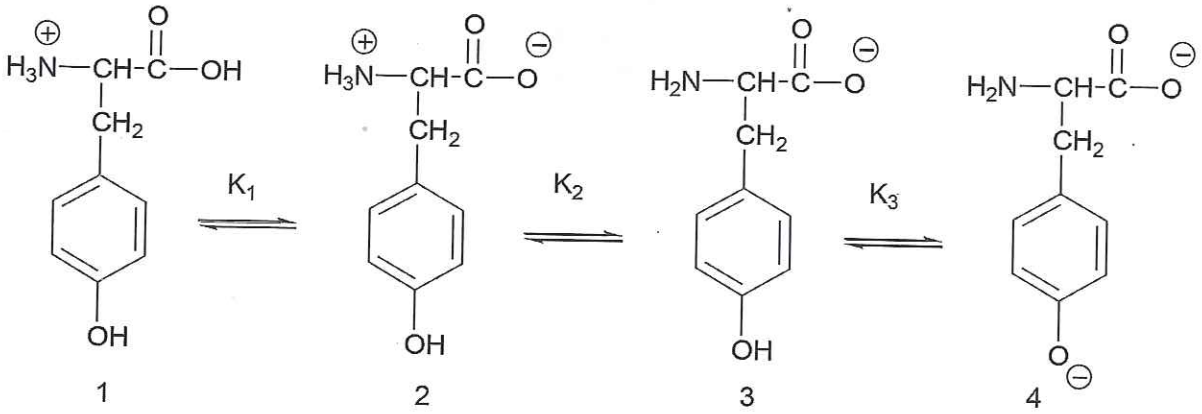
EXPERIMENT 6

SPECTROSCOPIC DETERMINATION OF A pKa VALUE

Introduction

Proteins absorb UV light around 280 nm. This is due to the presence of tyrosine residues in the side chains. The other aromatic residues (Phe, His, Trp) absorb at shorter wavelengths. The wavelength of absorption of tyrosine depends on the pH of the solution in which it is dissolved. This is because the fully deprotonated tyrosinate anion (**4**) absorbs at around 295 nm while the fully protonated form (**1**) has absorption at 278 nm. As the pH of the solution increases the majority of the sample will go from **1** via **2** and **3** to **4** and the UV spectrum will change accordingly. Each of the deprotonation reactions has a K (a value for the equilibrium constant) and the aim of this experiment is to determine pK_3.



Before you begin

Read the experimental procedure carefully.

Procedure

You will be provided with a tyrosine solution of unknown concentration and solutions of glycine/NaCl and sodium hydroxide. These solutions will allow you to prepare 9 different solutions of tyrosine all buffered to different pH values.

For solution 1: Pipette glycine/NaCl solution (8.50 cm^3) and 0.1 M sodium hydroxide (0.50 cm^3) into a boiling tube. Accurately pipette tyrosine solution (1.00 cm^3) into the tube and swirl gently to mix. Label as solution 1.

For the other 8 solutions follow the same procedure but use the quantities outlined in the table below:

Solution No.	Glycine/NaCl	0.1 M NaOH	Tyrosine soln
1	8.5	0.5	1.0
2	7.4	1.6	1.0
3	6.3	2.7	1.0
4	5.6	3.4	1.0

5	5.2	3.8	1.0
6	4.8	4.2	1.0
7	4.5	4.5	1.0
8	4.3	4.7	1.0
9	2.1	6.9	1.0

When all of your solutions have been made measure and record the pH of each of them using the pH electrode.

Then zero a spectrophotometer at 295 nm using a blank of distilled water. Record the absorbance of each of your 9 solutions at this wavelength. Plot a graph of absorbance against pH and use this to determine the pK (remember that pK=pH at 50% ionization). Have your graph marked as part of your in-class assessment.