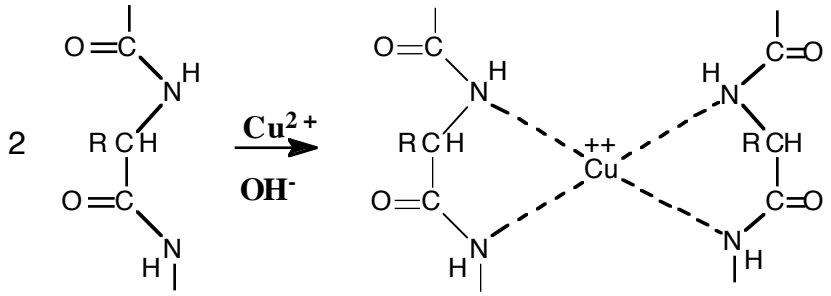
Experiment 11: Isolation and Characterization of Casein from Milk

Adapted from Experiment 21, "Isolation of Protein, Carbohydrate and Fat from Milk", in Mohr. S.C., Griffin, S.F., and Gensler, W. J. *Laboratory Manual for Fundamentals of Organic and Biological Chemistry by John McMurry and Mary E. Castellion*,: nglewood Cliffs, Prentice-Hall, 1994 and Wayne P. Anderson (4/2002)

You may recall the *Mother Goose* nursery rhyme, "Little Miss Muffet sat on a tuffet, Eating of curds and whey…." When milk is acidified, it is transformed into a solid component, called curd, and a liquid component called whey. The curds contain the butterfat and a protein called casein. The carbohydrate, lactose, is present in the whey. In this experiment you will isolate casein from milk and carry out some qualitative tests for protein.

The Biuret Test is a general test for protein. When the pale blue Cu^{2+} ion forms a complex with adjacent amide nitrogens of the peptide backbone, a very deep violet blue color forms.



The Xantoperoteic Acid Test on the other is a general test for the presence of the aromatic amino acids, tryptophan, phenylalanine and tyrosine, in proteins. Aromatic groups that have an amino group (tryptophan) or a hydroxyl group (tyrosine) are easily nitrate by concentrated nitric acid to form yellow (xantho, Greek for yellow) colored aromatic nitro compounds, thus the term xanthoproteic acids.

Procedure:

Determine the mass of a 125 mL Erlenmeyer flask. Add 50 mL of no-fat milk to the flask and re-weigh the flask to determine the mass of the milk. Check the label on the milk container and record the amount of protein per serving in your notebook. Prepare a water bath by placing 200 mL of water in a 600 mL beaker. Heat the water bath to 40ºC; as the temperature is critical for this experiment monitor the temperature with a thermometer. Place the flask containing the milk into the water bath. Slowly add 10 drops of glacial acetic acid to the milk while stirring with a glass rod. Continue to add acetic acid dropwise until no more precipitate is formed when a drop of acid is added. Allow the mixture to cool.

Filter the mixture into a 250 mL beaker by pouring it through cheesecloth that has been fastened to the beaker with a rubber band. Squeeze out as much liquid as possible from the solid. Then scrape the solid into a 100 mL beaker.

To remove the fat from the curd, add 25 mL of 95% ethanol to the solid in the 100 mL beaker. Stir the mixture for about 5 minutes; then let the solid settle. The fat will dissolve in the alcohol. Decant the liquid into another beaker.

Under a hood, add 25 mL of a 1:1 (v/v) mixture of diethyl ether and ethanol to residue. Be sure that there are no flames or sparks present as the diethyl ether is extremely flammable. Stir the mixture for about 5 minutes. This step dissolves additional fats. Collect the solid by vacuum filtration. Draw air through the solid for about 5 minutes. Ideally we would let the protein solid dry until next week but as next week is the last week of classes we do not have that luxury. Therefore, weigh your solid and determine the % yield.

Biuret Test:

Add a pea-sized amount of your casein to a small test tube and dissolve it in 4 mL of distilled water. Divide this protein solution into two 2 mL portions. Save one portion for the next test. Thoroughly mix 2 mL of the protein solution with 2 mL of 3 M sodium hydroxide solution. Add 1 drop of 0.1% copper sulfate solution. Note the color and record your observations in your notebook. Continue to add the copper sulfate solution one drop at time, noting and recording your observations. Stop after adding 10 drops of the copper sulfate solution. Repeat this test with a 1% casein solution

Xanthoproteic Acid Test:

To the second 2 mL protein solution carefully add 1 mL of concentrated nitric acid. Mix and not the appearance of any heavy white precipitate. Warm the mixture carefully in a hot water bath noting any change to a yellow colored solution. Cool the mixture in a stream of cold tap water and carefully add a few drops 3 M sodium hydroxide. A positive test is indicated by the yellow color deepening to orange. The entire tube does not have to turn to orange. Look for the color where the sodium hydroxide has entered the test solution, or on pieces of precipitate on the wall of the test tube. Repeat this test with a 1% casein solution.

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Name _____

Mass of 125 mL Erlenmeyer flask + milk: _____

Mass of 125 mL Erlenmeyer flask: _____

Mass of milk: _____

Mass of crude, partially dry casein: _____

% Yield (show calculation):

Using your crude mass determine the amount of protein in a serving of milk (show calculation):

Biuret Test:

Color of 0.1% copper sulfate solution:

Color of your casein + sodium hydroxide solution:

Color of your casein solution after addition of one drop of copper sulfate:

Color changes observed after adding additional drops of copper sulfate:

Color of 1% casein + sodium hydroxide solution:

Color of your casein solution after addition of one drop of copper sulfate:

Color changes observed after adding additional drops of copper sulfate:

Xanthoproteic Acid Test:

Observation for your casein mixed with concentrated nitric acid:

Observation for your casein with concentrated nitric acid after heating:

Observation after adding 3 M sodium hydroxide:

Observation for 1% casein mixed with concentrated nitric acid:

Observation for 1% casein with concentrated nitric acid after heating:

Observation after adding 3 M sodium hydroxide: