sensors used: A dosimeter (*DOSIMAT* XX, Metrohm) is used to add NaOH into the unknown solution. A pH electrode is used to measure the potential, which is proportional to the pH value in the solution. A magnetic stirrer and a stirrer bar are used to mix the solution.

Required material: Computer with spreadsheets for annotation of experimental data and calculations.

Safety in the lab:

- **Always** wear lab coats and goggles

- If you are handling chemicals, wear gloves

- Dispose of the chemicals in the appropriate container under the hood

- It is **forbidden** to drink and eat anything in the lab; ask the assistant for a break. After **removing** lab coats and goggles, go **outside** of the lab (10 min max per break). Highly recommended to *wash your hands* before eating and drink:-)!

You will handle several chemicals during the practical works. You should avoid contact with them on your skin or your eyes, and none of the chemicals should be ingested.

1. **Sodium hydroxide** (NaOH) 0.2 mol/L: it is very caustic and should be handled with care. Avoid spilling it in your skin, hands and eyes, or clothing. If NaOH gets on your skin, rinse immediately with plenty of water. In case of contact with your

eyes use the buffer solutions ready for emergencies. A treatment within 40 second is advised.

2. **Buffer solutions**: In general, not particularly hazardous. Handle with the usual precautions. In case of contact with skin, rinse with water.
3. **Oxalic acid** (HO_2CCO_2H) 0.08 mol/L: It is toxic if swallowed, wash your hands carefully before you drink or eat. It causes severe skin burns and eye damage. In case of contact with skin, wash with plenty of water.
4. **Malonic acid** ($CH_2(COOH)_2$) 0.08 mol/L: It is toxic if swallowed, wash your hands carefully before you drink or eat. It is highly corrosive, avoid any contact with skin, especially with eyes.
5. **Malic acid** ($HO_2CCH_2CH(OH)CO_2H$) 0.08 mol/L: Concentrated solutions are irritating to the skin and eyes. Mostly non-toxic, it is used as a general additive in animal drugs, feeds commercial applications.
6. **Glutaric acid** ($HOOC(CH_2)_3COOH$) 0.08 mol/L: Causes severe eye damage. In case of spilling in the eyes wash with plenty of water. If it is spilled on your skin or clothing, remove clothing immediately and use plenty of cold water to rinse skin.
7. **Glycine** (NH_2CH_2COOH) 0.08 mol/L: Essentially nontoxic. It should be handled with the usual precautions: do not ingest, get on your skin or eyes, and do not spill into the laboratory environment.
8. **β-Alanine** ($NH_2CH_2CH_2COOH$) 0.08 mol/L: Essentially nontoxic. It should be handled with the usual precautions: do not ingest, get on your skin or eyes, and do not spill into the laboratory environment.

For more information: https://pubchem.ncbi.nlm.nih.gov/

About the report:

Describe the aim of the experiment.

For the solutions provided by the assistant explain how you would prepare them with the chemicals, which you find in the laboratory.

Describe experimental setup and procedure performed, giving exact quantities of all relevant parameters. (Someone who is reading your report should be able to reproduce your experiment).

Give experimental results (raw data, i.e. potential measured, the volume of solutions added).

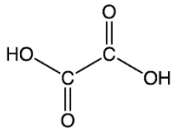
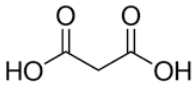
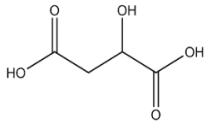
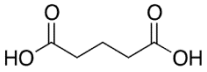
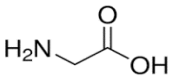
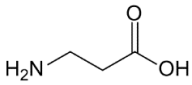
Evaluate raw data (i.e. calculate Z-plot) and discuss results and problems observed.

- equations
- pKa_s values of the ligand (H_2L)
- solution composition

Make concluding remarks.

The report needs to be delivered at 18:30 on the same day.

Compound list and info

Compound	Chemical formula	Structural formula	CAS number	Molecular Weight	pKa_1 (25°C)	pKa_2 (25°C)
Oxalic acid	HO_2CCO_2H		144-62-7	90.03	1.23	4.19
Malonic acid	$CH_2(COOH)_2$		141-82-2	104.06	2.83	5.69
Malic acid	$HO_2CCH_2CH(OH)CO_2H$		6915-15-7	134.09	3.40	5.15
Glutaric acid	HOOC(CH2)3COOH		110-94-1	132.11	4.34	5.41
Glycine	NH_2CH_2COOH		56-40-6	75.07	2.35	9.78
β-Alanine	$NH_2CH_2CH_2COOH$		107-95-9	89.09	3.55	10.24

General description:

Titration

In a titration, the concentration or amount of an unknown substance (*analyte*) is obtained by addition of a substance with a known, accurately determined, the concentration of titrant, named standard solution. Depending on the nature of the substances involved, the titration is called differently.
When the moles of the titrant added are stoichiometrically equivalent to the analyte moles initially present in the unknown solution, the equivalent point (Equivalent Point) has been met. This point is a theoretical value and corresponds to the equality of acid and base. The final point of a titration (End Point) depends on the indicator, that shows when the equivalent point has been met and overcome. It is an experimental measurement. A different type of titration method can be chosen based on the method used for the acquisition of the experimental to determinate the End Point.

Acid-Base titration type

In an acid-base titration, one acid and one alkaline solution are involved. A glass electrode, pH electrode, is mainly applied for this type of titration.
Strong acids are completely dissociated in water, while weak acids are only partially dissociated. The value of the equilibrium constant and the amount of the weak acid can be used to calculate its extent of dissociation.
Considering the dissociation reaction $HA \rightarrow H^+ + A^-$, Ka represents its dissociation constant.

$$Ka = \frac{(a_{H^+})(a_{A^-})}{a_{HA}} = \frac{[H^+][A^-]}{[HA]} * \frac{(\gamma_{H^+})(\gamma_{A^-})}{\gamma_{HA}}$$

where: a_{A^-} is the activity of the conjugate base, A^-

$[A^-]$ is the molar concentration of the conjugate base, A^-

γ_{A^-} is the activity coefficient of the conjugate base, A^-

The relative acidities of bases and acids are expressed in terms of pKa, $pKa = -\log_{(10)} Ka$.

Since $pKa \equiv -\log Ka$ & $pH \equiv -\log(a_{H^+})$, then

$$pKa = -[\log(a_{H^+}) + \log(a_{A^-}) - \log(a_{HA})]$$

$$pKa = pH - \log(a_{A^-}) + \log(a_{HA})$$

$$pKa = pH - \log\left(\frac{a_{A^-}}{a_{HA}}\right) = pH - \log\left\{\left(\frac{[A^-]}{[HA]}\right) * \left(\frac{\gamma_{A^-}}{\gamma_{HA}}\right)\right\}$$

The pK_a is $-\log(K_a)$ at the ionic strength of the solution and corresponds to the pH at which the activities of the acid HA and its conjugate base A^- are equal.

The approximation that $\left(\frac{\gamma_{A^-}}{\gamma_{HA}}\right) \cong 1$ yields an apparent $pH = pKa + \log\left(\frac{[A^-]}{[HA]}\right)^*$

In case of a diprotic acid (like the ligand in the unknown solution), its dissociation constants are defined as follows

1. $H_2A \rightarrow H^+ + HA^-$ $\quad Ka_1 = \frac{(a_{H^+})(a_{HA^-})}{a_{H_2A}}$; considering the approximation $\Rightarrow Ka_1 = \frac{[H^+][HA^-]}{[H_2A]}$

2. $HA^- \rightarrow H^+ + A^{2-}$ $\quad Ka_2 = \frac{(a_{H^+})(a_{A^{2-}})}{a_{HA^-}}$; considering the approximation $\Rightarrow Ka_2 = \frac{[H^+][A^{2-}]}{[HA^-]}$

*This equation is referred to as the Henderson-Hasselbalch equation. It is advantageous in the buffering region of the titration of a weak acid.

Example of diprotic acid titration curve

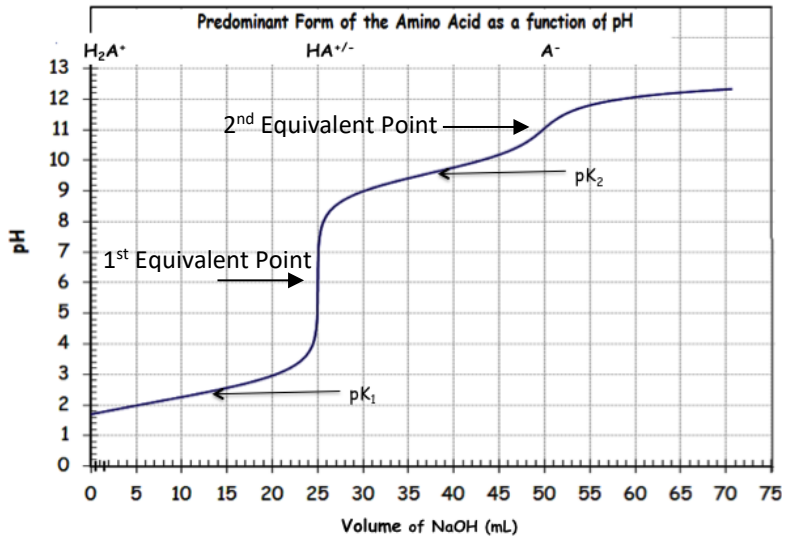


Figure 1: Titration of 25.0 mL of a 0.100 M glycine in the H_2L^+ form with 0.100 M NaOH

Potentiometric method

The potentiometric method consists of the measurement of the potential (E) as a function of the titrant volume added; the data are collected before and after the Equivalent Point. The plot of the potential values measured *vs* the volume of the titrant added gives the titration curve. The derivative of these values allows enhancing the Equivalent Point to look for more precise values. In these practical works, the potential of the H^+ ions in solution is measured. The Nernst Equation is used to correlate the potential measured with the concentration, or better activity, of H^+

$E = E_0 + b \log(a_{H^+})$, or using the previously applied approximation, we can write that

$$E = E'_0 + b \log [H^+]$$

where E_0 is the standard cell potential (V)

b is a constant related to T and other constant parameters

a_{ion} is the activity of the ion

$a_{ion} = [ion]^* \gamma_{ion}$

γ_{ion} = activity coefficient$_{ion}$. Among other parameters, it depends on ionic strength and size of the ion.

The Z formula

The Z formula represents the average number of H^+ ions released per ligand molecule and allows us to determine more accurately the constant dissociation values. We consider the case of a ligand H_2L,

$$Z = \frac{[H^+]_{\text{total protons seated on the ligand}}}{[H_2L]_{total}} = \pm \frac{H_{tot} - [H^+] + [OH^-]}{[H_2L]_{total}}$$

where: $[H^+]_{total}$ = total concentration of the H^+ ions released from the ligand in solution, obtained considering the amount of strong base (titrant, NaOH) added into the solution containing the ligand, and the eventual strong acid (HCl) added to protonate the amino group of an amino acid to obtain a diprotic acid.

$$[H^+]_{\text{total protons seated on the ligand}} = \frac{V_{HCl} * [HCl]}{V_{H_2L} + V_{NaOH} + V_{HCl}} - \frac{V_{NaOH} * [\text{NaOH}]}{V_{H_2L} + V_{NaOH} + V_{HCl}} - [H^+] + \frac{K_w}{[H^+]}$$

where $V_{total} = V_{H_2L} + V_{NaOH} + V_{HCl}$,

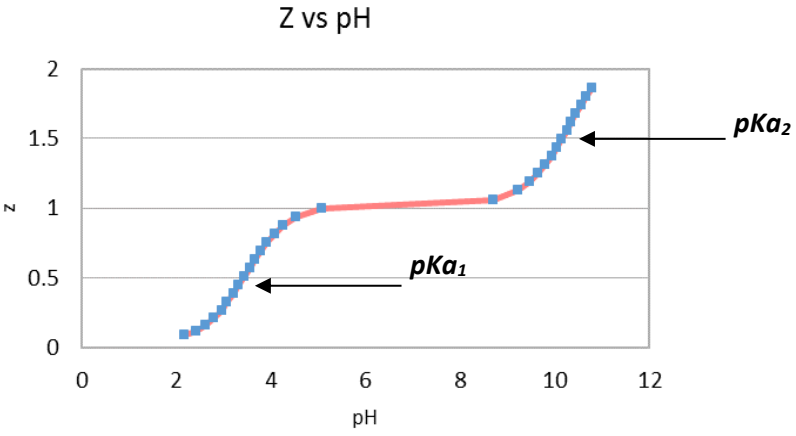
and V_{H_2L} correspond to the initial unknown solution volume

$[H_2L]_{total}$ = total concentration of the ligand in solution considering the dilution due to addition of NaOH and HCl into the starting unknown solution, $[H_2L]_{total} = \frac{V_{H_2L}[H_2L]}{V_{total}}$.

In the specific case of this practical works, the unknown solutions containing amino acids as a ligand has been already protonated (the ligand is a diprotic acid), then we do not consider the term related to HCl added. The simplified equation to use for the calculation is

$$Z = \frac{\left(\frac{V_{NaOH} * [\text{NaOH}]_{initial}}{V_{total}}\right) + [H^+] - \frac{K_w}{[H^+]}}{\left(\frac{V_{H_2L} * [H_2L]_{initial}}{V_{total}}\right)}$$

Plotting Z, as a function of pH, the pKa_s values of the ligand are represented by the two inflexion points.



If we write the Z express in terms of ligand speciation, we have:

$$Z = \frac{[\mathrm{HL^-}]+2\,[\mathrm{L^{2-}}]}{[\mathrm{H_2L}]+[\mathrm{HL^-}]+[\mathrm{L^{2-}}]}$$

Because

1. $H_2L \rightarrow H^+ + HL \qquad Ka_1 = \frac{[H^+][HL^-]}{[H_2L]} \qquad \Rightarrow \qquad [HL^-] = \frac{Ka_1\,[H_2L]}{[H^+]}$

2. $HL^- \rightarrow H^+ + L^{2-} \qquad Ka_2 = \frac{[H^+][L^{2-}]}{[HL^-]} \qquad \Rightarrow \qquad [L^{2-}] = \frac{Ka_2\,[H_2L]}{[H^+]}$

Expressing the ligands species in terms of equilibrium constants, we have

$$\mathrm{Z} = \frac{\frac{\mathrm{Ka_1}\,[\mathrm{H_2L}]}{[H^+]} + 2\frac{\mathrm{Ka_2}\,\mathrm{Ka_1}\,[\mathrm{H_2L}]}{[\mathrm{H^+}]^2}}{\mathrm{H_2L} + \frac{\mathrm{Ka_1}[\mathrm{H_2L}]}{[\mathrm{H^+}]} + \frac{\mathrm{Ka_2}\,\mathrm{Ka_1} * [\mathrm{H_2L}]}{[\mathrm{H^+}]^2}}$$

Moreover, expressing Z as Z = *f* (pH), the values of the dissociation constants of the ligands can derive graphically

$$\frac{\mathrm{Z}\,[\mathrm{H^+}]}{1-\mathrm{Z}} = \mathrm{Ka_1} + \frac{\mathrm{Ka_1Ka_2}}{[\mathrm{H^+}]} * \frac{2-\mathrm{Z}}{1-\mathrm{Z}}$$

Brief experimental description, data treatment and evaluation

1. Calibration of the pH electrode with three buffer solutions applying the Nernst equation, $E = E'_0 + b \log [H^+]$
    a. Determination of E_0 and K using the 3-points calibration method
    b. Make a table with experimental values and a graphical representation of **E** *vs* **pH**

pH	**E (mV)**	**T (°C)**
…	…	…
…	…	…

Remember: pH = -log $[H^+]$
Tip: Find the linear equation to determine slope (k), and intercept (E'_0).

2. Potentiometric determination of dissociation constant of the unknown ligand (**fast** titration) using *Z* formula
    a. Add 20 mL of unknown ligand solution (**S_x**);
    b. Titration with *NaOH* solution with additional steps of **0.5 mL each** until you find the dissociation constants pKa_1 and pKa_2;
    c. Make a table with experimental values and calculations, and graphical representation of **pH** *vs* **V_{NaOH} (mL)** and **Z** *vs* **pH**;
    d. Guess the identity of the ligand contained in your solution comparing your results with a table provided.

Volume NaOH [mL]	**Total Volume [mL]**	**E (mV)**	**pH**	**$[H^+]$**	**$[H^+]_{total}$**	**$[H_2L]_{total}$**	**Z**	**$\Delta Z/\Delta pH$**
	$V_L + V_{NaOH}$	…	…	…	…	…	…	…
	…	…	…	…	…	…	…	…

Remember $pH = \frac{E-E_0}{b}$; $pH = -\log[H^+] \Rightarrow [H^+] = 10^{-pH}$

3. Potentiometric determination of dissociation constant of the unknown ligand (**slow** titration) using Z formula
    a. Add 20 mL of unknown ligand solution (S_x);
    b. Titration with NaOH solution with additional steps of **0.5 mL each**, and **0.3 mL** close to the buffered zones related to pKa_1 and pKa_2 values;
    c. Make a table with experimental values and calculations, and graphical representation of **Z** *vs* **V_{NaOH} (mL)** and **Z** *vs* **pH**;
    d. Define the identity of the ligand contained in your solution.

Volume NaOH [mL]	Total Volume [mL]	E (mV)	pH	$[H^+]$	$[H^+]_{total}$	$[H_2L]_{total}$	Z	$\Delta Z/\Delta pH$
	$V_{H_2L} + V_{NaOH}$	…	…	…	…	…	…	…
	…	…	…	…	…	…	…	…

Tip: Define the Δ amplitude appropriate to interpret your experimental data.

In the graph of the derivative as a function of the pH, the maximum of the peaks represent the pKa_s values.