http://dx.doi.org/10.21577/0103-5053.20190150

J. Braz. Chem. Soc., Vol. 31, No. 1, 162-169, 2020
Printed in Brazil - ©2020 Sociedade Brasileira de Química

Article

Selective Inner-Filter on the Fluorescence Response of Chlorophyll and Pheophytin Molecules Extracted from *Caesalpinia echinata* Leaves

Jefferson M. S. Lopes,*[,a] Sanclayton G. C. Moreira[a] and Newton M. Barbosa Neto[a,b]

[a]*Programa de Pós-Graduação em Física, Instituto de Ciências Exatas e Naturais, Universidade Federal do Pará, 66075-110 Belém-PA, Brazil*

[b]*Programa de Pós-Graduação em Química, Instituto de Ciências Exatas e Naturais, Universidade Federal do Pará, 66075-110 Belém-PA, Brazil*

Photophysical processes in multicomponent solutions composed by natural pigments, extracted from *Caesalpinia echinata* (Brazil-wood) leaves, are investigated. Beacuse of the main fluorescent molecules in these solutions are the chlorophyll a, chlorophyll b and pheophytin a, all features are discussed regarding these three pigments. It is observed that the fluorescence bands of these macrocyclic molecules, located around ca. 673 nm, are progressively quenched due to the increase of the solutions' concentration. Due to a significant overlapping between their fluorescence and absorption Q-bands, it is concluded that the inner-filter effect causes such quenching. Through analysis of steady-state and time-resolved fluorescence, it is verified that the inner filter acts more effectively on the emission assigned to chlorophyll a. Invoking reported extinction coefficients for these pigments in their isolated forms, we can give a qualitative interpretation of such selectivity considering the effectiveness of the overlapping between the absorption and fluorescence bands.

Keywords: inner-filter, fluorescence spectroscopy, multicomponent solutions, chlorophylls, deconvolution

Introduction

Flora is a vast source of natural pigments, most of them with great potential for applications in different areas such as photovoltaic and health sciences.[1-3] Since these molecules are environment-friendly when compared to other artificial pigments, they stand out as possible substituents of well-established materials. Therefore, investigations on their structure, interaction mechanisms, ground, and excited state properties, and others are crucial to inspire and improve the potential uses of such molecules.

Various methods of extraction of pigments from plants have been developed,[4,5] however, until nowadays, the most straightforward and cheapest procedure is based on the emulsion of leaves in organic solvents.[6] This method, nonetheless, lacks selectivity and is very dependent on leaf-solvent interactions[7] and the content of pigments in each leaf species.[8,9] Such aspects imply that a great variety of pigments is released together in the solvent, resulting in multicomponent solutions.[6] According to the literature, the main classes of pigments available in leaves are the carotenoids and chlorophylls, both of them receiving considerable attention from the scientific community due to their photophysical,[10,11] photochemical,[11,12] and optical properties.[13-15]

Briefly, the carotenoids are pigments composed by a linear conjugated chain of around forty carbon atoms that due to their high conjugation length, present absorption and fluorescence bands spread over the near-UV and visible spectral region.[11,16] The chlorophylls are tetrapyrrole macrocyclic rings stabilized at its central position by a magnesium ion (Mg^{2+}), being the ring decorated with outlying substituents (e.g., phytol). These pigments, which are classified as natural metalloporphyrins, exist in six different structures known as chlorophylls a, b, c_1, c_2, d, and f. The two first types are the most commonly found in plants.[17] The substitution of the central metallic ion of chlorophyll by two hydrogen atoms results in the free base type structure, named pheophytin, which is also commonly found in vegetables.[10,17] Qualitatively, the photophysical properties of chlorophylls and pheophytins are explained by the four-orbital model proposed by Gouterman.[10,18] According to this model, their absorption spectrum is described by transitions from the ground state to the first

*e-mail: lopesjefferson01@yahoo.com.br

(Q-band) and second (Soret-band) excited states. Their luminescence is understood regarding the relaxation process from their first excited state, which results in significant fluorescence bands in the red portion of the spectrum.[10]

At first glance, the non-selectivity of the emulsion method for extraction of pigments might seem an undesirable issue. However, the obtained molecules can synergistically interact one each other, giving rise to solutions with unique features, regarding their photophysical and photochemical properties. Here, we investigate the fluorescence emitted by multicomponent solutions composed of pigments extracted from fresh *Caesalpinia echinata* (Brazil-wood) leaves emulsified in methanol. We observed that the inner-filter affects significantly the fluorescence bands located around ca. 673 nm as the concentration of extracted chlorophyll a (Chl a), chlorophyll b (Chl b) and pheophytin a (Pheo a) molecules increase. Using Voigt deconvolution of the steady-state spectrum and time-resolved fluorescence signatures, we verified that this effect is selective, acting mostly on the emission assigned to Chl a. This behavior is not straightforward since the fluorescence features of Chl b and Pheo a are expected to be affected too. We propose a qualitative model to explain this selectivity on the observed inner-filter.

Experimental

The pigments were extracted from fresh leaves of Brazil-wood (*Caesalpinia echinata*) at room temperature (ca. 23 °C), by the emulsion of 1.0 g of cut leaves in 35 mL of methanol (MeOH), used as received from Sigma-Aldrich (Saint Louis, USA). Before extraction of the pigments, the leaves were washed with distilled water and subsequently dried. The leaves were cut in strips of similar shapes and area of around 0.5 cm^2. During the extraction, the solution was stirred by a magnetic bar to ensure the homogeneity of the solution.

Stepwise extraction was performed during 240 min, where 12 aliquots of 2.0 mL were removed at every 20 min from the first bath and stored in a vial filled with extra 2 mL of solvent. This additional dissolution was adopted to avoid saturation problems during the acquisition of the absorption spectra. Before storing the aliquots into the vial, the samples were filtered by a filter paper method aiming to discard undesired solid residues.

UV-Vis absorption spectra were acquired in a Jasco spectrophotometer, model V-670. Steady-state fluorescence measurements were performed exciting the samples with a continuous wave (CW) laser (excitation wavelength at 473 nm) and detecting the photoluminescence signal in a 90° configuration with a portable spectrophotometer from Ocean Optics, model USB 2000. Time-resolved fluorescence signal was acquired using a time-correlated single photon counting system from Horiba, model Delta Flex, with 27 ps of temporal resolution. The excitation source employed was a pulsed light emitting diode (LED) with 8 MHz of repetition rate and excitation wavelength at 352 nm. All decays were probed at 673 nm. The spectroscopic measurements were performed at room temperature (ca. 23 °C), and the solutions were placed in a quartz cuvette with a 1 cm pathlength and four polished windows.

Results and Discussion

Absorption spectroscopy: second-derivative analysis, spectral deconvolution, and assignments of pigments

In Figure 1, we present the absorption spectra of the twelve aliquots obtained along 2 h of an emulsion prepared with leaves in methanol. From the spectra, it is verified that a great variety of different pigments is released in the solutions. A set of bands are observed in the spectral region below 400 nm and are attributed to electronic transitions of terpenoids[19] and flavonoids.[20,21] In fact, according to Bastos *et al*.,[22] *Caesalpinia echinata* leaves contain a high amount of these two classes of phenolic compounds. Unfortunately, we were not able to distinguish these molecules with the optical signatures presented here, and an accurate assignment is still elusive. Therefore, we focus our analysis on the bands located in the region of 400-750 nm, related to chlorophylls and carotenoids.[17]

Due to the multicomponent character of the solutions, formed along the extraction, intermolecular interactions can be favored implying in the rise of novel bands in the spectrum. Then, the assignment of the absorption bands of the mixture must be performed carefully. The normalization of the absorption spectra by their respective absorbance values at 412 nm reveals that the bands in the UV-Vis spectral region are not modified along the extraction, indicating that new species are not formed, see Figure S1 in the Supplementary Information (SI) section.

Considering previously reported assignments,[10,17] in Figure 1 we can identify the Soret bands of Pheo a, Chl a and Chl b at 412, 437, and 469 nm, respectively. A minor contribution coming from pheophytin b (Pheo b) is expected to occur between the Soret bands of Chl b and Pheo b,[17] however, no signal of this band is detected. Since features assigned to other structures, such as pheophorbide and chlorophyllins are not observed in our solutions, we assumed that the only chlorophyll-like molecules obtained along the extraction, with significant concentration, are

these three mentioned pigments. From the assignments of the Soret bands (412 nm for Pheo a, 437 nm for Chl a and 469 nm for Chl b), it is observed that the rates of extraction for the pigments are unchanged, presenting Pheo a the more significant rate (0.01 min^{-1}), followed by Chl a (0.008 min^{-1}) and Chl b (0.006 min^{-1}), see Figure S2 (SI).

Four bands, centered respectively at 507, 536, 610, and 663 nm are observed in the chlorophylls' Q-band spectral region. The attributions of these bands are not straightforward because some transitions in the Q-band of both chlorophylls considered and Pheo a can be overlapped between each other in the interval of 600-750 nm.[17]

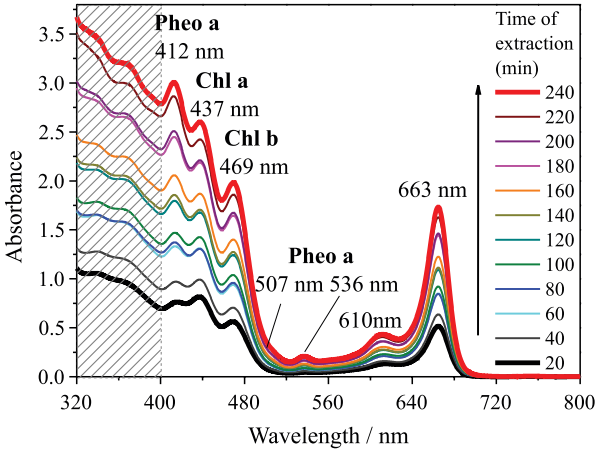

Figure 1. Absorbance spectra of the aliquots taken at different times from the emulsion of *Caesalpinia echinata* (Brazil-wood) leaves in methanol. Attributions of the Soret bands of Pheo a (412 nm), Chl a (437 nm) and Chl b (469 nm), as well as some of the unambiguous Q-bands of Pheo a (at 507 and 536 nm), are emphasized. The dashed area below 400 nm indicates the region related to the phenolic compounds.

According to the Gouterman's model,[10] the absorption bands of cyclic tetrapyrroles are due to transition dipoles oriented in two perpendicular directions of the macrocyclic ring. When a metal ion is placed in the ring's central positions, the transitions in both directions become degenerate.[10,18] This is the case for chlorophylls, where only one electronic transition and its vibronic counterpart form their Q-band.[10] However, when two hydrogen atoms substitute the Mg^{2+} ion, during the formation of pheophytins, the degeneracy of the electronic transitions is broken and the Q-band displays transitions occurring in the two distinct directions of the macrocyclic ring.[10] Following these considerations and the assignments previously proposed,[17,23] the main Q-bands of Chl a and Chl b dissolved in methanol, are expect to be located in ca. 663 nm and ca. 649 nm, respectively. Both electronic bands might present their respective vibronic bands at around 610 nm and 605 nm.[17] In the case of Pheo a (dissolved in methanol), four bands have been previously reported[17] at around 507, 536, 610 and 665 nm. These attributions confirm that from 600 to 750 nm the bands related to all these three pigments might be superposed between each other in our spectra.

Aiming to obtain a more accurate assignment of the absorption bands, we performed second-derivative analysis and deconvolution with Voigt functions on the absorption spectrum of the last aliquot, i.e., obtained after 240 min of an emulsion of leaves in methanol. It is worth to mention that Voigt functions are employed because their more general characteristics when compared with Gaussian and Lorentzian functions.[24] As shown in Figure 2, the Voigt functions centered at 437, 610 and 663 nm, present correspondence with the inflection points of the second-derivative spectrum, depicted in the bottom panel of Figure 2 and are assigned to Chl a. For Chl b, the assigned curves are centered at 469 and 649 nm. Our method does not allow us to resolve the vibronic Q-band of this molecule, at around 605 nm. Because of this, we are assuming that this feature is superposed with the Chl a's Voigt curve at 610 nm. It is important to highlight that the existence of the transition at 649 nm is not directly indicated by the second-derivative analysis since there is no inflection point at this wavelength. However, we infer its existence considering the asymmetric shape of the peak at 663 nm (for better visualization, see Figure S3, SI). Considering that Voigt distributions are symmetric around their central positions, the occurrence of asymmetry in its second-derivative peaks indicates the existence of hidden peaks, in other words, two bands with very close centers but with different magnitudes. Contributions of Pheo a are related to the Voigt curves centered at 412, 507, 536 and 665 nm. The vibronic progression of the electronic transition at 665 nm in the Q-band is expected to be located at around 610 nm and so is assumed to be overlapped with the Chl a's transition at 610 nm. Also, since the second-derivative peak at ca. 469 nm is very asymmetric (for a better visualization see Figure S3, SI), we propose the existence of an extra Voigt curve centered slightly higher than this value. As shown in Figure 2, by deconvolution analysis we obtain a Voigt curve centered at 484 nm. Following the literature, this feature can be assigned to the absorption band of some carotenoid related molecule.[16] Unfortunately, by absorption spectroscopy we are not able to elucidate exactly which specific carotenoid this curve is related.

Steady-state and time-resolved fluorescence of chlorophylls and pheophytin a molecules: description of the selective inner-filter

Although we have extracted a great variety of molecules from *Caesalpinia echinata* leaves, the main contribution in

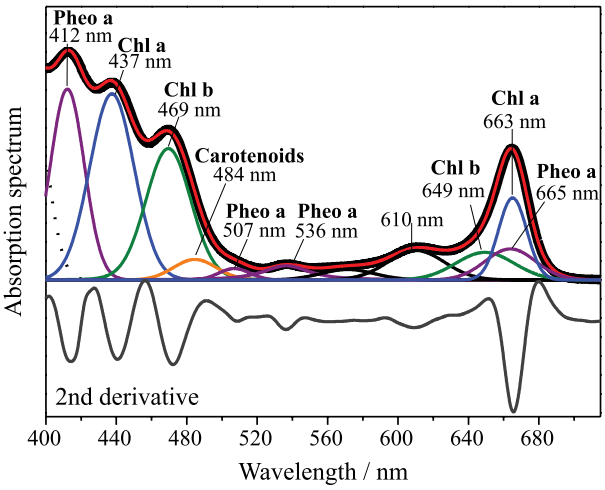

Figure 2. Voigt deconvolution (solid red line) and second derivative analysis (solid grey line at the bottom panel) applied to the absorption spectrum (solid black line) of the aliquot obtained after 240 min of an emulsion of leaves in methanol. The Voigt curves are attributed to the transitions of Pheo a (solid purple lines), Chl a (solid blue lines) and Chl b (solid green lines). The curve at 610 nm is interpreted as a superposition of the vibronic Q-bands of these three molecules. The curve at 484 nm (solid orange line) is assigned to carotenoids released in the solution.

the fluorescence spectrum of the aliquots comes from the chlorophylls (a and b) and Pheo a, as shown in Figure 3. This occurs because the fluorescence quantum yield of these macrocyclic structures, although small,[10,17,25] ca. 10^{-2}, is still much higher when compared to other obtained luminescent molecules such as carotenoids,[25,26] which present values of the order of 10^{-4}. As shown in Figure S4 (SI), we verified just minor contributions due to carotenoids, located at around 515 and 562 nm, in the fluorescence spectrum of the most concentrated aliquot. No accurate assignment is obtained for the band at ca. 515 nm but, following previous reports, the band at ca. 562 nm can be due to β-carotene molecules.[25,26] Because the carotenoid's signal is very low, we base all discussions in this work on the fluorescence features of Chl a, Chl b and Pheo a.

In Figure 3a, we compare the normalized absorption and fluorescence spectra (excitation at 473 nm) of the aliquot obtained after 20 min of an emulsion of leaves in methanol. A significant overlapping between the Q-absorption and the fluorescence bands of chlorophylls and Pheo a is observed at around 673 nm. In Figure 3b, we show the evolution of the normalized fluorescence spectrum along the extraction period. It is observed that the fluorescence bands, located in the region where occurs the overlap with absorbance spectrum (ca. 673 nm), are considerably quenched as the amount of extracted molecules increases. Moreover, an apparent relative increase of the bands located around ca. 720 nm as well as a redshift from 673 to 683 nm for the maxima of the fluorescence spectrum is also noted. Since the fluorescence bands become increasingly affected by the extraction time, i.e., by the increase in the concentration of pigments in the aliquots, we attribute all these behaviors to the inner-filter effect, which are triggered by the increase in the absorbance values at the overlapped region. As we will discuss later, a complex scenario takes place in our multicomponent aliquots, since the three emitters identified here act as quenchers for one each other as well as to themselves through the reabsorption mechanism.[27-30]

In order to explore in more details the roles of both chlorophylls (a and b) and Pheo a in the inner-filter effect, we performed Voigt deconvolution on the fluorescence spectra of the least (20 min) and the most (240 min) concentrated aliquots. As depicted in Figures 4a and 4b,

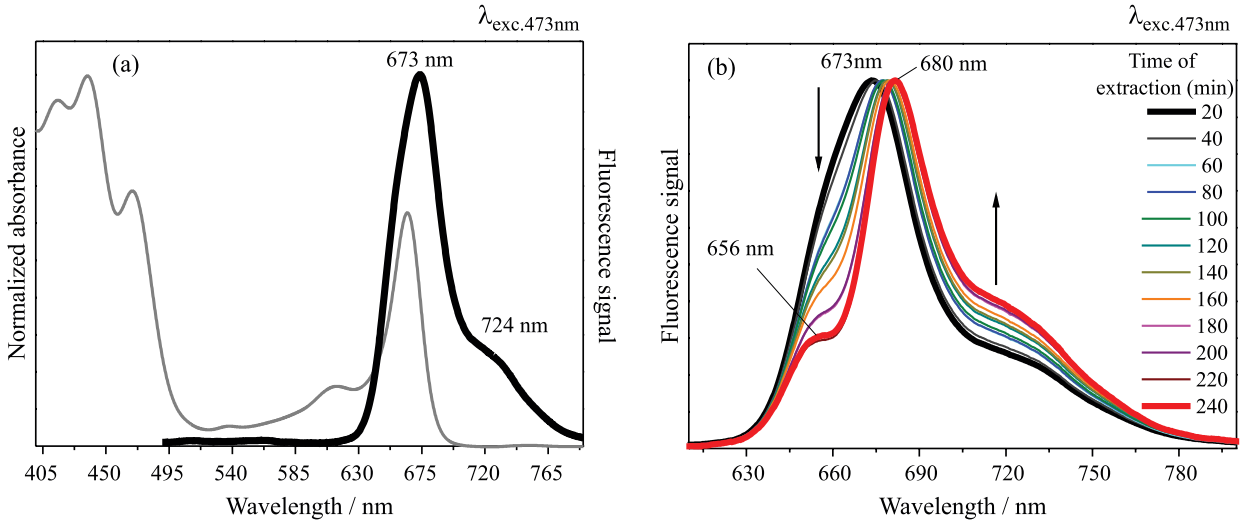

Figure 3. (a) Comparison between absorption (solid gray line) and fluorescence spectra (solid black line) (excitation at 473 nm) of the aliquot obtained after 20 min of an emulsion of leaves in methanol. (b) Evolution of the fluorescence spectrum (excitation at 473 nm) of aliquots acquired at intervals of 20 min. The changes in the relative intensity of bands around 653 nm indicate the occurrence of the inner-filter effect.

both spectra are well fitted adopting six Voigt functions, located at 653, 673, 683, 700, 720, and 745 nm. Assuming previous assignments, the Voigt curves at 653, 673 and 683 nm are attributed to the Q(0,0) fluorescence bands of Chl b, Chl a, and Pheo a, respectively.[17,31-35] The other three bands (700, 720, and 745 nm) are respectively assigned to the vibronic fluorescence bands Q(0,1) of these pigments following the same order.

Besides the central position of the bands, we support our analysis on the percentage contribution of each Voigt function to the total area of the normalized fluorescence spectra. This parameter is adopted because the area of an emission band is related to its corresponding quantum yield, which is very sensitive to the inner-filter.[36,37] As presented in Table 1, a significant decrease in the percentage contribution of the Q(0,0) fluorescence band of Chl a (at 673 nm) is verified, since this value changes from 43 to 8%. The contribution assigned to Chl b (at 653 nm) present minor change, going from 13 to 11%. Contrarily, the contribution of Pheo a to the fluorescence spectrum increased from 17 to 34%. In the case of the vibronic bands, it is verified an increase in the sum of their percentage contributions when we compare the first and the last aliquots, which furnish additional evidence of the inner-filter effect.

Following these results, the apparent shift presented in Figure 3b, can be interpreted assuming that in the least concentrated aliquot, the emission band of Chl a is the most intense, but after the occurrence of the inner-filter effect, the Pheo a's assumes this condition. This means that, no shift would be taking place at all, but a redistribution of the most intense emission bands, related to Chl a and Pheo a. Because these changes are so much more significant in the fluorescence of Chl a in comparison to the other pigments, this analysis suggests the existence of a selective inner-filter effect. This behavior is not expected, mainly because the bands of Chl b and Pheo a are also located in the overlapped area.

Beyond changes in the steady-state fluorescence spectrum, the inner-filter effect is expected to diminish the total rate of deactivation from the excited state of a molecule.[36,37] In other words, this effect increases the excited state lifetime.[36,37] Probing the fluorescence lifetime of all aliquots, at 673 nm, we observe that the decay follow

Table 1. Parameters obtained after deconvolution with Voigt functions of the fluorescence spectra of the least (20 min) and the most (240 min) concentrated aliquots

time of extraction / min	λ[a] / nm	Assignment	Area[b] / %
20	653	Q(0,0) → Chl b	13
	673	Q(0,0) → Chl a	43
	683	Q(0,0) → Pheo b	17
	700	Q(0,1) → Chl b	4
	720	Q(0,1) → Chl a	16
	745	Q(0,1) → Pheo a	7
240	653	Q(0,0) → Chl b	11
	673	Q(0,0) → Chl a	8
	683	Q(0,0) → Pheo b	34
	700	Q(0,1) → Chl b	13
	720	Q(0,1) → Chl a	20
	745	Q(0,1) → Pheo a	13

[a]Central position of each Voigt curve; [b]percentage contribution to the total area of the spectrum.

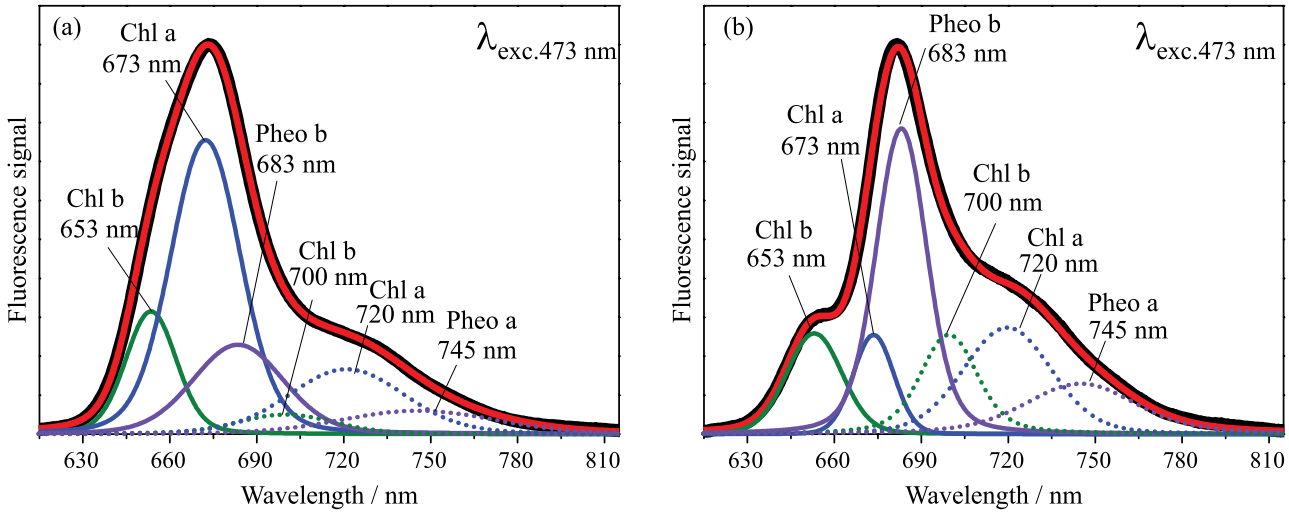

Figure 4. Fluorescence spectrum (solid black line) and theoretical Voigt-based deconvolution (solid red line) of (a) the least (20 min) and (b) the most (240 min) concentrated aliquots. The samples were excited at 473 nm. The bands at 653 nm (solid green line), 673 nm (solid blue line) and 683 nm (solid purple line) are attributed to Q(0,0) fluorescence bands from Chl b, Chl a, and Pheo a, respectively. The bands at 700 nm (dotted green line), 720 nm (dotted blue line) and 745 nm (dotted purple line) are attributed to the respective Q(0,1) fluorescence bands from these pigments.

bi-exponential kinetics, see Figure 5a. For the first aliquot, characteristic lifetimes of τ_1 = 2.35 ns and τ_2 = 5.79 ns, with respective percentage contributions of ca. 6% and ca. 94%, were obtained. The lifetimes of 2.35 and 5.79 ns can be assigned to chlorophylls b and a, respectively.[34,38,39] As pointed in the literature, in many solvents and concentration conditions, the excited state lifetime of Pheo a is usually very close to the value found for Chl a, being ca. 5.79 ns in *n*-hexane.[17,40] Assuming these reported results, we have tried to fit the decay kinetic with three exponential functions, but unfortunately, we could not distinguish between the decay of Chl a and Pheo a. Because of that, all considerations concerning the τ_2 lifetime can be assigned to contributions from Chl a as well as from Pheo a molecules.

Evaluation of the decay kinetics of posterior aliquots reveals a progressive increase on the lifetime τ_2, up to 6.14 ns, corresponding to a variation of ca. 350 ps, as the aliquots become more concentrated, i.e., as the inner-filter effect becomes more effective, see Figure 5b and Table 2. No changes in the τ_1 lifetime as well as in the percentage contributions of each decay were verified. This result together with the analysis of the percentage area of Voigt curves obtained for the emission spectra shows that the inner-filter is selective and affects mostly the excited states of Chl a molecules.

The mechanisms behind the inner-filter effect can be understood in two ways, described as the following: (*i*) in the overlapped region, each molecule can affect themselves by the reabsorption,[27-30] or (*ii*) the fluorescence bands of each molecule can be influenced by the absorption bands of other molecules present in the solution.[36,37] In both cases, the effectiveness of this photophysical process is dependent on the absorbance values at the overlapped region,[36,37] which are defined as the product between the corresponding extinction coefficient and the concentration of each pigment. In Figure 6, we compare the deconvoluted absorption and fluorescence spectra of the least concentrated aliquot. We observe that the absorption bands of Chl a, Chl b and Pheo a can both affect one each other as well as themselves. The fluorescence band of Chl b (centered at 653 nm) is overlapped with the blue-wing part of the absorption bands of Pheo a and Chl a molecules as well as with its own absorption band. A similar trend is observed for the other molecules, see Figure 6. In this scenario, the inner-filter effect should be efficient in the fluorescence response of all these molecules.

Table 2. Fluorescence lifetime parameters obtained by bi-exponential deconvolution of the decay signal

Time of extraction / min	τ_1 (Chl b)[a] / ns	τ_2 (Chl a/Pheo a)[b] / ns	$\Delta\tau_2$[c] / ps
20	2.35	5.79	0
40	2.35	5.79	0
60	2.35	5.79	0
80	2.35	5.79	0
100	2.35	5.83	40
120	2.35	5.87	80
140	2.35	5.95	170
160	2.35	6.02	230
180	2.35	6.05	260
200	2.35	6.07	280
220	2.35	6.11	320
240	2.35	6.14	350

[a]Lifetime attributed to chlorophyll b; [b]lifetime attributed to chlorophyll a and pheophytin a; [c]change of the τ_2 along the time of extraction of pigments.

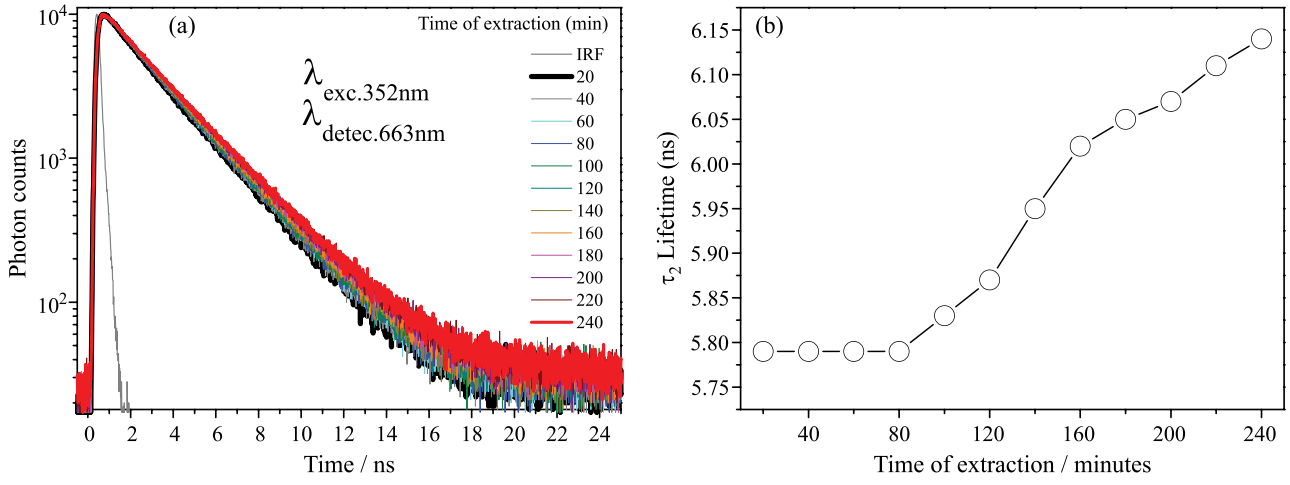

Figure 5. (a) Fluorescence lifetime of the aliquots acquired at different times of emulsion of leaves in methanol. Samples were excited at 352 nm, and the decays are detected at 673 nm. (b) Increase in the τ_2 lifetime along the time of acquisition of the aliquots.

Supporting our interpretation on the concentration and extinction coefficients at the overlapped bands, we propose a possible explanation for the observed selectivity of the inner-filter effect. Assuming that at a fixed wavelength, the variation in the absorbance spectrum is directly proportional to the change in the concentration, we verified increases of order of ca. 4.0, ca. 3.1 and ca. 3.5 times in the absorbance of the Soret bands of Pheo a (412 nm), Chl a (437 nm) and Chl b (469 nm), respectively. The absorbance of the Soret bands is considered in this discussion because only in this region we can distinguish unequivocally the contributions coming from these three molecules. Qualitatively, we observe that the concentration variation is almost the same for all these three pigments, reinforcing the expectation that the inner-filter should occur almost equally in all these three molecules. On the other hand, as described in the literature, the extinction coefficient of Chl b is ca. 3.5 times smaller than the values for Chl a and Pheo a molecules, at around 655 nm, in methanol environment.[17,41] Since the absorption of Chl a and Pheo a acts more effectively on the emission of Chl a and Chl b, while the fluorescence of Chl b is more effectively quenched by reabsorption mechanism,[27-30] we believe that the combination of factors like molecular concentration, extinction-coefficients, and band overlap can explain the selectivity of the inner-filter effect. Note that, due to the relative lower extinction coefficient presented by the Chl b, in this region, its absorbance would not achieve sufficient values to affect the fluorescence response of the other molecules in the aliquots.

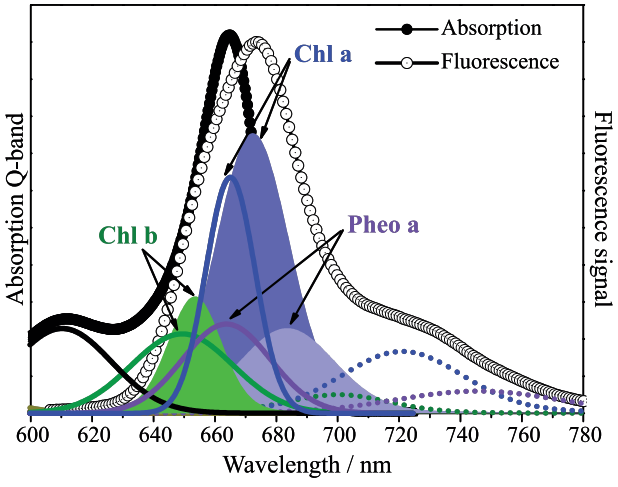

Figure 6. Overlapping between the deconvoluted absorption (full circles) and fluorescence (open circles) spectra of the least concentrated aliquot, acquired after 20 min of an emulsion of leaves in methanol. The Voigt curves with filled area represent the fluorescence bands of Chl b (green), Chl a (blue) and Pheo a (purple) while unfilled solid curves represent the absorption bands at the chlorophyll's Q-band and unfilled dotted curves represent emissions of the Chl a (dotted blue line) and Pheo a (dotted purple line).

Conclusions

We discussed the roles of chlorophyll (a and b) and Pheo a molecules in the fluorescence response of aliquots containing natural pigments extracted from *Caesalpinia echinata* (Brazil-wood), at distinct times of an emulsion of leaves in methanol. Besides the characterization of these solutions, combining Voigt deconvolution with time-resolved fluorescence technique, we were able to observe a selective inner-filter effect, where the features related to the Chl a molecule are the most affected among the characteristics of the other pigments. This selectivity is not straightforward because the absorption and fluorescence bands of Chl b and Pheo a are also significantly overlapped with their absorption Q-bands and should have also had been affected. The deconvolution analysis applied to both absorbance and fluorescence spectrum allowed us to propose an interpretation of this selectivity. The overlapping observed for absorption and emission bands indicates that the main mechanism responsible for the inner-filter in these three species is the reabsorption. However, both Chl b and Pheo a presents smaller extinction coefficients at Q-band when compared to Chl a. Thus, we believe that only for Chl a the absorbance at the overlapped region is large enough to promote an effective inner-filter effect.

Furthermore, by means of the steady-state absorption and fluorescence spectra, we were able to detect the presence of β-carotene molecule among the great variety of pigments released in the solutions. According to the literature, this carotenoid can effectively interact with Chl a and favor electron and energy transfer processes,[42,43] which would affect the chlorophyll's excited state lifetimes. However, under our experimental conditions, we were not able to observe these possible interactions. We believe that the concentrations of β-carotene adopted in these cited works[42,43] are higher than the concentrations extracted herein.

Supplementary Information

Supplementary information is available free of charge at http://jbcs.sbq.org.br as a PDF file.

Acknowledgments

The authors are indebted with Brazilian agencies: CNPq, FAPESPA, CAPES, PROPESP/UFPA (PAPQ program), and National Institute of Organic Electronic (INEO) for the financial support.

References

1. Syafinar, R.; Gomesh, N.; Irwanto, M.; Fareq, M.; Irwan, Y. M.; *Energy Procedia* **2015**, *79*, 896.
2. Hug, H.; Bader, M.; Mair, P.; Glatzel, T.; *Appl. Energy* **2014**, *115*, 216.
3. Davies, K. M. In *Plant Pigments and Their Manipulation, Annual Plant Reviews*; Davies, K. M., ed.; Blackwell Publishing: Oxford, UK, 2004.
4. Hagerthey, S. E.; Louda, J. W.; Mongkronsri, P.; *J. Phycol.* **2006**, *42*, 1125.
5. Rodríguez, M.; Mantell, C.; Macías-Sánchez, M.; de la Ossa, E. M.; *EJEAFChe, Electron. J. Environ., Agric. Food Chem.* **2008**, *7*, 3150.
6. Gross, J.; *Pigments in Vegetables: Chlorophylls and Carotenoids*, 1st ed.; Springer: New York, USA, 1991.
7. Eyster, H. C.; *Ohio J. Sci.* **1950**, *50*, 79.
8. Costache, M. A.; Campeanu, G.; Neata, G.; *Rom. Biotechnol. Lett.* **2012**, *17*, 7702.
9. Miazek, K.; Ledakowicz, S.; *Int. J. Agric. Biol. Eng.* **2013**, *6*, 107.
10. Gouterman, M. In *The Porphyrins*; Dolphin, D., ed.; Academic Press: New York, USA, 1978.
11. Truscott, T. G.; *J. Photochem. Photobiol., B* **1990**, *6*, 359.
12. Rosenberg, J. L.; *Annu. Rev. Plant Physiol.* **1957**, *8*, 115.
13. Kouissa, B.; Bouchouit, K.; Abed, S.; Essaidi, Z.; Derkowska, B.; Sahraoui, B.; *Opt. Commun.* **2013**, *293*, 75.
14. Corrêa, D. S.; de Boni, L.; dos Santos Jr., D. S.; Barbosa Neto, N. M.; Oliveira Jr., O. N.; Misoguti, L.; Zilio, S. C.; Mendonça, C. R.; *Appl. Phys. B: Lasers Opt.* **2002**, *74*, 559.
15. Bouchouit, K.; Derkowska, B.; Migalska-Zalas, A.; Abed, S.; Benali-cherif, N.; Sahraoui, B.; *Dyes Pigm.* **2010**, *86*, 161.
16. Zaghdoudi, K.; Ngomo, O.; Vanderesse, R.; Arnoux, P.; Myrzakhmetov, B.; Frochot, C.; Guiavarc'h, Y.; *Foods* **2017**, *6*, 4.
17. Kobayashi, M.; Sorimachi, Y.; Fukayama, D.; Komatsu, H.; Kanjoh, T.; Wada, K.; Kawachi, M.; Miyashita, H.; Ohnishi-Kameyama, M.; Ono, H. In *Handbook of Photosynthesis*, 3rd ed.; Pessarakli, M., ed.; CRC Press, Taylor & Francis Group. LLC: Florida, USA, 2016.
18. Gouterman, M.; *J. Mol. Spectrosc.* **1963**, *11*, 108.
19. Walker Jr., R. D.; Hawkiss, J. E.; *J. Am. Chem. Soc.* **1952**, *74*, 4209.
20. Solovchenko, A.; *Photoprotection in Plants: Optical Screening-Based Mechanisms*, 1st ed.; Springer: Heidelberg, Germany, 2010.
21. Voicescu, M.; Ionescu, S.; Gatea, F.; *J. Fluoresc.* **2014**, *24*, 75.
22. Bastos, I. V. G. A.; da Silva, G. K. C.; Rodrigues, G. C. R.; de Melo, C. M.; Xavier, H. S.; de Souza, I. A.; *Rev. Bras. Farm.* **2011**, *92*, 219.
23. Milenković, S. M.; Zvezdanović, J. B.; Anđelković, T. D.; Marković, D. Z.; *Adv. Technol.* **2012**, *1*, 16.
24. Di Rocco, H. O.; Cruzado, A.; *Acta Phys. Pol., A* **2012**, *122*, 666.
25. Kleinegris, D. M. M.; van Es, M. A.; Janssen, M.; Brandenburg, W. A.; Wijffels, R. H.; *J. Appl. Phycol.* **2010**, *22*, 645.
26. Kandori, H.; Sasabe, H.; Mimuro, M.; *J. Am. Chem. Soc.* **1994**, *116*, 2671.
27. Ahmed, S. A.; Zang, Z. W.; Yoo, K. M.; Ali, M. A.; Alfano, R. R.; *Appl. Opt.* **1994**, *33*, 2747.
28. Terjung, F.; *Z. Naturforsch., C: J. Biosci.* **1998**, *53*, 924.
29. Birks, J. B.; *J. Phys. B: At. Mol. Phys.* **1970**, *12*, 1704.
30. Sakai, Y.; Kawahigashi, M.; Minami, T.; Inoue, T.; Hirayama, S.; *J. Lumin.* **1989**, *42*, 317.
31. Boardman, N. K.; Thorne, S. W.; *Biochim. Biophys. Acta, Bioenerg.* **1971**, *253*, 222.
32. Thorne, S. W.; Newcomb, E. H.; Osmond, C. B.; *Proc. Natl. Acad. Sci. U. S. A.* **1977**, *74*, 575.
33. White, R. C.; Jones, I. D.; Gibbs, E.; Butler, L. S.; *J. Agric. Food Chem.* **1972**, *20*, 773.
34. Pfarrherr, A.; Teuchner, K.; Leupold, D.; Hoffmann, P.; *J. Photochem. Photobiol., B* **1991**, *9*, 35.
35. Goedheer, J. C.; *Biochim. Biophys. Acta* **1964**, *88*, 304.
36. Lakowicz, J. R.; *Principles of Fluorescence Spectroscopy*, 3rd ed.; Springer: New York, USA, 2006.
37. Valeur, B.; Berberan-Santos, M. N.; *Molecular Fluorescence: Principles and Applications*, 2nd ed.; Wiley-VCH: Weinheim, Germany, 2012.
38. Kaplanova, M.; Cermak, K.; *J. Photochem.* **1981**, *15*, 313.
39. Kaplanova, M.; Parma, L.; *Gen. Physiol. Biophys.* **1984**, *3*, 127.
40. Volkov, A. G.; Markin, V. S.; Leblanc, R. M.; Gugeshashvili, M. I.; Zelent, B.; Munger, G.; *J. Solution Chem.* **1994**, *23*, 223.
41. Porra, R. J.; Thompson, W. A.; Kriedemann, P. E.; *Biochim. Biophys. Acta, Bioenerg.* **1989**, *975*, 384.
42. Li, T.; Zhang, Y.; Gong, N.; Li, Z.; Sun, C.; Men, Z.; *Int. J. Mol. Sci.* **2016**, *17*, 978.
43. Chen, C.; Gong, N.; Li, Z.; Sun, C.; Men, Z.; *Molecules* **2017**, *22*, 1585.

Submitted: February 11, 2019
Published online: July 2, 2019