GENERAL BIOLOGY LABORATORY II

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Week 12

Detection of Isoelectric point (pI) of a protein

【Purpose】

1. Master the basic principle of the Isoelectric point (pI) of protein measuring
2. Learn how to determine the pI of protein with precipitation method

【Principle】

The isoelectric point (pI) of a protein is the pH where the net charge on the protein is zero.

Proteins tend to aggregate and precipitate at their pI because there is no electrostatic repulsion keeping them apart.

Proteins have different pI because of their different amino acid sequences (i.e., relative numbers of anionic and cationic groups), and thus they can be separated by adjusting the pH of a solution.

When the pH is adjusted to the pI of a particular protein, it precipitates leaving the other proteins in solution.

【Materials】

1. Apparatus

glass tubes (0.5×10), Rack for glass tube, Pipettes, pH meter.

2. Reagents

(1) 0.4% casein protein NaAc solution 200ml

0.4 g casein, add a small amount of water in the mortar grind carefully, will use the proceeds of protein gel suspension liquid into 200 ml conical flask, use a small amount of 40-50 ℃ warm water wash mortar, washing liquid will move inside the conical flask. Add 10 ml 1 mol/L sodium acetate solution. Put the conical flask to 50 ℃ water bath, and carefully rotating conical flask, until completely dissolved casein. The solution within the conical flask all move into 100 ml flask and add water to the scale, blending.

(2) 0.1 M HAc 300 ml

(3) 0.01 M HAc 50ml

(4) 1M HAc 100ml

【Procedure】

1. Take 4 test tubes and wash them with brush , write the number with marker pencil.
2. According to the following form, add the reagents.

Reagents(ml)	1	2	3	4
Distilled water	8.4	8.7	8.0	7.4
0.01mol/L Acetic acid	0.6	—	—	—
0.1mol/L Acetic acid	—	0.3	1.0	—
1mol/L Acetic acid	—	—	—	1.6
0.4 % casein NaAc solution	1.0	1.0	1.0	1.0

3. Shake the test tubes and wait for 10 minutes.
4. Observe and write down the results use “+” and “-” to express the quantity of precipitation.

【Results】

【Discussion】

【Conclusion】

【References】