S6: Conducting a Lysozyme Assay with the OPN Colorimeter

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We describe here the specific protocols that we followed for the lysozyme assay that we used to test both versions of the OPN Colorimeter. We adapted these procedures from the work of Stenesh (1984), whose text contains a number of useful experiments in biochemistry.1 Also, although there are no known hazards associated with the substances used in this exercise, readers should still exercise caution and use proper lab etiquette when working with these materials.

Lysozyme Assay

For the lysozyme assay, we first prepared a bacterial cell wall solution (our substrate) by adding 300 mg of Micrococcus Lysodeikticus ATCC No. 4698 (Sigma Aldrich Corp., St. Louis, MO; No. M3770) to 1 liter of 100 mM phosphate buffer (pH 7.0). We then prepared a separate solution of lysozyme by adding 20 mg of lysozyme purified from chicken egg white (Sigma Aldrich Corp., St. Louis, MO; No. L6876) to 200 mL of tap water.

As in the Bradford assay (S5), we placed a Pyle PLMT12 light meter and a Coast G20 flashlight into the wooden version of the OPN Colorimeter and turned them both on to give the light meter a sufficient time to warm up (S4), making sure to put the meter on the “200 lumens” setting. We also placed a blue cellophane filter in the front chamber of the OPN Colorimeter directly in front of the ⅛-inch hole (and, thus, in the path of the beam of light) before we closed the lid.

After waiting 10 minutes, we took a reading without the cuvette in place to obtain the value for the intensity of the incident light striking the cuvette (I). Specifically, we again moved the Coast G20 flashlight in the PVC tube of the OPN Colorimeter until the...
light meter read 120 lux (since prior testing had indicated that a value around 100 lux was sufficient to accommodate any increases or decreases in the readings as different enzyme concentrations were tested). We then taped the flashlight into place, so that it would not move during the experiment.

Next, for the lysozyme assay, we added 3.0 mL of the bacterial cell wall solution to a standard cuvette (external dimensions: 1.25 cm x 1.25 cm x 4.5 cm) and placed the cuvette in the OPN Colorimeter. We then added 25, 50, or 100 mL of the lysozyme solution to the cuvette using a plastic transfer pipette and gently suspended the mixture (three times) to ensure a thorough mixing. After that, we closed the lid of the OPN Colorimeter and recorded the value displayed on the readout at 30-second intervals (starting at the zero mark) for the next 5 minutes (which were the values for the transmitted light \(T\) at each time interval). We then cleaned the cuvette and repeated the above steps for the other lysozyme concentrations.

Next, we repeated the general steps set forth above (only without a 10-minute warm-up period) using the 3D-printed version of the OPN Colorimeter along with a Coast G20 flashlight, a blue cellophane filter, a PDV-8103 photocell by Advanced Photonics, Inc., which has a 500 kOhm “dark” resistance, and a Cen-Tech digital multimeter (S1). Specifically, we positioned the flashlight in the 3D-printed tube (S2C01, S3C01) until the multimeter read 350 Ohms (a value that, given our prior testing, allowed for sufficient increases or decreases in the readings as different samples were examined). Then, we taped the flashlight into place and carried out the lysozyme assay at the different enzyme concentrations as described above.

Also, in both versions of the OPN Colorimeter, we left the LED flashlight on throughout the experiments. In addition, as a control, we followed the same general steps set forth above, using a Hitachi U-1100 spectrophotometer. We then entered all
of our data into Microsoft Excel, calculated the corresponding Absorbance values, and graphed the results.

Hazards

Even though the bacterial cell wall solution (Micrococcus Lysodeikticus ATCC 4698) and lysozyme are not identified as hazardous substances in their Sigma-Aldrich Material Safety Data Sheets, teachers and students should still exercise caution when handling these substances, including wearing the proper protective equipment (e.g., gloves, goggles, etc.) and observing proper lab etiquette. Readers should further review the Material Safety Data Sheets for these substances before working with them, and both mixtures should be disposed of as chemical waste at the conclusion of any lab exercise or demonstration (and not poured down any drains).

REFERENCES