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CHAPTER 6

COMPARATIVE EVALUATION OF ORGANIC SOLVENTS IN THE EXTRACTION AND SOLUBILIZATION OF BIXIN AND NORBIXIN FROM ANNATTO (*BIXA ORELLANA* L.)

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Marco Aurélio da Silva Coutinho[1], Edmilson Miranda de Moura[2], Carla Verônica Rodarte de Moura[3] and Leanne Silva de Sousa[4]

Abstract

This chapter addresses the influence of different organic solvents on the solubility and spectroscopic properties of the natural dyes bixin and norbixin, extracted from the seeds of Bixa orellana L. Initially, general aspects of natural dyes are discussed, with emphasis on the carotenoids present in annatto and their industrial relevance in the food, cosmetic, and pharmaceutical sectors. Subsequently, the theoretical foundations related to solubility and UV–Vis spectrophotometry are presented, including the application of the Lambert–Beer Law for the quantification of compounds in solution.The experimental methodology involved pigment extraction, preparation of solutions in different solvents (acetone, ethanol, isopropanol, dimethyl sulfoxide, and chloroform), and spectrophotometric analysis in the 200–800 nm range. The results demonstrated that solubilization efficiency depends directly on the compatibility between solvent polarity and the molecular structure of the dye. Bixin, predominantly nonpolar, showed greater solubility in dimethyl sulfoxide and chloroform, whereas norbixin, more polar due to the presence of carboxylic groups, exhibited better performance in solvents of intermediate polarity, such as acetone.The UV–Vis

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[1] Master's degree in Materials Engineering from the Federal Institute of Piauí (IFPI), teacher at the State Department of Education of Piauí (SEDUC-PI, Teresina-PI, Brazil
E-mail: drmarcoaureliocoutinho@gmail.com
Lattes: http://lattes.cnpq.br/6930641108982221
ORCID: https://orcid.org/0000-0002-6703-2854

[2] PhD in Chemistry in the field of Inorganic Chemistry from the Federal University of Minas Gerais
Professor at the Federal University of Piauí
E-mail: mmoura@ufpi.edu.br
Lattes: http://lattes.cnpq.br/0624348825872858

[3] PhD in Chemistry in the field of Inorganic Chemistry from the Federal University of Minas Gerais
Professor at the Federal University of Piauí
Lattes: http://lattes.cnpq.br/8314351279360798

[4] PhD and Master's degree in Inorganic Chemistry from the Federal University of Piauí (UFPI), Professor and Coordinator of Research, Graduate Studies, and Innovation at the Federal Institute of Education, Science and Technology of Piauí (IFPI), Brazil
E-mail: leannesilva@ifpi.edu.br
Lattes: http://lattes.cnpq.br/6699468921628794
ORCID: https://orcid.org/0000-0003-3574-9313

spectra revealed characteristic bands associated with π → π* transitions, as well as solvatochromic effects, indicating that the chemical environment significantly influences the energy of electronic transitions. Solutions were prepared under the same conditions using the solvents acetone, ethanol, isopropanol, dimethyl sulfoxide, and chloroform. Absorbance was measured as a function of wavelength for each solution through UV–Vis spectral analysis in the 800–200 nm range. The data obtained from the UV–Vis analyses confirmed the higher effectiveness of dimethyl sulfoxide (DMSO) in dissolving bixin and of acetone for norbixin compared to the other solvents.

Keywords: Bixin, Norbixin, Solubility, UV–Visible.

INTRODUCTION

Dyes are organic or inorganic substances responsible for imparting color to a wide variety of materials, of both biological and synthetic origin, including foods, fabrics, paper, and beverages. According to their origin, these compounds may be classified as natural, obtained from plant, animal, or mineral sources, and synthetic, produced industrially from chemical precursors. The use of dyes dates back to Antiquity, with indigo being one of the first pigments widely used by humankind. Currently, there is growing interest in natural dyes due to their environmental advantages, such as biodegradability, lower toxicity, and potential application in sustainable systems (Yadav et al., 2022; Santos et al., 2021).

Annatto (*Bixa orellana* L.) is one of the main sources of natural dyes, containing carotenoid pigments responsible for the characteristic yellow-orange coloration, extracted from its seeds. Native to Central and South America, this plant has great industrial relevance and is widely used in the food, cosmetic, and pharmaceutical sectors. The main compounds present are bixin, which is lipophilic in character, and norbixin, its water-soluble form, whose versatility broadens its technological applications. It is estimated that annatto derivatives represent a significant share of the natural dyes employed in the food industry, consolidating their economic and scientific importance (Silva et al., 2023; Ribeiro et al., 2022).

Several recent studies have investigated the physicochemical, structural, and functional properties of bixin and norbixin, with emphasis on their antioxidant activities and stability under different conditions. However, the solubility of these compounds remains a critical aspect, since it directly influences their extraction, processing, and industrial application. Solubility, defined as the maximum amount of solute that can be dissolved in a solvent under specific conditions of temperature and pressure, is an essential thermodynamic parameter for the development of efficient processes (Souza et al., 2021; Yadav et al., 2022).

For the determination of solubility and quantification of dyes, modern analytical techniques have been widely employed, especially high-performance liquid chromatography (HPLC) and ultraviolet-visible (UV-Vis) spectrophotometry. UV-Vis spectrophotometry is based on the absorption of electromagnetic radiation by molecules at specific wavelengths, generally in the range of 200 to 800 nm, enabling the acquisition of characteristic spectra and the quantification of compounds in solution (Skoog et al., 2021; Harris, 2020).

The relationship between absorbance and the concentration of the absorbing species is described by the Lambert-Beer Law, widely used in quantitative analyses. This relationship may be expressed by the equation:

$$A = \varepsilon b c$$

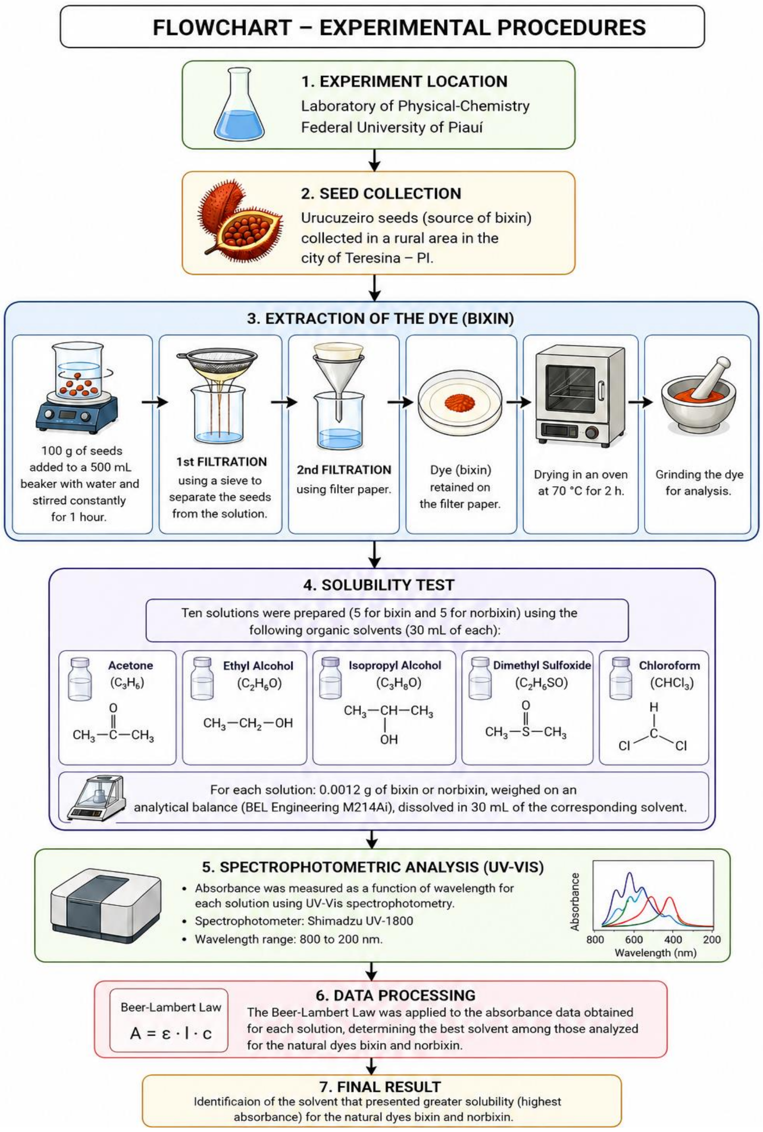
where A corresponds to absorbance, ε to the molar absorptivity coefficient, b to the optical path length of the cell, and c to the concentration of the solution. This law establishes that absorbance is directly proportional to the concentration of the absorbing species, provided that the radiation is monochromatic and the solution sufficiently dilute, and is widely applied in the quantitative determination of compounds in different solvents (Harris, 2020; Skoog et al., 2021).

Thus, the present study aims to evaluate the efficiency of different organic solvents — chloroform, ethanol, isopropanol, acetone, and dimethyl sulfoxide — in dissolving the natural dyes bixin and norbixin, through the determination of their concentrations using UV-Vis spectroscopy associated with the Lambert-Beer Law, contributing to the understanding of the factors that influence the solubility of these compounds and their application in industrial processes.

METHODOLOGY

Flowchart

Experimental procedures.



1. EXPERIMENT LOCATION
Laboratory of Physical-Chemistry
Federal University of Piauí

2. SEED COLLECTION
Urucuzeiro seeds (source of bixin) collected in a rural area in the city of Teresina – PI.

3. EXTRACTION OF THE DYE (BIXIN)
100 g of seeds added to a 500 mL beaker with water and stirred constantly for 1 hour.
1st FILTRATION using a sieve to separate the seeds from the solution.
2nd FILTRATION using filter paper.
Dye (bixin) retained on the filter paper.
Drying in an oven at 70 °C for 2 h.
Grinding the dye for analysis.

4. SOLUBILITY TEST
Ten solutions were prepared (5 for bixin and 5 for norbixin) using the following organic solvents (30 mL of each):
For each solution: 0.0012 g of bixin or norbixin, weighed on an analytical balance (BEL Engineering M214Ai), dissolved in 30 mL of the corresponding solvent.

5. SPECTROPHOTOMETRIC ANALYSIS (UV-VIS)
- Absorbance was measured as a function of wavelength for each solution using UV-Vis spectrophotometry.
- Spectrophotometer: Shimadzu UV-1800
- Wavelength range: 800 to 200 nm.

6. DATA PROCESSING
The Beer-Lambert Law was applied to the absorbance data obtained for each solution, determining the best solvent among those analyzed for the natural dyes bixin and norbixin.

7. FINAL RESULT
Identificaion of the solvent that presented greater solubility (highest absorbance) for the natural dyes bixin and norbixin.

Source: Author’s own work.

All experimental procedures were carried out at the Physical Chemistry Laboratory of the Federal University of Piauí (UFPI), Brazil. Annatto seeds (Bixa orellana L.) were obtained from the rural area of Teresina (PI, Brazil) and used as raw material for bixin extraction. Before the analyses, the seeds were visually inspected to remove impurities and stored under controlled conditions (25 ± 2 °C, protected from light and moisture), in order to minimize carotenoid degradation (Silva et al., 2023; Ribeiro et al., 2022).

EXTRACTION AND SAMPLE PREPARATION

A mass of 100.00 ± 0.01 g of annatto seeds was subjected to aqueous extraction using 500.0 ± 0.1 mL of distilled water under continuous stirring (400 rpm) for 60 min at room temperature (25 ± 1 °C). The mixture was subsequently filtered in two stages: initially through a stainless-steel sieve to remove coarse solid residues, followed by vacuum filtration using qualitative filter paper (Whatman No. 1). The retained material was dried in an oven with air circulation at 70 ± 1 °C for 2 h, until constant mass, ensuring moisture removal without causing significant degradation of thermosensitive carotenoids (Souza et al., 2021). After drying, the sample was macerated in a mortar and pestle until a homogeneous powder was obtained.

SOLUBILITY TESTS

Solubility tests were performed using five organic solvents with different polarities: acetone, ethanol, isopropanol, dimethyl sulfoxide (DMSO), and chloroform. For each solvent, bixin and norbixin solutions were prepared separately. Carefully weighed samples (0.0012 ± 0.0001 g) were dissolved in 30.00 ± 0.05 mL of solvent under magnetic stirring for 30 min, in order to ensure maximum solubilization. All experiments were performed in triplicate (n = 3), ensuring reproducibility and statistical reliability.

UV–VIS SPECTROPHOTOMETRIC ANALYSIS

Absorption spectra were obtained using a Shimadzu UV-1800 spectrophotometer, in the range of 200 to 800 nm, with a spectral resolution of 1 nm and quartz cuvettes with an optical path length of 1.00 cm. The wavelength of maximum absorption (λ_max) was determined for each solution and used in the quantitative analysis. Blank solutions of the respective solvents were employed for baseline correction. Instrument calibration was verified prior to measurements, ensuring analytical accuracy (Skoog et al., 2021; Harris, 2020).

The quantification of the dissolved pigments was based on the Lambert-Beer Law: $A = \varepsilon bc$

where A represents absorbance, ε the molar absorptivity coefficient ($L\cdot mol^{-1}\cdot cm^{-1}$), b the optical path length (cm), and c the concentration ($mol\cdot L^{-1}$). All measurements were performed within the linear range of the method ($A < 1.0$), ensuring compliance with the validity conditions of the Lambert-Beer Law (HARRIS, 2020).

STATISTICAL ANALYSIS

The results were expressed as mean ± standard deviation (SD). Statistical differences among the solvents were evaluated by one-way analysis of variance (ANOVA), followed by Tukey's test, adopting a 95% significance level ($p < 0.05$). Data normality and homogeneity of variances were verified prior to the application of ANOVA. Statistical analyses were performed using OriginPro 2023 software (OriginLab Corporation, USA).

EXPERIMENTAL UNCERTAINTY

Uncertainty contributions were estimated considering mass measurements (analytical balance precision ±0.0001 g), volumetric measurements (±0.05 mL), and instrumental variability (UV–Vis repeatability). The combined uncertainty was propagated according to standardized analytical procedures, ensuring the reliability and robustness of the results obtained.

RESULTS AND DISCUSSION

Table 1 presents the absorbance values of bixin as a function of wavelength in the different solvents evaluated by UV-Vis spectroscopy. The spectra obtained show characteristic bands associated with $\pi \rightarrow \pi^*$ electronic transitions, typical of highly conjugated systems such as carotenoids (Yadav et al., 2022; Silva et al., 2023).

Table 1

Results obtained from the UV-Vis spectra of bixin.

Solvent	Absorbance	Wavelength λ (nm)
Dimethyl sulfoxide	0,801	469
Chloroform	0,675	449
Acetone	0,433	455
Isopropyl alcohol	0,165	556
Ethanol	0,045	458

The higher absorbance observed for dimethyl sulfoxide (DMSO) indicates its superior efficiency in solubilizing bixin. This behavior may be explained by the dipolar aprotic character of the solvent, which has a high dielectric constant and high solvation capacity, allowing simultaneous interactions with polar regions (carbonyl groups) and nonpolar regions (conjugated chain) of the molecule (Ribeiro et al., 2022).

From a molecular standpoint, absorption in the visible region is associated with electronic excitation from the highest-energy occupied molecular orbital (HOMO) to the lowest-energy unoccupied molecular orbital (LUMO), characteristic of extensive conjugated π systems. The position of the absorption maxima (λ_max) may undergo shifts depending on the solvent (solvatochromic effect), reflecting changes in the relative stabilization of the ground and excited states (Souza et al., 2021).

Chloroform also showed high efficiency, which is associated with the lipophilic nature of bixin. Dispersion interactions (van der Waals forces) between the nonpolar solvent and the hydrocarbon chain favor solubilization. On the other hand, protic solvents such as ethanol and isopropanol showed lower efficiency, due to their lower compatibility with the predominantly nonpolar structure of bixin (Santos et al., 2021).

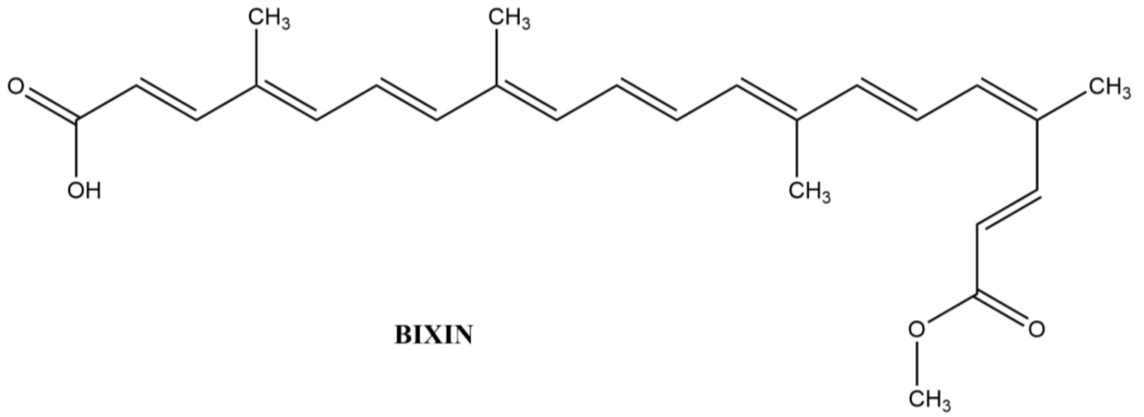
Statistical analysis by ANOVA revealed significant differences among the solvents ($p < 0.05$), indicating that solvent choice exerts a direct influence on bixin solubility. Tukey's test showed that DMSO differs statistically from the protic solvents, confirming its superiority as a dissolution medium.

The relationship between absorbance and concentration follows the Lambert-Beer Law: $A = \varepsilon bc$

Thus, higher absorbance values reflect a higher concentration of dissolved bixin, validating the experimental results.

In the graph of **Figure 1**, the absorption spectra in the UV-Vis region (800 to 200 nm) of bixin pigments in the different solvents used in the study are shown.

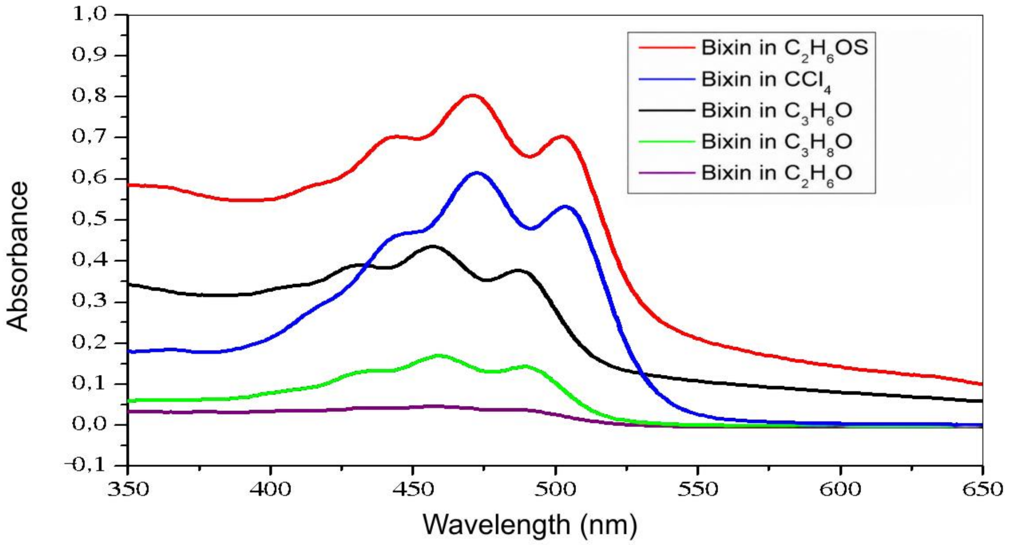
Bixin molecule



Source: Author's own work.

Figure 1

UV-Vis absorption spectrum of bixin in different solvents.



Source: Author.

The UV-Vis absorption spectra of bixin in different organic solvents show a broad and structured band in the 430–520 nm region, attributed to π → π* electronic transitions associated with the conjugated system of the molecule. This behavior is typical of carotenoids and apocarotenoids, whose optical properties are governed by the length of the conjugated chain and by interaction with the medium (Britton, 1995; Rodríguez-Amaya, 2001). The presence of poorly resolved vibronic structures indicates vibronic coupling, frequently observed in these highly conjugated systems.

The dependence of the intensity and position of the absorption maximum (λ_max) on the solvent evidences a significant solvatochromic effect. In highly polar solvents, such as dimethyl sulfoxide, a marked increase in absorbance is observed, indicating greater solubilization of bixin and a higher effective concentration of the chromophore in solution. In addition, polar aprotic solvents are known to stabilize excited states with higher dipole moments, favoring the intensity of electronic transitions (Reichardt; Welton, 2011). This behavior suggests that the excited state of bixin has greater polarity than the ground state, resulting in greater differential stabilization and a possible bathochromic shift.

In solvents of intermediate polarity, such as ethanol and isopropanol, the spectra show moderate intensity and good band definition. In these cases, specific interactions, such as hydrogen bonds between the solvent and the functional groups of bixin (ester and carboxylic acid), may influence the electronic distribution of the molecule, promoting small spectral variations (Silva et al., 2005). However, such interactions do not fully compensate for the lower solvation capacity compared with highly polar aprotic solvents.

On the other hand, less polar solvents, such as chloroform, show lower absorbance intensity and less defined bands. This behavior may be attributed to the low solubility of bixin and the possible formation of molecular aggregates in solution. Aggregation is a recurrent phenomenon in carotenoids and may lead to decreased absorbance and spectral changes, including band broadening and hypsochromic or bathochromic shifts depending on the type of intermolecular interaction (Hynninen, 1991; Delgado-Vargas et al., 2000).

The variations observed in λ_max values corroborate the influence of the solvent medium on the energy of the electronic transition. In general, bathochromic shifts in more polar solvents indicate greater stabilization of the excited state relative to the ground state, whereas hypsochromic shifts in less polar solvents reflect weaker solute–solvent interaction (Reichardt; Welton, 2011). This behavior is consistent with π-conjugated systems, in which electronic redistribution in the excited state is highly sensitive to the chemical environment.

Additionally, considering the Lambert-Beer Law ($A = \varepsilon \cdot l \cdot C$), the differences in absorbance among the solvents are not associated exclusively with the molar absorptivity coefficient (ε), but also with the effective concentration of bixin in solution, directly related to its solubility. Thus, solvents that promote better dissolution result in higher absorbance values, highlighting the importance of controlling the medium in spectrophotometric analyses (Skoog et al., 2014).

In general, the results demonstrate that the spectroscopic properties of bixin are strongly modulated by solvent polarity, intermolecular interactions, and the degree of solute dispersion. These factors are determinant both for the interpretation of spectroscopic data and for technological applications involving this natural dye, such as in food, pharmaceutical, and cosmetic systems.

For norbixin, the results presented in Table 2 show distinct behavior, due to the presence of carboxylic groups that confer greater polarity to the molecule.

Table 2

Results obtained from the UV-Vis spectra of norbixin.

Solvent	Absorbance	Wavelength λ (nm)
Acetone	2,215	486
Ethanol	0,873	487
Isopropyl alcohol	0,799	486
Dimethyl sulfoxide	1,267	500
Chloroform	0,104	472

Acetone showed the highest solubilization efficiency, which may be explained by its intermediate polarity and by the presence of the carbonyl group, capable of establishing dipole–dipole interactions and hydrogen bonding with the carboxylic groups of norbixin. This result is in agreement with recent studies that highlight the influence of solvent polarity on the solubilization of functionalized carotenoids (Silva et al., 2023; Yadav et al., 2022).

DMSO also showed high efficiency, reinforcing its universal solvation capacity. In contrast, chloroform showed low solubility, evidencing that nonpolar solvents are not suitable for compounds with greater polarity.

Statistical analysis (ANOVA) confirmed significant differences among the solvents ($p < 0.05$), with acetone being statistically superior to the other solvents tested.

From the perspective of Hansen solubility parameters, the greater efficiency of acetone may be attributed to the balance among dispersive, polar, and hydrogen-bonding components, resulting in greater compatibility with the amphiphilic structure of norbixin (Ribeiro et al., 2022).

In general, the results show that pigment solubility is directly related to the interaction between the structural properties of the dyes and the physicochemical parameters of the solvents. Bixin, predominantly nonpolar, has greater affinity for nonpolar or aprotic solvents, whereas norbixin, being more polar, is better solubilized by solvents of intermediate to high polarity.

These findings are relevant for the development of more efficient and sustainable industrial processes, allowing the rational selection of solvents for the extraction and application of natural dyes (Santos et al., 2021; Silva et al., 2023).

In the graph of **Figure 2**, the absorption spectra in the UV-Vis region (800 to 200 nm) of norbixin in the different solvents used in the study are shown.

Norbixin molecule

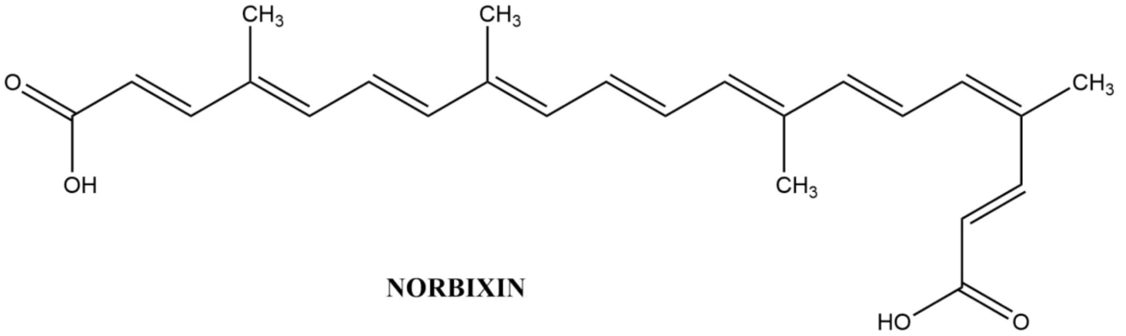
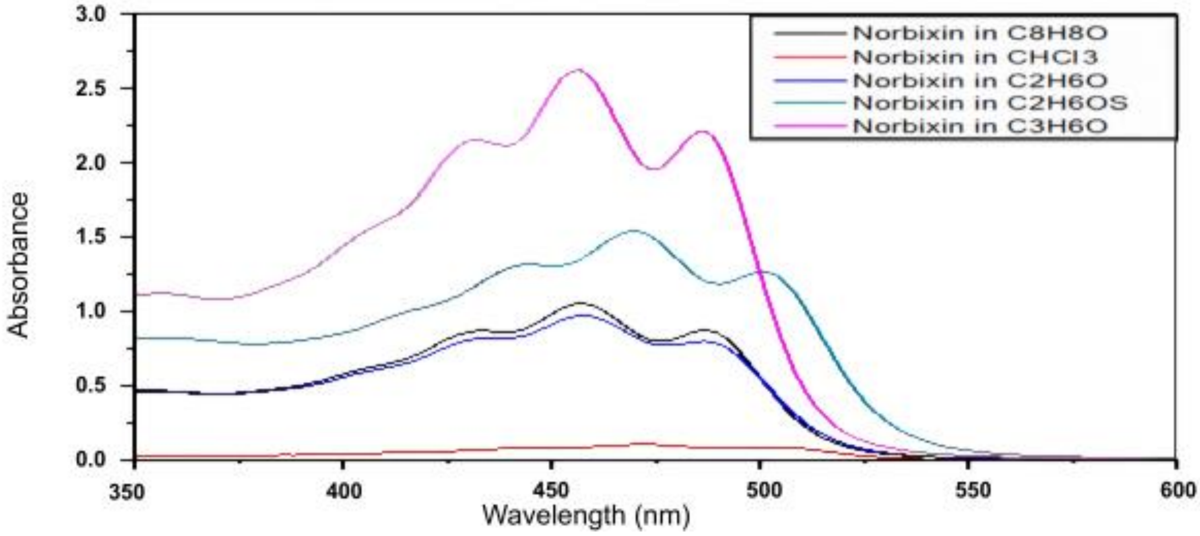


Figure 2

UV-Vis absorption spectrum of norbixin in different solvents



The UV-Vis absorption spectra of norbixin in different organic solvents show an intense and broad band in the region of approximately 430–520 nm, characteristic of π → π* electronic transitions associated with the extended conjugated system of the molecule. This behavior is typical of highly conjugated compounds, in which the extent of the π system determines the position of the absorption band and its spectral intensity (Mennucci et al., 2024).

The dependence of the intensity and position of λ_max as a function of the solvent evidences a pronounced solvatochromic effect. In the solvent of higher polarity, a significant increase in absorbance and a slight bathochromic shift are observed, indicating greater stabilization of the excited state relative to the ground state. This phenomenon is related to the reduction of the HOMO–LUMO energy gap in more polar environments, which favors the electronic transition (Sıdır & Sıdır, 2025). This behavior confirms that the excited state of norbixin has a greater polar character and is more sensitive to interaction with the solvent medium.

In solvents of intermediate polarity, such as alcohols, the spectra show moderate intensity and well-defined profiles. In these systems, in addition to the dielectric effect, specific interactions such as hydrogen bonding influence the electronic distribution of the chromophore, resulting in small variations in the position and intensity of the absorption bands (Mennucci et al., 2024). These interactions promote partial stabilization of the excited state, although less efficiently than in highly polar aprotic solvents.

On the other hand, less polar solvents show lower absorbance intensity and less defined bands, which may be attributed to the lower solubility of norbixin and the possible formation of molecular aggregates. Aggregation in π-conjugated systems reduces effective absorbance and may cause broadening of spectral bands or hypsochromic shifts, due to intermolecular interaction among chromophores (Pulvinic et al., 2023). This phenomenon is particularly relevant for carotenoids and structurally related compounds, whose spectral stability depends strongly on the state of molecular dispersion.

The variations observed in λ_max values among the different solvents corroborate the influence of the chemical environment on the energy of electronic transitions. In general, more polar solvents promote bathochromic shifts, whereas less polar solvents tend to induce hypsochromic shifts, reflecting the lower stabilization of the excited state (Recent Advances in Solvatochromism, 2024). This behavior is consistent with systems in which significant electronic redistribution occurs after excitation.

Additionally, the high absorbance values observed in certain solvents indicate high solubility of norbixin and a strong molar absorption coefficient. According to the Lambert-Beer Law, these variations do not reflect only intrinsic differences in the ε coefficient, but also changes in the effective concentration of the solute in solution, directly influenced by solute–solvent interaction (Sıdır & Sıdır, 2025). Thus, solvents that promote greater solvation result in higher spectral intensity.

In general, the results demonstrate that the spectroscopic properties of norbixin are highly dependent on the solvent medium, being modulated by polarity, intermolecular interactions, and degree of aggregation. The high sensitivity of norbixin to the chemical environment is directly related to its more polar nature compared with bixin, which amplifies solvation effects. These findings are relevant for analytical, food, and pharmaceutical applications, in which solvent choice directly influences the stability, color, and performance of the dye (Mennucci et al., 2024; Recent Advances in Solvatochromism, 2024).

CONCLUSION

This study demonstrated that the efficiency of organic solvents in solubilizing natural pigments is strongly governed by the interaction between solvent polarity and the molecular structure of the solute. UV–Vis spectroscopic analysis, combined with the Lambert-Beer Law and statistical evaluation (ANOVA, $p < 0.05$), enabled a reliable comparison of solvent performance.

Dimethyl sulfoxide (DMSO) showed the highest efficiency in solubilizing bixin, which may be attributed to its dipolar aprotic nature and high solvation capacity, allowing effective interactions with polar and nonpolar regions of the molecule. In contrast, acetone was identified as the most suitable solvent for norbixin, due to its intermediate polarity and its ability to promote dipole–dipole interactions and hydrogen bonding with the carboxylic groups present in the pigment structure.

The solvatochromic shifts observed in λ_max evidence the influence of the solvent medium on the electronic transitions ($\pi \rightarrow \pi^*$) of these carotenoids, reinforcing the importance of solvent choice in spectroscopic and industrial applications.

From an applied perspective, these results provide relevant information for optimizing extraction and formulation processes involving natural dyes. Bixin and norbixin stand out as promising alternatives to synthetic dyes, due to their low toxicity, biodegradability, availability, and relatively low cost, meeting growing demands for more sustainable and environmentally friendly technologies.

In general, this work contributes to a better understanding of solute–solvent interactions in carotenoid systems and supports the rational selection of solvents for industrial applications of annatto-derived pigments.

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