BOURNE TO MIX:

A TEACHING MODULE ON THE
MIXING EFFICIENCY OF DISSOLUTION TESTING
FOR PHARMACEUTICS AND ENGINEERING

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Module Topic: This module was created to introduce high school students to current Pharmaceutical Particulate Research and Pharmaceutical Engineering. The specific reactions involved in the investigations for compiling this unit are simple, straightforward and manageable. They lend themselves for study in an array of topics including but not limited to: Reaction rates, chemical stoichiometry, acid-base reactions, balancing equations, ionic/covalent bonding, solution concentrations and dilutions, Beers law, equilibrium, drug delivery, absorption and agricultural runoffs.

Appropriate Subject Area(s): Biology, Chemistry, Physics, Marine Science, Earth Science, Anatomy and Physiology, Physical Science

Rationale: Research complements scientific curricula. It allows students to develop and/or sharpen their analytical skills by providing them with the opportunity for intellectual investigation. It allows students to be more engaged in their own learning with the real-life situations they encounter during their studies. It is extremely important that students experience science through the research experience for two reasons. First, high school students do not have enough exposure to the fields available to them as a possible career. Being involved with a research curriculum would offer the students the opportunity to become familiar with these fields. Also, "*learning by doing*," is a strategy that captivates the adolescent mind. Our students need this exposure and guidance so that they can make better informed-decisions for future endeavors. Moreover, students need to physically manipulate equipment and materials in order to make relevant observations and collect data; use the collected data to form conclusions and verify hypotheses; and communicate and compare results and procedures in formal written reports, which

emphasize chemical calculations and the mathematical formulation of principles. With this in mind the unit on dissolution testing aims to enrich the high school student's curriculum by exposing him/her to some aspect of on-going research in the area of pharmaceutical particulate and engineering.

Learning Objectives:

Students will be able to:

1. Use appropriate laboratory safety procedure.
2. Use a thermometer, balance and graduated cylinder to measure temperature, mass and volume.
3. Demonstrate appropriate technique in transferring liquids.
4. Collect data and organize it in a table.
5. Calculate values from experimental measurements.
6. Use pH paper or pH probe.
7. Construct a calibration curve.
8. Graph data

Standards and Indicators:

STANDARD 5.1 (Scientific Processes) All students will develop problem-solving, decision-making and inquiry skills, reflected by formulating usable questions and hypotheses, planning experiments, conducting systematic observations, interpreting and analyzing data, drawing conclusions, and communicating results.

STANDARD 5.3 (Mathematical Applications) All students will integrate mathematics as a tool for problem-solving in science, and as a means of expressing and/or modeling scientific theories.

STANDARD 5.4 (Nature and Process of Technology) All students will understand the interrelationships between science and technology and develop a conceptual understanding of the nature and process of technology.

STANDARD 5.6 (Chemistry) All students will gain an understanding of the structure and behavior of matter.

STANDARD 5.7 (Physics) All students will gain an understanding of natural laws as they apply to motion, forces, and energy transformations.

STANDARD 5.10 (Environmental Studies) All students will develop an understanding of the environment as a system of interdependent components affected by human activity and natural phenomena.

Utilizing this module will address the following 6 of the 10 New Jersey Professional Standards for Teachers.

Standard 1: Subject Matter Knowledge

Standard 2: Human Growth and Development
Standard 4: Instructional Planning and Strategies
Standard 5: Assessment
Standard 6: Learning Environment
Standard 8: Communication

Background Information: In the pharmaceutical industry, dissolution testing is commonly used to examine the effectiveness of drug release (Bai, 2007). Dissolution testing can also be used to test for quality control. Using dissolution testing, companies can determine the reliability of drug delivery from one batch to another, before the drug is released to the public. The United State Pharmacopoeia (USP) has established four primary methods to examine dissolution testing. The first two involve the dissolution of solid compressed tablet dissolution.

Effectiveness of dissolution of solid drug delivery can be influenced by the fluid dynamics of the system. The current protocols for USP Dissolution testing does not take into account the effects of micromixing on the results. Akiti, et. Al. Showed that there is a great deal of variability of the turbulence produced in different locations throughout an unbaffled dissolution vessel (Akiti, 2005). Based on these findings, it could be predicted that the location in the vessel in which the pill is placed could greatly affect the dissolution rate. This could have major implications for the validity of dissolution testing and the consistency with which companies are testing the quality of their products.

One important consideration to take into account is that tablet dissolution testing is often measured as a function of time. Testing can be very inaccurate if the solution is not a homogeneous mixture. According to the standards set by the USP, samples can be taken from anywhere between the top of dissolution medium and the rotating impeller blade. The sample should not be taken less than 1cm from the vessel wall. (USP, 2005) If the sample is not homogeneous, then we could expect that there would be an unacceptable amount of variability between samples.

We can study the effects of micromixing in a beaker system utilizing a lozenge to simulate the drug dissolution testing protocol currently used in the pharmaceutical industry. The efficiency can be measured as the concentration of dissolved color in water. These values can be obtained via visible spectroscopy using a classroom spectrophotometer.

Materials:
Part I: Hotplate with stirring magnet, magnetic stirrer, 1000mL beaker, tripod for beaker, DPEE Boiling chips

Part II: Hotplate with stirring magnet equipped with graduations, magnetic stirrer, pH probe or pH paper with color-coded range, deionized water, 1000mL beaker, tripod for beaker, different colored-lozenges (red), spectrophotometer and cuvettes for readings, micro test tube holder for cuvettes, stop watch, plastic pipettes, 5—100mL beakers for samples and labels.

Approximate Time Required: Three sessions—One 40 minute lecture period for demonstration and background information and two—80 minute laboratory periods for student activity (experiment).

Classroom Activity Description: ***Day one: (Performed by teacher)***
Part I: ***Demonstration of mixing efficiency in a beaker system:***

1. Add 600mL of water in a 1000mL beaker.
2. Add ~25 DPEE boiling chips.
3. Drop a magnetic stirrer into beaker system.
4. Place the beaker and contents over a hotplate equipped with a magnet stirrer.
5. Turn stirrer on.
6. Point out the inefficiency of this mixing system
    i. (Point to the poor mixing of the boiling chips)
    ii. (Point to the vortex at top of system).
7. Next set up the system as described above.
8. Submerge a tripod in the beaker upside down to simulate a baffled system.
9. Point out the differences in the two systems.
    i. Baffled system lacks a vortex and has better mixing as shown by the boiling chips action.

Calibration Curve for Part II: ***Day One: (Performed by Students)***

1. Obtain a lozenge for use in Part II.
2. Obtain the mass of the lozenge and record it in your data sheet.
3. Completely dissolve the lozenge in 600mL of deionized water.
4. While the lozenge dissolves obtain a spectrophotometer and spectrophotometer cuvettes.
5. In a cuvette place 2.00mL of deionized water.
6. Wipe the cuvette and place it in the spectrophotometer.
7. Turn on the spectrophotometer.
8. Set the wavelength to 540 nm.
9. Turn the absorbance button until the needle reads: Transmittance 100% / 0 Absorbance.
10. Remove the Zeroing cuvette from the spectrophotometer and place it on a micro test tube holder for later use. (You may need it to re-zero later).
11. Transfer 2.00mL of the final solution you obtained in step 3 and place them into a cuvette, this will be standard 1.
12. Obtain a spectrophotometer reading of standard 1.
13. Transfer 1.00mL of the final solution and place it in a small beaker.
14. Add 9.00mL of deionized water. Call this standard 2.
15. Transfer 1.00mL of standard 2 to another beaker.
16. Add 9.00mL of deionized water. Call this standard 3.
17. Transfer 1.00mL of standard 3 to another beaker.
18. Add 9.00mL of deionized water. Call this standard 4.

19. Transfer 1.00mL of standard 4 to another beaker.
20. Add 9.00mL of deionized water. Call this standard 5.
21. Transfer 2.00mL of standard 2 to another cuvette and obtain a reading.
22. Repeat step 21 for all standards
23. Record your observations and measurements in your data sheet.
24. Plot a graph of Absorbance against sample.

Part II: ***The Dissolution Solution: A laboratory experience in effective mixing.***

Day Two: (Performed by students).

1. Label 5 – 100mL beakers 1 – 5.
2. Label beaker 1 (4 minutes), beaker 2 (8 minutes), beaker 3 (12 minutes), beaker 4 (16 minutes) and beaker 5 (20 minutes).
3. Place 600mL of deionized water in a 1000mL beaker.
4. Add a magnetic stirrer into the beaker and run the magnet stirrer at the lowest setting (1).
5. After 1.00min record the temperature of the system.
6. Record the pH of the water.
7. Obtain a lozenge equal in mass to the one from the calibration curve.
8. Drop the lozenge into the side of a beaker making sure not to disturb the magnetic stirrer at the bottom of the beaker.
9. Immediately start the stopwatch.
10. After 4 minutes insert a pipette 2.5cm below the surface of the water level and draw 5mL of the solution. Make sure to note where you obtained the sample. You will need to draw more samples from this very same spot. Be careful not to disturb the beaker system.
11. Place the 5mL of solution into beaker 1.
12. Repeat this process every four minutes until all 5 beakers have been utilized.
13. Turn the stirrer off
14. Obtain 6 cuvettes for the spectrophotometer.
15. Prepare 5 cuvettes for spectrophotometer readings by placing 2.00mL of each sample into the cuvettes.
16. Prepare 1 cuvette with 2.00mL of deionized water.
17. Make sure to label and track your samples.
18. Standardize the spectrophotometer
    a. Remove all fingerprints from the cuvettes by wiping them with tissue paper.
    b. Turn on the spectrophotometer.
    c. Set the wavelength to 540nm.
    d. Insert the cleaned deionized water cuvette into the spectrophotometer cuvette chamber.
    e. Turn the absorbance button until the needle reads 100% Transmittance / 0 Absorbance.

   f. Remove the Zeroing cuvette from the spectrophotometer but keep it in a test tube holder for later use. (In case you may have to re-zero the spectrophotometer).

19. Place the first sample cuvette into the spectrophotometer 20 cuvette chamber.
20. Record the Absorbance on your data sheet.
21. Repeat steps 19 – 20 with each of the other 4 samples.
22. Obtain concentration of your samples by correlating them with the calibration curve.
23. Repeat steps 1- 22 with the following change: In step 3, invert a beaker tripod into the 1000mL beaker such that it will function as a baffle.
24. Compare the concentrations of your samples and draw your conclusions.

Homework/Exercises/Problems:

Day Three: (Performed by students).

1. Design your own system to improve the mixing efficiency of dissolution. You may want to try changing the pH or temperature of the system.
2. Remember you must include quantitative data. (Compare a measurable quantity (concentration) to a standard)).
3. You may use the unbaffled system as a control.

Bourne to Mix

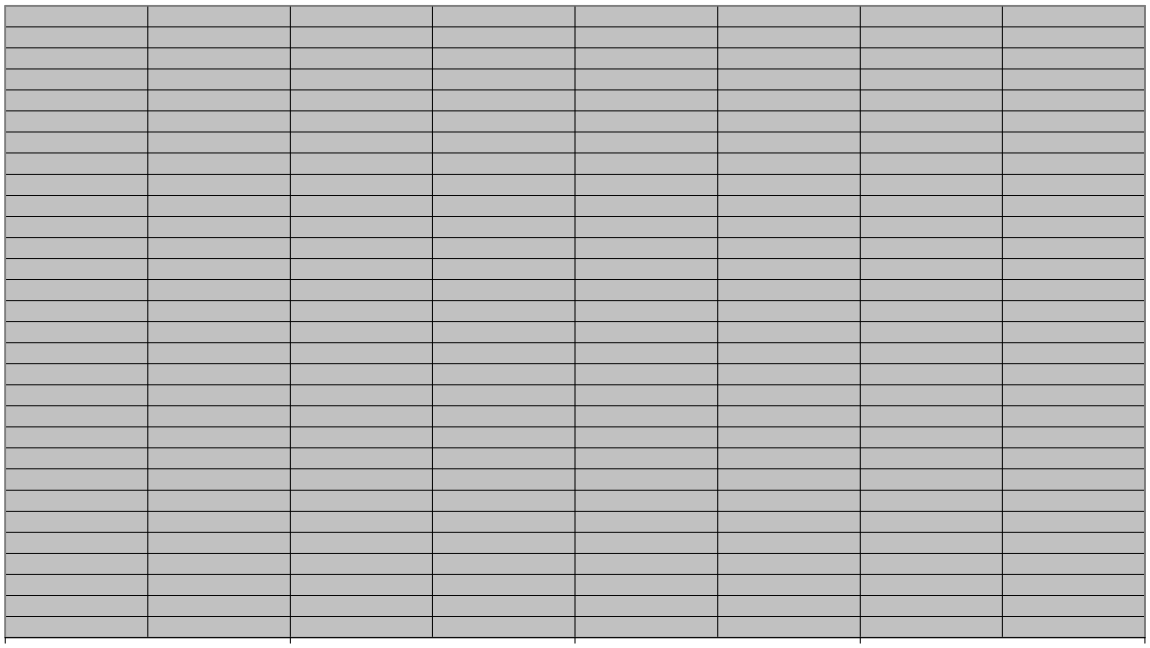
Data Sheet 1:

Name:_____ Date:_____

Calibration Curve:

1) Mass of lozenge: _____ milligrams
2) Concentration of lozenge: _____ mg/L = ppm
3) Absorbance for Standard 1: _____ Conc. _____ ppm
4) Absorbance for Standard 2: _____ Conc. _____ ppm
5) Absorbance for Standard 3: _____ Conc. _____ ppm
6) Absorbance for Standard 4: _____ Conc. _____ ppm
7) Absorbance for Standard 5: _____ Conc. _____ ppm
8) Plot a graph of Absorbance on the Y – axis and Standard number on the X – axis

Calibration Curve



Data Sheet 2:

1) Water temperature: _____ deg Celsius
2) Water pH: _____
3) Mass of lozenge _____mg

Unbaffled System

4) Sample 1 Absorbance: _____
5) Sample 2 Absorbance: _____
6) Sample 3 Absorbance: _____
7) Sample 4 Absorbance: _____
8) Sample 5 Absorbance: _____

Baffled System

9) Sample 1 Absorbance: _____
10) Sample 2 Absorbance: _____
11) Sample 3 Absorbance: _____
12) Sample 4 Absorbance: _____
13) Sample 5 Absorbance: _____

Calculations:

1) Determine the concentration of the initial standard solution.
    a. Concentration = _____mg / 0.600L solution
    b. mg/L also = ppm
    c. Concentration = _____ppm

2) Determine the concentration for the diluted standards.
    a. Concentration from 1c / 10 = _____ ppm
    b. Standard 2 = _____ ppm

3) Determine the concentration for the diluted standards.
    a. Concentration from 2b / 10 = _____ ppm
    b. Standard 3 = _____ ppm

4) Determine the concentration for the diluted standards.
    a. Concentration from 3b / 10 = _____ ppm
    b. Standard 4 = _____ ppm

5) Determine the concentration for the diluted standards.
    a. Concentration from 4b / 10 = _____ ppm
    b. Standard 5 = _____ ppm

Assessment of Learning Outcomes:

1. Prepare and hand in a formal laboratory report.
    a. Must address the learning objectives cited above.
2. Pre and Post Laboratory Questions.

References:

Bai, G., Armenante, P.M., Plank, R., 2007. Experimental and Computational Determination of Blend Time in USP Dissolution Testing Apparatus II. Journal of Pharmaceutical Sciences 00, 1-15.

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The United States Pharmacopeia & The National Formulary. The Official Compendia of Standards, USP 29-NF 24 2005, Pharmacopoeial Convention Inc., November, 2005

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Akiti, O., Armenante, P.M., 2004. Experimentally-Validated Micromixing-Based CFD Model for Fed-Batch Stirred-Tank Reactors. American Institute of Chemical Engineers Journal 50, No. 3.