What if It's an Acid and a Base?

Food Additives

Introduction

Any change in the pH of a protein's environment will cause observable changes in the solubility of the protein. Solubility changes, in turn, reflect changes in the three-dimensional structure of the protein. The effect of pH on protein solubility explains why most enzymes function well at an optimum pH, and why their activity decreases substantially at pH values other than the optimum. This demonstration examines the effect of pH on the solubility and structure of casein, a milk protein.

Concepts

- Protein
- Solubility
- Isoelectric point
- pH

Materials (for each demonstration)

Casein, 1 g
Hydrochloric acid, HCl, 2 M, 10 mL
Sodium hydroxide, NaOH, 0.1 M, 25 mL
Sodium hydroxide, NaOH, 2 M, 10 mL
Universal indicator, 2 mL (includes pH color chart)
Balance, centigram
Beaker or flask, 600-mL
Magnetic stirrer and stirring bar
Pipets, Beral-type, graduated, 2
Water, distilled or deionized

Safety Precautions

Hydrochloric acid solution is a corrosive liquid and is toxic by ingestion and inhalation. Sodium hydroxide solution is corrosive and is especially dangerous to the eyes. Universal indicator is a flammable liquid—keep away from flames and heat. Avoid contact of all chemicals with eyes and skin. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Please review current Material Safety Data Sheets for additional safety, handling, and disposal information.

Procedure

1. Add 25 mL of 0.1 M sodium hydroxide and a stirring bar to a 600-mL beaker or flask and place the beaker on a magnetic stirrer. Add 225 mL of distilled or deionized water and stir at moderate speed. Add 1 g of casein and stir to dissolve. *(The solution will be slightly cloudy or translucent.)*
2. *(Optional)* Add 1–2 mL of universal indicator to observe pH changes, if desired. Consult the universal indicator color chart for pH values.
3. With rapid stirring, add 2 M hydrochloric acid in 0.5-mL increments using a graduated, Beral-type pipet. *(The solution will turn cloudy, but will clear up again as the hydrochloric acid is dispersed. After 1–2 mL of acid has been added, the cloudiness will reach a maximum—this is the isoelectric point. The pH at the isoelectric point is 4–5.)*
4. Once the isoelectric point has been reached, pause just long enough to record observations (15–20 seconds.) Continue adding 2 M hydrochloric acid in 0.5-mL increments with stirring until the solution is clear again. *(The cloudiness will fade and the precipitate will redissolve after the addition of another 2–3 mL of acid, when the pH drops below the isoelectric point, pH ≤ 2.)*
5. Continue to stir the solution. Reverse the process by adding 2 M sodium hydroxide in 0.5-mL increments using a clean, graduated, Beral-type pipet. *(After 2–3 mL of sodium hydroxide has been added, the protein will precipitate out again at the isoelectric point.)*
6. Continue adding sodium hydroxide in 0.5-mL increments with stirring until the solution is clear again. (*The solution will clear up after an additional 1–2 mL of sodium hydroxide has been added and the pH > 10–12.)*
7. The process may be repeated using the same casein solution. This will give time to explain the observations.

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Disposal

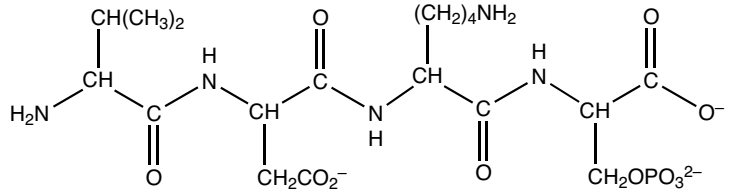
Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures governing the disposal of laboratory waste. The casein solution may be stored at basic pH for several months. Alternatively, the solution may be rinsed down the drain with excess water according to Flinn Suggested Disposal Method #26b.

Discussion

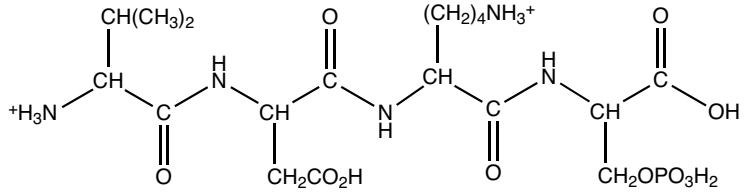
Casein is the principal protein in milk (80% of the total protein content). Casein is a phosphoprotein—it contains a large number of phosphate groups attached to the amino acid side chains in its polypeptide structure. The negatively charged phosphate groups are balanced by positively charged calcium ions and are responsible for the high nutritional calcium content in milk. Casein is almost completely insoluble in water at neutral pH (pH = 7).

Casein, like other proteins, is an ionic species containing amino groups and carboxyl groups on its terminal amino acids. It also contains a variety of other acidic and basic groups on the side chains of its non-terminal amino acids. The effect of pH on the solubility of casein reflects the ionization of the acidic and basic groups in its structure.

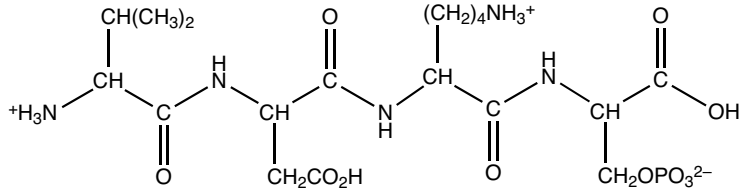
At high pH, casein will have a net negative charge due to ionization of all the acidic side chains (—CO_2^-) in its structure. Because casein is ionized at high pH values, it is soluble in dilute sodium hydroxide solution.



At low pH, casein will have a net positive charge due to protonation of all basic side chains (—NH_3^+) in its structure. Because casein is ionized at low pH values, casein is also soluble in strongly acidic solutions.



At intermediate pH values, casein will contain roughly equal numbers of positively and negatively charged groups and the protein will have a net charge of zero. Casein is insoluble in neutral solutions because it is not charged under these conditions.



The solubility of a protein is usually at a minimum at its isoelectric point. The isoelectric point is defined as the pH at which a protein has a net charge of zero. For casein, due to the attached phosphate groups, the isoelectric point is close to pH = 4.

Connecting to the National Standards

This laboratory activity relates to the following National Science Education Standards (1996):

Unifying Concepts and Processes: Grades K–12
Evidence, models, and explanation
Constancy, change, and measurement
Content Standards: Grades 9–12
Content Standard B: Physical Science, structure and properties of matter
Content Standard C: Life Science, matter, energy, and organization in living systems

Answers to Worksheet Questions

Data Table

Chemical Added to Casein	Observations	Approximate pH
25 mL Sodium Hydroxide	*The solution is slightly cloudy.*	*Greater than 10–12*
1–2 mL Hydrochloric Acid	*At first, the solution gets slightly more cloudy and then clears up. After 1–2 mL however, the solution is very cloudy.*	*4–5*
2–3 mL Hydrochloric Acid	*The solution is again only slightly cloudy.*	*2*
2–3 mL Sodium Hydroxide	*The solution is very cloudy.*	*4–5*
1–2 mL Sodium Hydroxide	*The solution is again only slightly cloudy.*	*Greater than 10–12*

Discussion Questions

1. Casein has both acidic side chains and basic side chains. At a high (basic) pH, ionization occurs in the acidic chains. At a very low (acidic) pH, protonation occurs in the basic chains. Do you think casein is most soluble with a net charge that is positive, negative, or around zero? Why?

   Casein is most soluble when it has either a highly positive or a highly negative charge. Bases ionize its acidic chains, resulting in a negative charge, and acids protonate its basic chains, resulting in a positive charge. During the demonstration, there was the least amount of solid in the solution at the pH extremes. Therefore, casein is the least soluble when the net charge is zero.

2. A protein's isolectric point is the pH at which the protein has a net charge of zero. Approximate the isolectric point of casein.

   The isoelectric point of casein is probably around 4-5, because that is when the solution was the cloudiest during the demonstration.

Reference

This activity was adapted from *Flinn ChemTopic™ Labs*, Volume 20, Biochemistry; Cesa, I., Editor; Flinn Scientific: Batavia IL (2002).

Flinn Scientific—Teaching Chemistry™ eLearning Video Series

A video of the *What if It's an Acid and a Base?* activity, presented by John Little, is available in *Food Additives*, part of the Flinn Scientific—Teaching Chemistry eLearning Video Series.

Materials for *What if It's an Acid and a Base?* are available from Flinn Scientific, Inc.

Materials required to perform this activity are available in the *pH and Protein Solubility* available from Flinn Scientific. Materials may also be purchased separately.

Catalog No.	Description
AP6692	pH and Protein Solubility—A Reversible Demonstration Kit
AP1349	Magnetic Stirrer
H0034	Hydrochloric Acid Solution, 3 M, 500 mL
S0149	Sodium Hydrochloric Solution, 0.1 M, 500 mL
U0009	Universal Indicator Solution, 35 mL

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.

What if It's an Acid and a Base? Demonstration Worksheet

Data Table

Chemical Added to Casein	Observations	Approximate pH
25 mL Sodium Hydroxide		
1–2 mL Hydrochloric Acid		
2–3 mL Hydrochloric Acid		
2–3 mL Sodium Hydroxide		
1–2 mL Sodium Hydroxide		

Discussion Questions

1. Casein has both acidic side chains and basic side chains. At a high (basic) pH, ionization occurs in the acidic chains. At a very low (acidic) pH, protonation occurs in the basic chains. Do you think casein is most soluble with a net charge that is positive, negative, or around zero? Why?

2. A protein's isolectric point is the pH at which the protein has a net charge of zero. Approximate the isolectric point of casein.