BMOL2201/6201 PRACTICAL 1
Amino acid titrations

AM	PM	Outline
10:05	2:05	Introductory talk
10:15	2:15	Pre-lab Quiz and get started
10:20	**2:20**	**Titration of amino acids**
12:20	4:20	Cleanup
12:30	4:30	Go to iLearn and complete Prac Quiz
12:55	4:55	Prepare to leave lab

Practical 1 Aims

Using acid-base titrations,

1. determine the pK_a values for a simple amino acid provided: glycine.
2. determine the pK_a values for an unknown amino acid, and identify it using the reference table provided.

- *Associated Lecture for revision*:
  - Lecture 2: Amino Acids 1

Theory for Prac 1

pH:

- pH = $\log_{10} 1/[H^+] = - \log_{10} [H^+]$; if $[H^+] = 10^{-3}$, pH = 3
- Gives an idea of H^+ concentration in the solution
- pH scale:

→ pH low: $[H^+]$ high; acidic

physiological; neutral

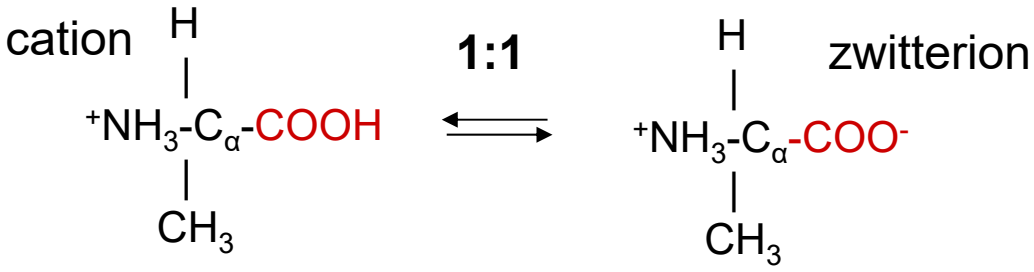
pH high: ← $[H^+]$ low; basic

Titration of an amino acid

pK:

- is the pH where protonation of the titrated group is 1:1
  e.g. for the α-COOH group, COOH and COO^- are in equal concentrations

e.g. alanine

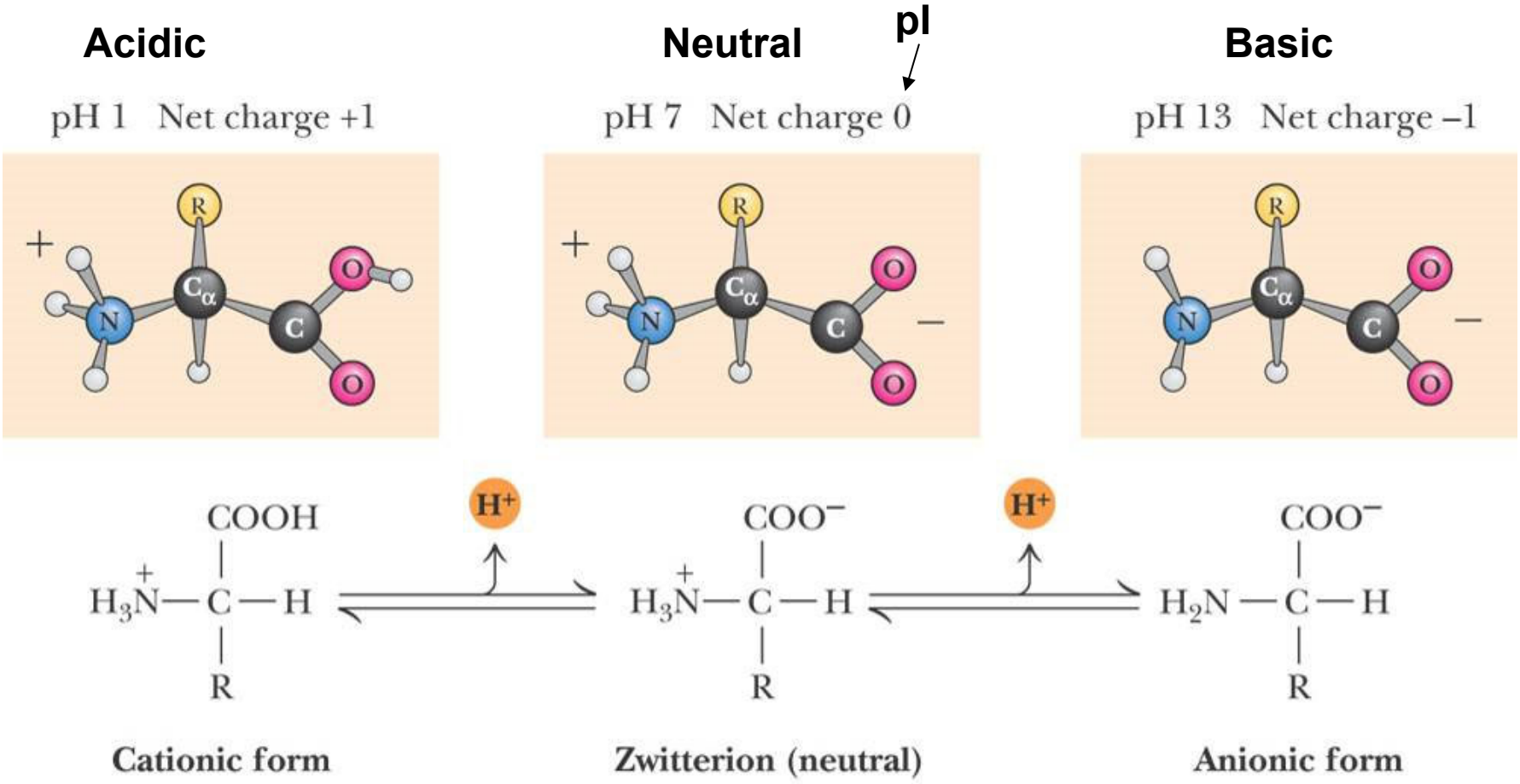


- All free COOH and NH_2 groups can be titrated: **two pK values** for a simple amino acid.
- pK of α-COOH is ~ pH 2 and pK of α-NH_3^+ is ~ pH 9.
- Some R-groups can be titrated; these amino acids have **three pK values**:
  - Acidic (i.e. COOH) groups in Aspartate (D) and Glutamate (E).
  - Basic (i.e. amino) groups in Arginine (R), Lysine (K) and Histidine (H).

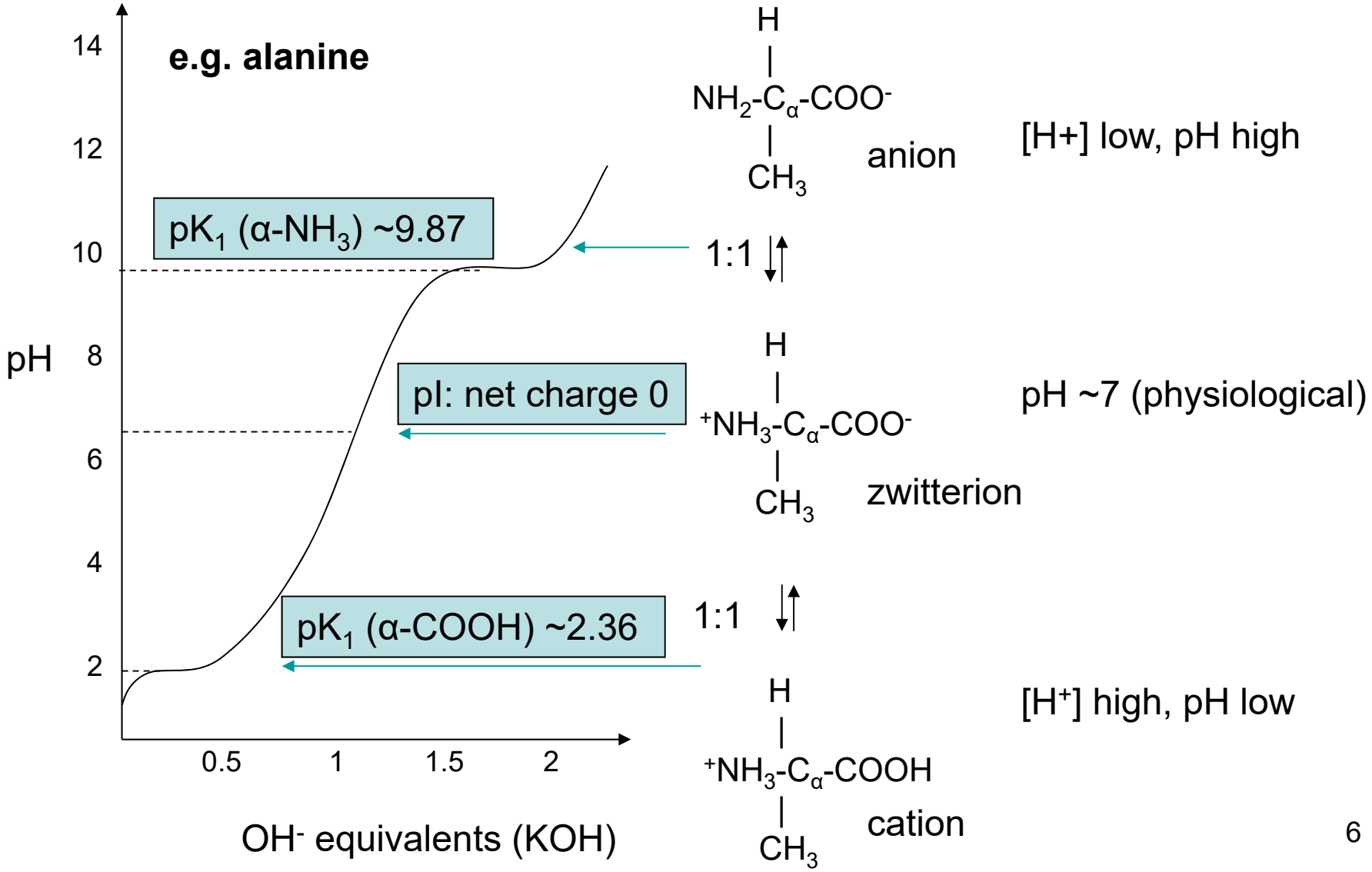
pI:

- is the net charge of the amino acid is zero (zwitterion).
- is characteristic of an amino acid and can be used to identify unknown amino acids.

Most amino acids have two ionizable groups!



Titration curve for a simple amino acid



Example: Glycine

Titration of glycine, a simple amino acid.

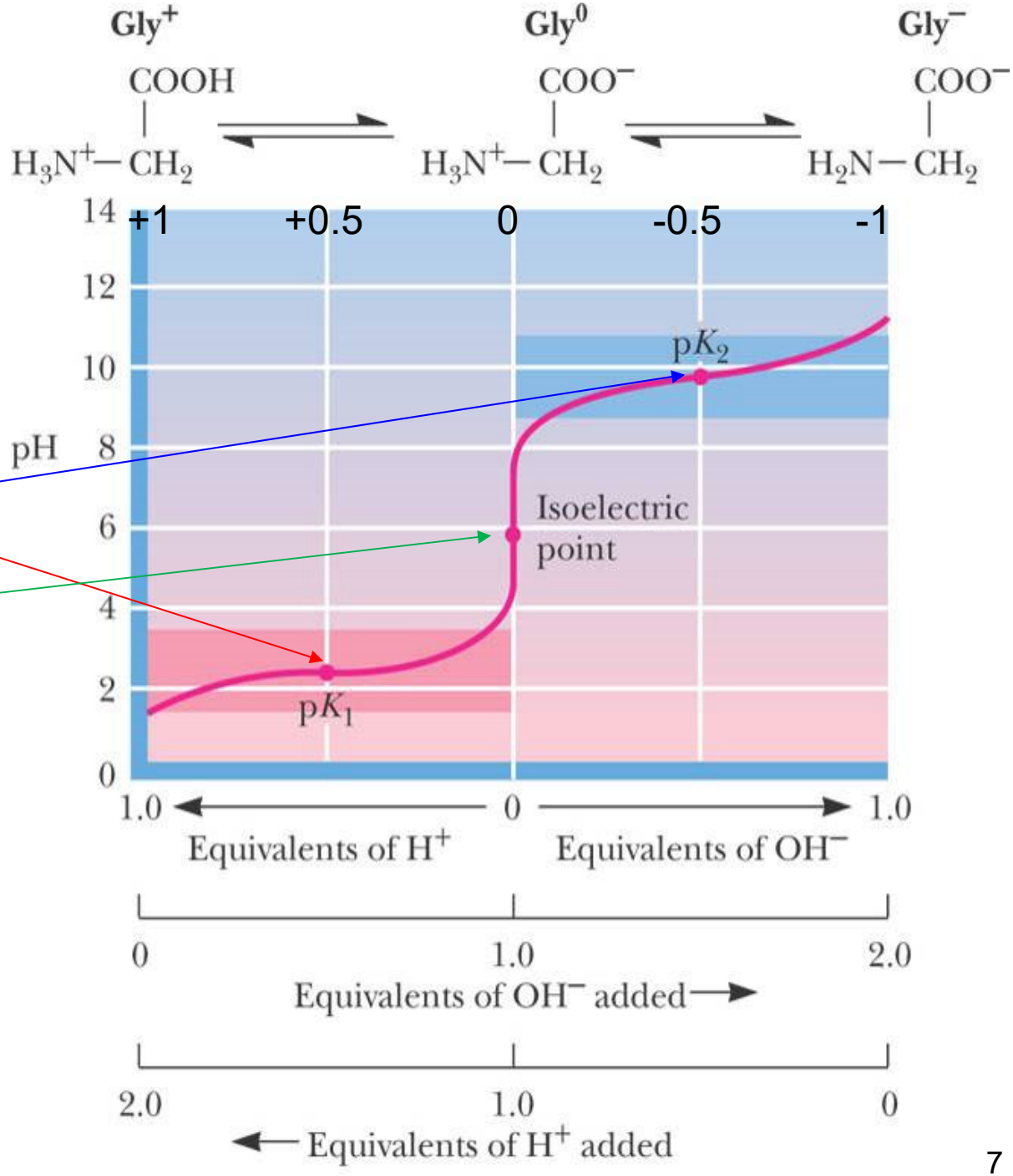
pK_1 ~2

pK_2 ~10

pI ~6

the isoelectric point, is the pH where the molecule has a net charge of 0, and is calculated as

pI = (pK_1 + pK_2)/2.



Titration of amino acids - Summary

Experiment: Determine pK's and pI for a known amino acid (glycine) and an unknown amino acid, by titration with KOH.

1.Titration of glycine **CORROSIVE!!!**

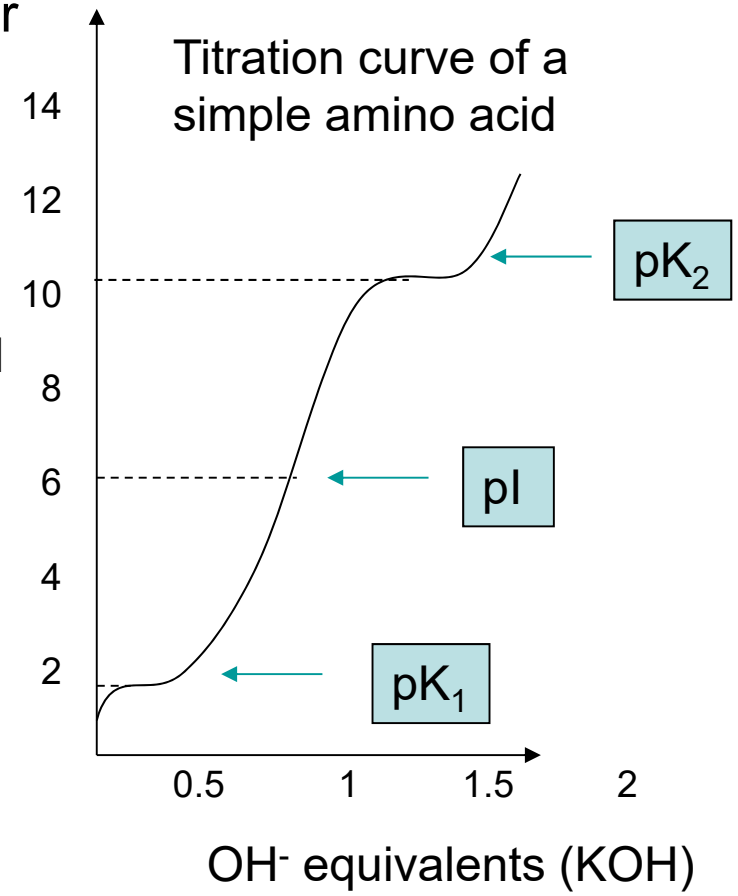
- Put glycine and water in beaker with stirrer
- Fill burette with KOH up to zero mark
- Place pH meter in solution
- Add KOH 0.2 ml at a time.
- Record pH & volume of KOH (excel file)

2. Titration of unknown amino acid

- Repeat steps 2-4 with unknown amino acid instead of glycine

To complete

- Generate titration curves & save excel file
- Determine pK's and pI from the graphs
- Identify unknown amino acid
- Answer all questions and save.



Before you start the experiment

- View the Lab Safety video

Prac 1 relevant videos guides for setting up and using equipment:

- Burette video
- Magnetic stirrer video
- pHmeter video
- Waste disposal video

Liquid dispenser

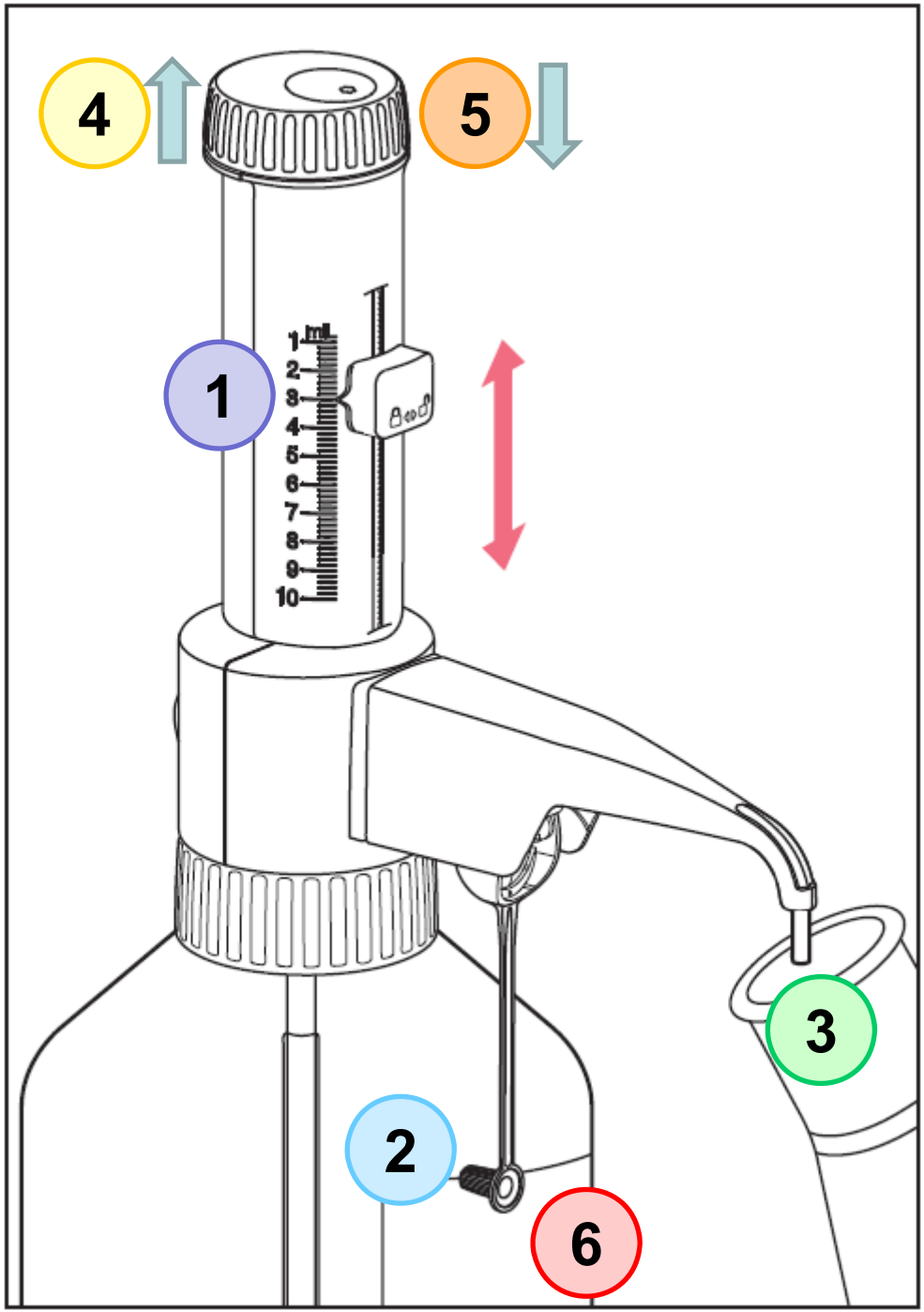
- Safe and reliable method of extracting fixed volumes of liquids
- Take your beaker to collect the liquids

Part A: You will need

1. 50 ml of glycine
2. 20 ml of water.

Part B: You will need

1. 20 ml of water.



How to use the liquid dispenser

1. Check the required volume is correctly set.
2. Remove the safety cap.
3. Hold your beaker here.
4. Gently lift the piston ***all the way up*** with the other hand.
5. Push down gently to collect the liquid in the beaker.
6. Replace the safety cap at the end…

STUDENTS TO PROCEED ON THEIR OWN FROM HERE

Procedure: Step 0

What to do - all students

1. You need covered shoes!
2. No bags on lab bench – place in the under-bench storage
3. Tie up your hair, if applicable
4. Button up your lab coats
5. Put on safety glasses.
6. No food or drink please!

Need help?

- Lab Safety video

Procedure: Step 1a: Getting started

1. Decide who from each group is going to do the titration first. We will call this person **Student 1**.
2. The team will have the other student (s): **Student 2** (and optionally **Student 3**).

Student 1 (no gloves)

a. Login to iLearn and complete the Pre-Lab Quiz.

Student 2 (no gloves)

A. Collect an empty tray from the backbench/shelves and put into it:
   1. a **wash bottle**
   2. a box of **Kimwipes**
   3. a **liquid waste container**
   4. a **Pasteur pipette**

B. Bring the tray back to your desk.

Procedure: Step 1b: More to get

Student 1 (gloves on)

a. From the backbench, collect:
   1. a **beaker** and
   2. a **stir bar** – put into the beaker.

b. Add 50 ml glycine and 20 ml water into this beaker using liquid dispensers.
   - Remember ***to pull the dispenser up all the way up***, before pushing down, to get the right volume!

c. Then, pick up
   3. a bottle of **1M KOH**.

d. Carry these carefully back to your desk – ***make two trips if needed***.

e. Place the beaker and the KOH bottle in the tray.

Student 2 (no gloves)

A. Login into iLearn and complete your Pre-Lab Quiz. (Also **Student 3** if applicable)

Procedure: Step 2

Student 1 (gloves on)

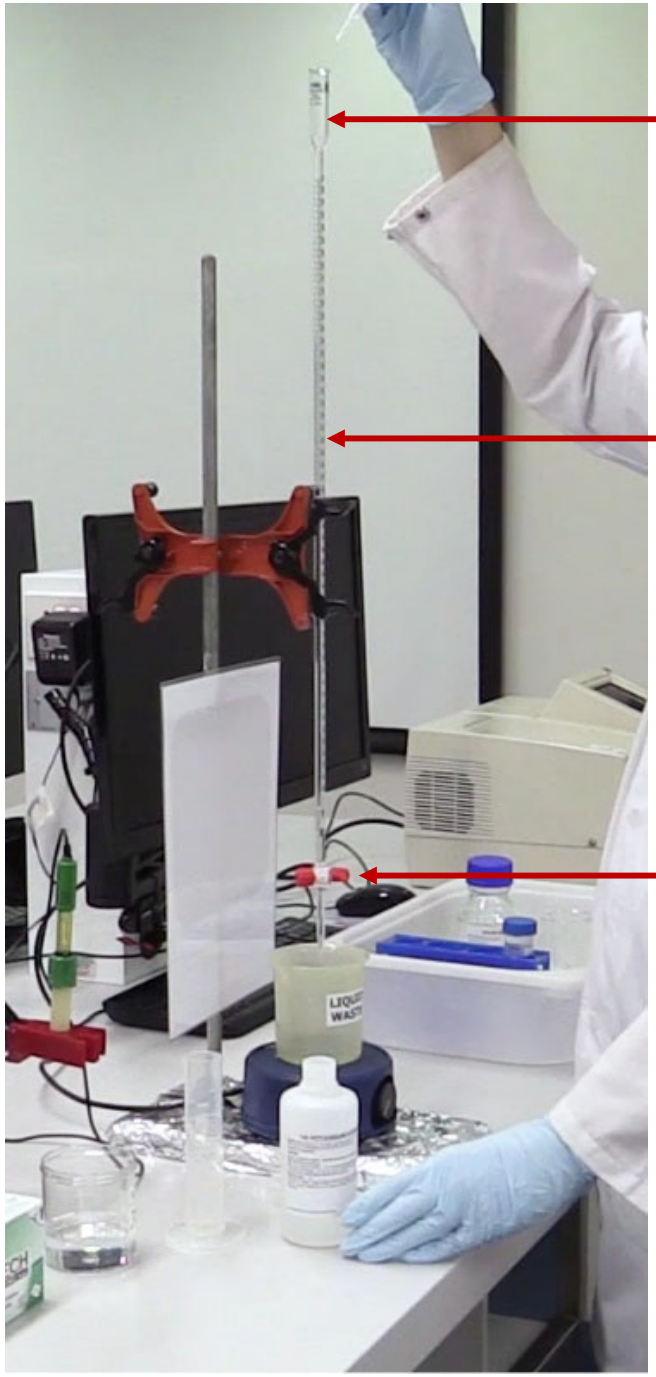
a. Place the liquid waste container on the magnetic stirrer.
b. Fill the burette with KOH up to the zero or a convenient mark at eye level, with the Pasteur pipette (careful!). Put the pipette back in the tray.
c. Ensure there are no air bubbles in the burette and clear the nozzle.
d. Keep the burette closed.
e. Swap the liquid waste container with the beaker containing Glycine and water.
f. Turn ***on*** the **magnetic stirrer** to stir the contents of the beaker *gently*.
g. Place the pH electrode in the solution - *far from the burette tip and the stir bar!*

Student 2 (no gloves)

1. Download and open ***Prac 1 data*** excel file from iLearn and **save on Desktop** with your **name/SID**.
2. Fill in the **desk number** and your **group details** on the first worksheet. Save.

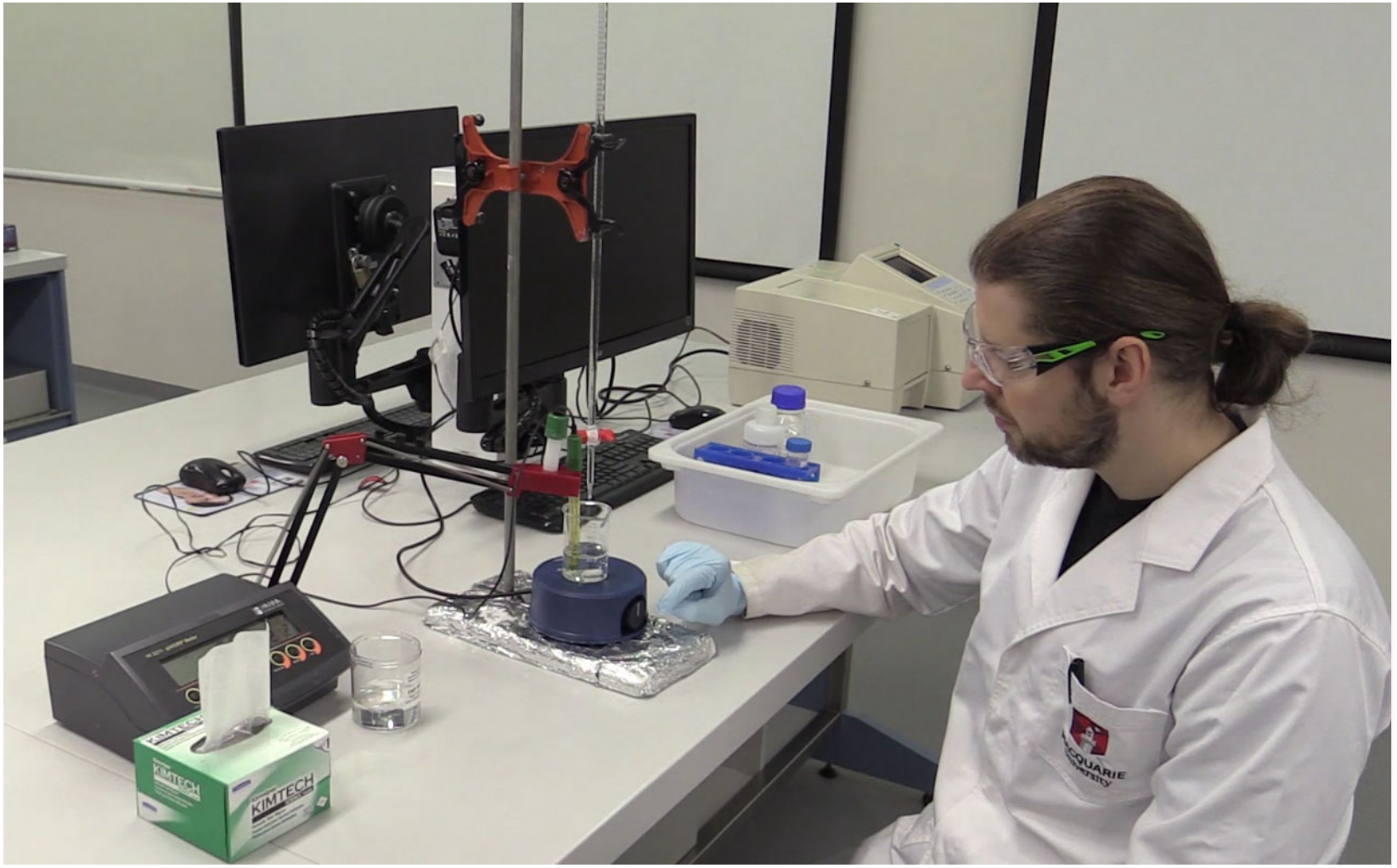
Need help?

- Stirrer video
- Burette video
- pHmeter video



- Burette top – fill with dropper pipette
- Burette capacity: 10 ml: **each graduation is 0.2 ml**
- Stop cock: turn slowly and make sure all air bubbles are removed.

Check final setup with your tutor!



Procedure: Step 3

Student 1 (gloves on)

- Add **KOH** **0.2 ml at a time (one large marking to the next)**, from the burette.
- When you reach **10.0 ml**, **stop** to refill the burette with KOH and then continue titration.
- Keep adding **0.2 ml** KOH at a time, till **pH 12.0** is reached! Turn the **magnetic stirrer *off***.
- Remove the **pH electrode**, rinse into liquid waste container and place it in tap water container.

Student 2 (no gloves)

- Enter the **pH** & **volume of KOH** in the excel file for **each addition of KOH.**
- Keep **saving** with each entry (Ctrl+S).
- The graph should draw itself.
- Complete the graph and axes labels, in consultation with the other student(s) and save.

Need help?

- pHmeter video

Procedure: Step 4

Student 1 (gloves on)

- Place the *liquid waste container* on the magnetic stirrer and take the <u>beaker with the stir bar</u> to the backbench,.
- Remove the stir bar with the magnetic rod and place the rod with the stir bar in the tray. Then, tip the beaker contents carefully into the **<u>Chemical waste container</u>**.
  - ➢ *If the magnet falls in by mistake, use the rod to pull it out.*
- Rinse the beaker and the stir bar (at the end of the magnetic rod) separately with the wash bottle right next to the Chemical waste container and tip the rinsing also into the same container.
- Repeat the rinsing and then put the stir bar from the magnetic rod back into the beaker.

❖ Add 20 ml water into the beaker using <u>liquid dispenser</u> for the next titration, and return to your desk.

Need help?

➢ Liquid waste disposal video

Student 2 (no gloves)

- Complete the worksheet and save.

Step 5: Unknown Amino Acid

Student 1 and **Student 2** to swap places.

Student 1

(no gloves)

A. Go to the **Unknown amino acid** worksheet in the Data file.

B. Enter the **label** of the **unknown amino acid** and save.

Student 2 (gloves on)

a. Fill the burette with KOH, using the Pasteur pipette. If you need more KOH, pick a fresh bottle from the back bench and place it in the tray.

b. From the backbench, collect:
   1. one bottle of the unknown amino acid and
   2. a measuring cylinder.

c. Carry these carefully back to your desk.

d. Add **50 ml** of the unknown amino acid into the rinsed beaker (with the stir bar and the 20 ml water), using the measuring cylinder.

e. Place the beaker on the magnetic stirrer.

Procedure: Step 6

Student 2 (gloves on)

- Add **KOH** **0.2 ml at a time (one large marking to the next)**, from the burette.
- When you reach **10.0 ml**, **stop** to refill the burette with KOH and then continue the titration.
- Keep adding **0.2 ml** KOH at a time, <u>till **pH 13.0**</u> is reached! Turn the **magnetic stirrer *off.***
- Remove the **pH electrode**, rinse into liquid waste container and place it in tap water container.
- Release <u>ALL the remaining KOH</u> into the liquid waste container.
- Fill the burette all the way to the top with **RO water** from your **wash bottle** and let it in run completely out of the burette. Repeat this step once more.
- Leave the stopcock of the burette open.

Student 1 (no gloves)

- Enter the **pH** & **volume of KOH** in the excel file for <u>**each addition of KOH.**</u>
- Keep <u>**saving**</u> with each entry (Ctrl+S).
- The graph should draw itself.
- Complete the graph and axes labels, in consultation with the other student(s) and save.

Step 7: Packing up

Student 2 (gloves on)

- Wipe your desk clean with a Kimwipe, in case of spills.
- Take **your tray to the backbench** and put the following back in their respective locations:
  1. the KOH bottle,
  2. the Kimwipes,
  3. the wash bottle and
  4. the unknown amino acid bottle.
- Put the used Pasteur pipette in the ***Chemical Waste bin***.
- Take the **stir bar** out using the **magnetic rod**, rinse into the liquid waste container and return it to the labelled tray.
- Empty the **beaker** and the **liquid waste container** into the **Chemical waste container**.
- **Wash** the following **using the RO water taps** and put them in the drying trays next to the sink:
  1. the beaker,
  2. the liquid waste container and
  3. the measuring cylinder.
- Leave your **empty tray** on the back bench.

Procedure: Step 8

All team members

- Complete all the calculations in the Data file and save.
- Use Table 3 p*K* values and Slides 25-28, to identify the unknown amino acid.

Finding pK values from the pH graph for amino acids

- For **simple amino acids** (e.g. glycine), look for **one steep rise** (charge 0 and pI) about the centre of the graph
- Corresponding volume of base (on x-axis) is **V** for 1 proton
- To get **pK_1**, look for pH (on y-axis) corresponding to **V/2** (on x-axis).
- Similarly, **pK_2** is pH (on y-axis) at the volume (**3V/2**; on the x-axis).
- To calculate **pI**, calculate mean of **pK_1** and **pK_2** ($pK_1 + pK_2 / 2$)
- Important: use this **V** value and the general shape of this graph to figure out **which** of the **3 types of unknown amino acids** you have been assigned (see slides 21-23)!

Example: Glycine

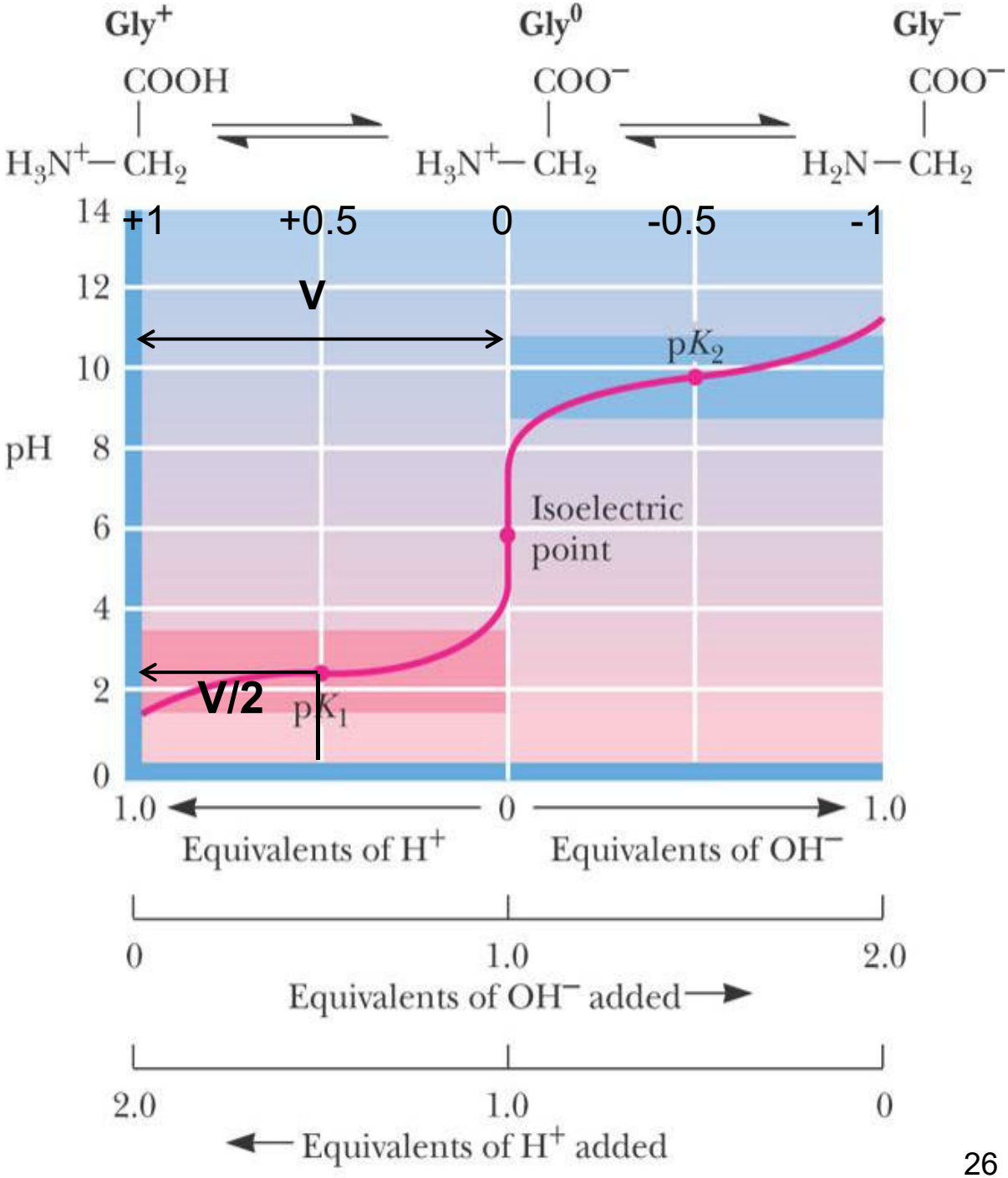
Titration of glycine, a simple amino acid.

The isoelectric point, **pI**, the pH where the molecule has a net charge of 0, is defined as (pK_1+ pK_2)/2.

pK_1 = 2.35; pK_2 = 9.78

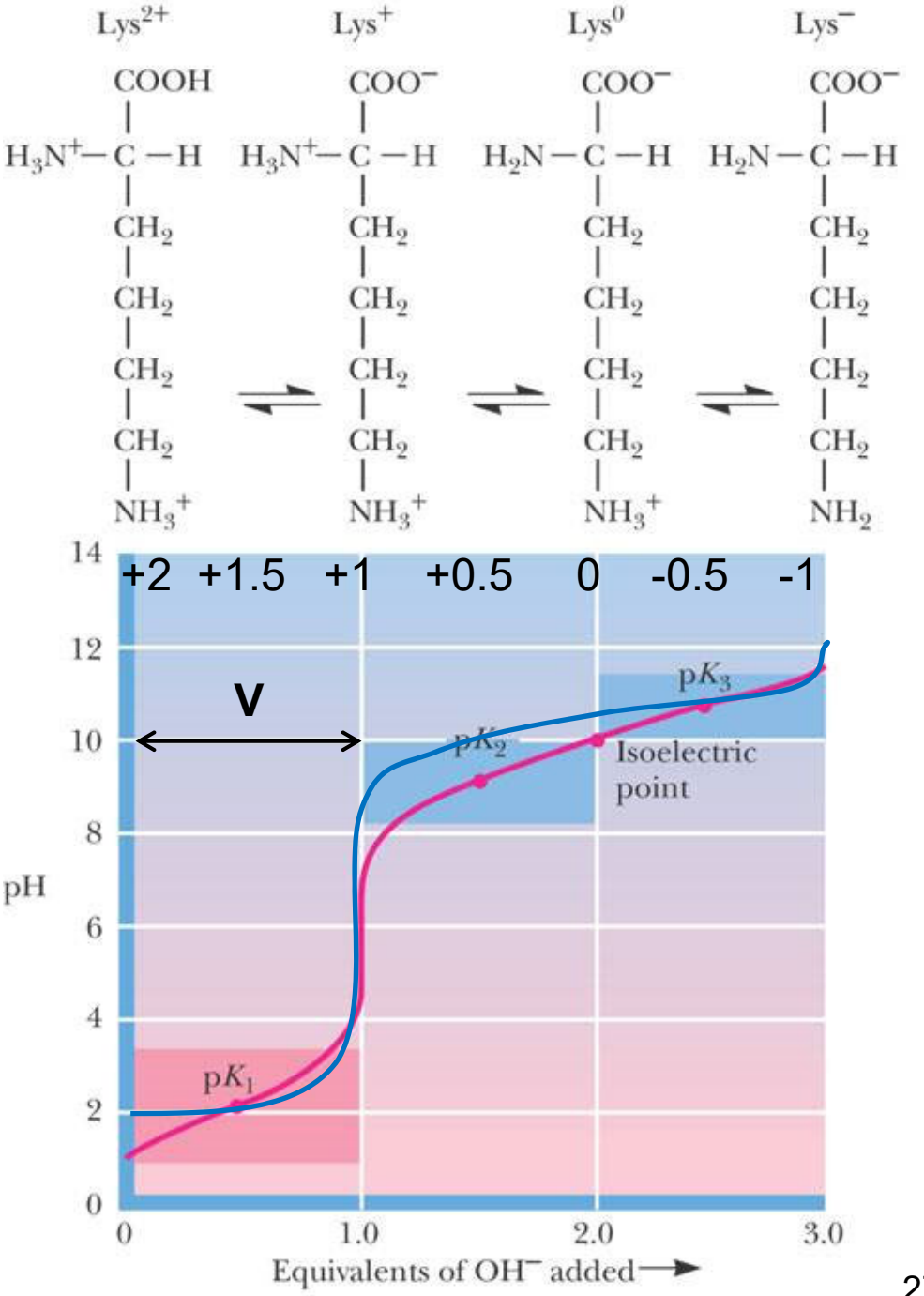
So, pI = (2.35+9.78)/2

= 6.06



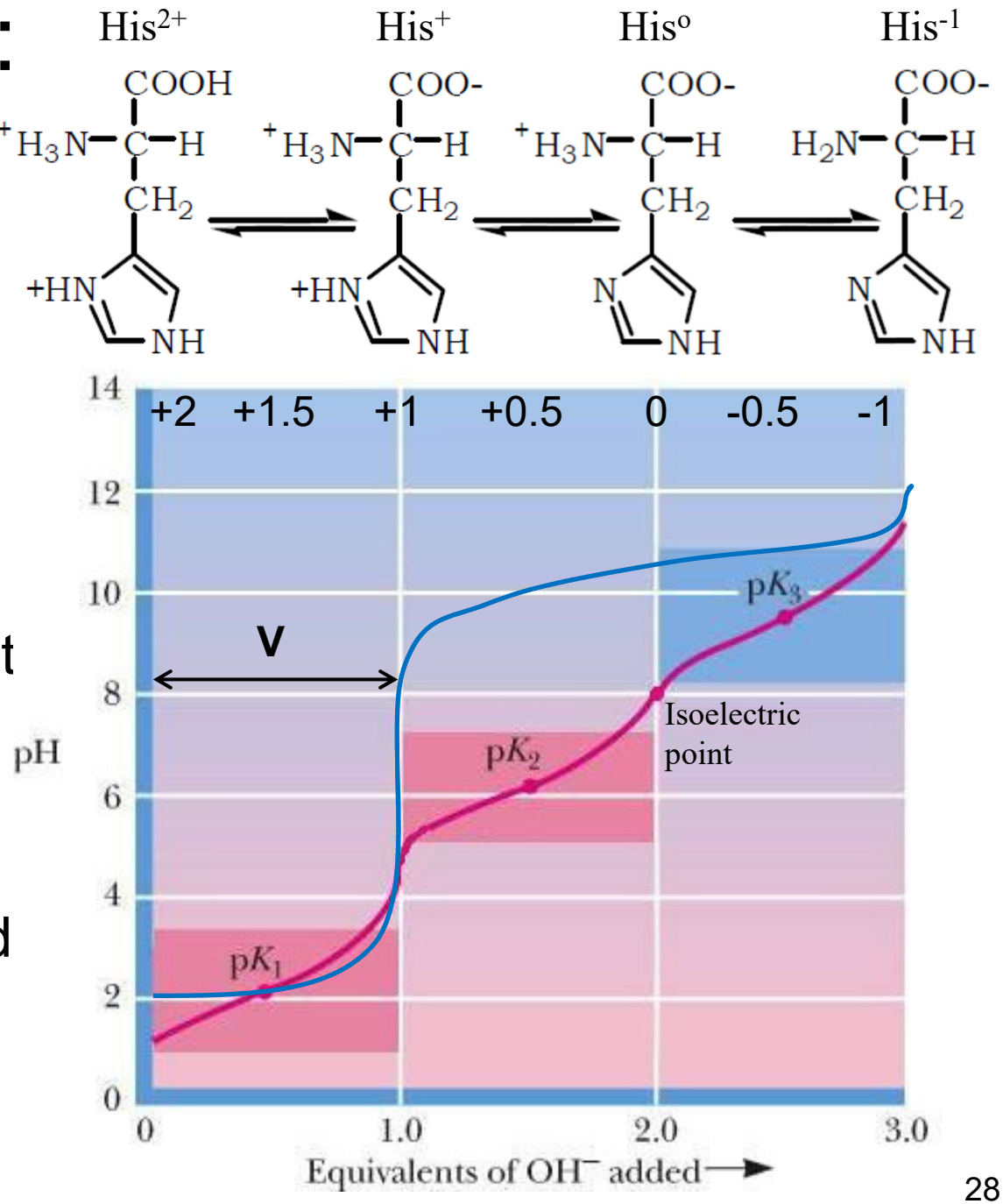
Basic R groups: arginine, lysine

- 3 p*K*'s
- Compare graph for unknown aa with **blue line** for glycine, which is flatter.
- ***Smaller steep rise*** in pH at volume **V,** which is similar to that of glycine, followed by a long tail
- "Halfway" points (**V/2, 3V/2, 5V/2**) correspond to p*K* values
- **pI** is the average of pK_2 and pK_3.



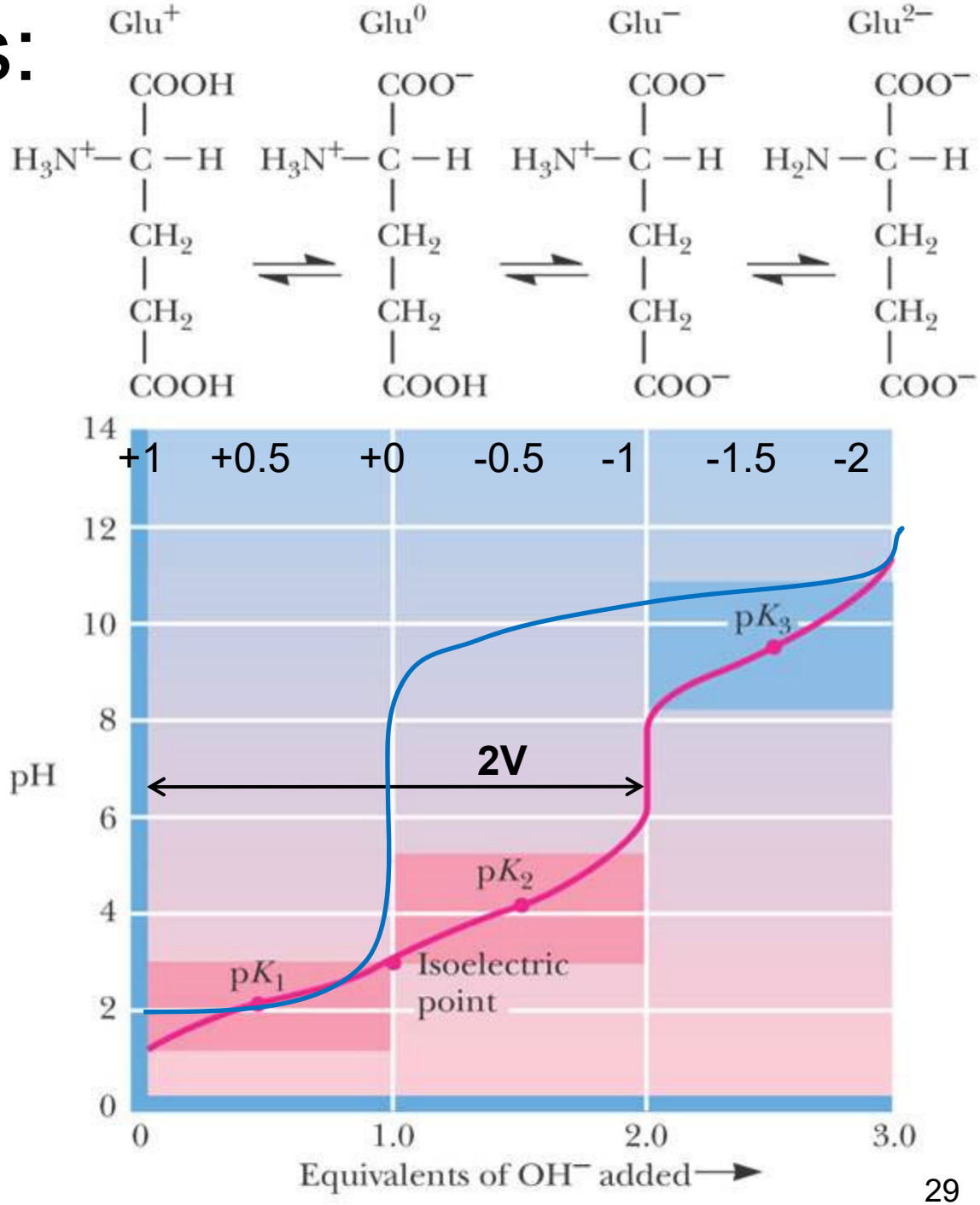
Basic R group: histidine

- 3 p*K*'s: ***step-wise rise*** when each proton is titrated: 2 in acidic pH, third in basic pH
- Compare graph for unknown aa with blue line for simple aa's.
- First sharp rise in pH at volume **V,** has a value similar to that of glycine
- "Halfway" points (**V/2, 3V/2, 5V/2**) correspond to p*K*'s.
- **pI** is the average of pK_2 and pK_3



Acidic R groups: glutamic or aspartic acid

- 3 p*K*'s
- Compare graph for unknown aa with blue line for glycine.
- **Steep rise** after a ***long trail***, when 2 protons are titrated, at almost twice the volume for glycine (now measured as **2V**)
- "Halfway" points (**V/2, 3V/2, 5V/2**) correspond to p*K*'s
- **pI** is the average of pK_1 and pK_2



Completing Prac 1

- Save the excel file on the desktop.
- Email the file to all team members, just in case.
- **Backup your data:** Save the Excel file and email it to yourself and to your lab partner(s). Also copy to your OneDrive! Just in case!

Submitting your data and results

- Each student to login to iLearn and follow the instructions for completing the prac.
- Upload your Prac 1 Data file individually to iLearn.
  - You will then get access to Prac 1 Quiz
  - Complete the Prac Quiz individually before you leave the lab!
  - *We need your data file and completed Quiz for grading!*