UV DETERMINATION OF CAFFEINE CONTENT

Introduction:
Caffeine is a common organic molecule found in many beverages such as coffee, tea, and cola. It is a stimulant to the central nervous system. That is why many students drink coffee or soda to help them feel alert.

Like many conjugated organic molecules, caffeine absorbs radiation with a wavelength around 260 nm. A conjugated system is one containing 2 double bonds separated by a single bond. This conjugated pattern may be repeated several times in the molecule. If a series of caffeine standards are analyzed in this region of absorption and a Beer's law is plot prepared, then the amount of caffeine in another substance can be determined. One should be aware that the assumption is being made that the unknown contains no other substances which absorb at this wavelength.

Purpose:
The purpose of this experiment is to determine the amount of caffeine in selected soft drinks.

Equipment/Material:

spectrophotometer	iron rings
quartz cuvets	separatory funnels
caffeine standards	50 mL graduated cylinders
degassed soft drink samples	Erlenmeyer flasks, with stoppers
methylene chloride	automatic pipets
droppers	50 mL volumetric flasks (opt.)
ring stands	waste container

Safety:
• Apron and goggles must be worn in the lab.

Procedure:
1. Prepare 50.00 mL of the assigned caffeine standard (50, 100, 150, 200, or 250 ppm) by a quantitative dilution of the 1000 ppm stock solution.

2. Place 10mL of a caffeine standard in a separatory funnel. Add 25 mL of methylene chloride.

3. Extract the caffeine by inverting the funnel at least 3 times. Vent the separatory funnel after each inversion.

4. Remove the methylene chloride layer, which is the bottom layer, and save in a clean, stoppered Erlenmeyer flask.

5. Add another 25 mL of methylene chloride to the separatory funnel.

6. Extract twice more by repeating steps 3-5. Combine the methylene chloride layers.

7.   Put 10mL of degassed soda to a clean separatory funnel (the sep funnel may be rinsed with methylene chloride to be certain that no caffeine from the previous sample remains).

8.   Extract the soda 3 times with 25 mL portions of methylene chloride as above. Save the methylene chloride layers in another clean, stoppered Erlenmeyer flask.

9.   With the sample compartment empty, turn on the power switch. Let the instrument initialize and run through the self-diagnostic.

10.   Use the down arrow to select "UV" from the menu, and then press the Enter button.

11.   Press the green button under "Run Test."

12.   Fill a clean cuvet with methylene chloride. Wipe the unfrosted sides with a Kimwipe. Place the cuvette in the sample compartment with the unfrosted sides facing the front and back. Close the lid.

13.   Press the green button under "Collect Baseline" and wait for the collection to be completed. (The baseline will be stored in the instrument, but will not appear on the screen. "Baseline Collected" will appear when the scan is complete.)

14.   Remove the blank and dump the contents into a waste container. Rinse the cuvette into the waste container twice with small amounts of sample. Fill the cuvette ¾ full with sample. Return the cuvette to the sample compartment, making certain to orient it correctly.

15.   Press the green button under "Measure Sample" to begin the scan.

16.   When the scan is completed, press the button under "Edit Graph."

17.   Press the green button under "Math."

18.   Press the green button under "Peaks & Valleys." A pop-up menu will appear.

19.   Press Enter to turn on "Label Peaks."

20.   Press ESC to exit the menu. The wavelength of maximum absorbance (λ_{max}) will be labeled. Record the λ_{max} and its absorbance in the data section.

21.   Press the green Print button at the bottom right corner of the keypad. Label the print-out.

22.   Repeat steps 14 -- 21 with the other standards and unknown solutions. Remember to label the printouts with the sample name.

23.   Upon completion of the lab, be sure to put all waste in the appropriately labeled container.

24.   Plot a Beer's law curve of absorbance (y) vs concentration (x) for the caffeine standards.

Data Table:

Caffeine Standards

Concentration (ppm)	Absorbance
0	
50	
100	
150	
200	
250	

Soda

Brand	Absorbance	Concentration ppm	Caffeine mg/L

Calculations:

1.　　Using the graph, determine the concentration of caffeine in each soda in ppm.

2.　　Calculate the mg of caffeine in a 12 oz serving (253 mL) of soda.

Questions:

1.　　Why was it necessary to extract the standards?

2.　　Why was it necessary to degas the soda?

3.　　Why was it necessary to extract the soda?