Name:

Experiment: The pH dependence of Pepsin

(Why do antacids hinder digestion?)

Background: As you know from reviewing the Pepsin Report, pepsin is an enzyme that hydrolyzes the peptide bond of a substrate. As a result, the protein is cleaved into smaller units. The reaction (as is most enzyme reactions) is pH dependent. It is the purpose of **this experiment to investigate the pH dependence of the pepsin reaction.**

Objective: In this experiment you will investigate the action of pepsin on homogenized denatured egg white, a protein source. You will use Biuret Reagent to identify which solutions have protein and which have no protein after the reaction. The Biuret reagent changes from blue to violet and is pink in the presence of short chain peptides.

Solutions- (Note: All solutions are aqueous)

A. 1% Pepsin
B. 1% Pepsin in 0.4% HCl
C. 0.4% HCl
D. 1% Pepsin in 0.5% $NaHCO_3$
E. Protein Suspension – (Albumin) - Homogenize the white of a hard boiled egg in 250 mL water.
F. Biuret Reagent

Procedure:

Arrange a series of test tubes. Label and dispense the following solutions into each:

Test Tube No.	Protein Suspension (mL)	Pepsin Solution (mL)	HCl Solution (mL)	$NaHCO_3$ Solution (mL)	Water (mL)
1	10.0	3.0	3.0	0	0
2	10.0	3.0	2.5	0	0.5
3	10.0	3.0	2.0	0	1.0
4	10.0	3.0	1.5	0	1.5
5	10.0	3.0	1.0	0	2.0
6	10.0	3.0	.5	0	2.5
7	10.0	3.0	0	0	3.0
8	10.0	3.0	0	.5	2.5
9	10.0	3.0	0	1.0	2.0
10	10.0	3.0	0	2.0	1.0

Using a fresh square of Parafilm on the top of each test tube, invert several times to mix. Return each tube to its place on the rack. Measure the pH of each tube by inserting a pH strip into each tube. Record the pH. Cover the tubes and allow to stand overnight.

The next day, observe the appearance of each tube and note any changes that may have occurred. Add 2.0 mL Biuret reagent to each tube, invert to mix. Observe the color of each tube. From the color of the Biuret, indicate the presence or absence of protein. Complete the data chart and answer the lab questions.

Results:

Tube	pH	Appearance	Biuret Test Color	Presence of protein?
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

Finally: Pour away the contents of ALL the tubes down the sink, then rinse the tubes with a gentle flow of cold water before placing them in the washing up bowl.

Analysis of Results:

In which test tube(s) was the protein consumed? In which tubes did the protein remain essentially unchanged?

Using what you have learned about pepsin, are your results consistent with the theory of pepsin enzyme reactivity? Explain fully.

What would happen to the digestion of proteins in your stomach if you took a large dose of antacid? Explain.

http://www.biotopics.co.uk/nutrition/pepsin.html