香港教育大學
The Education University of Hong Kong

The Education University of Hong Kong
2021-2022 Quality Education Fund Thematic Network – Tertiary Institutes
STEM Project Team

SCHOOL: CHRISTIAN AND MISSIONARY ALLIANCE SUN KEI SECONDARY SCHOOL (S4)

TOPIC: MATERIAL SCIENCE WITH SPECTROPHOTOMETER 1

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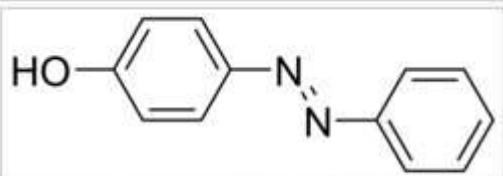
TEST 03

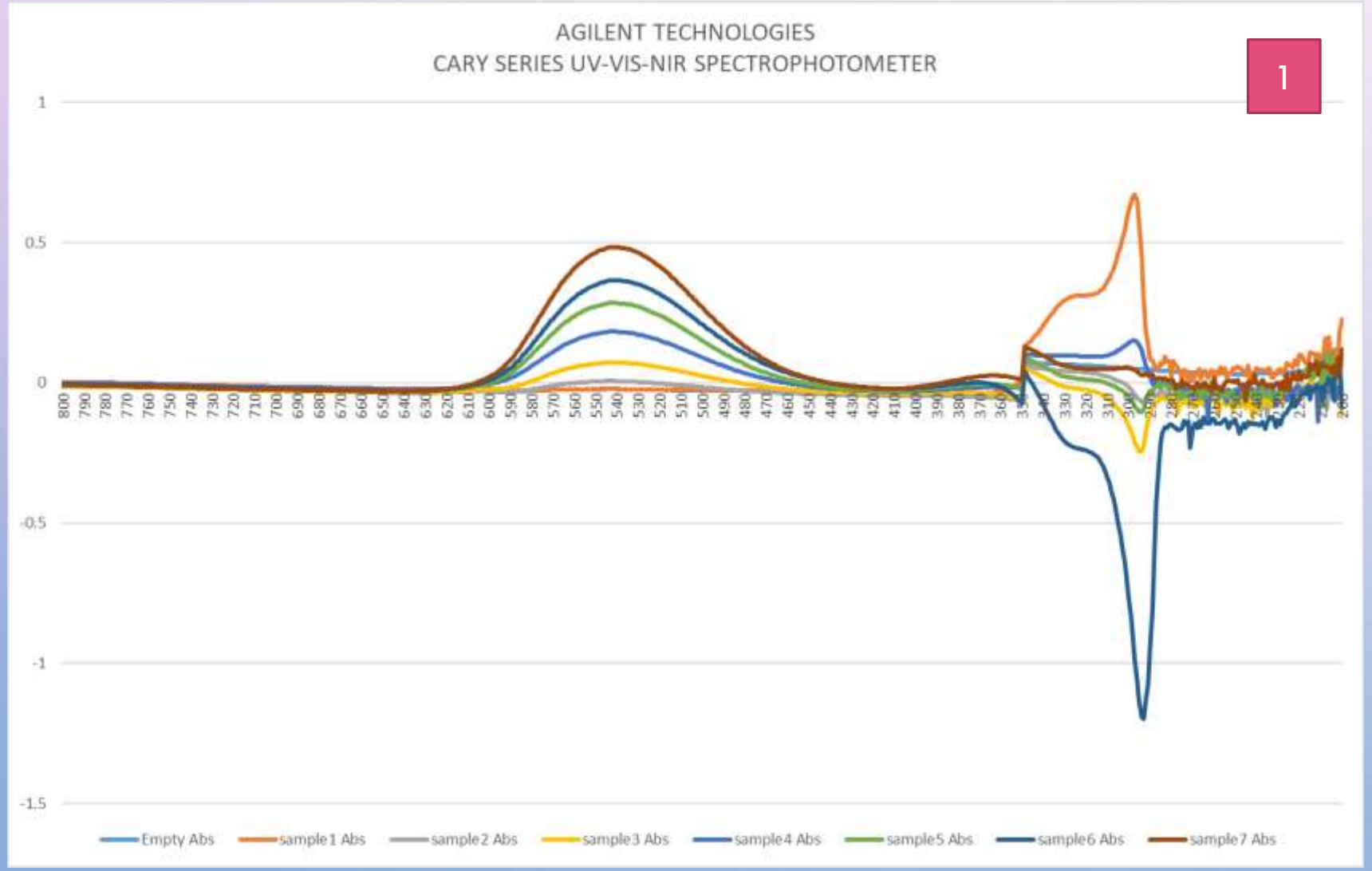
Sample	ppm
1	0.00
2	0.04
3	0.10
4	0.20
5	0.30
6	0.40
7	0.50

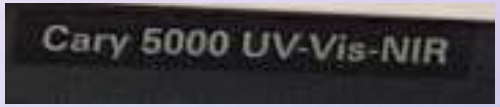
Azo dye, 542nm
Organic compounds,
Function group R-N=N-R'



Chemical structure of an orange colored azo dye.

AGILENT TECHNOLOGIES
CARY SERIES UV-VIS-NIR SPECTROPHOTOMETER





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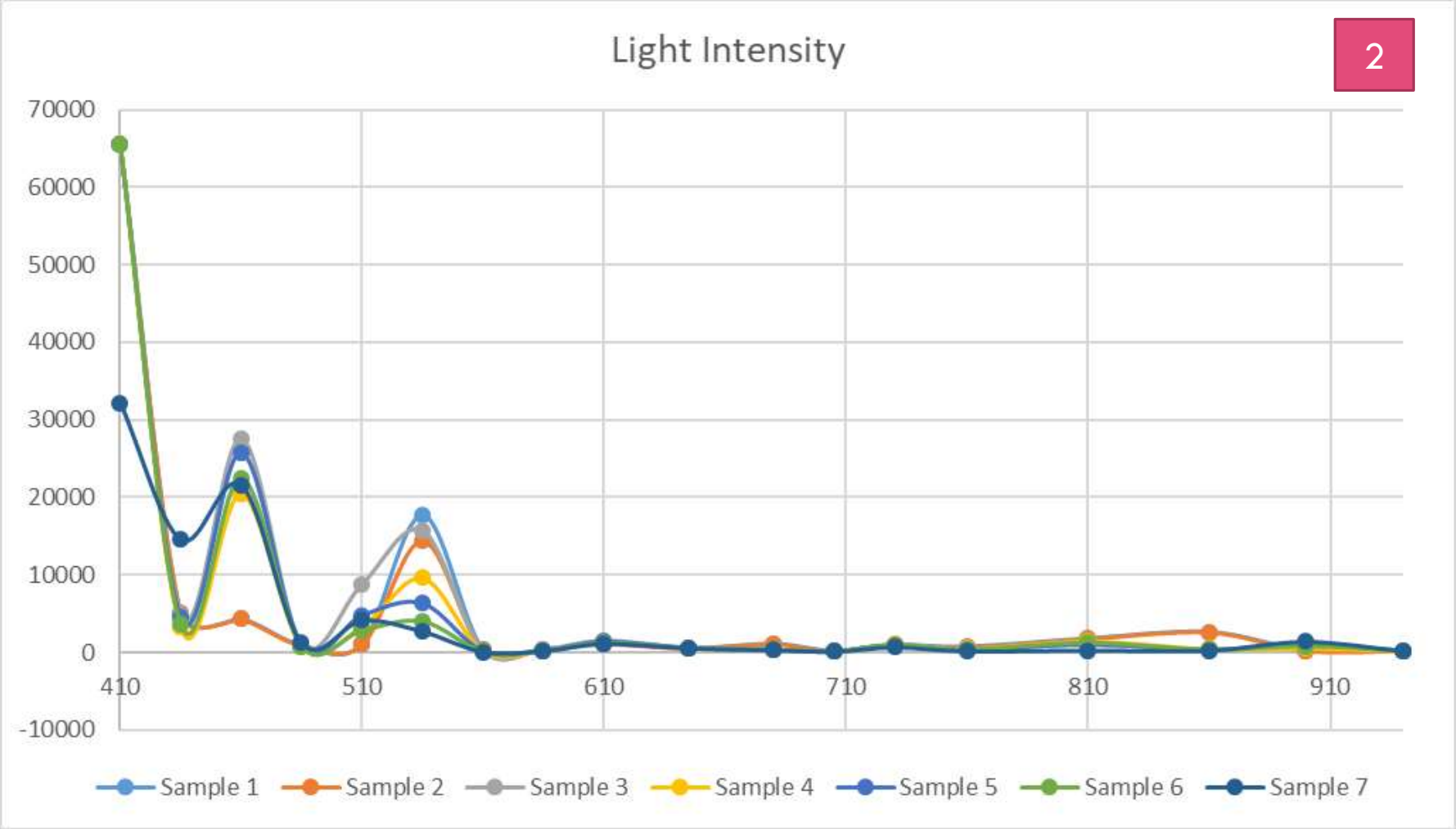
SPARKFUN LIGHT INTENSITY



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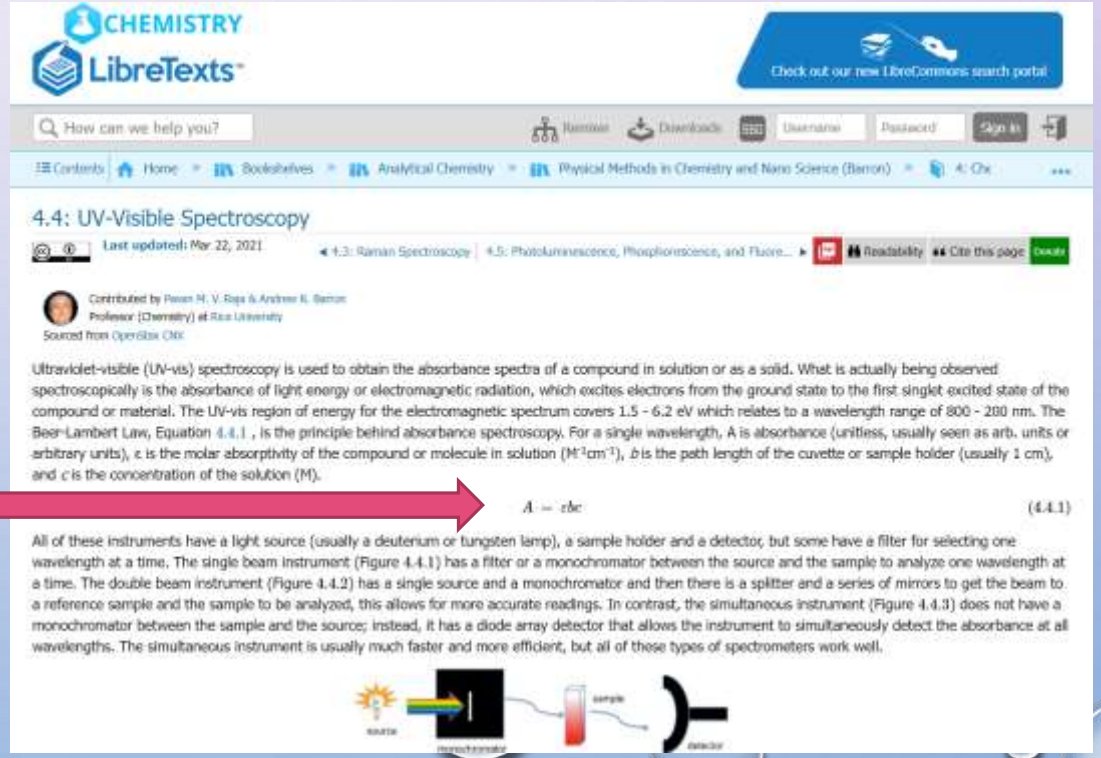
MORE INFO (ABSORBANCE AND BEER'S LAW)

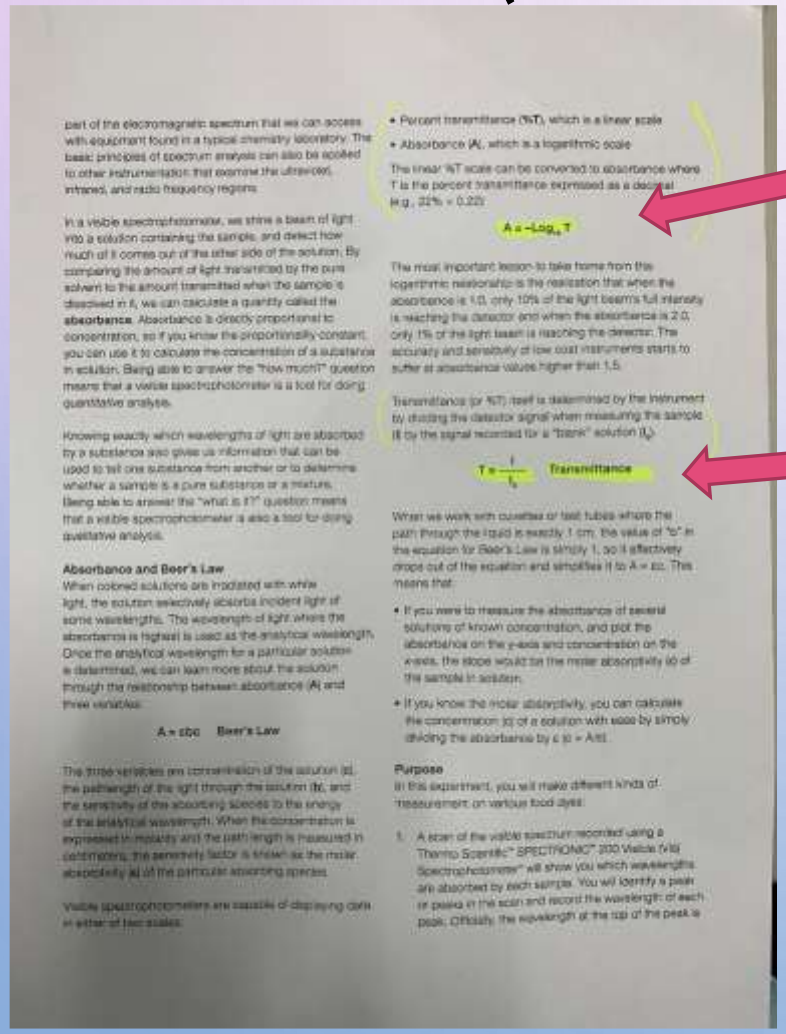
- https://chem.libretexts.org/Bookshelves/Analytical_Chemistry/Physical_Methods_in_Chemistry_and_Nano_Science_(Barron)/04%3A_Chemical_Speciation/4.04%3A_UV-Visible_Spectroscopy

$$A = \varepsilon bc$$



A = absorbance (logarithmic scale)
B = the path length of the sample holder
C = the concentration of solution ($M^{-1}cm^{-1}$)

MORE INFO (ABSORBANCE AND BEER'S LAW)



part of the electromagnetic spectrum that we can access with equipment found in a typical chemistry laboratory. The basic principles of spectrum analysis can also be applied to other instrumentation that examine the ultraviolet, infrared, and radio frequency regions.

In a visible spectrophotometer, we shine a beam of light into a solution containing the sample, and detect how much of it comes out of the other side of the solution. By comparing the amount of light transmitted by the pure solvent to the amount transmitted when the sample is dissolved in it, we can calculate a quantity called the **absorbance**. Absorbance is directly proportional to concentration, so if you know the proportionality constant, you can use it to calculate the concentration of a substance in solution. Being able to answer the "how much?" question means that a visible spectrophotometer is a tool for doing quantitative analysis.

Knowing exactly which wavelengths of light are absorbed by a substance also gives us information that can be used to tell one substance from another or to determine whether a sample is a pure substance or a mixture. Being able to answer the "what is it?" question means that a visible spectrophotometer is also a tool for doing qualitative analysis.

Absorbance and Beer's Law

When colored solutions are irradiated with white light, the solution selectively absorbs incident light of some wavelengths. The wavelength of light where the absorbance is highest is used as the analytical wavelength. Once the analytical wavelength for a particular solution is determined, we can learn more about the solution through the relationship between absorbance (A) and three variables:

$A = \varepsilon bc$ **Beer's Law**

The three variables are concentration of the solution (c), the pathlength of the light through the solution (b), and the sensitivity of the absorbing species to the energy of the analytical wavelength. When the concentration is expressed in molarity and the path length is measured in centimeters, the sensitivity factor is known as the molar absorptivity (ε) of the particular absorbing species.

Visible spectrophotometers are capable of displaying data in either of two scales:

- Percent transmittance (%T), which is a linear scale
- Absorbance (A), which is a logarithmic scale

The linear %T scale can be converted to absorbance where T is the percent transmittance expressed as a decimal (e.g., 22% = 0.22):

$A = -\text{Log}_{10} T$

The most important lesson to take home from this logarithmic relationship is the realization that when the absorbance is 1.0, only 10% of the light beam's full intensity is reaching the detector and when the absorbance is 2.0, only 1% of the light beam is reaching the detector. The accuracy and sensitivity of low cost instruments starts to suffer at absorbance values higher than 1.5.

Transmittance (or %T) itself is determined by the instrument by dividing the detector signal when measuring the sample (I) by the signal recorded for a "blank" solution (I_0):

$T = \frac{I}{I_0}$ Transmittance

When we work with cuvettes or test tubes where the path through the liquid is exactly 1 cm, the value of "b" in the equation for Beer's Law is simply 1, so it effectively drops out of the equation and simplifies it to $A = \varepsilon c$. This means that:

- If you were to measure the absorbance of several solutions of known concentration, and plot the absorbance on the y-axis and concentration on the x-axis, the slope would be the molar absorptivity (ε) of the sample in solution.
- If you know the molar absorptivity, you can calculate the concentration (c) of a solution with ease by simply dividing the absorbance by ε ($c = A/\varepsilon$).

Purpose

In this experiment, you will make different kinds of measurement on various food dyes:

1. A scan of the visible spectrum recorded using a Thermo Scientific™ SPECTRONIC™ 200 Visible (Vis) Spectrophotometer will show you which wavelengths are absorbed by each sample. You will identify a peak or peaks in the scan and record the wavelength of each peak. Officially, the wavelength at the top of the peak is

$A = -\text{Log}_{10} T$

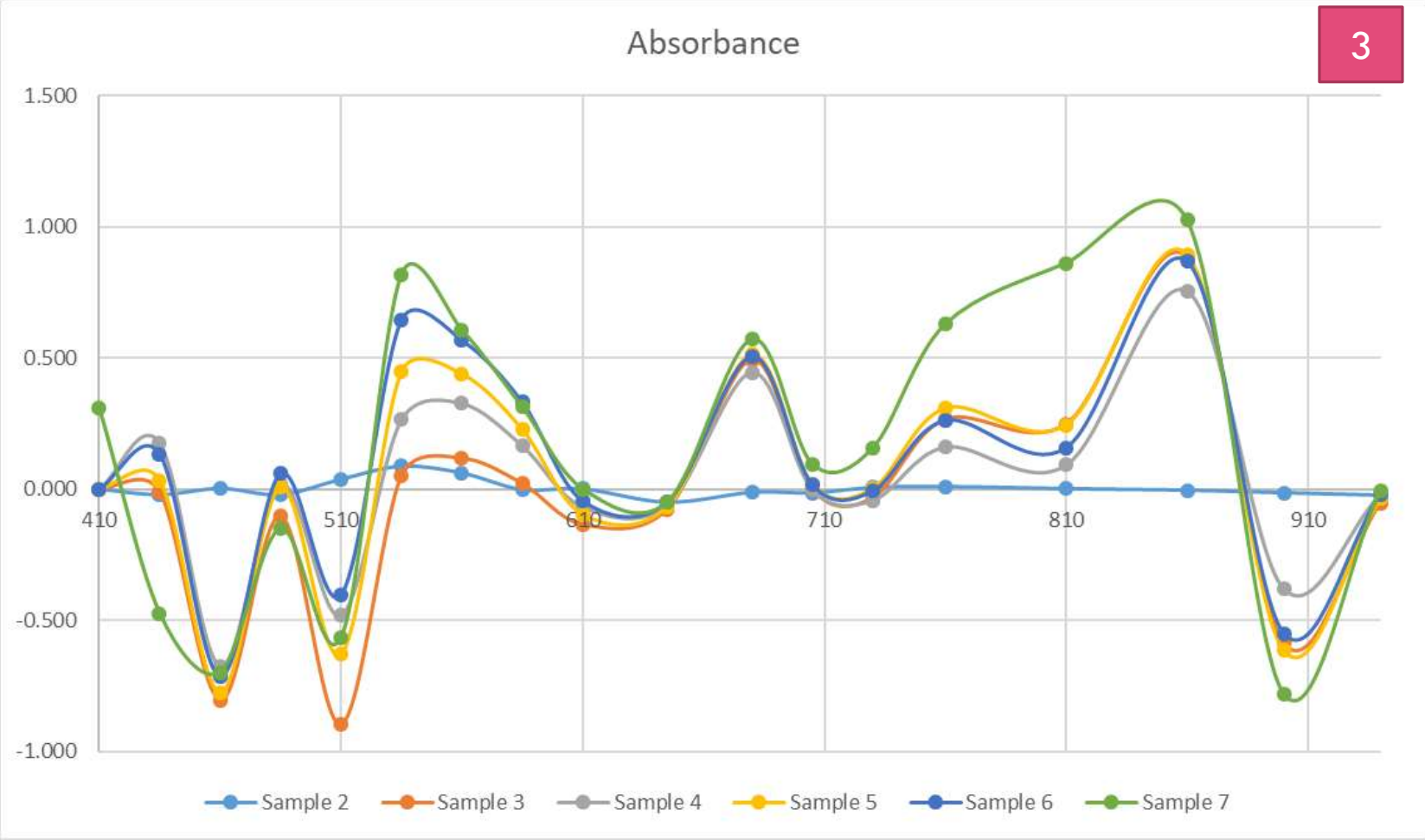
$T = \frac{I}{I_0}$ Transmittance

T = Percent transmittance(%T) (linear scale)

The linear %T scale can be converted to absorbance where T is the percent transmittance expressed as a decimal (e.g., 22% = 0.22)

Transmittance (or %T) itself is determined by the instrument by dividing the detector signal when measuring the sample (I) by the signal recorded for a "blank" solution(I_0)

SPARKFUN ABSORBANCE



Azo dye, **542nm**
Organic compounds,
Function group R-N=N-R'

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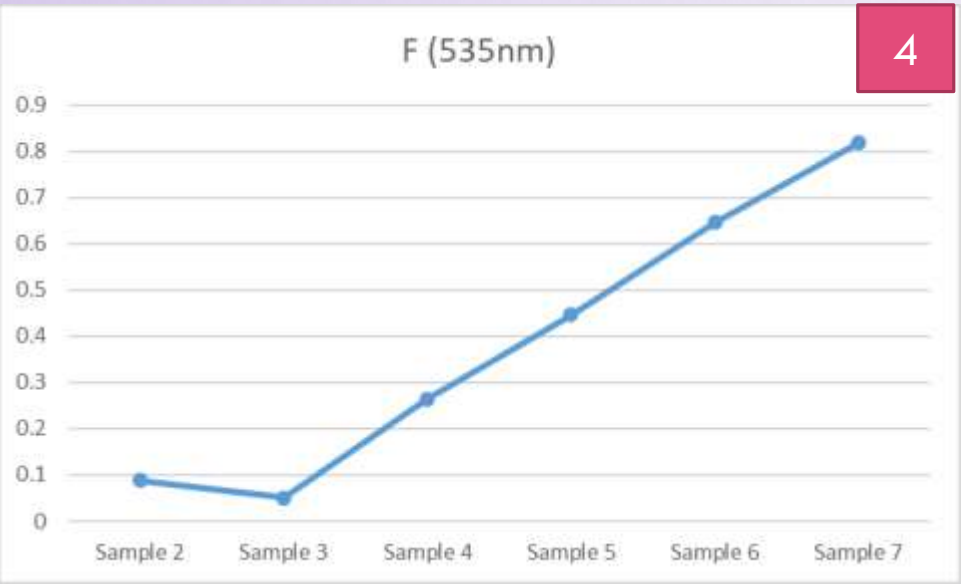

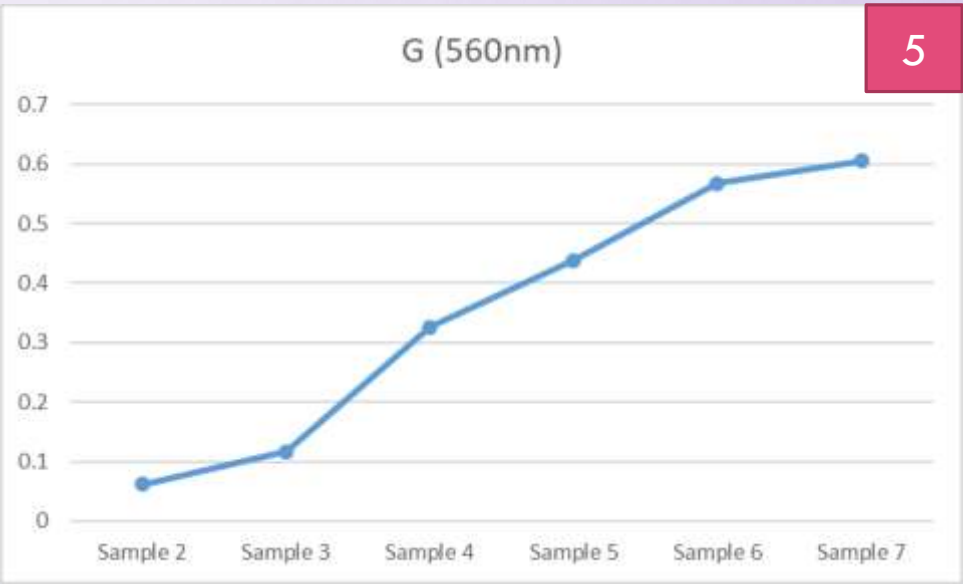

ABSORBANCE WITH CALIBRATION

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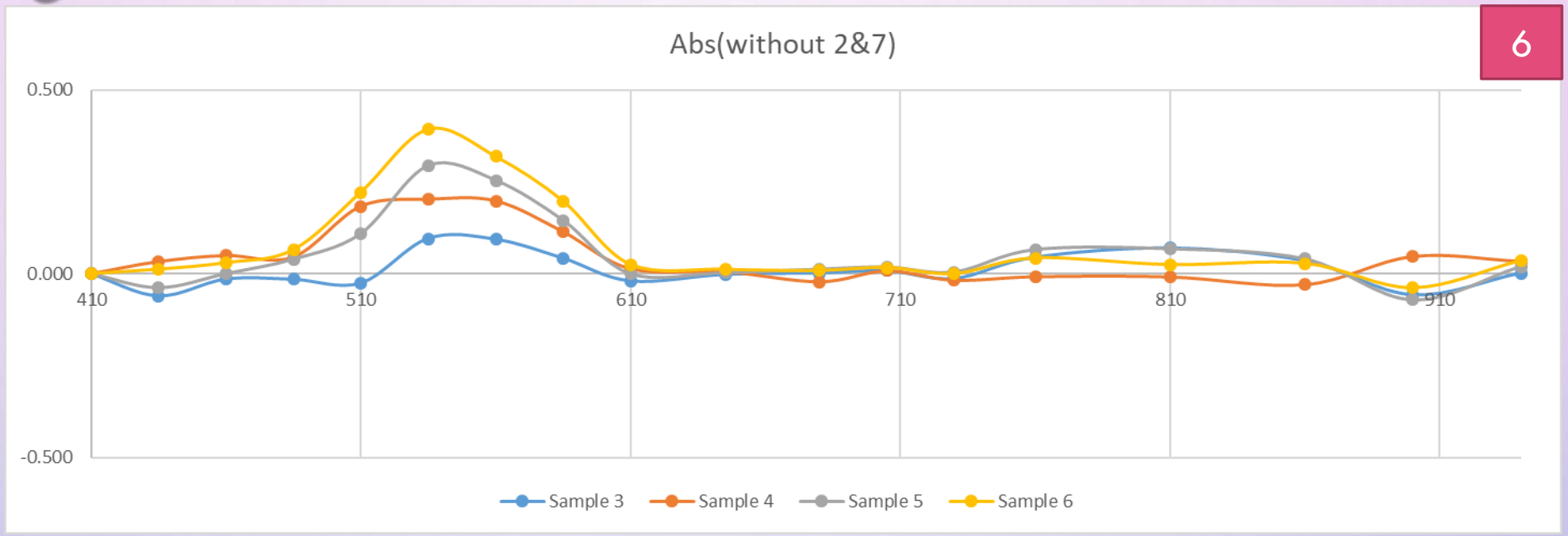

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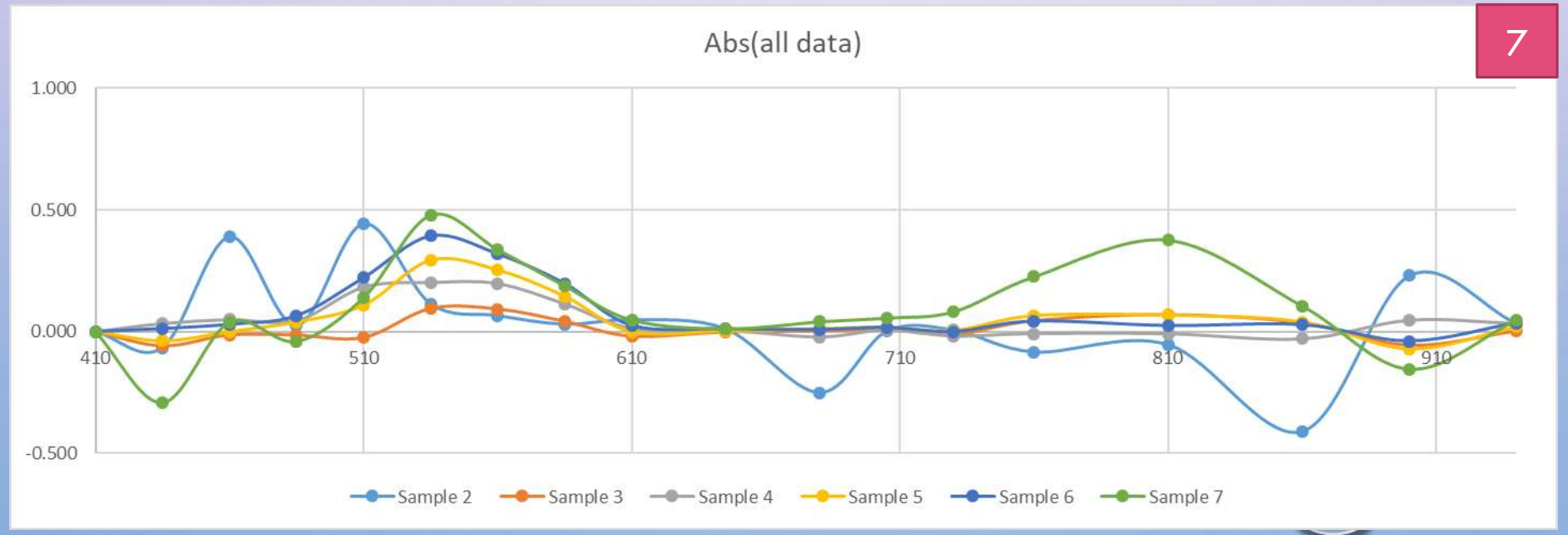
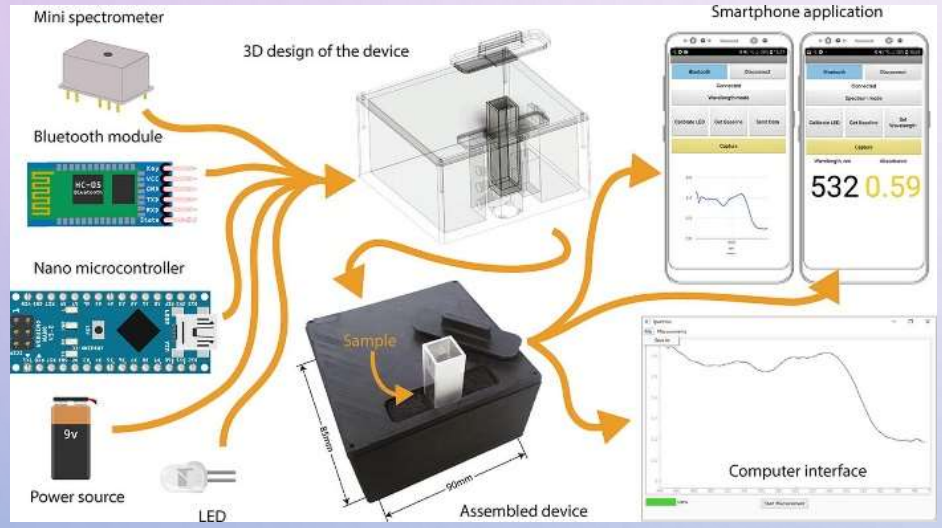
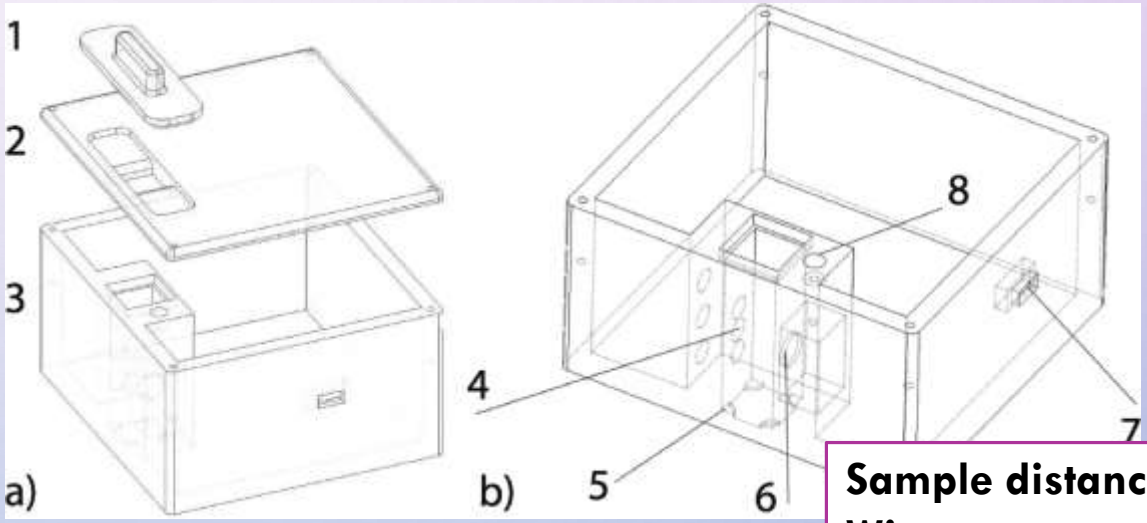


PORTABLE SPECTROPHOTOMETER

https://www.sciencedirect.com/science/article/pii/S246806722030016X





Sample distance ~3cm
Wire
Arduino
Mirror
Sample container
Multi-function?

SPECTROPHOTOMETER TEST IN COLOUR MEASUREMENT

Sample distance ~3cm
Wire
Arduino
Mirror
Sample container
Multi-function?

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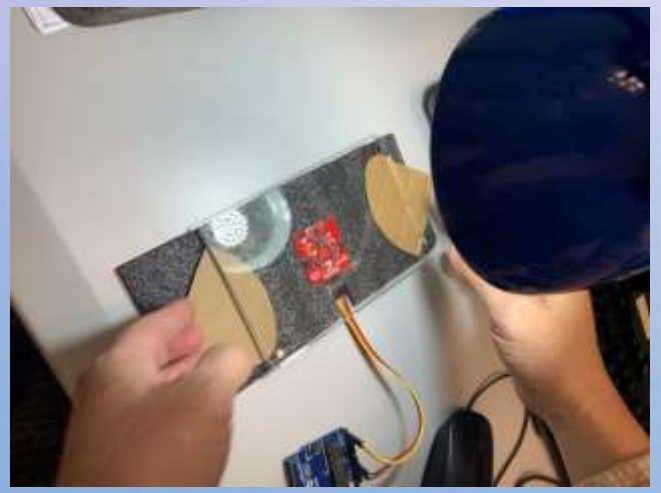
SPECTROPHOTOMETER TEST IN MATERIAL ANALYSIS



Sample distance ~3cm
Wire
Arduino
Mirror
Sample container
Multi-function?

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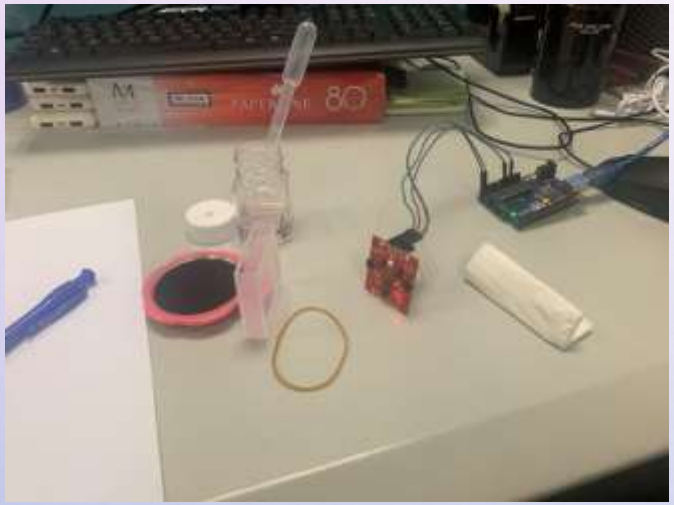
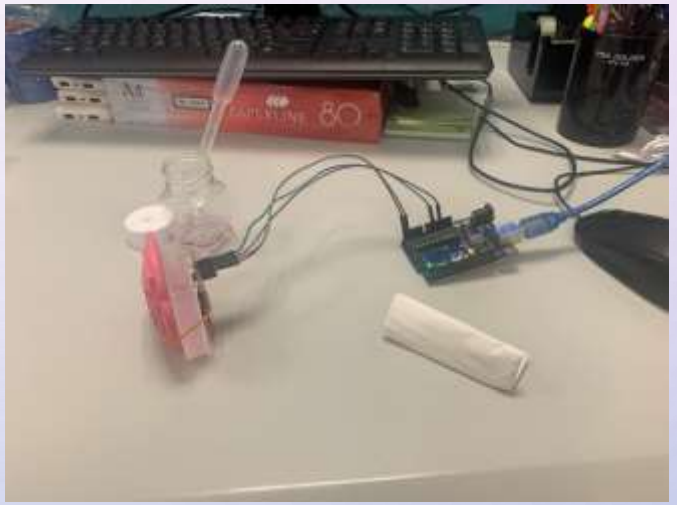
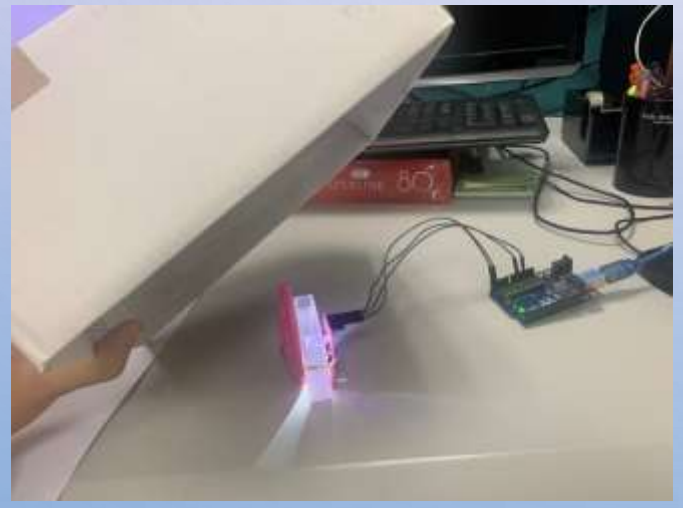
SPECTROPHOTOMETER TEST IN DETERMINE CONCENTRATION OF ABSORB SOLUTION







Sample distance ~3cm
Wire
Arduino
Mirror
Sample container
Multi-function?

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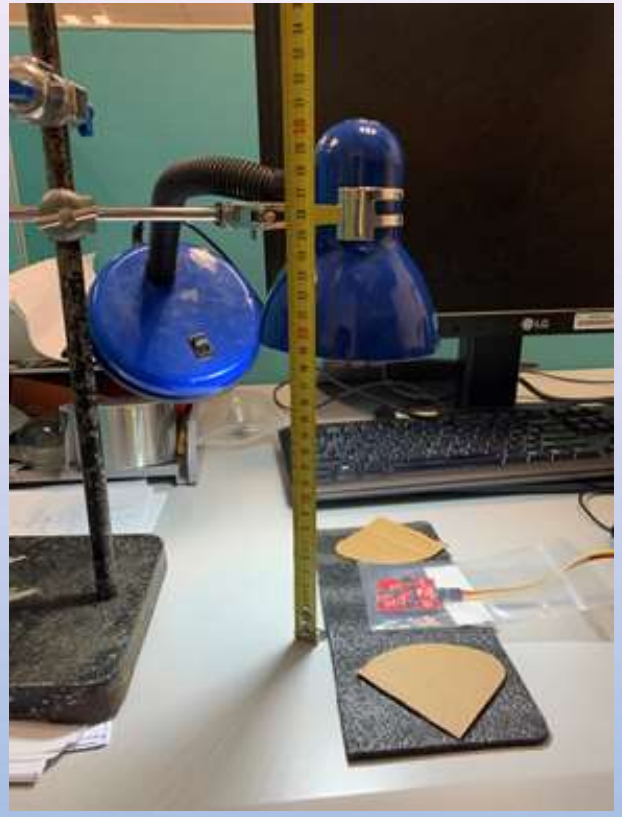
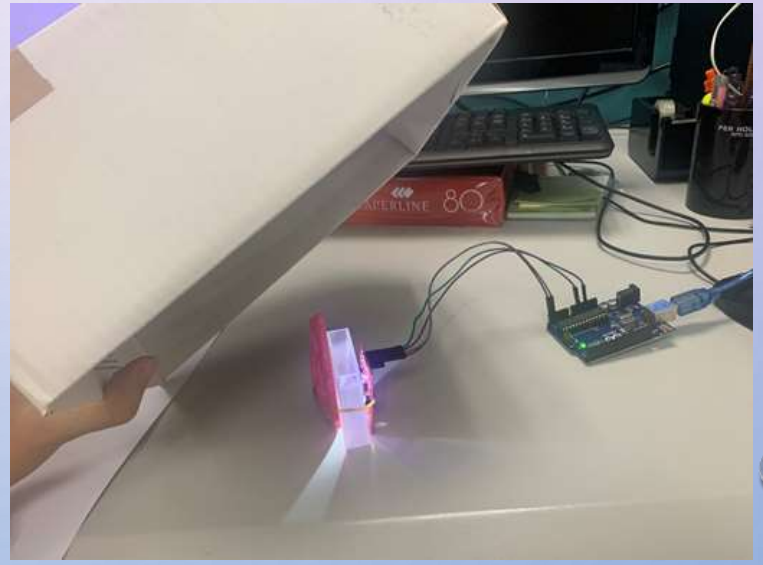
WORKSHOP: DEVELOP PORTABLE SPECTROPHOTOMETER WITH DATA LOGGING





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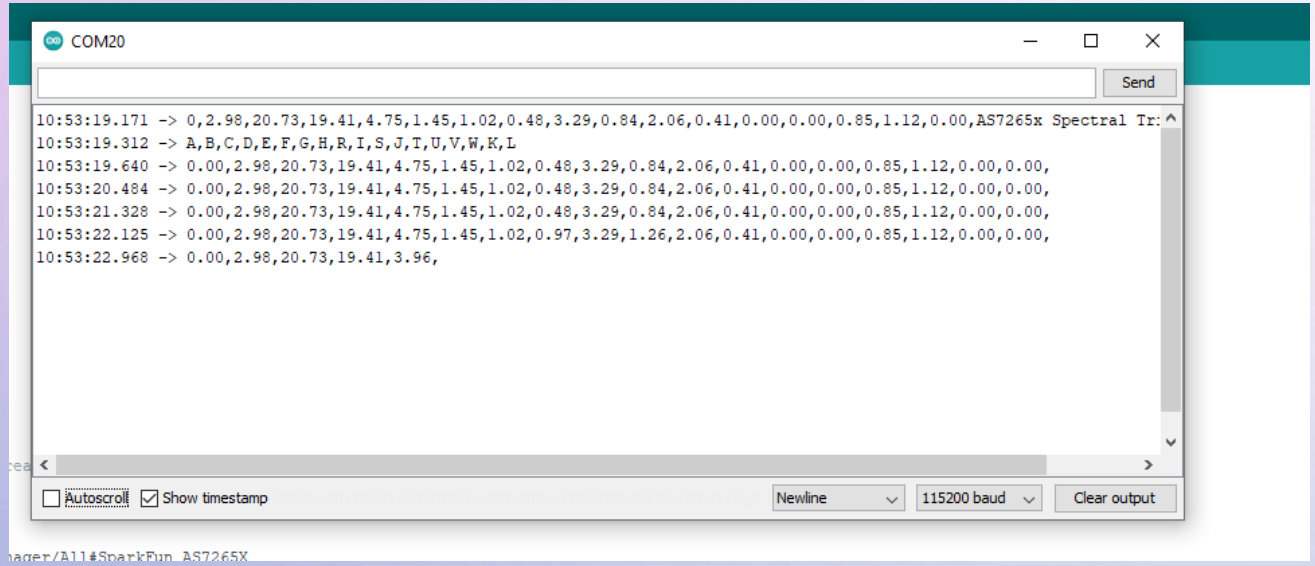
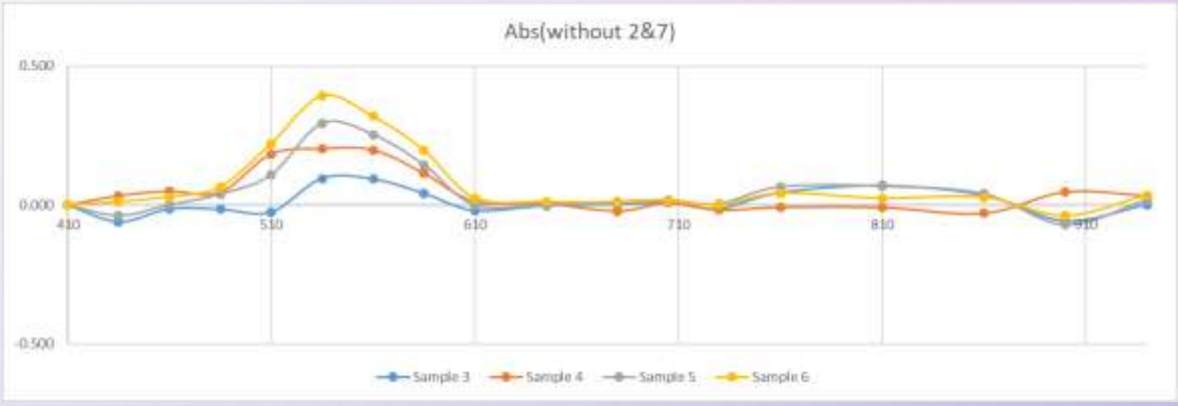
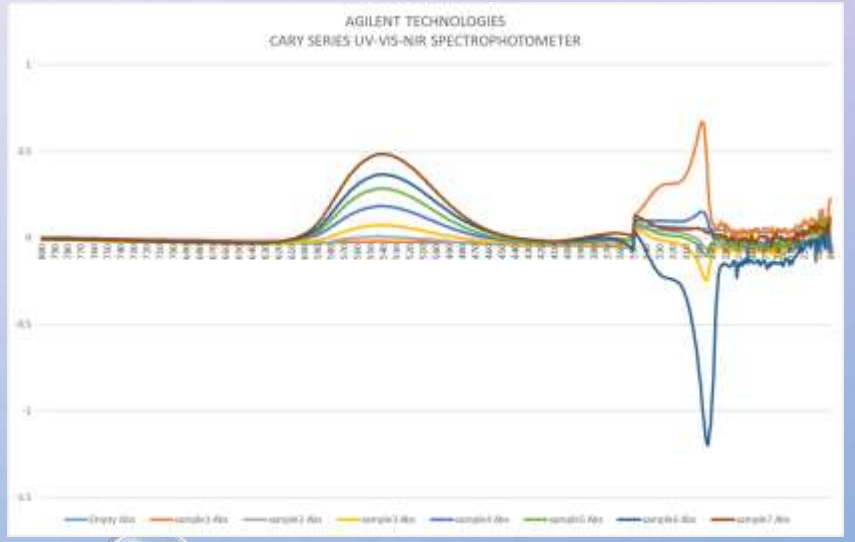
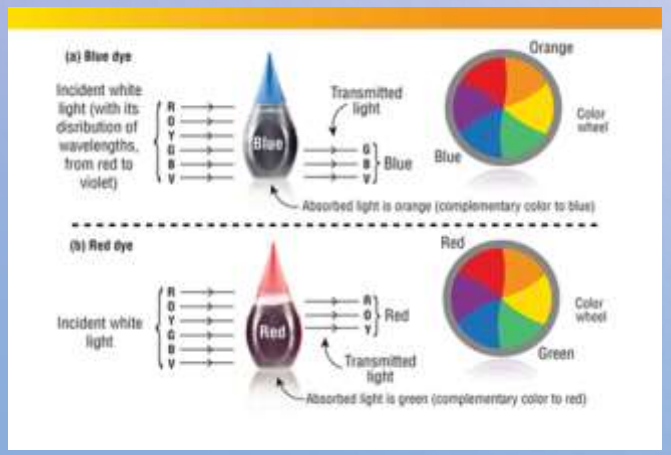
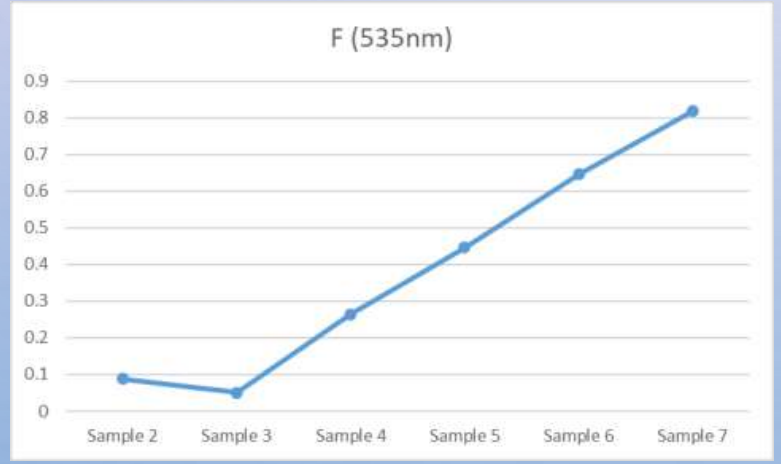
WORKSHOP: DATA LOGGING & ANALYSIS











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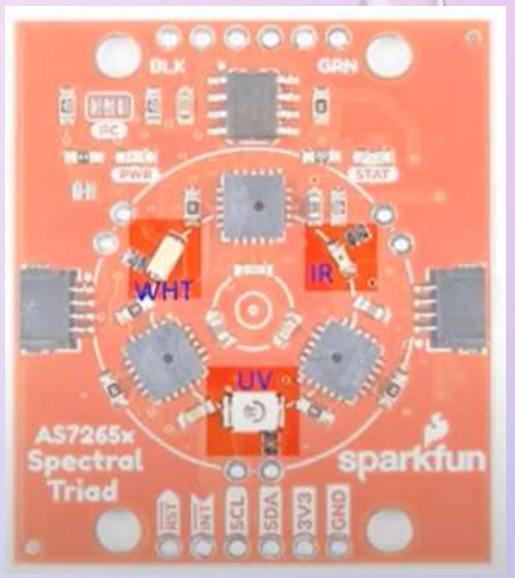
REFERENCE: MATERIAL LIST(HKD)



Spectroscopy Sensor
SEN-15050 SparkFun Triad Spectroscopy Sensor - AS7265x - ~$550

玻璃比色皿
~ $70*2

小鏡子
~ $15