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# **Non-linear Correlation of Absorbance with Respect to Concentration of Sugar in Aqueous Solutions of High Purity Laboratory Chemical Reagent Sucrose and Ordinary Cane Sugar**

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## **Abstract**

We show the trend for absorbance with concentration of sugar in aqueous solution deviates from theory. The aqueous solutions of sugar were prepared using High Purity Laboratory Chemical (HPLC) reagent sucrose in grade-3 de-ionized water. The sucrose solution was found to have an absorption band between 450 – 550 nm with a maximum peak at 490 nm. The absorbance rate ( $\epsilon$ ) using HPLC, was found to be 0.3 m<sup>-1</sup> per % concentration, 0.8 m<sup>-1</sup> per % concentration and 0.1 m<sup>-1</sup> per % concentration at low, middle and higher concentration respectively. This is shown to have striking difference from results calculated from Beer's law. Comparison with results obtained with Ordinary Cane Sugar (OCS) instead of HPLC exhibited a similar trend. This is shown to occur due to particulate kinetics of a solute in solution.

**Keywords:** non-linear; correlation; HPLC; OCS; sucrose; absorbance.

## **1. Introduction**

Several analytical techniques have been used to determine the concentration of sugars in food sample [1-6].

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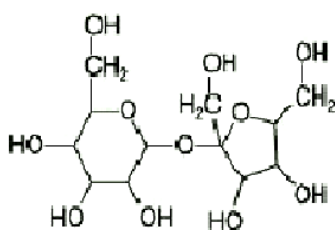
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These methods are relatively tedious and require complex pretreatment of samples as compared to Electronic Spectroscopy (ES). The organic reagents used in these pretreatment processes are hazardous and require high cost for storage and disposal.

Electronic Spectroscopy (ES) is a technique routinely used in analytical chemistry for the quantitative determination of different analytes [7]. Electronic transitions are responsible for the strong absorption of the UV-Vis spectral region (200 nm – 780 nm) by biological materials [8]. ES has advantage over other techniques and provides a rapid, noninvasive quantitative analytical method to assess the quantity of an analyte in various aqueous samples. Tayone (2015) has applied ES to determine chromium (VI) content in canned fruit juices [9], Atomic Absorption Spectroscopy (AAS) for the determination of the Chromium content in selected foods [10], and Near-Infrared studies of glucose and sucrose in aqueous solution [11].

Measuring the concentration of an absorbing species in a sample solution is accomplished by applying Beer-Lambert's law [12]. From the result, Frank-Condon principle helps explain the intensity of vibronic transition that simultaneously changes the vibrational and electronic quantum numbers due to absorption and emission of a photon [13].

Unlike other carbohydrates, sucrose (table sugar) is the only non-reducing common disaccharide. Consequently, most test of sugar detection utilizes such reagents as Benedict's solution, Fehlings solution, and DNS solution results in negative readings for sucrose. The sucrose molecule ( $C_{12}H_{22}O_{11}$ ) shown in Figure 1 has a molecular weight of,  $M_{\text{sucrose}} = 342$ . This molecule dissolves in water to form a clear aqueous solution and is quantized by measuring its absorbance at its wavelength of maximum absorption.



**Figure 1:** Structural Formula of sucrose molecule

From the above discussion, monitoring concentration of sugar in aqueous solution is an important industrial process in order to produce products with right measure. This study has been motivated by the possibility of providing a direct, noninvasive approach to measuring concentration of an absorbing species in aqueous solution. The obtained absorbance curve is a useful reference tool in determining the concentration of unknown sucrose in aqueous solution and the procedure is applicable to other water soluble materials.

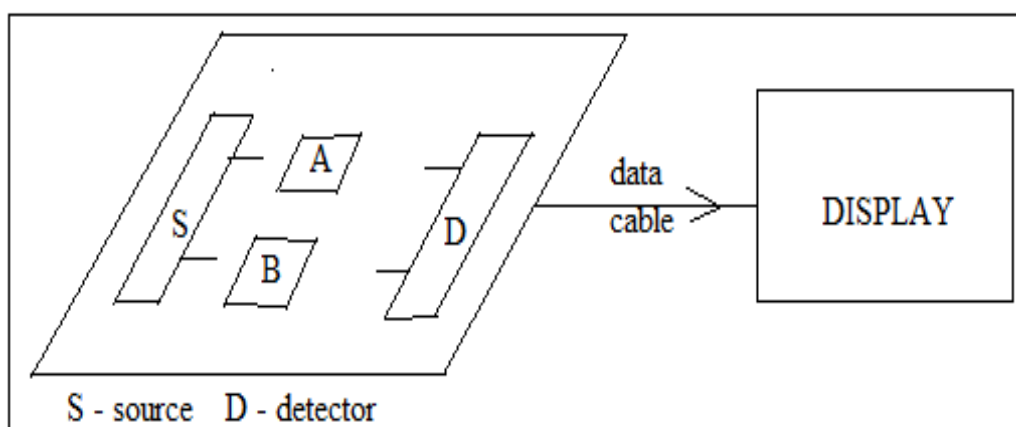
## 2. Materials and Methods

### 2.1. Preparation of sugar solution

Five different aqueous sugar solutions of concentrations (in percentage) 0, 8.2, 22.5, 41.8, and 57.1 were prepared through the procedure as outlined by [14]. Mass of sugar was measured using an electronic balance – model XL-3100D and solutions prepared using HPLC reagent sucrose in grade-3 de-ionized water. The mixture was stirred in a glass beaker to dissolve all the sugar granules to form a homogeneous solution. To ensure equilibrium, the solution was allowed to stand for 30 minutes and checked for sedimentation. The concentration is a measure of the amount of sucrose by mass in 100 g of the aqueous solution with de-ionized water. In this case, de-ionized water is considered 0% concentration. The proportion of sugar in 100 g of the solution is taken as the percentage concentration. Similar procedure was applied to prepare sugar solutions of OCS.

**2.2. Determination of maximum wavelength absorption of sugar in aqueous solution**

The UV-spectrophotometer instrumentation used in this study was set up as shown in Figure 2. The baseline was first run so as to correct deviation due to dust and air particles. De-ionized water in a cuvette was placed in pocket A to act as the standard solution. A cuvette of width 10 mm made of quartz was used since ordinary glass would absorb the visible region. Since aqueous samples for electronic spectroscopy are required very dilute [15], 10 ml of 22.5% concentration of sucrose solution was poured into 100 ml volumetric flask and de-ionized water added up to the 100 ml graduation mark. The mixture was thoroughly shaken in the flask to ensure homogeneity. A sample solution was put in a cuvette and placed in pocket B. The absorbance was scanned in spectrum mode from 190 nm to 800 nm against the de-ionized.



**Figure 3:** Schematic diagram of a UV-spectrophotometer; pocket A holds the standard solution while the solution under analysis is put in pocket B

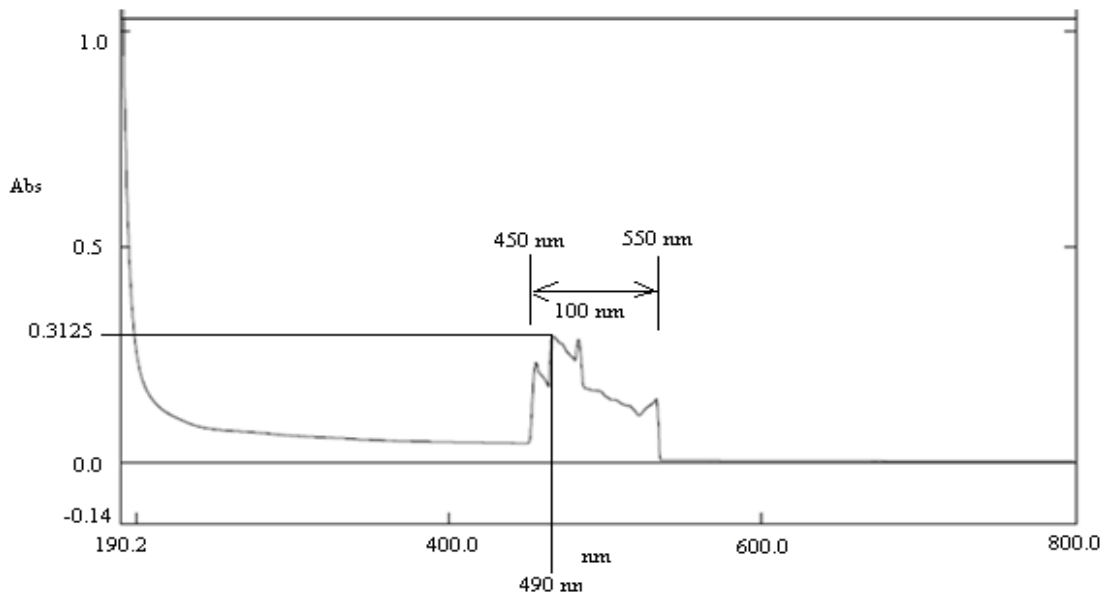
**2.3. Measurements of absorbance of sugar in aqueous solution**

10 ml each of the prepared concentration solutions was poured in 100 ml in a volumetric flask and diluted with de-ionized water up to 100 ml. After 30 minutes, the absorbance of the solutions was determined at maximum wavelength using UV-1800 Shimadzu instrumentation. Absorbance was measured in a quartz cell against de-ionized water as the reference solution for all measurements. All the measurements were taken at room temperature (about 298 K). Reproducibility and stability of the measurement were tested before proceeding with the study.

### 3. Results and Discussion

#### 3.1. Absorbance of sugars in aqueous solution

Figure 3 displays the absorption spectrum ranging from 190 nm to 800 nm of HPLC sucrose in aqueous solution for the 22.5% concentration. The absorbance initially decays exponentially from 1 to 0.0625 at 450 nm wavelength. A major absorption band is observed with the bandwidth ranging from 450 – 550 nm. Seven peaks are observed with the highest peak estimated at 490 nm with absorbance 0.3125.



**Figure 3:** Absorbance spectrum of HPLC sucrose solution at 22.5% concentration

The value of absorption coefficient ( $\alpha$ ) was extracted from the measured absorption spectrum at 490 nm (Figure 3) using Beer-Lambert’s formula:

$$\alpha(w, c) = \frac{A(w, c)}{b} \dots\dots\dots 1$$

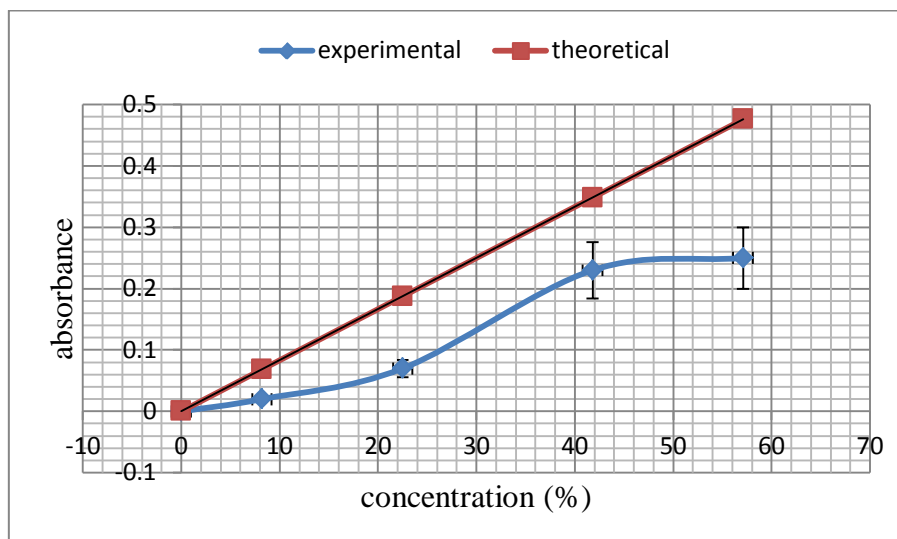
The obtained value of  $\alpha$  was used to evaluate the value of molar absorptivity ( $\epsilon$ ) that was further used to calculate theoretical absorbance of the prepared sugar solution according to Beer-Lambert’s law (equation 1).

$$A = -\log(T) = \epsilon cb = \alpha b \dots\dots\dots 2$$

where  $A$  is the absorbance,  $T$  is the measured transmittance,  $\epsilon$  is the molar absorptivity,  $c$  is the concentration,  $\alpha$  is the absorption coefficient and  $b$  is the width of the sample solution.

Figure 4 displays curves obtained from Shimadzu instrumentation and theoretical measurement (as given by equation 1). The experimental data points are an average of four measurements with an error of 5% as shown by

the error bars. From the graph, it is observed that there is an overall increase in absorbance with concentration. Absorbance is fairly gradual up to 20% concentration for experimental (blue with diamond). Beyond this, a steep slope is observed up to around 40 % concentration after which the change in absorbance with concentration was not remarkable. In contrast, the theoretical slope (red with squares) is linear through the origin.



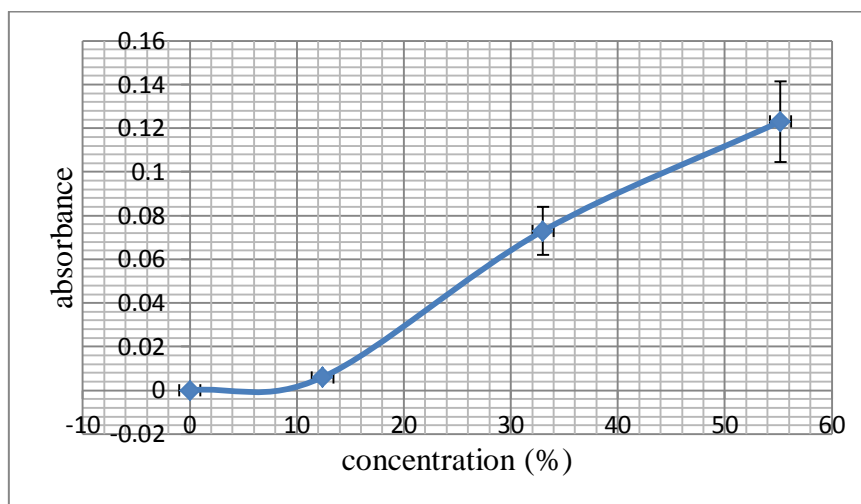
**Figure 4:** Comparison of the experimental versus theoretical absorbance curve using HPLC sucrose in aqueous solution

Similar procedure and analysis was carried out with OCS using the instrumentation of Figure 2 and the results are shown in Figure 5. The slope of this result is fairly horizontal between 0 to 10% after which it rises linearly and steeply. The absorbance by the OCS sugar solution is relatively lower than the absorbance by the HPLC solution even though the overall trend is similar.

The absorption band (Figure 3) occurred only in sucrose solution but was absent in pure solvent. This strong absorbance band in visible spectral region can be attributed to electronic transitions due to absorption of light energy by sugar molecules [8]. The amount of light energy absorbed at this wavelength will increase as the number of atoms of the selected solute in the light path increases. The overall quantitative concentration dependent trends are very similar (figures 4 and 5). Comparing the absorbance of HPLC and OCS sucrose, the absorbance of HPLC is higher than the OCS. This means that OCS contains some non-sucrose elements which are removed during purification to produce HPLC.

The absorbance curve displayed by this Shimadzu instrumentation is attributed to fundamental deviations due to limitations of Beer –Lambert’s law at low and high concentration [7, 16]. By comparing the absorbance rate ( $\epsilon$ ) using HPLC, we found  $\epsilon$  to be  $0.3 \text{ m}^{-1}$  per % concentration,  $0.8 \text{ m}^{-1}$  per % concentration and  $0.1 \text{ m}^{-1}$  per % concentration at low, middle and higher concentration respectively. The value of  $\epsilon$  at low and high concentration is lower than the calculated  $\epsilon$  ( $0.8 \text{ m}^{-1}$  per % concentration). At middle range (20% up to 40%) concentration the experimental  $\epsilon$  is equal to the theoretical. The absorbance rate ( $\epsilon$ ) using OCS at low concentration (< 10) is not

remarkable after which it attains  $0.2 \text{ m}^{-1}$  per % concentration. At low concentration ( $< 10$ ), there is limited association between solute molecules so the orientation of these molecules is sparsely apart hence high transmittance observed. At high concentration ( $> 20$ ), there is a shift in chemical equilibrium as a function of concentration. Solute molecules cause different charge distribution on their neighboring species in solution due to electrostatic interaction between molecules in close proximity. Since ES absorption is an electron phenomenon, the absorptive coefficient is adversely affected and deviates from linearity of Beer's law. This has been attributed to the particulate chemical dynamics according to kinetic-molecular theory.



**Figure 5:** Standard absorbance curve for OCS in aqueous solution

#### 4. Conclusion

The absorbance rate ( $\epsilon$ ) using HPLC, was found to be  $0.3 \text{ m}^{-1}$  per % concentration,  $0.8 \text{ m}^{-1}$  per % concentration and  $0.1 \text{ m}^{-1}$  per % concentration at low, middle and higher concentration respectively. At low concentration ( $< 10$ ) and high concentration ( $> 20$ ), is lower than the theoretical. This is attributed to particulate chemical dynamics of a solute in solution according to limitations of Beer-Lambert's law.

#### 5. Recommendation

Beer-Lambert's law is a useful tool in quantitative analysis of many substances in aqueous solution. However, it does not hold as a universal law for all substances. More work should be carried out to analyze calibration performance of commonly used substances in the laboratory considering the shift in chemical equilibrium as a function of concentration.

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