Assessing the Fluorescent Properties of Anthraquinone-Based Dyes

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Abstract

Fluorescence is the ability of compounds to absorb a photon of light to excite an electron, then emit a photon of light at a different wavelength as the electron returns to its original state. This simple yet beautiful phenomenon in nature can be utilized for many applications. Fluorescence can be used in cell staining to track organelles or to visualize when two proteins interact. Not all fluorophores are created equally; certain fluorophores have a larger difference between the wavelength they absorb at and emit at; this gap is called the Stokes shift; however, other dyes may have an extremely efficient rate of absorbing and releasing photons, called the quantum yield. However, the dyes used in applications are sought to have both traits and also to emit light for an extended length of time, which is the photostability of the fluorophore. Utilizing organic chemistry techniques, our lab has produced many fluorophores based upon the anthraquinone core. These fluorophores have large Stokes shifts (> 70 nm) and are compared to several commonly used commercial dyes (specifically fluorescein and DAPI) and dyes of similar structure found in creation (specifically purpurin and alizarin) in the following three categories: Stokes shift, quantum yield, and photostability. These comparisons resulted in showing the advantages of our dyes over ones in creation and the current market, but they also revealed the traits where our dyes are lacking. Our dyes possess Stokes shifts that are comparable or larger than those of the current commercial dyes, and preliminary results revealed a similar photostability to fluorescein. However, preliminary results also show that our dyes have a low quantum yield. This work shows the potential of our class of fluorophores for cell staining and other applications but also reveals the direction of future research.

Introduction/Research Question

This research seeks to synthesize or discover the ideal anthraquinone-based fluorescent dye that possesses optimal values for its Stokes shift, quantum yield, and photostability. Novel anthraquinone dyes have been produced and their fluorescent properties and possible biomedical applications are currently being investigated.

Methods

A literature review was completed to obtain the data regarding the commercially produced dyes.

For all measurements measuring fluorescence, the Jasco FP-8300 Spectrofluorometer was used with quartz cuvettes. For the absorbance measurements, the Evolution 300 UV/Vis Spectrophotometer was utilized. The fluorimeter was turned on and zeroed. The “Spectrum Measurement” function was used. After this test was done, the optimal emission wavelength was observed using the “Spectrum Analysis,” by recording data cross a range of values to see the greatest intensity of fluorescence. Utilizing the optimal emission wavelength from the Spectrum Measurement test, the photostability of each compound was tested running each trial for 1800 seconds, recording data every 10 seconds. For measuring absorbance, the emission range for each compound was inputted onto the UV/Vis spectrophotometer. The machine was blanked with DMF, then the compound was analyzed, and the absorbance value was obtained from the resulting graph.

Lastly, the quantum yield of each dye was calculated by implementing the following equation:

$$\varphi x = \varphi s(As/Ax)(Fx/Fs)(Dx/Ds)$$

Where the subscript x is used for the unknown compound and the subscript s is used for the standard. A represents the value of the intensity of the optimal absorbance, F is the value of the peak area obtained after correction, and D is the dilution. The standard used was fluorescein and it has a known quantum yield of .872 in DMF. In this method, the dilution ratio portion of the equation was flipped since molarity was used not a ratio (for example, .001 M vs. 1:1000).

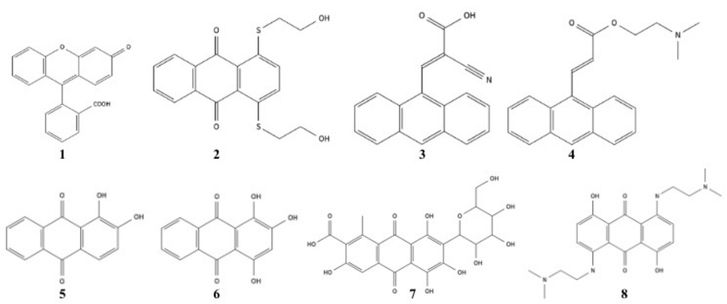

Figure 1. Structures of Fluorescein (1). ADS-7 (2). RRT-21a (3). ADS-20 (4). Alizarin (5). Purpurin (6). Carmine (7). DRAQ 5 (8).

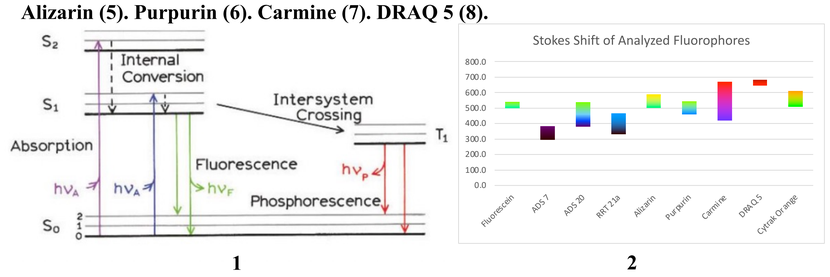

Figure 2. (1) Jablonski Diagram describing fluorescence at electronic level [1]. (2) Graph showing stokes shift of various fluorophores [2-6].

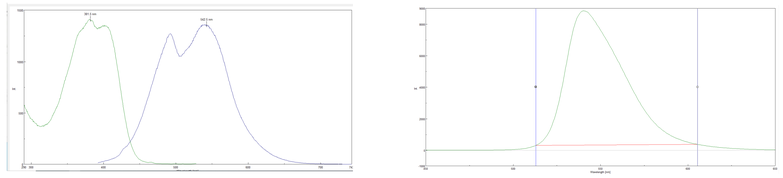

Figure 3. Excitation and emission of ADS 20 (a.) at 381.5 nm and 542.5 nm and fluorescein (b.) at optimal excitation wavelength [7].

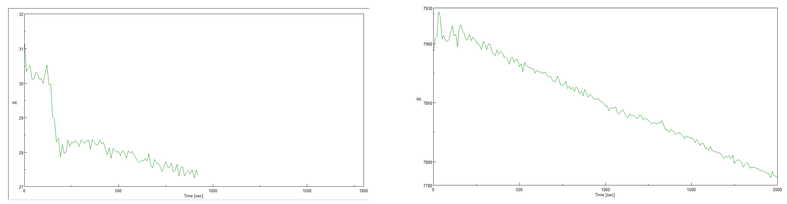

Figure 4. Photostability of ADS 20 (left) and fluorescein (right) measured at optimal excitation and emission wavelength [7].

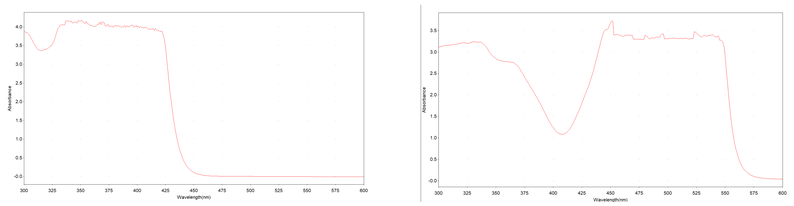

Figure 5. Absorbance of ADS 20 (left) and fluorescein (right) from 300-600 nm, to analyze how much fluorescent photons are absorbed by fluorophore [7].

Figure 6. Photo of madder root [8]. The source for purpurin and alizarin.

Carmine

Figure 9. Photo of Porphyrophora hamelii [9].

Alizarin

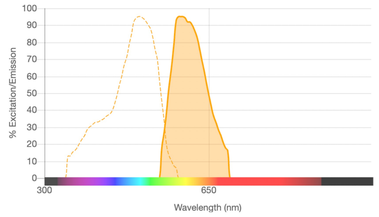

Figure 6. Excitation/emission of alizarin (502 nm/586 nm) [3]

Purpurin

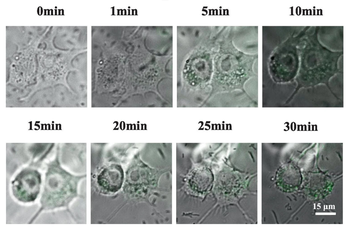

Figure 8. Time lapse fluorescent image of Purpurin being used to visualize phagocytosis [4]

Table 1. Quantum Yield of Studied Compounds

Compound	Quantum Yield
Fluorescein (in DMSO) [10]	0.87
ADS-7 (in DMSO)	N/A
RRT-21a (in DMSO)	0.000007772
ADS-20 (in DMSO)	0.036508641
Alizarin [11]	0.12
Purpurin [11]	0.0875
Carmine [12]	0.021
DRAQ 5 [13]	0.003

Results and/or Conclusion

1. ADS20 displayed an extremely wide Stokes shift of 161 nm (Figure 3) but a low photostability (Figure 4).
2. Dyes found in nature have a variety of biomedical applications and desirable qualities, such as antibacterial or anti-tumor [4].
3. Purpurin can be used to visualize acidic intracellular environments, such as the lysosome (Figure 8), and it displays a moderate photostability with its ability to image intracellular organelles and processes over time [4].
4. Carmine can be utilized to stain glycogen, nuclei, and acid mucopolysaccharides [7].
5. The dyes produced by this lab have wider stokes shifts than other anthraquinone-based dyes used commercially and found in creation (besides carmine).
6. The dyes produced by this lab do not have a competitive photostability with either the commercially used dyes nor the dyes found in creation.
7. ADS-20 has a competitive quantum yield when compared to DRAQ 5 and the dyes found in creation.

Future Work

Future work involves further synthesis and analysis of the novel fluorescent dyes. Biomedical applications could be analyzed using flow cytometry, cell staining, and DNA gel electrophoresis. Further analysis of the effect of a glycosidic group (as seen by carmine) should be analyzed. Lastly, analyzing how to develop a stronger quantum yield is necessary.

References

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