

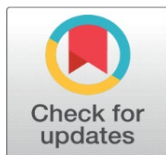
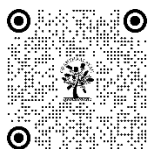
A REVIEW ON SPECTROPHOTOMETRIC ANALYSIS FOR DRUGS

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ABSTRACT

Through this review article focusing on Application of Spectrophotometric analysis for both Qualitative and Quantitative assay in drug, medicine and remedy standardization. Thereafter it plays a major role in the quality assessment in drug substances.

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1. INTRODUCTION

Absorption spectroscopy is a practical method frequently employed to determine the concentration of colored substances in a sample. Due to the straightforward correlation between concentration and the quantity of light absorbed, UV-visible spectroscopy finds extensive use in fundamental chemistry laboratories, biomedical studies, and clinical diagnostics. For instance, in biochemistry laboratories, enzyme assays are typically formulated to monitor the presence of a colored substance resulting from the reaction to evaluate enzyme performance or detect a specific substrate. This principle underpins the enzyme-linked immunosorbent assay (ELISA), wherein a colored substance is generated when an enzyme binds to a target analyte.[1-2] The output from an ELISA plate is measured as an absorbance value of the colored product. Enzymatic reactions that result in color shifts of a solution have been adapted for clinical applications to identify a diverse range of targets.

Given the widespread use of absorbance spectroscopy in chemical, biochemical, and clinical fields, it is essential to provide education to students who will pursue careers in these domains. Fundamental spectroscopy techniques are integral components of the undergraduate curriculum within chemistry departments and related degree programs such as materials science, biology, and bioengineering. However, mastering this laboratory skill requires hands-on training

and practice for students to become adept and informed in its application. This level of proficiency is challenging to achieve in a single afternoon in a college laboratory; hence, introducing the principles and practices to students early and consistently throughout their education is beneficial. Unfortunately, this can be challenging and financially burdensome for many institutions.

In the past, several economical, DIY (do-it-yourself) solutions have emerged to address this issue. Many of these are LED-based colorimeters that connect absorption to concentration without providing a direct spectrum output.[3-10] While this is acceptable for numerous applications, it has limitations in teaching students how to interpret, comprehend, and quantify visible spectral characteristics. Other instruments are made to showcase a visible spectrum but may not be suitable for conducting analytical chemistry measurements.[11-13] Various other device designs have been reported that produce a spectrum and incorporate the fundamental elements of a research-quality spectrometer for performing analytical chemistry experiments. [14-18]

2. THEORY

An absorbance spectrophotometer evaluates the strength of light throughout the visible spectrum both with and without a sample present. In a typical setup a white light source is distributed onto a detector after passing through the sample. The measured intensity, I_0 , of the light traveling through the apparatus without the sample is reliant on wavelength, $I_0(\lambda)$. $I_s(\lambda)$ is a representation of the intensity of light at each wavelength that results from some light being absorbed by the sample. The absorbance, A , is the negative base-10 logarithm of the transmission, and the transmission at each wavelength is just the ratio of these two values. slit, cuvette, grating, and camera in order to do repeatable spectral observations. SolidWorks software was used to create the drawing, which was then exported as a 3D printable file for manufacturing. The following is a list of the design's main components. The author's website has 3D print files, additional assembly instructions, and information on how to get a print file.[20] The NIH 3D Print Exchange website also offers the print file.[21]

3. THE TEACHING METHOD

Wavelength and Light, We provide a few easy exercises in this part that can be completed by students of different skill levels. Investigating how white light splits into its distinct colors is the first example.

To illustrate this using the SpecPhone, take out the cuvette and cover the orifice to keep out unwanted light. Point the SpecPhone toward different light sources in the classroom while the iPhone camera app is open. For instance, a rainbow image will be created by diffused sunlight coming in through a window. Three bands are frequently visible in computer display light: red, green, and blue. Fluorescent lights above will display distinct lines that represent lanthanide and mercury luminescence.

The Beer-Lambert Law and other fundamental concepts in visual spectroscopy are taught with the SpecPhone. This requires a quantitative analysis of the spectra. In Students will learn how to convert the through this exercise. image of the light scattered into a spectrum that shows wavelength versus intensity. This process is a chance. enable pupils to understand the relationship between data

gathering and evaluation. In addition, it serves as a useful activity for introducing introducing students to the fundamental data analysis tools of spreadsheet programs similar to Excel. We use this method to get the pixel values for the image. recommend using the free program ImageJ, developed by Health National Institutes.[22] It was made to look at data from medical imaging, which is currently used for a variety of applications.[23] An assignment with these peaks is intriguing for the curious student. accessible through the Wikimedia Commons.[24]

4. THE BEER-LAMBERT LAW

It outlines the connection between the concentration of a specific analyte and its absorbance, A , represented by the formula: $A = \epsilon(\lambda) \cdot l \cdot c$. As mentioned in the earlier Introduction and Theory sections, this relationship is widely used in both laboratory and clinical environments. In this section, we modify a student laboratory procedure for use with the SpecPhone spectrophotometer.[25] The primary objective of this exercise is to create an absorptivity calibration plot using various dilutions of a colored analyte. For the sake of demonstration, we examine the absorbance of a cherry-

flavored drink powder (e.g., Kool-Aid) since it is both safe and affordable. Several other sample protocols have been developed that are compatible with the SpecPhone.[26-28]

5. CONCLUSION

This Spectrophotometer plays a major role for both Quantitative and Qualitative analysis of drugs substances in standardization.

CONFLICT OF INTERESTS

None.

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REFERENCES

- Engvall, E.; Perlmann, P. Enzyme-linked immunosorbent assay (ELISA) quantitative assay of immunoglobulin G. *Immunochemistry* 1971, 8 (9), 871–874.
- Lequin, R. M. Enzyme Immunoassay (EIA)/Enzyme-Linked Immunosorbent Assay (ELISA). *Clin. Chem.* 2005, 51 (12), 2415–2418.
- Anzalone, G.; Glover, A.; Pearce, J. Open-Source Colorimeter. *Sensors* 2013, 13 (4), 5338–5346.
- Jaffar, M.; Zahid, Q. A low-cost precision colorimeter. *J. Chem. Educ.* 1988, 65 (12), 1099.
- Crump, J.; Sandwick, R. K. A Simple Microwell Colorimeter for Use in an Introductory Chemistry Lab. *J. Chem. Educ.* 1994, 71 (8), A199.
- Hamilton, J. R.; White, J. S.; Nakhleh, M. B. Development of a Low-Cost Four-Color LED Photometer. *J. Chem. Educ.* 1996, 73 (11), 1052.
- Mozo, J. D.; Galan, M.; Roldán, E. Application of Light Emitting Diodes to Chemical Analysis: Determination of Copper in Water. *J. Chem. Educ.* 2001, 78 (3), 355.
- Gordon, J.; Harman, S. A Graduated Cylinder Colorimeter: An Investigation of Path Length and the Beer-Lambert Law. *J. Chem. Educ.* 2002, 79 (5), 611.
- Gordon, J.; Tye, S. A LED Microtiter Plate Reader. *J. Chem. Educ.* 2005, 82 (6), 903.
- Vacas-Jacques, P.; Linnes, J.; Young, A.; Gerrard, V.; Gomez- Marquez, J. Portable digital lock-in instrument to determine chemical constituents with single-color absorption measurements for Global Health Initiatives. *Rev. Sci. Instrum.* 2014, 85 (3), 033103.
- Kiisk, V. An educational spectrograph using a digital camera as a training aid for physics students. *Eur. J. Phys.* 2014, 35 (3), 035013.
- Public Lab Spectrometer. <http://publiclab.org/wiki/spectrometer> (accessed Oct 2015).
- Starlab Project Star Spectrometer. <http://www.starlab.uk.com/Spectrometer/Spectrometer.htm> (accessed Oct 2015).
- Vanderveen, J. R.; Martin, B.; Ooms, K. J. Developing Tools for Undergraduate Spectroscopy: An Inexpensive Visible Light Spectrometer. *J. Chem. Educ.* 2013, 90 (7), 894–899.
- Knagge, K.; Raftery, D. Construction and Evaluation of a LEGO Spectrophotometer for Student Use. *Chem. Educ.* 2002, 7 (6), 371–375.
- Yu, H.; Tan, Y.; Cunningham, B. T. Smartphone Fluorescence Spectroscopy. *Anal. Chem.* 2014, 86 (17), 8805–8813.
- Long, K. D.; Yu, H.; Cunningham, B. T. Smartphone instrument for portable enzyme- linked immunosorbent assays. *Biomed. Opt.Express* 2014, 5 (11), 3792–3806.
- Arafat Hossain, M.; Canning, J.; Ast, S.; Cook, K.; Rutledge, P. J.; Jamalipour, A. Combined dual absorption and fluorescence smartphone spectrometers. *Opt. Lett.* 2015, 40 (8), 1737–1740.
- Scheeline, A. Teaching, Learning, and Using Spectroscopy with Commercial, Off-the-Shelf Technology. *Appl. Spectrosc.* 2010, 64 (9), 256A–268A.

- Smith, A. W. The SpecPhone: A 3D-Printed Smartphone Spectrophotometer for Research and Education. <http://smithlab.uakron.edu/specphone/> (accessed Oct 2015).
- NIH 3D Print Exchange. <http://3dprint.nih.gov> (accessed Oct2015)
- Rasband, W. S. ImageJ. <http://imagej.nih.gov/ij/> (accessed October 29, 2015).
- Schneider, C. A.; Rasband, W. S.; Eliceiri, K. W. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 2012, 9 (7), 671–675.
- (24)WikimediaCommons.https://commons.wikimedia.org/wiki/File:Fluorescent_lighting_spectrum_peaks_labelled.gif (accessed Oct2015).
- Stevens, K. E. Using Visible Absorption To Analyze Solutions ofKool-Aid and Candy. *J. Chem. Educ.* 2006, 83 (10), 1544.
- Harmon, K. J.; Miller, L. M.; Millard, J. T. Crime SceneInvestigation in the Art World: The Case of the Missing Masterpiece.*J. Chem. Educ.* 2009, 86 (7), 817.
- Sigmann, S. B.; Wheeler, D. E. The Quantitative Determination of Food Dyes in Powdered Drink Mixes. A High School or General Science Experiment. *J. Chem. Educ.* 2004, 81 (10), 1475.
- Klotz, E.; Doyle, R.; Gross, E.; Mattson, B. The EquilibriumConstant for Bromothymol Blue: A General Chemistry Laboratory Experiment Using Spectroscopy. *J. Chem. Educ.* 2011, 88 (5), 637–639.