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# Characterization of photophysical properties of curcumin for theranostics of neurodegenerative diseases

Marcelo Saito Nogueira[1,2,3], Fabio Francisco Pinto Junior[3], Raphael Antonio Caface[3], Kleber Thiago de Oliveira[4], Vanderlei Salvador Bagnato[3], Francisco Eduardo Gontijo Guimarães[3]
[1]Tyndall National Institute, Lee Maltings, Dyke Parade, Cork, Ireland (present affiliation)
[2]Department of Physics, University College Cork, College Road, Cork, Ireland (present affiliation)
[3]São Carlos Institute of Physics, University of São Paulo, PO Box 369, 13560-970, São Carlos, SP, Brazil
[4]Department of Chemistry, Federal University of São Carlos, São Carlos 13565-905, São Paulo, Brazil

## ABSTRACT

Curcumin is a natural and biocompatible compound that has been used for a variety of medical applications. These applications include treatment of several tumor cells, skin diseases, wound healing, and inflammation. Moreover, curcumin has potential to be used for theranostic of neurodegenerative diseases involving formation of Aβ plaques, as it can stain amyloid-β (Aβ) plaques and slightly improve the cognitive function in elderly. However, the diagnosis contrast and the treatment efficiency curcumin can provide are dependent on its molecular microenvironment, as it can change curcumin physical and chemical properties. In this paper, we characterize these properties for two types of curcumin formulations and suggest a quantum yield approach to enhance the detection of Aβ plaques with one of these formulations. The first formulation is synthetic curcumin (100% curcumin) and the second is Sigma Aldrich curcumin has 94% of curcuminoid content and 80% curcumin. Our measurements show that solutions containing only curcumin provided highest fluorescence signal with relatively lower optical densities, i.e., an increase of 73.2% (375 nm excitation) and 55% (445 nm excitation) in the quantum yield for the concentration of 20 μg/ml (54.30 μM). This suggests the synthetic curcumin formulation may be more efficient when used as a biomarker for diagnostics purposes or monitoring the efficiency of curcumin treatments using fluorescence spectroscopy.

**Keywords:** Curcumin, neurodegenerative diseases, Alzheimer disease, optical spectroscopy, diagnostics, photodynamic therapy, medical applications, fluorescence spectroscopy.

## 1. INTRODUCTION

Current methods of diagnosing neurodegenerative diseases associated with the formation of amyloid fibrils or plaques in the brain are based on the clinical exclusion of other causes of mild cognitive impairment and dementia or on biomarkers of structural MRI, molecular neuroimaging with PET, and cerebrospinal fluid analyses. However, the clinical criteria leads to insufficient diagnostic specificity, PET and MRI scans are expensive, and biochemical analysis of the cerebrospinal fluid is still invasive. Then, current techniques do not provide cheap and non-invasive diagnostic options to the patient. Fluorescence spectroscopy can be used for detection of amyloid plaques associated with certain neurodegenerative diseases and has potential to provide the necessary methods for the theranostics of these diseases, i.e., disease treatment on site right after the diagnosis.

Fluorescence spectroscopy is a method to investigate molecular interactions involving endogenous biological tissue components and exogenous agents. Previous studies have reported that curcumin and curcumin-based probes have been used as fluorescent contrast agent capable of staining amyloid-β (Aβ) plaques[1,2], assess the status of *in vivo* human skin [3], and monitoring the mass change of brown adipose tissue and browning of white adipose tissue.[4] Studies using curcumin

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have mostly used the curcumin formulation of Sigma Aldrich (containing 94% of curcuminoid content and 80% curcumin).

In addition to the possibility of staining Aβ plaques, curcumin may slightly improve the cognitive function in elderly people[5,6], is able to lower and inhibit aggregation of Aβ, has antioxidant and anti-inflammatory properties[7,8], and has potential to be used for photodynamic therapy and microbial inactivation[9–20]. Since curcumin can be included in the diet as a spice (present in curry, for example), diagnostics and therapeutical applications of curcumin would not cause discomfort to the patient relatively to other theranostics methods.

Curcumin localization and efficiency will depend on its molecular microenvironment in the formulation it is going to be used. Characterization of each formulation is necessary for comparison among studies. Previous studies investigated curcumin photophysical properties for different solvents, temperature, concentrations and nanoformulations. However, studies are lacking in different curcumin types. We provide a characterization of two curcumin formulations and suggest a quantum yield approach for diagnostic purposes.

Our technique has potential to provide an enhanced detection of Aβ plaques by using a correction of the fluorescence emission based on the curcumin absorption and curcumin with a higher quantum yield. In this approach, fluorescence spectroscopy can be used together with optical techniques that retrieve absorption coefficients (such as diffuse reflectance spectroscopy) to allow real-time, cheap, and in situ diagnosis of neurodegenerative diseases related to the formation of curcumin-binding components such as amyloid plaques. We propose to use a curcumin formulation containing only curcumin in order to achieve a higher quantum yield and better discrimination compared to previous studies. We compared the absorbance and fluorescence emission for formulations of Sigma Aldrich curcumin (SigAC) and synthetic curcumin (SynthC) to calculate the relative quantum yield of between the two curcumins.

## 2. METHODOLOGY

### 2.1 Absorbance determination

The absorbance measurements were performed by using a Varian Cary 50 UV-Vis containing a full spectrum Xe pulse lamp single source and a beam splitter for simultaneous reference beam correction. All the measurements were performed in the fast mode with double beam.

### 2.2 Fluorescence spectroscopy measurements

Our fluorescence system contains 375 nm and 445 nm lasers (BDL-375-SMC and BDL-445-SMC, Becker and Hickl, Berlin, Germany). They were set for emission in continuous wave mode. The system has a 400-μm-diameter core bifurcated fiber (BIF400-UV-VIS, Ocean Optics, Dunedin, Florida, USA) that delivers the excitation light to the sample in a common end and collects the fluorescence emission and backscattered light. The backscattered light is removed by longpass filters (405 nm longpass for the 378 nm laser and 475 nm longpass for the 445 nm one). The fluorescence is sent to a portable spectrometer (USB2000-FLG, Dunedin, Florida, USA) and the captured signal is sent to a computer that displays the fluorescence spectra. More details about the system were reported in previous studies by our group [21–39]. Figure 1 shows a scheme of the assembled experimental setup.

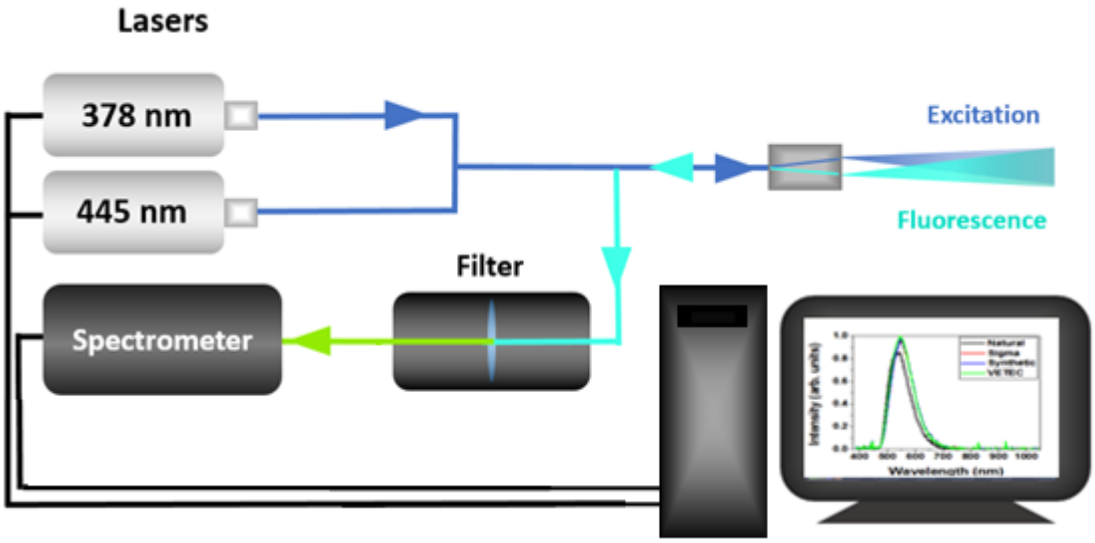


**Figure 1:** Instrumentation for the fluorescence spectroscopy measurements of curcumin.

### 2.3 Sample preparation

A stock solution of 1 mg/ml was prepared for each type of curcumin. This solution was diluted to the concentrations of 10 and 2 μg/ml by using phosphate buffer saline (PBS) to keep the solution at pH 7. The solutions were centrifuged in order for appropriate homogenization. The solution was transferred to a cuvette and measured at the fluorescence spectroscopy system and the UV-Vis spectrophotometer. Measurements compared the synthetic curcumin (100% curcumin) and the Sigma Aldrich curcumin (94% of curcuminoid content and 80% curcumin).

### 2.4 Data processing and analysis

The spectral data was analyzed in Origin software (OriginLab Corporation. Northampton, Massachusetts, USA). The relative quantum yield (QY) of synthetic curcumin compared to the Sigma Aldrich curcumin was calculated by using the equation:

$$\text{Relative quantum yield} = \frac{QY_{synthetic}}{QY_{Sigma}} = \frac{\eta^2_{synthetic}\, I_{synthetic}\, A_{Sigma}}{\eta^2_{Sigma}\, I_{Sigma}\, A_{synthetic}}$$

## 3. RESULTS AND DISCUSSION

### 3.1 Absorbance of curcumin formulations

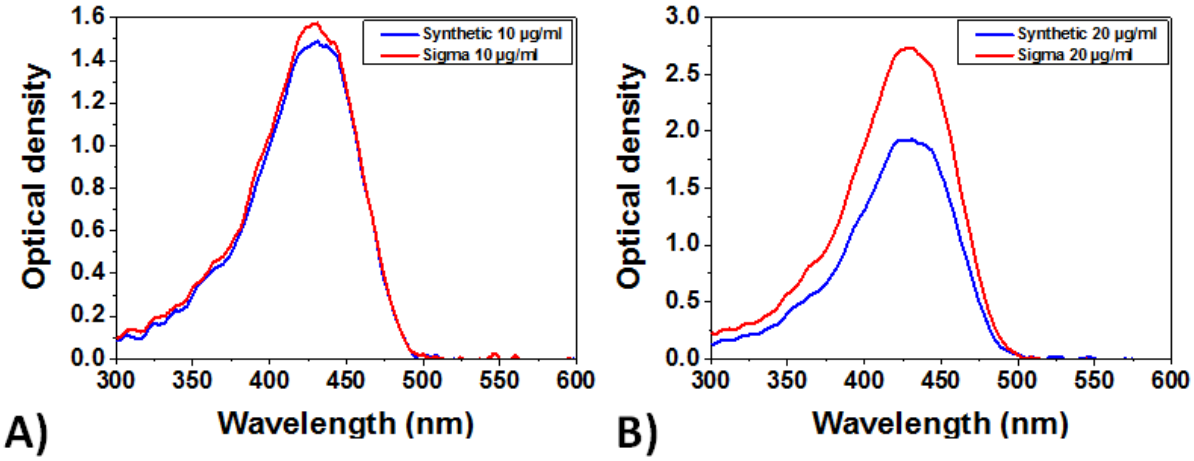


**Figure 2:** Absorbance of SigAC and SynthC diluted in PBS in the concentrations of 10 and 20 µg/ml.

The absorbance of curcumin (figure 2) shows both SigAC and SynthC formulations can be excited in optimum wavelengths between 420 nm and 450 nm. The absorbance of both formulations was similar for the concentration of 10 µg/ml, while the absorbance of SynthC is considerably lower than the one of SigAC at 20 µg/ml.

### 3.2 Fluorescence of curcumin formulations

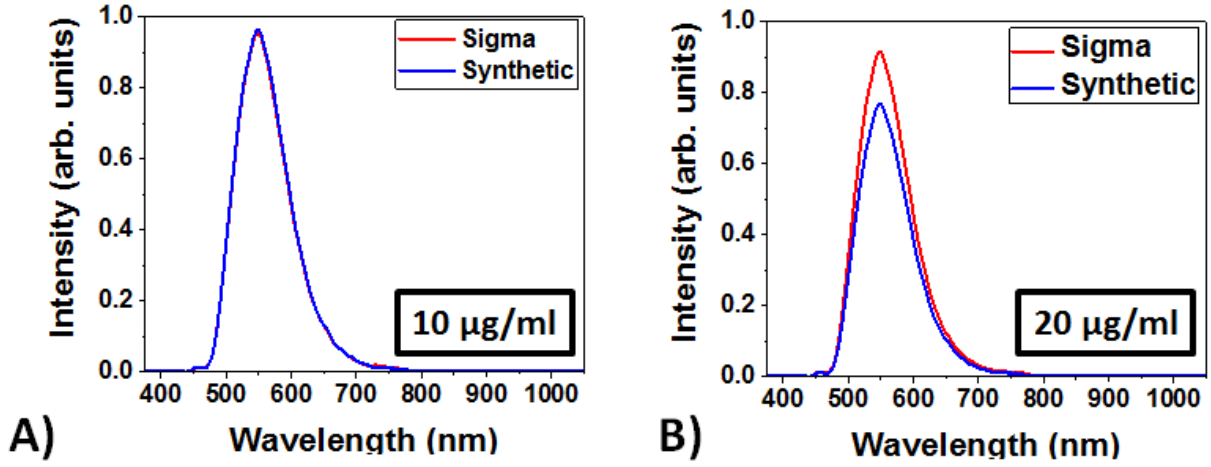


**Figure 3:** Fluorescence spectra of SigAC and SynthC using the 375 nm excitation.

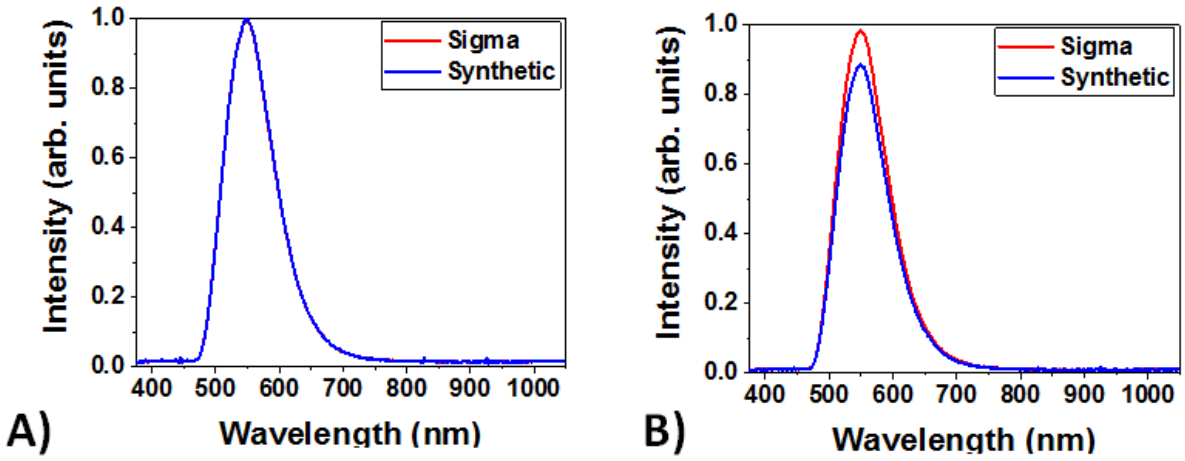


**Figure 4:** Fluorescence spectra of SigAC and SynthC using the 375 nm excitation.

The fluorescence emission for both excitations (375 nm and 445 nm) occurs between 450 nm and 750 nm (figures 3 and 4). The fluorescence intensity shows the same tendency as for the absorbance values at the respective concentrations. However, in this case, the fluorescence emission of SynthC at 10 µg/ml is higher than the one for SigAC. In addition, the relative difference of the fluorescence emission of SynthC and SigAC is less pronunciated than the absorbance.

**Table 1:** Relative quantum yield ($QY_{SynthC}/QY_{SigAC}$) for each concentration and excitation wavelength.

| Excitation wavelength | Concentrations | |
|---|---|---|
| | 10 µg/ml (27.15 µM) | 20 µg/ml (54.30 µM) |
| 375 nm | 1.074 | 1.732 |
| 445 nm | 1.026 | 1.550 |

The quantum yield for SynthC is relatively higher than SigAC for the concentration of 20 µg/ml (table 1).

## 4. DISCUSSION

The Stokes shift was 4951 $cm^{-1}$ for both curcumin formulations (peak at 431.04846 nm for the absorbance spectrum and 548 for the fluorescence spectrum). This indicates the transition between the ground and excited state of curcumin in PBS is due to change in dipole moment in polar environments or vibrational shifts, as opposed to excited state reactions observed in Stokes shifts between 8000-10000 $cm^{-1}$. This agrees with the literature, as curcumin absorption maximum, fluorescence intensity maximum, and Stokes shifts vary from 408 to 430 nm, from 460 to560 nm, and from 2000 to 6000 $cm^{-1}$ , respectively, in experiments with different solvents[40,41]. Our result also agrees with prior fluorescence lifetime findings, which suggest the curcumin singlet excited states decay by nonradiative processes[40,42]. Then, this result shows features of our curcumin formulations that can be used for comparison with other formulations reported in the literature.

The quantum yield for SynthC is relatively higher than SigAC at all concentrations analyzed. This suggests the SynthC may have a higher quantum yield than SigAC as the concentration increases and can be used to improve the detection of neurodegenerative diseases depending on the bioavailability of curcumin in the amyloid β plaques. Previous studies show that the mouse curcumin bioavailability in brain after administration of curcumin in chow was reported to be 0.469 ± 0.220 µg/g of tissue (or 1.276 µM) and 0.525 ± 0.125 (or 1.428 µM) for 2.5 mg of curcumin/day and 10 mg/day during 4 months, respectively.[43] This bioavailability was higher (1% or 13.57 µM) in rat plasma, as reported by a

study where freely moving rats had oral administration of curcumin of 500 mg/kg of chow) [44]. On the other hand, another study in rats showed a peak of 15 ng/mL in blood plasma at 50 ± 32 minutes after the oral administration of curcumin (1,000 mg/kg)[45]. Peak of curcumin levels observed in human blood plasma was 0.41- 1.75 μM after 1 hour of oral dosing[46]. Also, curcumin nanoformulations are reported to have relatively higher bioavailability than curcumin in brain and plasma at and after 30 minutes of their administration in rats[47].

Even though the curcumin concentrations we obtained (27.15 μM and 54.30 μM) are relatively low compared to the bioavailability previously reported in plasma (0.41- 1.75 μM for humans and 13.57 μM for rats) and brain (between 1.276 μM and 1.428 μM for mice) after oral curcumin administration, SynthC still shows a potential to improve curcumin detection in amyloid β plaques in the brain, which preferentially bind to curcumin. By using a quantum yield approach, SynthC may show more potential than nanoformulations, since their photophysical properties with respect to quantum yield are still under study.

The quantum yield is higher for the 375 nm excitation. This suggests that UV excitation can improve the detection of SynthC by using our proposed technique. Previously reported studies use excitation in visible (close to the curcumin absorption maximum) or near-infrared (to increase the light penetration in tissues) wavelengths[1–4]. UV excitation needs to be tested and has potential to allow better detection of amyloid β plaques based on a quantum yield approach.

Previous studies analyzed the photophysical properties of curcumin with respect to temperature, concentration, the use of different solvents, and curcumin-based nanoformulations. These nanoformulations are shown to have increased bioavailability compared to curcumin. Concentrations of the solutions prepared in this study are lower than what is the bioavailability observed in human and animal models. Even though the bioavailability and concentrations are lower than what is reported in previous studies, our characterization of curcumin absorbance and fluorescence-related properties at the concentrations of 27.15 μM and 54.30 μM shows the potential of a quantum yield approach for diagnosis of neurodegenerative diseases involving amyloid β plaques. This characterization allows comparison with properties of other formulations. Our dataset is still limited in properties shown for each concentration. Then, more investigation is required in order to understand the efficiency of the formulation and suggest improvements for better theranostic procedures. Nanoformulations based on SynthC may further improve the results obtained in this study by increasing the bioavailability of the formulation, especially if the focus is based on contrast of fluorescence intensity instead of quantum yield.

In case quantum yield is the main focus, further characterization of the new formulations is necessary. In addition, the quantum yield approach would require at least two types of spectroscopy and/or imaging methods. One is based on fluorescence and the other should retrieve the curcumin absorption properties. Diffuse reflectance spectroscopy is one example of technique that can provide scattering and absorption properties of tissue chromophores[48]. Optical techniques such as diffuse reflectance spectroscopy and fluorescence spectroscopy can contribute to additional biochemical data that can even improve the detection of amyloid β plaques and related diseases. In the future, the detection can be further improved by including other optical techniques such as Raman spectroscopy. [49–53]

## 5. CONCLUSIONS

In this study, we obtained the absorbance and fluorescence properties for two curcumin formulations and calculated their relative quantum yield values for concentrations of 27.15 μM and 54.30 μM. Our study of reports photophysical properties of curcumin formulations that are not nanoscale-based. Moreover, we propose a new approach for detection of Aβ plaques based on the quantum yield of the compounds that preferentially bind to the plaques. Future applications of our approach could improve the theranostics of neurodegenerative diseases, as curcumin can be used to stain Aβ plaques and treat these diseases due to its Aβ-lowering, Aβ aggregation inhibition, antioxidant, anti-inflammatory properties.

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