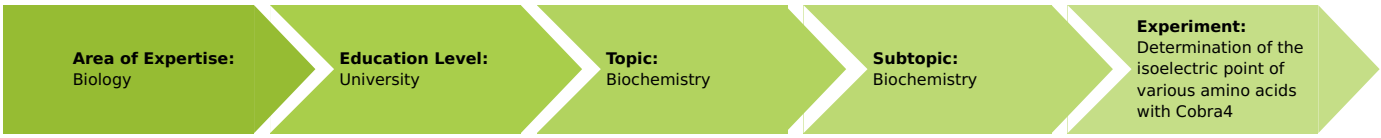
Determination of the isoelectric point of various amino acids with Cobra4 (Item No.: P4120160)

Curricular Relevance



Difficulty	Preparation Time	Execution Time	Recommended Group Size
Difficult	10 Minutes	50 Minutes	2 Students

Additional Requirements:

- PC
- Phenylalanine
- Serine
- Proline

Experiment Variations:

Keywords:

Isoelectric point, Acidic anions, Basic cations, Zwitterions, Equivalence (inflection) points, pKs value, Titration

Overview

Short description

Related topics

Strong and weak acids, strong and weak bases, pK_a value, pK_b value, equivalence point, half-equivalence point, and isoelectric point

Principle

In this experiment, the amino acids glycine, proline, phenylalanine, and serine are examined by way of pH titration. Following acidification with hydrochloric acid and subsequent titration with a 1M sodium hydroxide solution, the carbonyl and amino groups of the amino acids are ionised so that they are a zwitterion at the first equivalence point and a monoanion of the respective amino acid at the second equivalence point. As a result, the amino acids, which differ strongly in terms of their structure, become comparable. In addition, the process of a titration on a molecular level can be comprehended and the similarities can be pointed out.

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Fig. 1: Experiment set-up

Equipment

Position No.	Material	Order No.	Quantity
1	Cobra4 Wireless/USB-Link incl. USB cable	12601-10	2
2	Cobra4 Sensor-Unit Drop Counter	12636-00	1
3	Cobra4 Sensor-Unit Chemistry	12630-00	1
4	pH-electrode, plastic body, gel, BNC	46265-15	1
5	USB-Ladegerät für Interface-SystemLink	07932-99	2
6	Holder for Cobra4 with support rod	12680-00	2
7	Magnetic stirrer without heating, 3 ltr., 230 V	35761-99	1
8	Burette, 50 ml, grad. 0.1 ml	47151-01	1
9	Magnetic stirring bar 30 mm, cylindrical	46299-02	1
10	Graduated pipette, 5 ml : 0,1	36599-00	1
11	Watch glass, dia.60 mm	34570-00	1
12	Pipettor,bulb,3 valves, 10ml max.	47127-01	1
13	Wash bottle, plastic, 500 ml	33931-00	1
14	Beaker, low, BORO 3.3, 100 ml	46053-00	1
15	Glass beaker DURAN®, tall, 50 ml	36001-00	3
16	Volumetric flask 50 ml, IGJ12/21	36547-00	4
17	Funnel, glass, top dia.60 mm	34458-00	1
18	Spoon, special steel	33398-00	1
19	Precision Balance, Sartorius ENTRIS623-1S, 620 g / 0.001 g	49294-99	1
20	Buffer solution, pH 4.01, 1000 ml	46270-12	1
21	Buffer solution, pH 10.01,1000 ml	46272-12	1
22	Caustic soda solution, 1.0 m, 1000 ml	48329-70	1
23	Hydrochloric acid, 1.0 mol/l, 1000 ml	48454-70	1
24	Glycocoll /glycine/ 100 g	31341-10	1
25	Water, distilled 5 l	31246-81	1
26	Burette clamp, roller mount.,1pl.	37720-01	1
27	Retort stand, 210mm × 130mm, 500mm	37692-00	1
28	Right angle clamp	37697-00	2
29	Software Cobra4 - multi-user licence	14550-61	1

Tasks

1. Record the titration curves of various amino acids and explain the individual sections.
2. Work out the differences and similarities between the amino acids.

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Safety information

Always wear suitable protective gloves, safety goggles, and suitable clothes when handling chemicals. The appendix includes detailed information concerning the various chemicals.




Hazard symbol, signal word	Hazard statements	Precautionary statements
Water		
-		
Glycine		
-	-	-
Phenylalanine		
-	-	-
Serine		
-	-	-
Proline		
-	-	-
Hydrochloric acid		
 **Danger**	H314: Causes severe skin burns and eye damage. H335:May cause respiratory irritation.	P261: Avoid breathing dust/fume/gas/mist/vapours/spray. P280: Wear protective gloves/protective clothing/eye protection/face protection. P305+P351+P338:IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing. P310: Immediately call a POISON CENTRE or doctor/physician.
Sodium hydroxide solution		
 **Danger**	H314:Causes severe skin burns and eye damage.	P280: Wear protective gloves/protective clothing/eye protection/face protection. P305+P351+P338:IF IN EYES:Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing. P310: Immediately call a POISON CENTRE or doctor/physician.

Set-up and procedure

Set-up

- Set the experiment up as shown in Figure 1. This means the drop counter as well as the pH sensor must be connected to the computer via an own Wireless-Link and Wireless-Manager. Position the beaker on the magnetic stirrer and arrange the equipment so that the pH sensor as well as the tip of burette aim into the beaker through the drop counter.
- Prepare 50 ml of an acidified amino acid solution ($c_{HCl} = c_{amino\ acid} = 0.1$ mol/L). To do so, weigh in succession 0.378 g of glycine, 0.826 g of phenylalanine, 0.576 g of proline, and 0.525 g of serine. Fill each of these amino acids into its own volumetric flask via a funnel.
- Add 50 ml of the 1M hydrochloric acid into the volumetric flasks and top them up to 50 ml.
- The amino acids should be completely dissolved.
- Put the magnetic stirrer bar into the beaker (100 ml), add the content of one of the volumetric flasks, and place the beaker on the magnetic stirrer and switch the magnetic stirrer on.
- Fill the burette via a funnel with 20 ml of the 1M sodium hydroxide solution. Prior to doing so, check whether the stopcock of the burette is closed.

Procedure

- Start the PC and Windows.
- Connect the Cobra4 Wireless Manager to the USB port of the PC.
- Start the "measure" software package on the PC.
- Attach the Cobra4 Sensor-Unit Chemistry to the Cobra4 Wireless-Link and connect it to the pH electrode.
  Tip: As an alternative to the Sensor-Unit Chemistry, it is also possible to use the Sensor-Unit pH.
- Connect the drop counter to the second Cobra4 Wireless-Link.
- Switch the two Wireless-Links on and wait until the program identifies the measurement sensor. Select "Unknown titration volume" via the drop counter overview (Fig. 2).

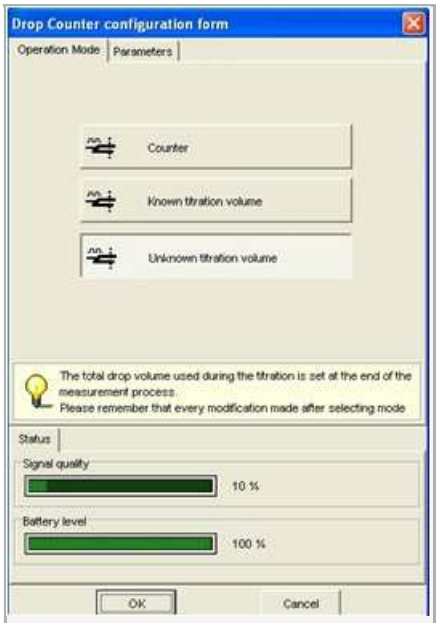

Fig. 2: Drop counter overview for ad-justing the titration volume

- The program will automatically create the virtual channel for the determination of the volume.
- Prior to the start of the experiment, the temperature inputs of the Cobra4 Sensor-Unit Chemistry must be deactivated. To do so, select "Temperature T1" and then "Temperature T2" in the "Navigator" and switch them to "inactive" by deselecting the active field (Fig. 3).

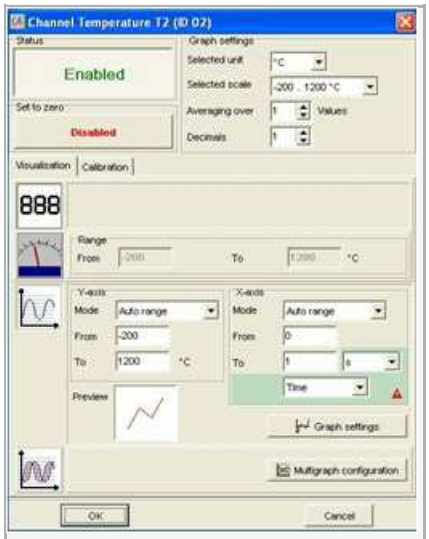

Fig. 3: Window of a temperature measuring channel

- Start the measurement data recording process in "measure" ( ). Then, start the titration by carefully opening the stopcock of the burette. The dropping rate should be low with one drop per second at maximum.
- After the end of the titration, stop the measurement data recording process ( ).
- Enter the sodium hydroxide solution quantity that has been used into the window that pops up and confirm the value.
- After the graph has been displayed, it will take some time before the program completes the calculation of the data and before the y-axis can be adapted accordingly (click the graph).
- The pH value can then be plotted as a function of the volume in "measure" (copy the data of the calculated table → in "measure" → Measurement → Import measurement values ...).
- Double-click the graph in order to change the axis labels.

Dsiposal

The solutions which contains any monochloracetic acid or propionic acid have to be collected in a container. The diluted and neutralised solutions of the other used acids and bases can be disposed by rinsing into the drain.

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Result and evaluation

Results and evaluation

Figure 4 shows the graph of the titration of glycine. The curves for proline, serine, and phenylalanine have been recorded in the same manner. They can be seen in a completely evaluated manner in Figures 10, 11, 12, and 13.

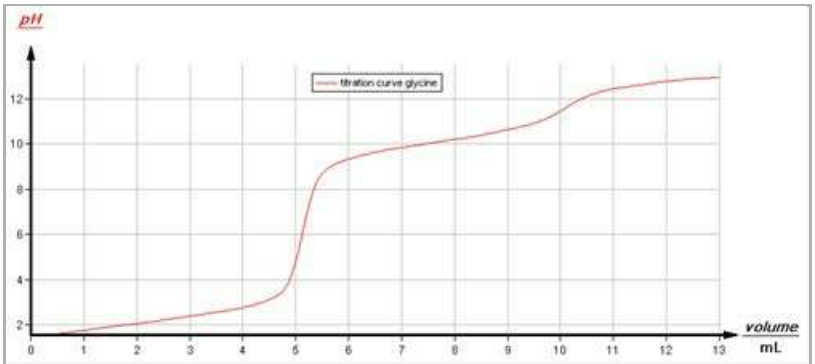

Fig. 4: Titration curve of glycine

Evaluation

The following points of the curve should be labelled:

- 1^{st} pK_a value
- 1^{st} equivalence point (1^{st} EP)
- 2^{nd} pK_a value
- 2^{nd} equivalence point (2^{st} EP)

To do so, click the button for the equivalence point determination on the toolbar (see the arrow in Figure 5). Then, select all of the options and close the window (Fig. 5).

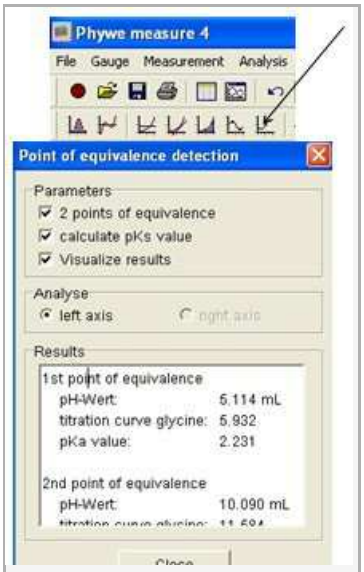

Fig. 5: Top: Button for the equivalence point calculation
Bottom: Tab for the equivalence point calculation

The following section includes the explanation of a titration curve with two equivalence points based on the example of glycine.

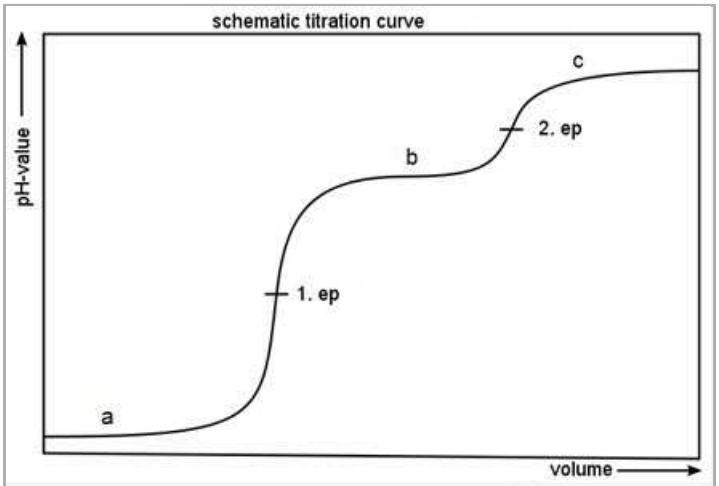

Fig. 6: Schematic diagram of a titration curve with two EPs

At the start of the measurement, the amino acid is present in an acid environment. Due to this excess of protons, the cation of the amino acid is formed (see Figure 7, area a).

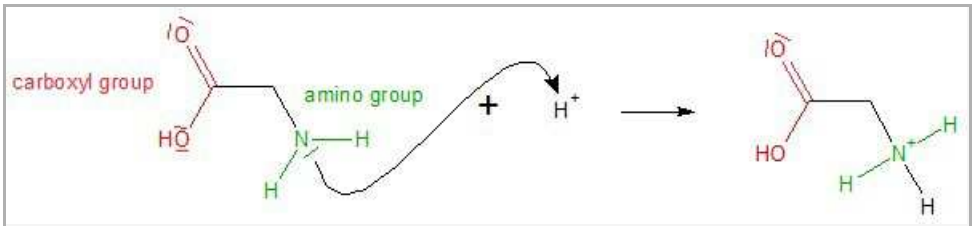

Fig. 7: Amino acid at the start of the measurement

When the sodium hydroxide solution is added, the number of hydroxide ions increases, thereby leading to the formation of the zwitterions of the amino acid. At the first equivalence point, all of the amino acid molecules are present in the form of zwitterions (see Figure 7, area of the 1st EP).

After the first equivalence point, there is an excess of hydroxide ions, causing the zwitterion to release a proton, thereby forming water. The result is the glycinate monoanion (Fig. 8).

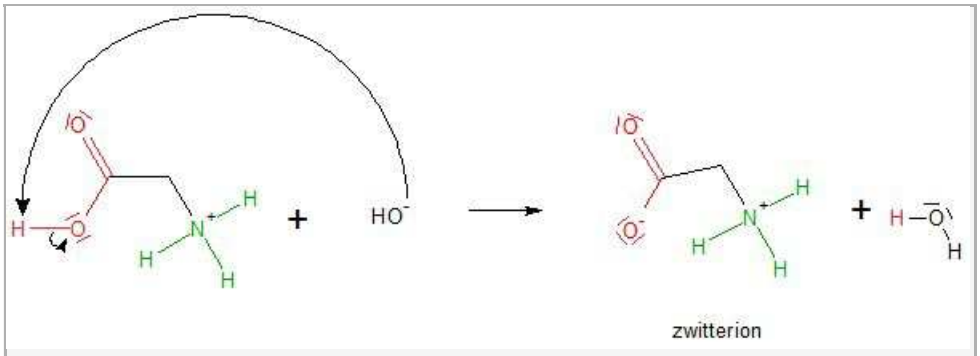

Fig. 8: At the first equivalence point, the amino acid molecule is present in the form of a zwitterion

Figure 9: After the first equivalence point, the amino acid molecule is present in the form of a monoanion

At the second equivalence point, all of the amino acid molecules are present in the form of glycinate monoanions. From this point on, the pH value increases due to the continuing addition of hydroxide ions (Fig. 9).

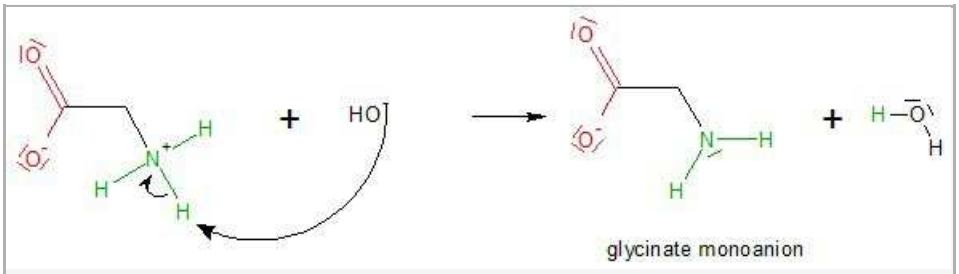

Fig. 9: After the first equivalence point, the amino acid molecule is present in the form of a monoanion

At the equivalence point of an acid-base-titration, there are equivalent substance quantities of the acid and base that are part of the titration.

The isoelectric point of a titration of amino acids is reached at the pH value that occurs when the number of acid groups with a negative charge corresponds to the number of amino groups with a positive charge. At this point, the total charge of the molecules is neutral.

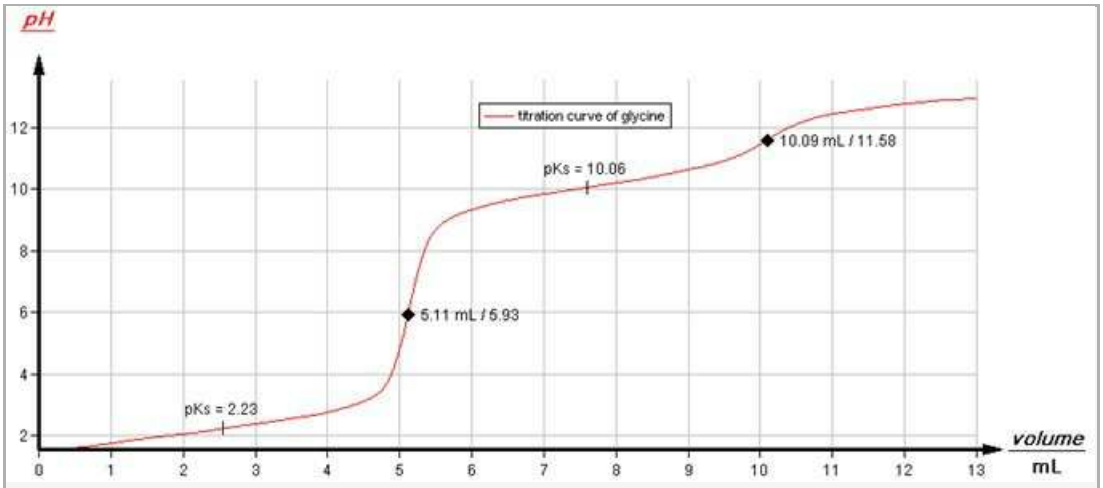

Fig. 10: Titration curve of glycine with the data for the pKa values and equivalence points

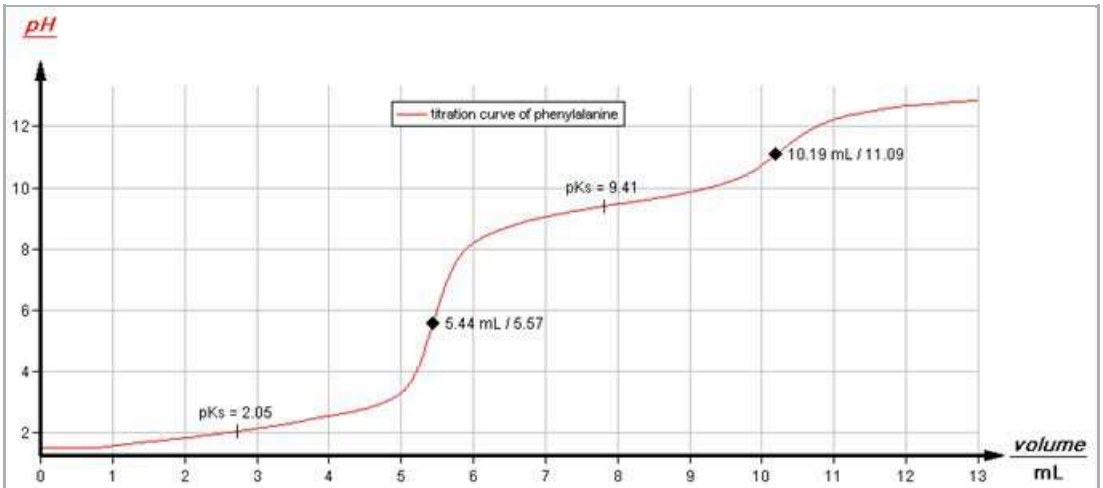

Fig. 11: Titration curve of phenylalanine with the data for the pKa values and equivalence points

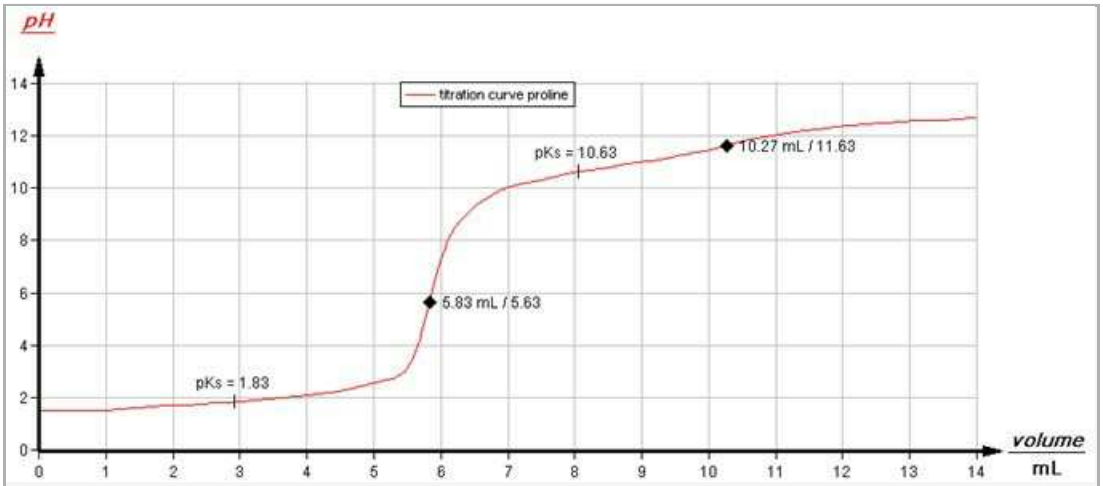

Fig. 12: Titration curve of proline with the data for the pKa values and equivalence points

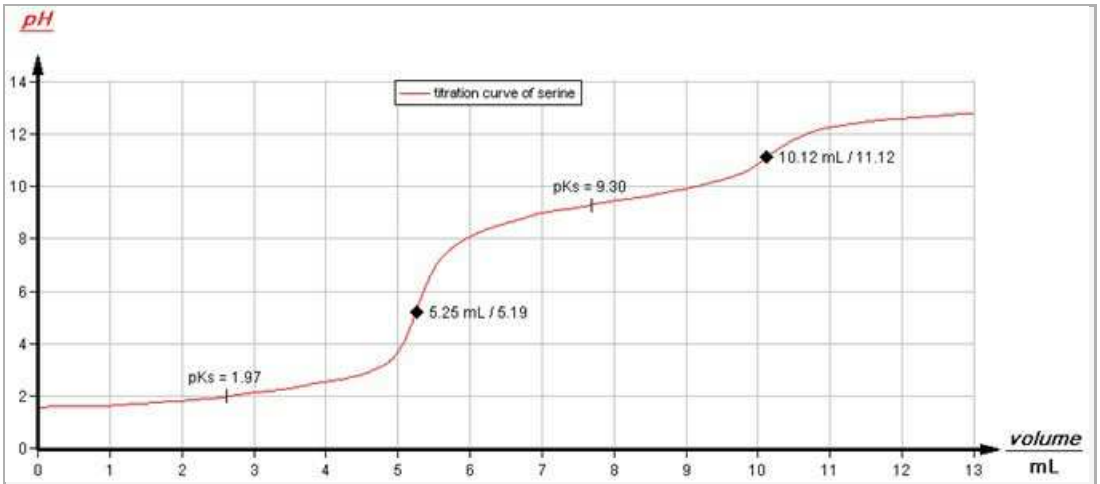

Fig. 13: Titration curve of serine with the data for the pKa values and equivalence points