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Spectral Characterization and True Color Analysis of Different Natural Dyes such as *Bixa Orellana*, *Brassica oleracea var. Capitata*, and *Indigofera suffruticosa*

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— Abstract—

This study evaluated the process of obtaining dyes from plant species such as *Bixa Orellana* (Achiote) and *Brassica oleracea, var. capitata* (red cabbage). The absorbances of the obtained dyes were compared with *Indigofera suffruticosa* (indigo) and methylene blue, commercially acquired, using ultraviolet (UV), visible (Vis), and near-infrared (IR) spectroscopy, achieving absorbances greater than 2.5 in the high-energy wavelength range, which is useful in the medical industry due to its disinfectant properties. Linear regression models were determined using dilutions of these dyes to obtain the true color, achieving determination coefficients greater than 95%.

Keywords:

Dyes; extraction; UV-Vis spectroscopy; true color.

The low availability of water for human consumption is one of the most significant health issues today since it is a resource used for supply, food, recreation, and economy, among others. In Mexico, about 80% of aquatic ecosystems have some degree of pollution. The main pollutants observed are organic matter, nutrients such as nitrogen and phosphorus, and microorganisms (fecal coliforms) (Hernández, et al. 2020). There are different conventional and unconventional techniques for the elimination of microorganisms in water such as the application of chemicals, UV sterilization, and reverse osmosis (Faroon, et al. 2023), for the first case and nanofiltration (Nasir, et al. 2022), electrocoagulation (Gamero, et al. 2020), photosensitization (Santos, et al. 2023), among others, as a second case. The latter represents a low-cost alternative for the disinfection of water using solar radiation and dyes, which are chemical structures used for their coloring properties. They are classified as natural by their vegetable, animal, or mineral origin (dyes) and artificial due to physical or chemical modifications, they absorb light and give color in the visible region of the spectrum (400-800 nm). Due to their characteristics, they are also used in substrates to give them color and can resist discoloration when exposed to water, oxidizing agents, sweat, and microbial attack, so they are also used in industries such as textiles, food, printing, cosmetics, medicine, plastics, concrete, and paper. One of the most widely used dyes in the industry is methylene blue, which is commonly applied to color silk, wool, cotton, and paper (Rodríguez-Basantes, et al. 2019).

This paper presents the extraction process of two different organic dyes, as well as the determination of the true color, comparing them with Indigofera suffruticosa and methylene blue.

MATERIALS AND METHOD

The procedure to follow for the extraction of the colorant is the selection of a determined mass of the chosen vegetal species (seeds or leaves), if this one is composed of leaves, it has to be cut to diminish the time of obtaining the colorant. Next, two extraction techniques are presented, the first uses water at boiling temperature with the chosen plant species and with constant agitation at 200 RPM for 10 minutes, it is allowed to cool and the liquid is filtered using filter paper, the extract is concentrated by boiling the solution or putting it in a water bath, this process is repeated in some cases up to 4 times to obtain the largest possible amount of dye. The second is using a solution of sodium hydroxide or potassium hydroxide (alkaline medium) at a concentration of 1.5% or also using concentrated ethyl alcohol to extract the dye under constant agitation at 150 RPM in times ranging from 10 minutes to 1 hour, depending on the plant species, in the case of using

the alkaline medium, the solution is acidified using phosphoric acid at 10% (H_3PO_4) with constant agitation until reaching a pH between 2 and 2. 5, finally, the extract is concentrated by decanting the material and drying in an oven at an optimum temperature so that the properties of the dye are not modified. For its preservation, the concentrated material can be frozen or ethyl alcohol can be added in a 1:8 ratio (Rodríguez-Basantes, et al. 2019). The final mass of dye obtained is measured using an OHAUS analytical balance model PIONEER TJ2611 with a precision of 0.1 mg to determine the extraction efficiency.

The spectral analysis follows the procedure described in NMX-AA-017-SCFI-2021, for the measurement of true color in natural, waste, treated waste, and marine waters, using spectral absorption coefficients at three different wavelengths in the visible range of the spectrum (436, 525, and 620 nm) using

$$\propto (\lambda) = \frac{A}{d} f$$

Where A is the absorbance of the water sample at wavelength λ, d is the distance in mm of optical path through the cell containing the water sample and f is a factor to obtain the spectral coefficient in m^{-1} (f=1000). The study was carried out with a HACH model DR6000 UV/VIS spectrophotometer. The true color is expressed as a function of the spectral absorption coefficient at the analyzed wavelength, as well as the pH value of the sample. If the sample is diluted, the volume of water used in the final calculation of the true color is evaluated.

RESULTS

Three different raw materials such as indigo, annatto, and purple cabbage were used to obtain organic dyes, which were contrasted with methylene blue. The following is a description of the process of obtaining each of them.

For Indigo, from *Indigofera suffructicosa*, we had a colorant with a solid presentation, so for its preparation, it was ground to obtain granules smaller than 0.5 mm in diameter. Next, a mass of 800 mg of indigo was placed in 400 mL of distilled water and kept in agitation for 10 minutes at 200 RPM to homogenize the mixture until a concentration of 2 g/L was obtained, with an average pH of 7.0.

From this concentration, 9 dilutions were prepared, decreasing the concentration in periodic values, until a minimum concentration of 200 mg/L was reached.

In the case of *Bixa Orellana* (Annatto), two procedures were performed to obtain the dye, in the first one a mass of 100 g of annatto seeds was placed with 150 mL of distilled water in an Erlenmeyer flask, boiled at a temperature of 150°C with constant agitation at 200 RPM in a Thermo Scientific model Cimarec heating and stirring rack for 15 minutes, making sure that the seeds did not adhere to the walls of the flask. Once the time had elapsed, the mixture was filtered to eliminate the washed annatto seeds using a regular nylon mesh strainer. The liquid obtained was deposited in three porcelain capsules distributed in equal volumes for subsequent drying. Since the first process did not completely remove the colorant from the annatto seeds, we washed them a second time under the same conditions as the first one. The porcelain capsules with the liquid obtained were placed in a Biobase drying oven, model FCD-3000 Serials, previously conditioned at a temperature of 60°C for approximately 8 hours.

Figure 1. Enlarged view of annatto seeds. a) first wash, b) second wash, the removal of dye between washes is observed by obtaining a larger dark surface on the seeds

Because the extract obtained, despite the washes performed, presented a thick consistency that inhibited the extraction process, it was necessary to use a larger volume of water, a smaller mass of annatto seeds, or a larger number of washes. Once the dye extraction process was completed, the mass and its removal efficiency were evaluated, obtaining 0.9397g of dye, which represents a removal efficiency of 93.32%.

For the second procedure, 10 g of annatto seed and 30 mL of an alkaline medium (NaOH or KOH) were used, both solutions at a concentration of 1.5%, the mixture was kept under constant stirring at 140 RPM for 1 hour, then it was left to digest for 24 hours, after which the seeds were separated and a second wash was carried out repeating the process described above. The extracts obtained for each wash were combined and acidified by adding drops of 10% phosphoric acid (H_3PO_4) with constant stirring AT 150 RPM until a pH of 2 to 2.5 was reached. Finally, the material for the separation

of the seeds was decanted, allowing the liquid to dry for three days at room temperature in a controlled space. The mass obtained using sodium hydroxide was on average 1.2 g, while using potassium hydroxide it, was 1.8 g.

For *Brassica oleracea, var. capitata* (purple cabbage), 463.85g of purple cabbage (6 leaves) was finely chopped and divided into four blocks of approximately 115g. To each block contained in beakers, we added 460 mL of distilled water previously boiled for 30 minutes with magnetic stirring. Two batches were allowed to cool to room temperature; then 1/8 of their volume of concentrated ethyl alcohol was added for preservation and evaluation, and to prevent the proliferation of microorganisms. The other two batches were reduced by evaporation at an average temperature of 250°C for one hour, resulting in a decrease of 100 and 55 mL, respectively.

Absorbance

With the dyes obtained from each plant species, a concentration of these and a solution of methylene blue was determined in such a way that when measuring the absorbance they had values close to each other, obtaining the curves presented in Figure 2.

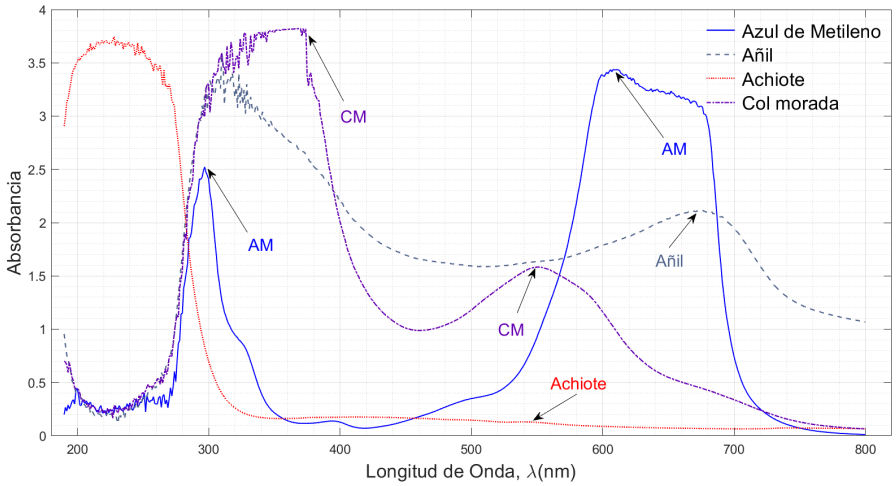

Figure 2. Absorbance curves of the dyes analyzed

From Figure 2, the highest absorbance for methylene blue at a concentration of 10 mg/L was at 610 nm, which indicates higher temperature but lower energy, similar to that observed in other works such as Amaya, 2023, which presented an analysis from 390 to 800 nm. However, in this case, a scan was performed from 190 nm presenting a second absorbance peak at 297 nm, which corresponds to a high frequency of light. This wavelength range with higher energy allows its use in medicine to eliminate microbial

population, and in water treatment (Santos, et al. 2023). There was a higher absorbance around 310 nm and 675 nm for Indigo at a concentration of 2 g/L, according to what was observed by Basuki, 2018. In the case of annatto with a concentration of 22.5 mg/L, an absorbance peak was determined at a wavelength below 300 nm which corresponds to ranges of higher energy useful in medicine, the characteristic absorbance bands between 250, 370 and 500-545 nm are indicated for anthocyanins. The absorption of the latter band varies with the pH of the medium which makes it possible to detect the type of anthocyanin. The purple cabbage had two absorbance peaks at 370 nm and 550 nm, values close to those observed by Paez-Cartaya, 2018.

True Color

To determine the true color of the substances dissolved in water, the spectral absorption coefficient was calculated at 436, 525, and 620 nm, all samples when diluted in distilled water raised their pH to values close to 7.0. The calibration curve was then determined using methylene blue at different concentrations ranging from 0.1 to 10 mg/L.

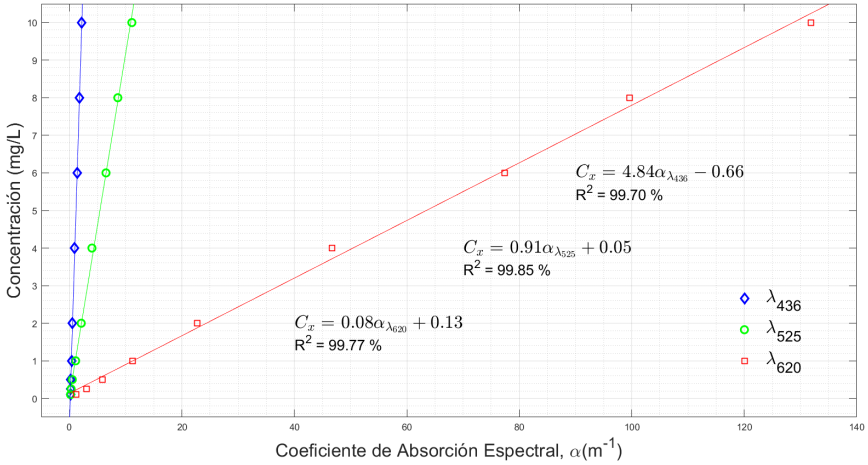

Figure 3. Calibration curve of Methylene Blue with the spectral absorption coefficient at different concentrations

Figure 3, presented the linear relationship between the spectral absorption coefficient and the dye concentration at different dilutions. The value of the spectral absorption coefficient for each wavelength represented the true color considering the dilutions made for each concentration. In the graph, different slopes were observed for each wavelength analyzed, the one corresponding to 620 nm which is the one at which the maximum absorbance of the dye was obtained, implies greater sensitivity of the analytical method due to the prolonged slope found and greater control of color gradients. A coefficient of determination R^2 greater than 99% was deter-

mined in all the wavelengths analyzed; which indicated a strong linear relationship between the variables. The expressions found will make it possible to ensure that the final application given to the dye is the desired one because the required tone or color will be exact by controlling the concentration of the same.

Figure 4 shows the indigo calibration curve, where no significant difference was observed between the wavelengths analyzed, which is inferred not to be within some maximum absorbance peak, so the true color will vary only in brightness.

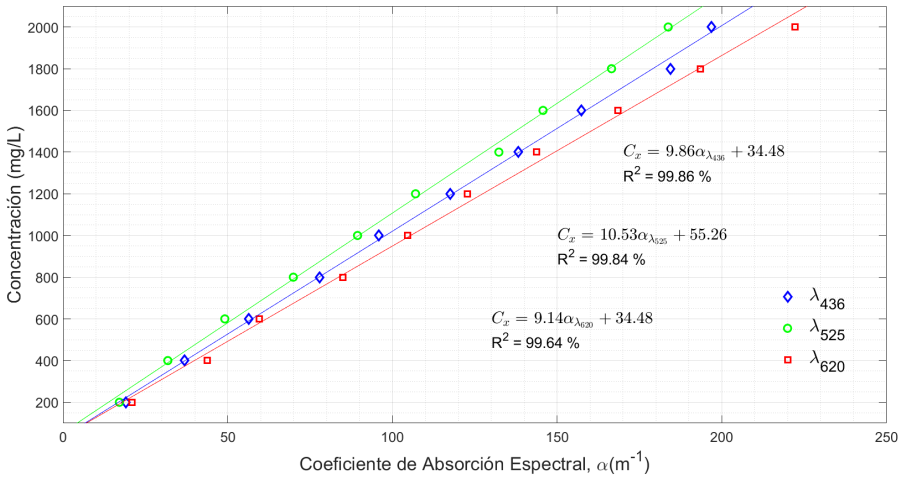

Figure 4. Indigo calibration curve with the spectral absorption coefficient at different concentrations

It should be noted that when the indigo was diluted in distilled water, it did not present a homogeneous solution, i.e., sedimentation or residues of indigo were observed scattered in the container, which implied variations in the concentration that depended on the initial conditions of the dye.

For the curve in Figure 5, 4 dilutions of the dye obtained from annatto were prepared decreasing the concentration from 22.5 to 12.5 mg/L, higher sensitivity was observed at 436 nm, in addition to the best approximation with an R^2 above 98%. There were also low values in the spectral absorption coefficient, which indicates that an intense color was obtained in the solution with low concentrations of annatto.

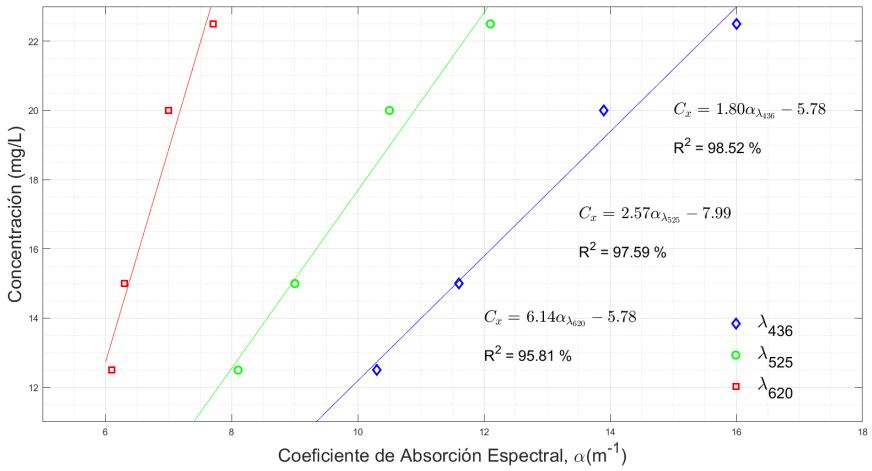

Figure 5. Calibration curve of the Annatto-based dye with the spectral absorption coefficient at different concentrations

For this dye, the obtaining method chosen was the second one based on sodium hydroxide at a concentration of 1.5% and phosphoric acid at 10%, because the first method presented sedimentation when diluted in water, while for the second one, a homogeneous mixture was obtained.

For the colorant obtained from purple cabbage, 10 dilutions were prepared, decreasing the initial concentration in periodic values until a minimum of 5% was obtained, as presented in Figure 6. Higher sensitivity was observed at 525 nm. It is important to point out that this dye has been proposed as a pH indicator due to its sensitivity through visible changes in color, which is effective in the development of biosensors for food preservation (Molina-Arteaga, et al. 2022).

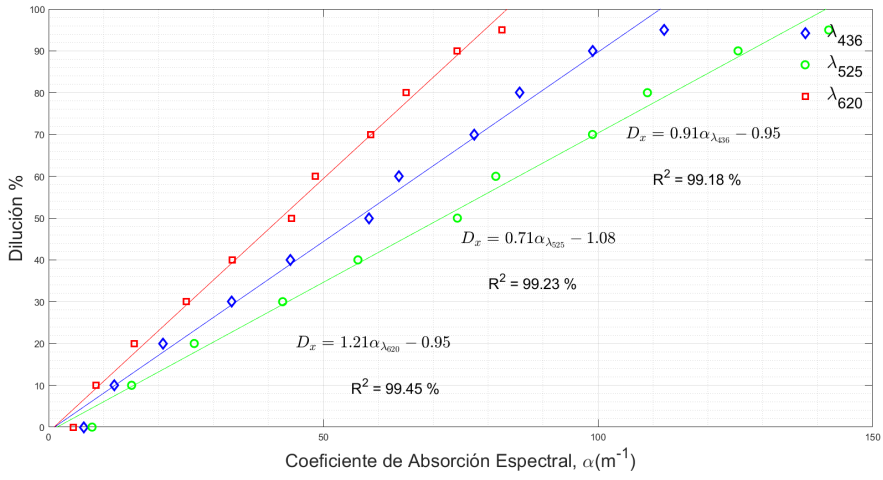

Figure 6. Calibration curve of the dye obtained from Purple Cabbage with the spectral absorption coefficient at different dilutions

With the equations obtained for each of the dyes, it was possible to determine the spectral absorption coefficient at an unknown concentration of these with an R^2 superior to 99% at the three wavelengths; only in the case of annatto was the coefficient of determination lower than 96% at 620 nm. These equations will help to simplify the monitoring system in the case of not having a spectrometer that scans the visible spectrum, by using a color sensor that evaluates the absorbances at wavelengths of 436, 525, and 620 nm, respectively.

CONCLUSIONS

Processes for obtaining water-soluble dyes were presented using potassium hydroxide and sodium hydroxide as solvents for the extraction of annatto colorant for its economic advantages and water for purple cabbage.

The absorbances of different organic dyes were determined at concentrations of 2 g/L and 12 mg/L, corresponding to indigo and annatto, respectively. The dyes analyzed showed high absorbances at wavelengths below 400 nm, which corresponds to high energy, making them viable in areas such as medicine and tertiary water treatment.

Linear relations were obtained between the spectral absorption coefficient and the concentrations of the dyes used, observing variations in the color gradient in the case of methylene blue and variations in brightness in the case of organic dyes.

Coefficients of determination R^2 greater than 99% were observed in most cases, indicating a high relation of the linear regression model to the actual data and allowing simplification of the true color procedure with a color detector covering the wavelengths proposed in NMX-AA-017-SCFI-2021.

REFERENCES

Amaya Vesga, Á. A., Carvajal Grimaldos, A. C., Gómez Tarazona, R. A. y Mendoza Castellanos, Y. R. (2023). Demostración práctica de la importancia de la longitud de onda en la espectroscopía Ultravioleta Visible. *Educación Química, 34*(2), 40-49. http://dx.doi.org/10.22201/fq.18708404e.2023.2.83275

Basuki, Suyitno and Kristiawan, B. Absorbance and electrochemical properties of natural indigo dye. 2018. *AIP Conf. Proc*. 1931, 030067. https://doi.org/10.1063/1.5024126.

Faroon, M., ALSaad, Z., Albadran, F., & Ahmed, L. (2023). Review on Technology-Based on Reverse Osmosis. *Anbar Journal of Engineering Sciences, 14*(1), 89-97. DOI: 10.37649/aengs.2023.139414.1047

Gamero-Quijano, A., García, P., & La Rosa-Toro-Gómez, A. (2020). Remoción de bacterias patógenas del agua mediante electrocoagulación con ánodos de aluminio / Removal of pathogenic microorganisms from water by electrocoagulation with aluminum electrodes. *Tecnología Y Ciencias Del Agua, 11*(4), 136-178. https://doi.org/10.24850/j-tyca-2020-04-05

Hernández Vargas, O., Mancilla Villa, O. R., Palomera García, C., Olguín López, J. L., Flores Magdaleno, H., Can Chulim, Álvaro, Ortega Escobar, H. M., & Sánchez Bernal, E. I. (2020). Evaluación de la calidad del agua y de la ribera en dos cuencas tributarias del río Tuxcacuesco, Jalisco, México. *Revista Internacional De Contaminación Ambiental, 36*(3), 689–701. https://doi.org/10.20937/RICA.53595

Molina-Arteaga, N.I., Toala-Zambrano, A.N., Sanchéz, F., Macías-Pro, M.A., Rosero-Delgado, E.A. (2022). Antocianinas como biosensores en la conservación de alimentos. *Revista Bases de la Ciencia, 7*(2), 15-32. https://doi.org/10.33936/revbasdelaciencia.v7i2.4449

Nasir, A. M., Adam, M. R., Mohamad Kamal, S. N. E. A., Jaafar, J., Othman, M. H. D., Ismail, A. F., Aziz, F., Yusof, N., Bilad, M. R., Mohamud, R., A Rahman, M., & Wan Salleh, W. N. (2022). *A review of the potential of conventional and advanced membrane technology in the removal of pathogens from wastewater. Separation and purification technology, 286*, 120454. https://doi.org/10.1016/j.seppur.2022.120454

Paez-Cartaya, I., Rodríguez-Sánchez, J. L., Cruz-Viera, L. (2018). *Optimización de la extracción de antocianinas de Hibiscus sabdariffa L. y su caracterización cromática. Ciencia y Tecnología De Alimentos, 28*(2), 17-21. https://revcitecal.iiia.edu.cu/revista/index.php/RCTA/article/view/56

Rodríguez-Basantes, A. I., Esparza-Bonilla, C. J., & Huacho-Chávez, I. F. (2019). Obtención de antocianinas de la Brassica oleracea var.Capitata para el uso en alimentos. *Dominio De Las Ciencias, 5*(1), 652–666. https://doi.org/10.23857/dc.v5i1.1067

Santos, A. D., Gonçalves, D., Martins, R. C., Gmurek, M., Nogueira, A., Castro-Silva, S., Castro, L. M., & Quinta-Ferreira, R. (2023). Homogeneous Photosensitized Oxidation for Water Reuse in Cellars: A Study of Different Photosensitizers. *Sustainability, 15*(8), 6861. https://doi.org/10.3390/su15086861