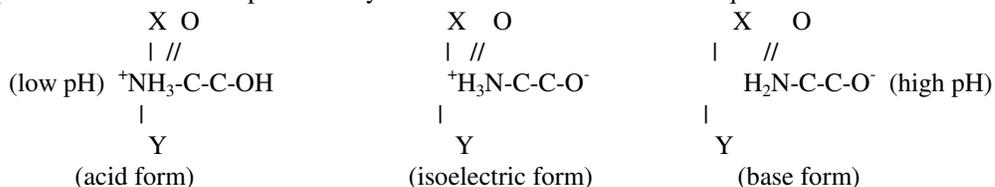


***BUT WHAT IF IT'S AND ACID AND A BASE?***  
**A Demonstration of Organic Amphiprotism**

**INTRODUCTION**

Students may be familiar with acids and bases, and the use of indicators to test for their presence, but amino acids present a special problem in that they have characteristics of both. The same molecule will contain an amino group (-NH<sub>2</sub>) which acts as a base (a proton acceptor), and a carboxylic acid function (-COOH) which behaves as an acid (a proton donor). In the presence of acids the amino group is converted to -NH<sub>3</sub><sup>+</sup>. Bases will convert the carboxylic acid to the carboxylate anion, -COO<sup>-</sup>. The **isoelectric point** for an amino acid is the pH at which both functional groups are in their ionized state, a so-called "inner salt." Under these conditions, the negative carboxylate anion of one molecule exerts an attractive force on the positive ammonium group of another molecule, and a precipitate is observed. The process may be considered sort of a double equilibrium:



where X and Y are the groups attached to the alpha-carbon; it is they which distinguish one amino acid from another. Even though the demonstration is fairly obvious in terms of a single system responding both to acids and to bases, for beginning classes especially it might be more suitable (if not truly accurate) to refer to the middle form as being neither cation nor anion, rather than as a zwitterion, with cation at one end and anion at the other; you must judge your audience.

Like all proteins, casein, the chief protein in milk, is a polymer made up of a large number of amino acids. As such, it too contains both the amino and carboxylic acid functional groups, and so has its own isoelectric point. In this demonstration, the students will observe significance of the isoelectric point for casein and see evidence of the approximate pH at which it occurs. You may wish to extend the discussion to include the role that pH plays in digestion.

**DEMONSTRATION**

Half-fill a 250- or 400-mL beaker with the alkaline casein solution (size to be determined by the size of the room and distance to the audience). With rapid, automatic stirring, add 2M HCl dropwise. A cloudiness will appear, but will clear up again as the acid is distributed throughout the system. Eventually it will reach a maximum as the isoelectric point is achieved. The system will then begin clear up as addition of acid continues and the casein structure is converted to its acid (protonated) form. Once the isoelectric point is reached, as indicated by failure of the cloudiness to fade, pause long enough to point the fact out to observers, but do not allow the system to remain cloudy for more than 15-20 seconds, as it soon becomes impossible to get the solid to redissolve. Continued addition of HCl will cause the system to clear up again, as the pH drops below the isoelectric point. The change can be reversed by adding 2M NaOH dropwise. As before, the cloudy appearance will persist at the isoelectric point, then clear as the pH is raised by further addition of NaOH.

Repeat the demonstration as many times as desired, then, using the same casein solution, run it once more, but this time add a few drops of bromothymol blue indicator. The color change of the indicator occurs between pH 6.0 and 7.6; by noting the color at the point of maximum cloudiness, students can get a rough idea of the pH at casein's isoelectric point. A more precise value may be obtained by using a pH meter, but the judgement of when the mixture is at its most-opaque is still entirely subjective. Materials are inexpensive enough, and easy enough to prepare in quantity that the process may be suitable as an experiment for an advanced or AP class.

**MATERIALS**

**Casein solution**--dissolve 0.1 g (1 pellet) of NaOH in 250 mL of distilled or deionized water. Add 1 gram of purified casein powder. Stir well until the solution is clear. (A slight haziness is not uncommon.)

**2M HCl**--Add 4 ml of concentrated acid, slowly and with stirring, to 21 mL of deionized water.

**2M NaOH**--Dissolve 2 grams of solid in 25 mL of deionized water.

**0.1% Bromothymol blue indicator**--Use a prepared solution or dissolve 0.1 g of powder in 100 mL of deionized water.

{The concentrations of hydrochloric acid and sodium hydroxide are not critical, but they should be reasonably close to equal; 10% solutions work just fine, too. For 10% HCl, dilute 10 mL of the concentrated (38%) hydrochloric acid to 38 mL total, by adding the acid to 28 mL of water; for 10% NaOH, add 4 grams of pellets to 40 mL of water.}

[This demonstration was originated by Dr. Jerry Bell of Simmons College. For an expanded discussion of the chemistry involved, see: Shakhshiri, Bassam, CHEMICAL DEMONSTRATIONS: A Sourcebook for Teachers, Vol. III, University of Wisconsin Press, 1989.]