

Objectives

1. To gain experience in preparing buffers and other solutions
2. To use simple model systems to study factors that affect the rate of the Maillard reaction.

Apparatus and Equipment

1. pH meter
2. Pipettes, 10 mL
3. Volumetric flasks, 50 and 100 mL
4. Beakers, 50, 100, 250, and 600 mL
5. Test tubes
6. Hot plates
7. Graduated cylinders, 10 and 50 mL
8. Pasteur pipettes
9. Vortex mixer and glass stirring rods
10. Top loading balance
11. Spectrophotometer
12. Permanent marker
13. Water bath, boiling
14. Boiling chips
15. Aluminum weighing dishes
16. Oven, set at 125°C

Reagents and Materials

To be prepared by the student

1. Phosphate buffer, 1/15 M, pH 8.0
2. Glucose, 0.5 M, in phosphate buffer, pH 8.0
3. Glycine, 0.5 M, in phosphate buffer, pH 8.0

Prepared by TA

1. Crystalline glucose
2. Crystalline glycine
3. HCl, 0.1 N
4. HCl, 2N
5. NaOH, 2N
6. KH_2PO_4 , 1/15 M
7. Na_2HPO_4 , 1/15 M
8. Fructose, 0.25 M + glycine, 0.25 M, in phosphate buffer, 1/15 M, pH 5 and 8
9. Sucrose, 0.25 M + glycine, 0.25 M, in phosphate buffer, 1/15 M, pH 5 and 8
10. Lactose, 0.25 M + glycine, 0.25 M, in phosphate buffer, 1/15 M, pH 5 and 8
11. Glucose, 0.25 M, in phosphate buffer, 1/15, pH 5 and 8

12. Fructose, 0.25 M, in phosphate buffer, 1/15, pH 5 and 8
13. Sucrose, 0.25 M, in phosphate buffer, 1/15, pH 5 and 8
14. Lactose, 0.25 M, in phosphate buffer, 1/15, pH 5 and 8
15. Glycine, 0.25 M, in phosphate buffer, 1/15, pH 5 and 8
16. Ascorbic acid, 0.25 M + glycine, 0.25 M, in phosphate buffer, 1/15 M, pH 5 and 8
17. Sorbitol

Procedure

Preparation of Glucose-Glycine Model System

(Purpose: To prepare buffered glucose-glycine solutions with identical concentrations but different pH values)

1. Prepare 100 mL 1/15 M phosphate buffer, pH 8.0 (prepare from 1/15 M KH_2PO_4 and 1/15 M Na_2HPO_4). See Table 1 for volumes to mix.
2. Prepare 50 mL 0.5 M glucose solution in the phosphate buffer. The MW of the glucose is 180.16. Add the glucose a little at a time, stirring, to about 30 mL buffer in a beaker; transfer to a volumetric flask; and dilute to volume with buffer.
3. Prepare 50 mL 0.5 M glycine in the phosphate buffer. The MW of glycine is 75 g/mol.
4. Mix 50 mL glucose solution with 50 mL glycine solution to form a glucose-glycine solution. What are the molar concentrations of glucose and glycine in this solution?
5. Transfer 20 mL glucose-glycine solution to each of two 50-mL beakers and treat as follows (one treatment per beaker):
 - a. Adjust the pH to 5.0 and add water to bring the total volume to 40 mL. Recheck the pH.
 - b. Adjust the pH to 8.0 and add water to bring the total volume to 40 mL. Recheck the pH.

(Use 2 N HCl or NaOH to adjust the pH. Record but do not adjust the pH after bringing to final volume.)

Heating Experiment

1. Transfer 10 mL aliquots of the solutions listed below to test tubes. You should have one tube for each of the treatments. Cap the tubes loosely and label with a permanent marker.
 - a. Glucose-glycine, pH 5
 - b. Glucose-glycine, pH 8
 - c. Fructose-glycine, pH 5
 - d. Fructose-glycine, pH 8
 - e. Sucrose-glycine, pH 5
 - f. Sucrose-glycine, pH 8
 - g. Lactose-glycine, pH 5
 - h. Lactose-glycine, pH 8
 - i. Sorbitol-glycine, pH 5
 - j. Sorbitol-glycine, pH 8

- k. Glucose, pH 5
 - l. Glucose, pH 8
 - m. Fructose, pH 5
 - n. Fructose, pH 8
 - o. Sucrose, pH 5
 - p. Sucrose, pH 8
 - q. Lactose, pH 5
 - r. Lactose, pH 8
 - s. Glycine, pH 5
 - t. Glycine, pH 8
2. Prepare a boiling water bath in a 1-L beaker by adding a few boiling chips and bringing to a boil on a hot plate. Place all tubes in a boiling water bath for 30 min. Transfer the tubes to a beaker of tap water to cool.

Measurement Extent of Browning

1. After the tubes from the *Heating Experiment* have cooled, measure the pH in each tube.
2. Turn on your spectrophotometer and allow it to warm up. Turn the wavelength selector to 430 nm. Use water to set 0 absorbance.
3. Measure the absorbance of each of your solutions. You may have to dilute (with water) the darker solutions to keep them on the scale (absorbance readings are most accurate when they fall between 0.20 and 0.80). To calculate the absorbance of the original undiluted solutions, multiply the absorbance of the diluted solution by the dilution factor.

Table 1. Buffer preparation using 1/15 M KH_2PO_4 and 1/15 M Na_2HPO_4 .

Mixing formula: x mL 1/15 M KH_2PO_4 + $(100 - x)$ mL 1/15 M Na_2HPO_4		
pH	mL 1/15 M KH_2PO_4	mL 1/15 M Na_2HPO_4
5.0	99.2	0.8
5.2	98.4	1.6
5.4	97.3	2.7
5.6	95.5	4.5
5.8	92.8	7.2
6.0	88.9	11.1
6.2	83.0	17.0
6.4	75.4	24.6
6.6	65.3	34.7
6.8	53.4	46.6
7.0	41.3	58.7
7.2	29.6	70.4
7.4	19.7	80.3
7.6	12.8	87.2
7.8	7.4	92.6
8.0	3.7	96.3