An elegant science experiment for young (and old) students – the digestion of egg white (albumen) by a common protease enzyme, pepsin.

We did this experiment in our non-profit private school, the Las Cruces Academy, working up some calculations in one week and doing the experiment on the following Monday, 15 April 2019.

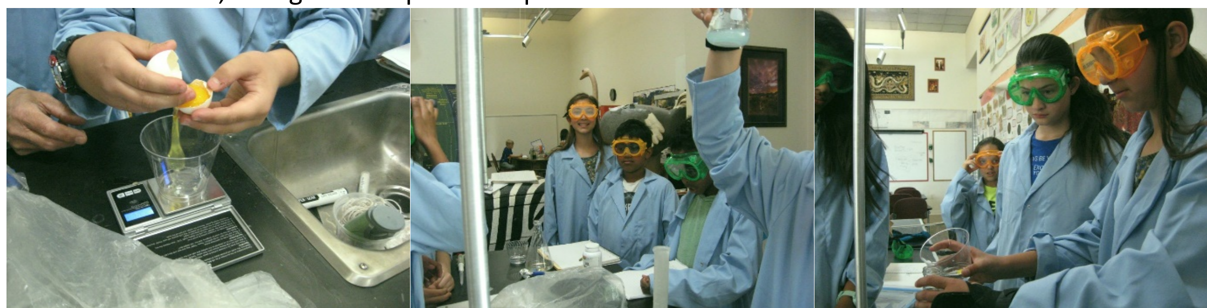
We do serious STEM education. I'm mostly disappointed in what I see presented as STEM, where there's little or no explicit math. We do real math in our science demos and experiments, as you see below. The math here does extend beyond the grasp of the students in the class; the advanced part is here for readers who want to go more deeply into the interesting concepts brought up in the experiment and its data analysis.

Sixth-grade student Erika suggested that we try an experiment to see how fast an enzyme, pepsin, can break down egg white protein. Pepsin is a protease, which breaks bonds in proteins by hydrolysis, adding water to the peptide bond between amino acids in a protein. The bond being broken, the protein falls into two parts – and more, as the hydrolysis continues.

We tried this with bulk egg white and a pepsin solution, finding almost no changes in properties over several days; the amount of protein exposed to pepsin action is too small a fraction of the whole mass, because the albumen is in a gloopy mass as if one big lump, to use a simple non-technical term we all appreciate.

Erika found a procedure in the literature in which the egg white or albumen is dispersed into fine droplets, making a colloid. Now the enzyme has a chance to act on a vast surface area of albumen. The colloid is heated to condense the albumen into white droplets that can scatter light strongly, thus becoming detectable by the amount of light left unscattered in passing through the solution.

Before the details, let's get a few pictures up:



The procedure is (a bit modified for our case):

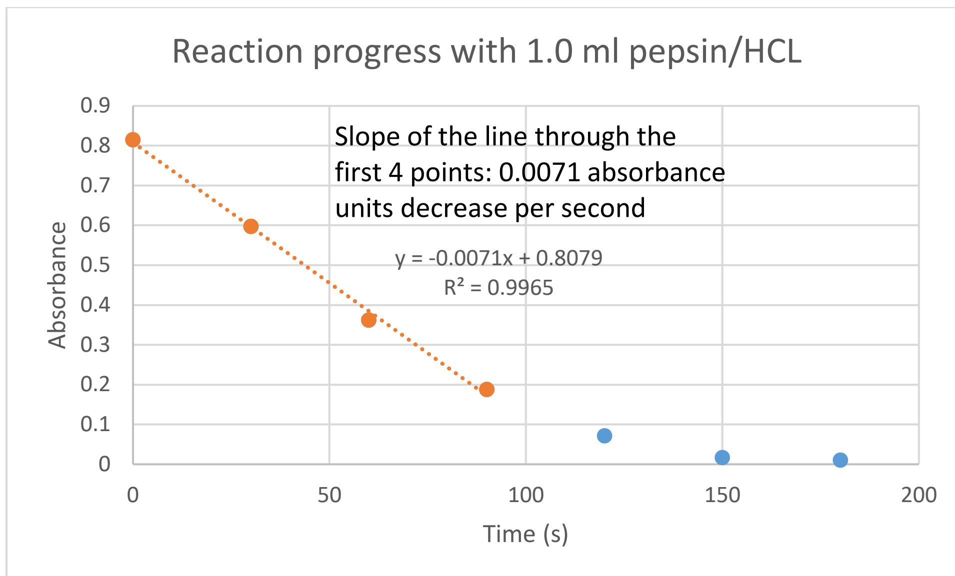
- Make up a colloidal solution of egg white in four times its volume of water. Strain the solution to remove big particles. We used a coffee filter, obtaining a clear solution.
- Heat this solution carefully, with strong stirring. We put it on a stirring hot plate with a standard Teflon-coated magnetic stir bar. It was going to be a test, since we hadn't located a thermocouple thermometer yet (it was at Vince's home) to measure the temperature, which is supposed to be between 55°C and 60°C. However, it proved to be unnecessary – there was a sharp turbidification of the solution, at which point we removed the solution and let it cool. As the write-up claimed, it looks like diluted milk. This was planned as a test but the solution proved stable over a weekend, so we used it in the final test.

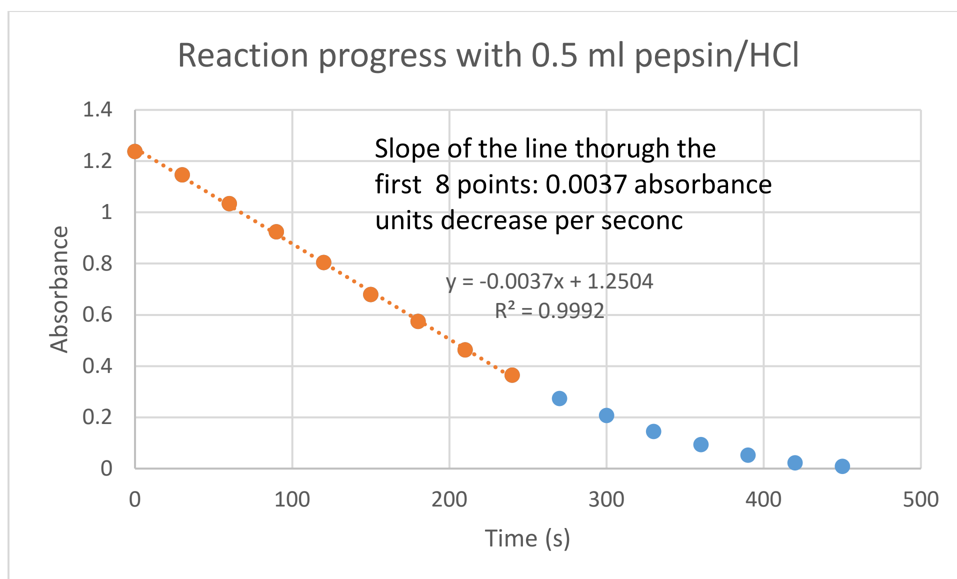
- Make up a solution of pepsin – actually, of pepsin with hydrochloric acid (HCl) and a stabilizing base, betaine. To make a long story shorter, we used the claimed ingredients in commercial pepsin/HCl capsules sold in health food stores to figure out that a capsule cracked and its contents dissolved in 32.4 ml of water should be 0.5% by mass in pepsin and 0.123M in HCl, which is just in the range that the published procedure calls for. Calculations are in an appendix at the end here.
- Filter this solution with fine chemical filter paper; this removes fine particles (silica?) that appear to be in the capsule to keep the contents free-flowing.
- Make up a control solution of just HCl of the same strength. This should not cleave albumen but should have the same optical properties. This was 1.2 ml of concentrated (36%) HCl in 100 ml of water.
- Have purer water ready as a diluent.
- Warm up a spectrophotometer ("spec"), which measures light transmission at a wavelength (color) one selects. They suggest 650nm, in the red.
- Set the zero light transmission of the spec with its button that cuts off light.
- Set the 100% light transmission of the spec with a solution that has pepsin/HCl (1 ml) and water (2.6 ml; less than the 3.0 ml in the write-up, since that fills the spec's cuvette too high). Adjust the control over transmission to attain 100%.
- Make up a control solution with HCl and the albumen colloid but no pepsin: 1.0 ml of the HCl solution, 2.0 ml of the colloidal solution, and 0.6 ml of water. Read the absorbance of the mixture. Read it again later, at some time, to see if it has changed.
- Make up a reacting solution, with 1.0 ml of the pepsin/HCl solution, 2.0 ml of the colloidal solution, and 0.6 ml of water. Read its absorbance quickly and continue to do so every 30 s or so.

What you observe is a rather rapid fall in "absorbance" (really, scattering) of the reacting solution. In a few minutes it's clear. Plot the data vs. time.

We repeated the procedure with only half as much pepsin: 0.5 ml of the pepsin/HCl solution, 0.5 ml of the HCl solution (so, the same total HCl content), 2.0 ml of the colloidal solution, and 0.6 ml of water.

Here's what we found:





The rate of decline of absorbance is just a constant, independent of time and of the amount of colloid left. It does depend directly with the concentration of pepsin – that is, in essentially direct proportion to the pepsin concentration (our measurements of the volume of pepsin/HCl added were not of high resolution). These are all very nice data! Nice suggestion, Erika! Good work, all! The others involved were Anish, Isaac, Janaki, Kaleb, Mariah, Mohammed, Samantha, and Syan.

This rate is unusual, to a chemist like me, Vince. Considering absorbance as analogous to the concentration of a chemical reactant, this looks like a zero-order reaction. That is, the rate is constant, independent of the "concentration" of the reactant, the colloid. One expects at least a first-order reaction, in which the rate of disappearance of the "reactant" falls off in proportion to the amount remaining – fewer reactants, slower reaction.

The explanation lies in analyzing the origin of the light scattering. Scattering is not done per molecule of albumen; it's done per area of albumen particles. I provide an appendix with the arguments why a progression of layers that each occlude a small fraction of light passing through gives us an attenuation of light that is proportional to a negative exponential of the total number of layers and the fraction occlusion per layer. Then, with absorbance in the spectrophotometer calculated as the (negative of the) natural logarithm of light transmission, that reading as absorbance is proportional to the total area of light scatterers in the path – not their number, not their mass (if they are spheres), but their areas.

The section that follows, short of the appendices, is for advanced students and researchers. I haven't seen a concise analysis of this type, so it may be of interest.

Now into a bit more math:

- It's a good assumption that the rate at which mass is lost from any one particle is proportional to the area exposed to the enzyme. If a sphere has a diameter L, then it has an area πD^2 and the rate at which the sphere loses mass is proportional to this – let's write it as $k\,\pi D^2$. Here, k is a rate constant that depends on the activity of pepsin, which will be its concentration multiplied by factors that depend upon the solution temperature and acidity.

- The sphere has a mass equal to its density times its volume, or $m = \rho \frac{\pi}{6} D^3$. It's rate of change is the time derivative,

$$\frac{dm}{dt} = \frac{d}{dt}\left(\rho \frac{\pi}{6} D^3\right)$$
$$= \rho \frac{\pi}{2} D^2 \frac{dD}{dt}$$

  This is basic calculus. The factor dD/dt is just the time rate of change of the diameter of the particle. An appendix below can get you started on differential calculus, with the explanation of the derivative.
- A key finding: We have from above that

$$\frac{dm}{dt} = k\pi D^2$$

  by chemical reaction, but we also have

$$\frac{dm}{dt} = \frac{\rho\pi D^2}{2}\frac{dD}{dt}$$

  from geometry. Equating the two forms we get
-

$$k\pi D^2 = \frac{\rho\pi D^2}{2}\frac{dD}{dt}$$

  Both sides are proportional to D^2, so we can cancel out that factor. We get:

$$\frac{dD}{dt} = \frac{2k\pi}{\pi\rho}$$
$$= \frac{2k}{\rho}$$

  That is, the rate of shrinkage of the diameter of a particle is independent of its size!
- The fractional occlusion by all the particles in a small volume is the sum of their squared diameters. Let's assume that there is a probability distribution, p(D), for the probability that particles have diameters between D and D+δD is just p(D) δD.
- Consider a thin layer of solution with blockers. The total occlusion, O, is then summed over diameters, with their probabilities of occurrence, p(D), or integrated over a continuous distribution of diameters:

$$O = \int_0^{D\max} dD\, p(D)\, \pi D^2$$

- We can estimate a form of the probability distribution – say, p(D) as a power law, aD^{-2}. Using

$$O = \int dD\, aD^{-2}\, \pi D^2$$

  that, we get

$$= a\pi \int dD$$
$$= a\pi D_{\max}$$

- What's interesting is the rate at which occlusion changes, dO/dt:

$$\frac{dO}{dt} = \int dD\, p(D) \frac{d}{dt}\left(\pi D^2\right)$$
$$= \int dD\, p(D)\, \pi 2D \frac{dD}{dt}$$

- Using the constancy of dD/dt, we get
$$\frac{dO}{dt} = \frac{dD}{dt} * 2\pi \int dD\, p(D)\, D$$
- Both of the factors on the right-hand side are constant. Thus, we expect occlusion (and the light "absorbance" of the colloidal solution to decline linearly with time – an apparent zero-order reaction.
- The same argument holds trivially if there is a uniform size distribution – all spheres are the same size.

I must admit that the explanation here is a bit facile. I treated light scattering as if it removes light from the path, but if light is truly scattered more than absorbed it can be secondarily scattered back into the forward direction rather than all lost. This effect is probably minor for the nicely collimated light beams in a spectrophotometer. In the more general case where we count up light reaching the "end" of the path but traveling at any angle, not just vertical, the attenuation of light is more complex. I have a mathematical model for how light goes through layers of scatterers. It's not just simple exponential attenuation. I haven't split out just this model, but it's in a posting on my website for a detailed model of how light reaches all leaves in an orchard (and how it drives the rate of photosynthesis).

Appendix I. Calculating the amounts of pepsin and of HCl in a commercially available capsule.

One capsule of pepsin HCl has a reported 650 mg of betaine HCl and 162 mg pepsin
To get 0.5% pepsin, we need a volume of water that's 200x larger, or
200 x 162 mg = 32.4 g; that'll be 32.4 ml of water.
We're also getting HCl in the capsule – how much?
Betaine HCl has a formula mass of 153.6; HCl has a formula mass of 36.5
→ Betaine HCl is a fraction 36.5/153.6 = 0.23 as HCl
Since the total mass of betaine HCl is 650 mg, the mass of HCl is then 0.23*650mg = 149.5 mg
This mass of HCl represents how many moles of HCl? It's 149.5 mg/(36.5 mg /mol) = 4 mmol, closely
If we put 4 mmol of HCl into 32.4 ml = 0.0324 L, the concentration is 0.004 mol /0.0324 L = 0.123 mol/L, or molar. That's right where we want it.

Appendix II. A bit of differential calculus: the derivative of a function, with the derivative of x^2 as an example.

The derivative of some function of x can be considered as the slope of the graph of that function. Here's a graph of the function $f(x)=x^2$.

The derivative at any value of x is just the change in the function over a small step in x, divided by that small step in x, taking the limit of a step size going to zero. Let's do that for $f(x)=x^2$. We have its value at x as just x^2. At a small step δx further, at $x+\delta x$, its value is $(x+\delta x)^2 = x^2+2x\,\delta x+(\delta x)^2$. The derivative looks like

$$\begin{aligned}\frac{d}{dx}x^2 &= \lim_{\delta x \to 0}\frac{x^2+2x\delta x+(\delta x)^2-x^2}{\delta x}\\ &= \lim_{\delta x \to 0}\frac{2x\delta x+(\delta x)^2}{\delta x}\\ &= \lim_{\delta x \to 0}\left(2x+\delta x\right)\\ &= 2x\end{aligned}$$

If you're new to calculus, you've just done your first derivative.

Appendix III. Why thin layers of occluding objects give us an exponential falloff of the amount of light making it through.

In the path of light are scatterers or absorbers than decrease the amount of light getting through. Let's look at them in a sequence of thin layers. Let the whole path have a total area A of these guys per unit area of the path (such as the path through a square-sided cuvette used in the spectrophotometer). We can even have A greater than the unit area of the path!....but this won't mean total exclusion of light, because the blockers, as we can call them, are in random places. Let's use X to represent the fraction of blocked area per unit area, and X can be greater than 1. If the blockers are placed randomly – and we certainly expect molecules or colloids in a solution be be so – then we can slice up the path in layers along the direction of the light. Let's make a lot of thin layers, n of them, each with a portion X/n, of the blockers. The fraction of light getting through the first layer is 1-x. This fraction reaches the second layer. This does NOT subtract another fraction, x, to give 1-2x. Instead, consider the first layer's transmitted light smeared out evenly, with no regard to the placement of blockers in the second layer. The fraction passing both layers is the product (1-x)(1-x) = $1\text{-}2x\text{+}x^2$. That positive part is important. Let's take x = 0.1 (10%) and 10 layers. Instead of 1-10x = 1-10*0.1 =0, we get $(1\text{-}0.1)^{10} = 0.9^{10}$ = 0.349, to 3 figures! We can divide layers even further, in an arbitrary number of them, N. We get

$$Fraction\,transmitted, T = \left(1-\frac{X}{N}\right)^N$$

If we take the limit of very large N (→∞), we get the exponential, exp(-X), also written as e^{-X}. This is a mathematical function readily calculated, as on any but the simplest modern calculator, or in Excel, or in any programming language. This formula is one of several equivalent definitions of the exponential (the negative exponential; change the minus to plus for the positive exponential, e^X). I have more details on my website (where there's much more – check out science-technology-society.com). This version is handwritten and, thus, a bit messy, though it is right on this topic. A treatment that starts from more specialized cases, so, not so directly applied, is nicely typeset.