

CATALySES Development Award

Incorporating the Science of Emerging Pathogens in Secondary School Curricula

Title: Analytical Chemical Techniques to DIScover Antibiotics for Resistant Microbes

(ACT to DISARM)

Principle Investigator: Sharon Karackattu, Ph.D.

Oak Hall School

Gainesville, FL

skarackattu@oakhall.org

617-504-4056

Abstract: Student in AP Chemistry are often asked to solve complex chemistry problems or learn difficult concepts and techniques without couching them with appropriate real-world scenarios. This action proposal seeks to contextualize several of the experimental and analytical techniques that AP Chemistry students must be familiar with in terms of their utility in studying antibiotic resistance and the drug discovery and development process. Students will learn about each technique through a series of short readings or videos, then come prepared to discuss or solve problems with their classmates. Assessments will include a student satisfaction survey on the delivery of the content, a multiple choice quiz and scoring of their performance on related AP Chemistry style problems. It is expected that students will improve their understanding of these techniques and have a better experience throughout the course when understanding how the content can benefit human health and welfare.

Rationale:

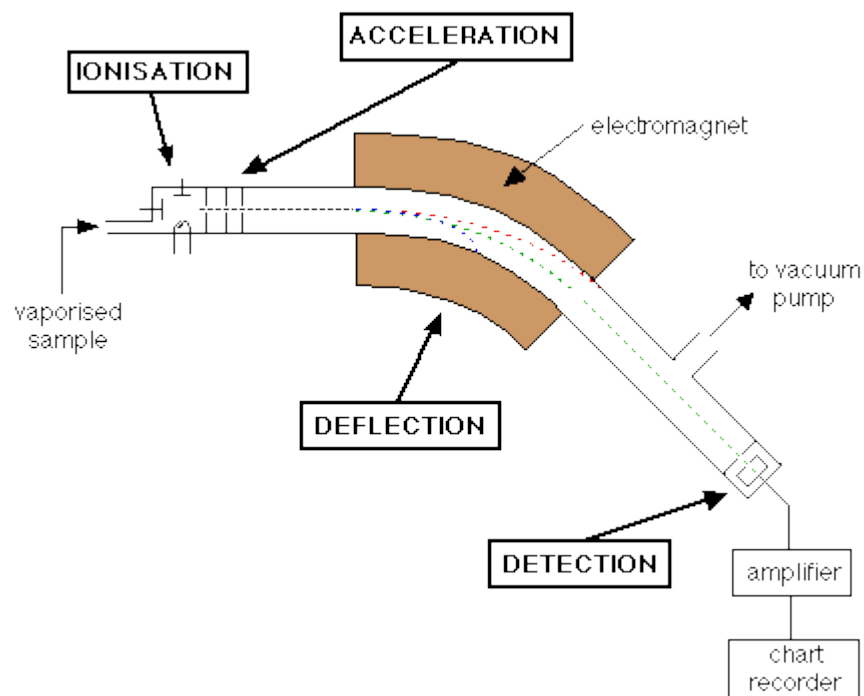
The AP Chemistry curriculum requires that the “course provides students with opportunities to apply their knowledge of AP Chemistry concepts to real-world questions or scenarios (including societal issues or technological innovations) to help them become scientifically literate citizens. Significant components of the AP Chemistry Course Exam and Description include discussion of the ways in which chemistry contributes to society through the creation of new materials and compounds that benefit the health and welfare of people in the community.

Students pursuing advanced science courses in high school are often unaware of how the content and methods they learn in class are applicable to real-world problems. In addition, though students are somewhat informed of conventional career paths in medicine and engineering, they aren't generally knowledgeable about the specific types of jobs available in these fields or their interdisciplinary nature. Discussion of these techniques and how they are used to solve scientific problems also addresses the coverage of scientific and engineering practices in the Next Generation Science Standards for high school physical science. This learning module seeks to better teach students in AP Chemistry about the how analytical chemical techniques are utilized in the drug discovery and development process with particular emphasis on the societal need for the expansion of the current anti-microbial arsenal. The lesson plans in this action proposal are targeted towards advanced chemistry students taking a second year chemistry or Advanced Placement Chemistry course generally in 11th and 12th grade.

Module on Mass Spectrometry and Drug Discovery and Development: 1-2 Days

Unit 1.2 SPQ 5 on 2019 AP Chemistry

Students will learn the general concept of separating atoms and molecular fragments using mass spectrometry. They will then view a video demonstrating the concept and read a short excerpt on how mass spectrometry can be used to identify both resistance in bacteria as well as novel potential drugs.

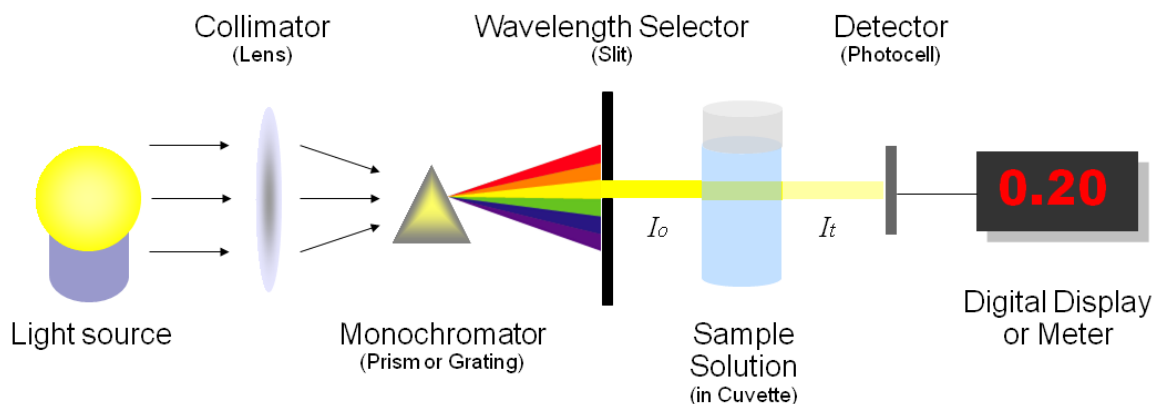


Module on Spectrophotometry and Beer's Law (color intensity, turbidity, absorbance, transmittance).

Unit 3.13 SAP 2 from AP Chemistry

Lesson plan for this module was developed as part of the action plan. The lesson should take 2-3 days. Day 1 includes viewing the videos in the enclosed Powerpoint presentation. Days 2-3 involve using the PhET simulation and the activity worksheet to investigate Beer's Law and Spectrophotometry.

This module was inspired by a visit to Dr. Paul Gulig's laboratory in which he discussed the high-throughput screens by which hundreds of thousands of small molecule inhibitors are tested for their ability to increase the sensitivity of resistant bacterial strains to antibiotics by inhibiting a transporter that signals upregulation of the beta-lactamase gene. The first step of the screen involves incubating microplates of resistant bacteria with a combination of amoxicillin and the test small molecules. Molecules are assayed for their ability to retard bacterial growth in combination with amoxicillin (or by themselves) through a spectrophotometric assay for turbidity. The lesson will include a discussion of Beer's Law and interpreting standard curves of absorbance or transmittance vs. concentration.

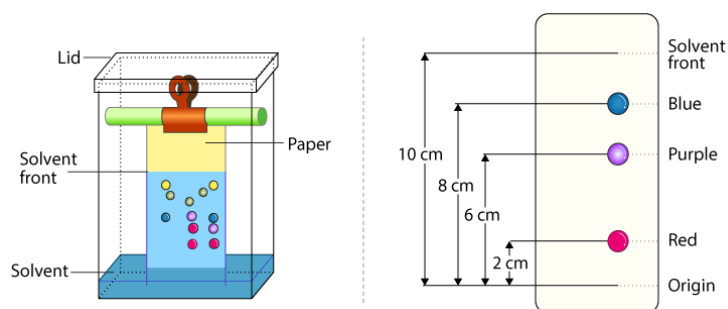


Module on chromatography and separation techniques:

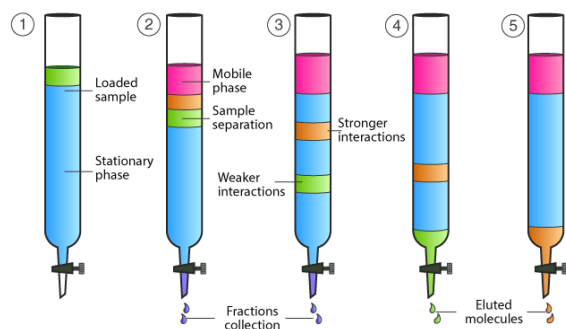
Unit 3.9 SPQ 2 in AP Chemistry

Students in chemistry are taught that mixtures of pure substances can be separating using their physical properties. In first-year chemistry, we discuss these separation techniques briefly within the context of their home (kitchen science) or basic industrial processes with which they are familiar (distillation of alcoholic spirits). They may have separated plant pigments from spinach leaves or inks using basic chromatography. This module will include a chromatography lab or virtual laboratory and a discussion of how these techniques are used in laboratories to separate and purify proteins or small molecules in the drug discovery and development process.

Thin Layer



Column Chromatography.



Expected Outcomes: Students will gain an appreciation of how these analytical chemical methods are utilized in an interdisciplinary manner in conjunction with biology and human health sciences to positively impact society.

Assessments: Assessments may include short multiple choice quizzes, asking students to discuss the merits of each technique to solve a particular problem and questions modified or developed to simulate problem associated with emerging pathogens.

How this differs from how I previously taught the unit: Prior to this module, this material was taught through a lecture on each technique and students solving pre-written problems without a real-world context. This new module attempt to incorporate aspects of Team-Based Learning, Flipped Classroom and actual case studies or lectures to contextualize these lab-based problems in AP Chemistry. Ideally, I would like to create a self-paced WISE unit to allow students to learn about these techniques.

Budget: \$0 if only using the Powerpoint and Spectrophotometry Simulation Worksheet

References:

2014 AP Chemistry Course and Exam Description

AP Chemistry Resource Requirements

<https://apcentral.collegeboard.org/courses/ap-chemistry/course-audit?course=ap-chemistry>

Spectrophotometry Simulation to Accompany PhET Beer's Law

Part I: Introduction to Using the Spectrophotometer

1. Click on the "Beer's Law Simulation" PhET in the Spectrophotometry Powerpoint. This will take you to the PhET web simulator.
2. Click on the right icon to start the "Beer's Law" Simulation.
3. In the "Green Readout Box" on the top right of the screen, switch the reading from "Transmittance" to "Absorbance".
2. Select the potassium permanganate (KMnO_4) in the dropdown box at the bottom of the simulation window where you can select the substance (it is preset to "Drink Mix").
3. The spectrophotometer will automatically preset the wavelength for maximum absorbance of the KMnO_4 substance. Switch the dropdown box to each of the other substances. Write down the Maximum Absorbance Wavelength for each substance in the chart below.
4. Click on the red button on the "Spectrophotometer Icon" in order to shine light on the sample and get a reading in the "Green Readout Box". For each substance at 100 mM (control the concentration using the slider below the dropdown box that sets the substance), record the Absorbance in Column 2 in the chart below.
5. In the "Green Readout Box" on the top right of the screen, switch the reading from "Absorbance" to "Transmittance". For each substance at 100 mM (control the concentration using the slider below the dropdown box that sets the substance), record the Transmittance in Column 4 in the chart below.
6. In the Column 5 of the chart, calculate the absorbance for each substance at 100 mM using the equation $\text{Absorbance} = -\log_{10}(\text{Transmittance})$. Verify that the calculated value matches the measured value in Column 3.

Column 1	Column 2	Column 3	Column 4	Column 5
Substance	Maximum Absorbance (wavelength)	Absorbance (Value for [] = 100 mM)	Transmittance (Value for [] = 100 mM)	Calculated Absorbance $\text{Abs} = -\log_{10}(T)$
KMnO_4				
Drink Mix				
$\text{Co}(\text{NO}_3)_2$				
CoCl_2				
$\text{K}_2\text{Cr}_2\text{O}_7$				
K_2CrO_4				
NiCl_2				
CuSO_4				

Part 2: Absorbance Spectra and Absorbance vs. Concentration

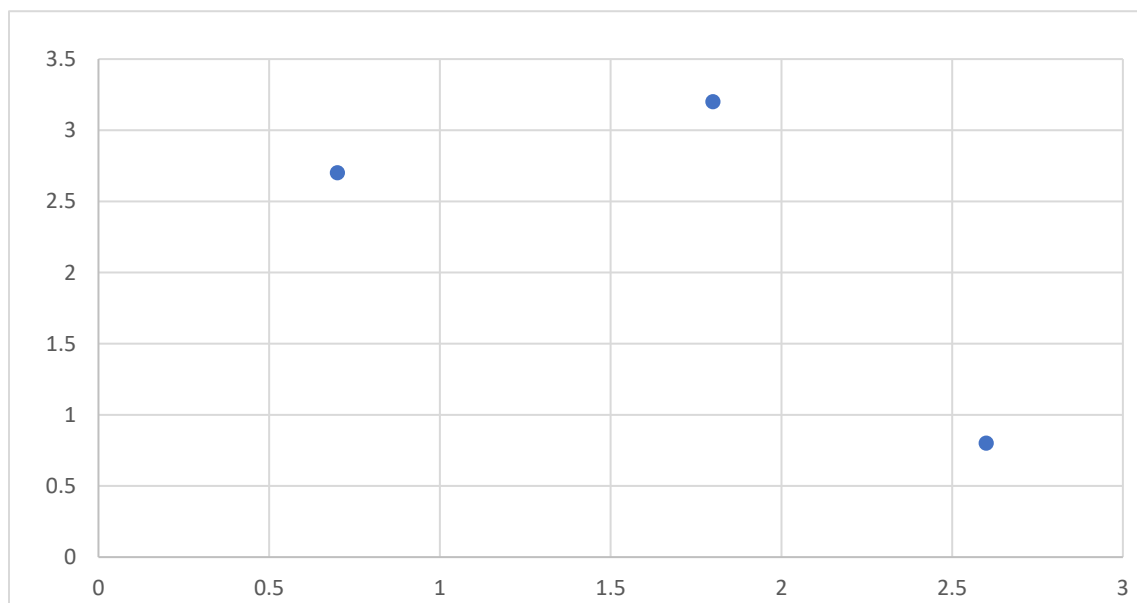
1. Set the dropdown box to potassium permanganate at 100 mM again. (Tip: Remember that KMnO_4 is purple → it shows up a lot!). In the “Green Readout Box” on the top right of the screen, switch the reading from “Transmittance” to “Absorbance”.
2. Go to the spectrophotometer icon on the left side and click the radio button from “preset” to “variable”. Try moving the slider on the “Spectrophotometer Icon” to different wavelengths and record the absorbance of the indicated wavelengths in the chart below. Press the red button on the “Spectrophotometer Icon” to get an Absorbance value in the “Green Readout Box”.

This chart below gives yields the absorbance spectrum for KMnO_4 and indicates which wavelengths are absorbed the most and the least by KMnO_4 .

Absorbance Spectrum for KMnO_4 at 100 mM									
Wavelength	390 nm	450 nm	500 nm	525 nm	580 nm	610 nm	650 nm	700 nm	750 nm
Absorbance									

3. Set the dropdown box to potassium permanganate at 100 mM. Go to the spectrophotometer icon on the left side and click the radio button from “variable” to “preset”. Press the red button on the “Spectrophotometer Icon” to get an Absorbance value in the “Green Readout Box”. Change the concentration of the potassium permanganate by using the slider below the dropdown box used to set the substance. Record the absorbance of potassium permanganate for each of the concentrations given in the chart below.

Absorbance vs. Concentration for KMnO_4									
Concentration	25 mM	50 mM	100 mM	150 mM	200 mM	250 mM	300 mM	350 mM	400 mM
Absorbance									

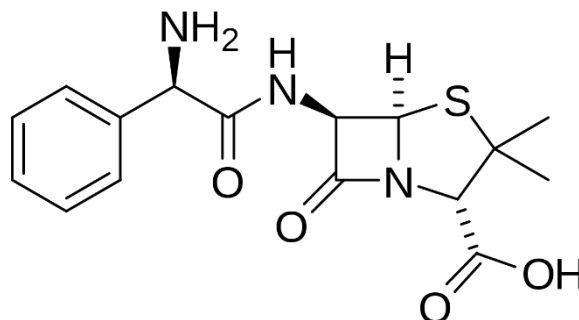
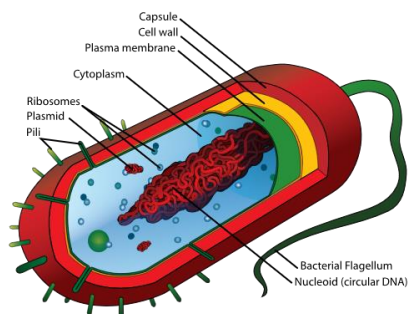


- Plot a graph of absorbance vs. concentration for potassium permanganate using data in the chart above. Remember to label your axes and title the graph. Right clicking on the graph will bring up a menu that will allow you to edit the data and enter your X and Y values.
- Find the equation for the line relating absorbance to concentration.
- Find the concentrations of the following unknown KMnO_4 samples. The absorbance for each sample is given:

Absorbance Spectrum for KMnO_4				
Absorbance	0.77	0.28	1.30	1.92
Concentration				

Part 3: Spectrophotometry in Determining Bacterial Growth

One of the major challenges facing human health and medicine is the emergence of antibiotic resistant strains of bacteria responsible for millions of infections in the U.S. each year and tens of thousands of deaths. Though fewer pharmaceutical companies are participating in the development of novel antibiotics, those that are use a variety of analytical chemical techniques. Many antibiotics are small molecules, like ampicillin shown below, that interfere with cellular processes required for bacterial cells to survive and multiply. For example, medicines based on penicillin disrupt the process by which bacterial cells form stable cell walls which are required for them to grow and reproduce.



Spectrophotometry can be used to screen thousands of small molecules at a time for their potential effectiveness as antibiotic drugs.

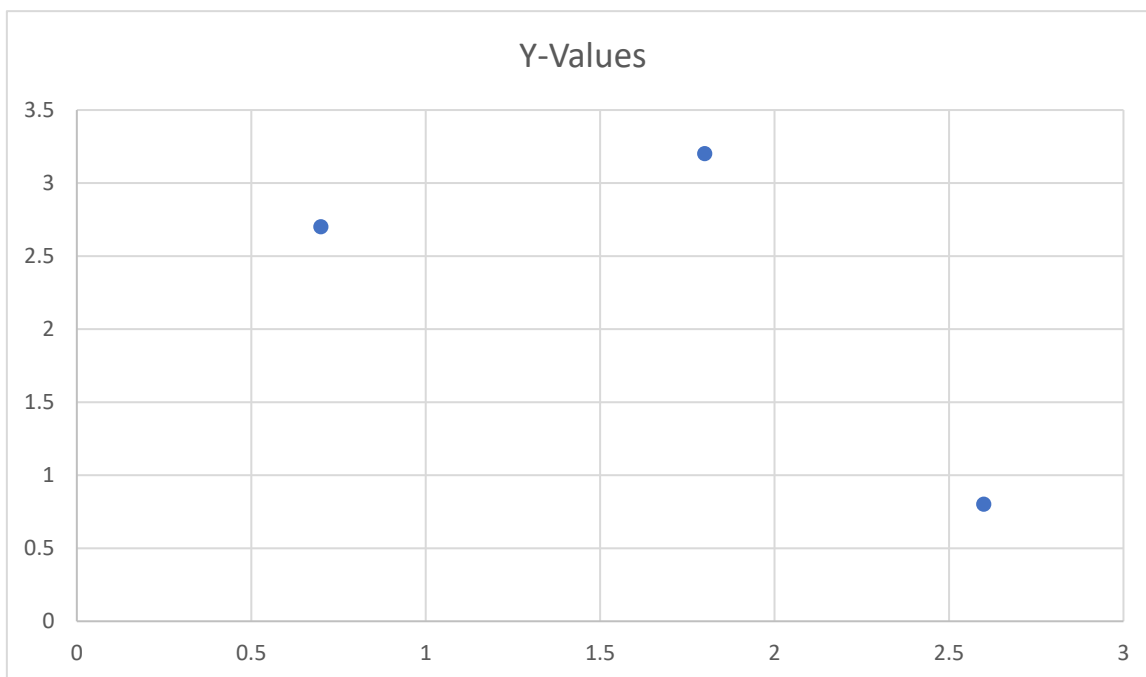
Screening small molecules to determine whether they might make good antibiotics is done by growing bacteria in the presence of the prospective drug. If a culture of the bacteria in the presence of the drug being investigated is cloudy (turbid), that means that the particular drug does not inhibit bacterial growth and is not a good candidate for an antibiotic. Conversely, if the culture of bacteria grown in the presence of a prospective drug is clear (not turbid), then the drug under study prevents bacterial growth and could be an effective antibiotic. The level of turbidity of a bacterial culture can be determined by reading absorbance of the culture at 600 nm in a spectrophotometer.

1. The chart below shows the data for a stock sample of bacteria at 1.5×10^8 bacterial cells/mL and subsequent dilutions of the stock. For example, the $\frac{1}{2}$ stock sample would have 7.5×10^7 bacterial cells/mL. Fill in the rest of the bacterial cell counts in the table below.

Sample	Agilent 8354	Bacterial Cell Count
Stock	1.1588	1.5×10^8 cells/mL
Stock 1:2	0.5696	7.5×10^7 cells/mL
Stock 1:4	0.2794	
Stock 1:8	0.1372	
Stock 1:16	0.0684	
Stock 1:32	0.0326	
Stock 1:64	0.0140	

<https://www.denovix.com/pdf/168-OD600.pdf>

2. Construct a graph below showing bacterial cell count vs. absorbance. The absorbance for each sample is given in the column labeled “Agilent”



3. Test Molecule A is added to a sample culture of bacteria. Another culture contained Test Molecule B. Both samples were incubated overnight and the absorbance read at 600 nm. Sample A has an absorbance of 0.986 whereas Sample B had an absorbance of 0.026. Find the bacterial count in the unit of cells/mL in each sample and determine which molecule could be a potential antibiotic.

Introduction

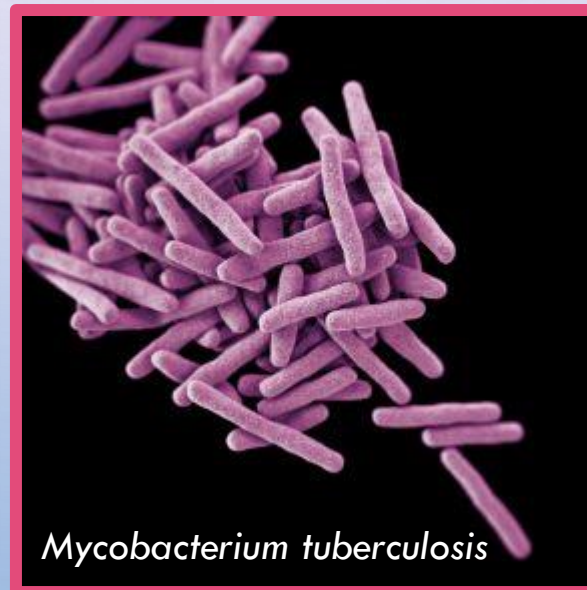
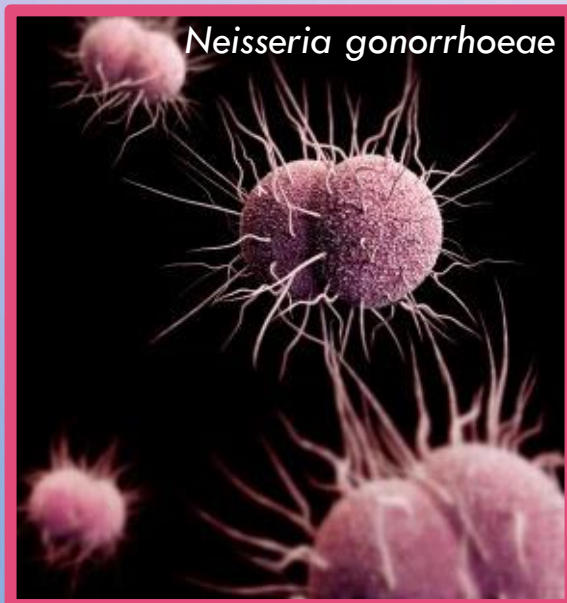
In AP Chemistry, you will learn about several quantitative analytical techniques. These techniques are utilized for a variety of applications including the discovery and development of new medicines.

One of the most significant issues threatening human health is that of the emergence of antibiotic resistant strains of pathogenic bacteria. According to the CDC, each year in the US, antibiotic resistant strains are responsible for at least 2 million infections per year and 23,000 deaths.

([HTTPS://WWW.CDC.GOV/DRUGRESISTANCE/BIGGEST_THREATS.HTML](https://www.cdc.gov/drugresistance/biggest_threats.html))

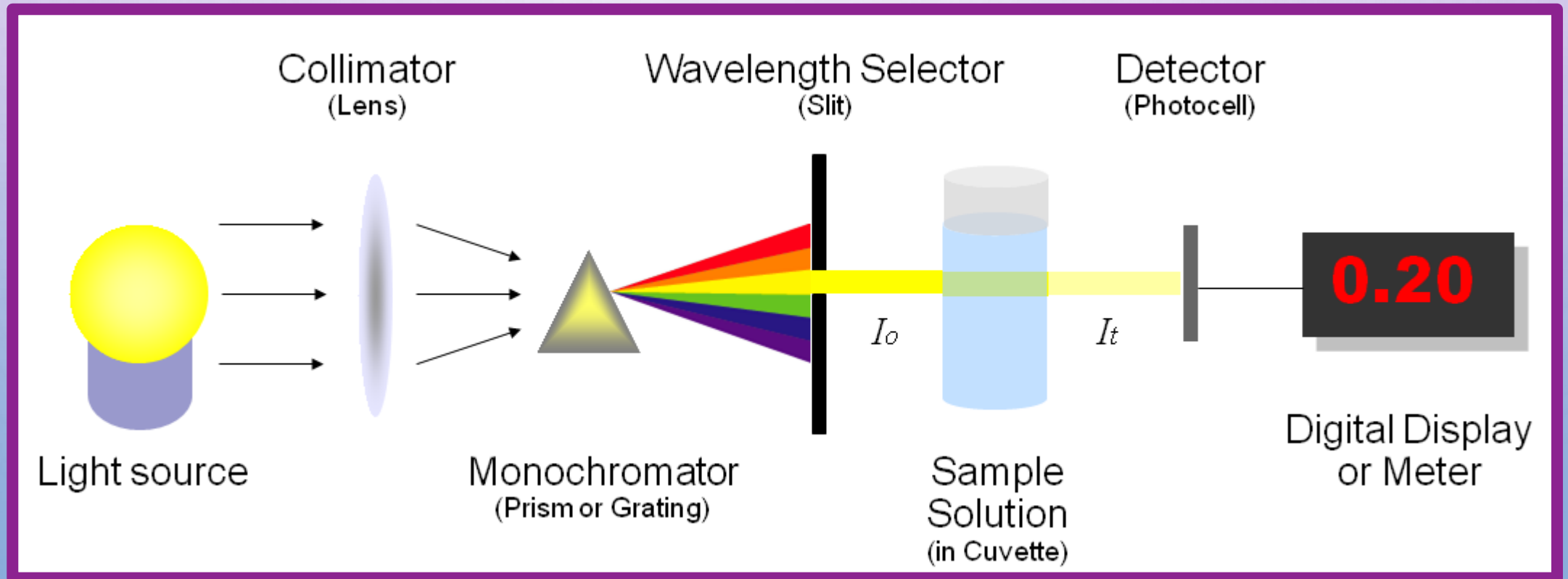
Antibiotic Resistant Bacteria

Resistant bacteria contain genes that allow them to counteract or inactivate a variety of common antibiotics. It is imperative that chemists working for pharmaceutical companies understand how chemical techniques can help in the discovery of novel antimicrobial agents.



Spectrophotometry

One very useful analytical technique is called **spectrophotometry** and involves passing wavelengths of UV or visible light through a colored or opaque sample to detect the percent **transmittance** or **absorbance (abs)** of the light. The greater the concentration of the sample, the less light is transmitted and the more light is absorbed.



Beer-Lambert Law

Light absorbance is directly proportional to the concentration of the sample. A plot of absorbance vs. concentration yields a straight line. If you have several known concentration of a sample, you can use them to generate a linear curve. You can then use this curve to determine the concentration of an unknown sample.

The principle that relates light absorbance to concentration is called the **Beer-Lambert Law (aka Beer's Law)**. The equation is below and explained in the next slide.

$$A = abC \text{ or } A = \epsilon lC \quad \text{and} \quad A = -\log T$$

Absorbance = molar absorptivity x path length x concentration = -log transmittance

Transmittance is a measure of how much light can pass through the sample.

Transmittance (T) = $\frac{I}{I_0}$ where I is the intensity of light passing through the sample.

Beer-Lambert Law


$$A = abC \text{ or } A = \epsilon lC$$

(This is the common form used in AP Chemistry)

- **Absorbance = molar absorptivity x path length x concentration**
- **Molar absorptivity (a or l) is a constant specific to the sample substance (units are L/mol•cm)**
- **Path length is the width of the cuvette usually in cm (sample tube)**
- **C = concentration of the sample**

View the videos on the next two slides to see how this is done.

Click to start Video

						
Conc	2.00	1.00	0.50	0.25	0.13	

② Do dilutions of standard and measure absorption in spectrometer (colourimeter)

Click to start Video

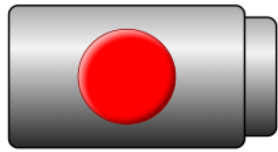
Problem A solution of KMnO_4 has an absorbance of 0.539 when measured at 540 nm in a 1.0-cm cell. What is the concentration of the KMnO_4 solution? In determining the absorbance for the unknown solution, the following calibration data were collected for the spectrophotometer.

Concentration of KMnO_4 (M)	Absorbance
0.0300	0.162
0.0600	0.330
0.0900	0.499
0.120	0.670
0.150	0.840

From Kotz, Treichel, and Townsend Chemistry and Chemical Reactivity

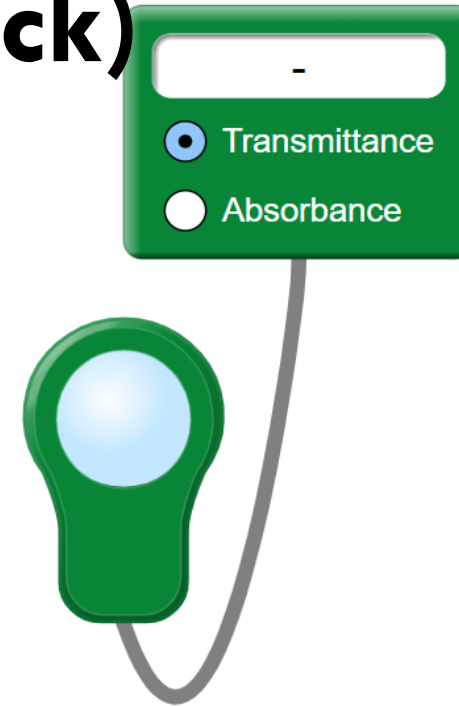
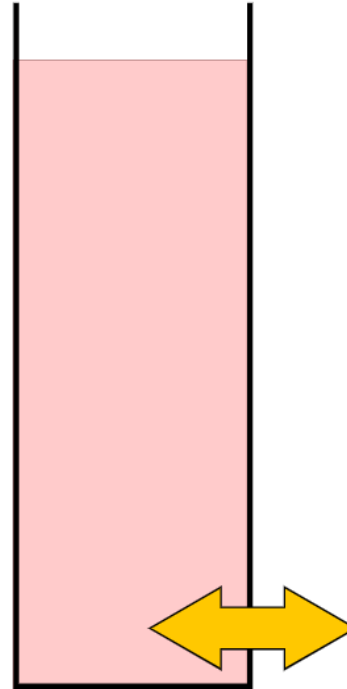
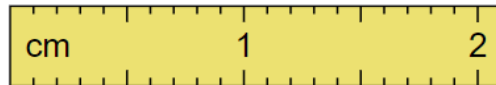
So this 540 nanometers is the wavelength of light that we're

PhET Beer's Law Simulation (click)



Wavelength: 508 nm

preset variable



-
 Transmittance
 Absorbance

Solution: ■ Drink mix

Concentration: 100 mM

