FOOD ANALYSIS

DETERMINATION OF RELATIVE FLUORESCENCE QUANTUM YIELD USING THE AGILENT CARY ECLIPSE

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ABSTRACT

This article describes a solution for the determination of the relative quantum yield of food dyes by comparison with curcumin - a reference standard which has a known quantum yield.

INTRODUCTION

The fluorescence quantum yield is defined as the ratio of the number of photons emitted to the number of photons absorbed1. Experimentally, relative fluorescence quantum yields can be determined by measuring fluorescence of a fluorophore of known quantum yield.

$$\text{Quantum Yield} = \frac{\text{Number of photons emitted}}{\text{Number of photons absorbed}}$$

There are two methods for calculating the fluorescence quantum yield - single point and comparative. The comparative method involves the creation of a calibration curve using multiple standards of a compound with known fluorescence quantum yield. Plotting the area of fluorescence against the absorbance for different concentrations of the fluorophore, this method provides high accuracy by calculating the slope of the line generated.

In the comparative method, the quantum yield can be calculated using:

$$Q = Q_r \times (m/m_r) \times (n^2/n_r^2)$$

Where:

- m is the slope of the line obtained from the plot of the area of fluorescence vs. absorbance.
- n is the refractive index of solvent and the subscript r refers to the reference fluorophore of known quantum yield.

A typical quantum yield will be less than 1.

INSTRUMENTATION

The Agilent Cary Eclipse was used to measure the fluorescence spectra in this application. This spectrometer is sensitive, accurate and flexible, and enables the user to collect data in a variety of different modes - Fluorescence, Phosphorescence, Chemi/Bio-luminescence and time resolved Phosphorescence measurements can all be easily made without changing a single hardware component. The unique design of the Cary Eclipse features 'room light immunity' allowing the instrument to operate with the front and top panels removed for easy access to the sample and fast installation of large accessories. Optimized design, based on the use of a high intensity Xenon flash lamp and highly sensitive photomultiplier tube (PMT) detectors, ensure that the Cary Eclipse gives the required performance right across the spectrum, with the ability to collect data at rates of up to 80 points per second (one data point every 12.5ms) or collection of full range spectral scans in less than 3 seconds.

An Agilent Cary 60 UV-Vis spectrometer was used to accurately measure the absorbance of each of the samples and standards used in this experiment.

The instrument operating parameters for the Eclipse are summarized below in Figure 1.

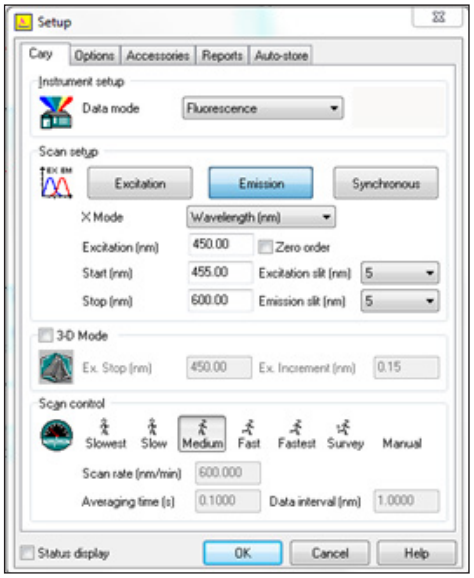

Figure 1. *Instrument operating parameters.*

Standard and sample preparation

- Prepare 3-4 standards of curcumin with absorbances between 0.01-0.1 at the excitation wavelength and prepare samples of the food dye using the same solvent
- Curcumin has an excitation wavelength around 420-421nm and the food dye has an excitation wavelength around 449-450nm
- Measure the fluorescence spectrum of the curcumin standards from 425nm to 600nm using an excitation wavelength of 420-421nm in a 10mm pathlength cuvette
- Measure the fluorescence spectrum of the food dye samples from 455nm to 600nm with the prepared samples using an excitation wavelength of 449-450 nm in a 10mm pathlength cuvette
- Calculate the integrated fluorescence intensity from the spectrum and plot the magnitude of the integrated area of fluorescence against the absorbance of the corresponding solution

RESULTS AND DISCUSSION

The standards should be chosen to ensure they absorb at a similar excitation wavelength compared to the test sample and, if possible, emit in a similar region to the test sample. The standard samples must be well characterized and suitable for such use.

In this example, curcumin was chosen as the standard as it has a similar region of excitation and emission as the food dye sample. In literature, the quantum yield of curcumin in acetone is reported to be 0.174.

There are a few considerations that need to be taken into consideration before starting the analysis:

- The user must work within a carefully chosen concentration range, ensuring that the samples and standards have absorbances within the range of 0.01-0.1 Abs.
- The solvent refractive indices must be known as these values are included in the ratio calculation

The fluorescence spectra of the samples and standards (Figure 2) are obtained using the Cary Eclipse after recording the absorbance values of each. The resulting linear fits are then plotted (Figure 4) using measured absorbance vs. the area of fluorescence obtained from Table 1.

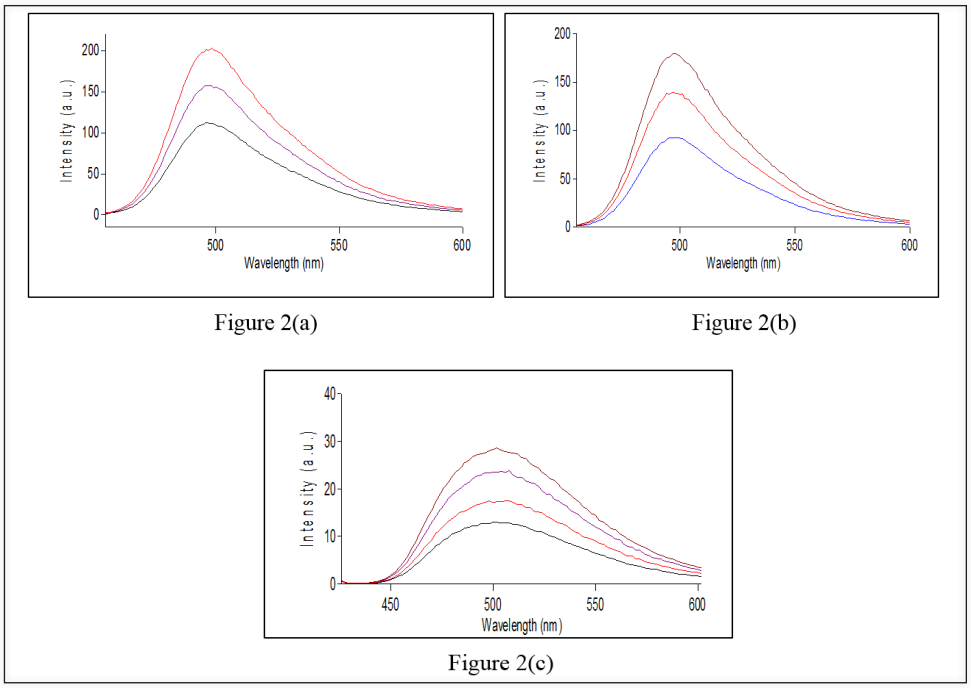

Figure 2. *Fluorescence spectrum 2(a) food dye batch 1, 2(b) food dye batch 2 and 2(c) curcumin.*

The area under the curve of each spectrum is calculated using the 'integrate' function within the WinFL software (Figure 3) and the values obtained are shown in Table 1, together with the corresponding absorbance value for that sample.

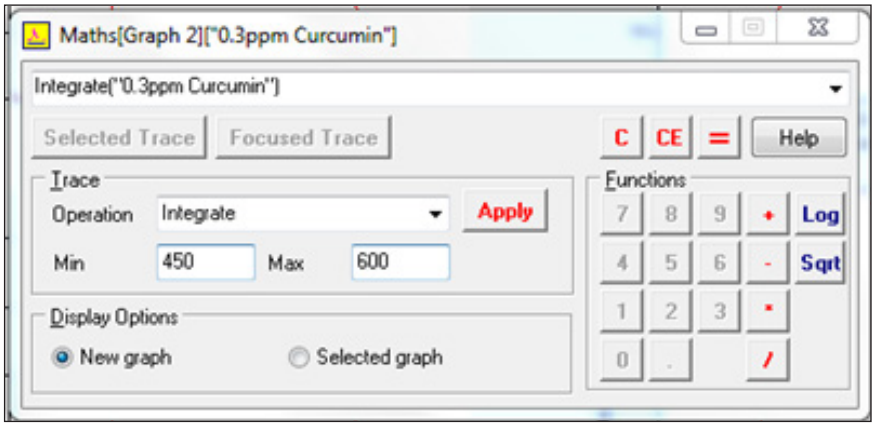

Figure 3. *'Integrate' function used to determine the total area under the curve*

Description	Integrated peak area of standard/sample in DMF	Absorbance
Curcumin	1101.2	0.035
	1495.5	0.047
	2016.2	0.066
	2412.1	0.080
Batch - 1	6222.8	0.054
	8945.0	0.082
	11214.0	0.100
Batch - 2	5175.0	0.048
	7746.1	0.071
	9925.0	0.095

Table 1. *Area of fluorescence of samples and standard with corresponding absorbance values*

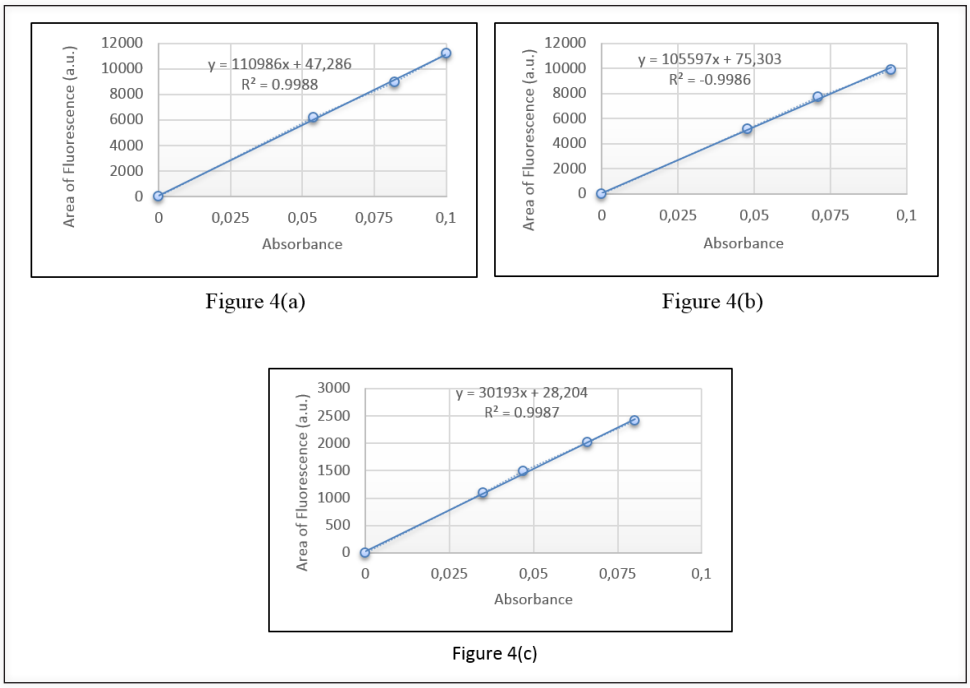

Figure 4. *Linear fits (Absorbance vs. Area of Fluorescence) obtained from Table 1 for 4(a) Food Dye Batch 1, 4(b) Food Dye Batch 2 and 4(c) Curcumin.*

By substituting the values in the equation for Quantum Yield:

$$Q=Q\,(r) \times (m/m_r) \times (n^2/n_r)$$

Quantum yield of curcumin = 0.174

Refractive Index of dimethylformamide (DMF) and Acetone are 1.43 and 1.36 respectively.

Using values of m and m_r derived from the plots of Absorbance vs Area of Fluorescence of the sample and curcumin, the fluorescence quantum yield of the food dye samples can be calculated and the results are given in Table 2.

Description	Quantum Yield
Batch 1	0.70
Batch 2	0.67

Table 2. *Quantum Yield of food dye samples.*

CONCLUSIONS

The fluorescence quantum yield of food dye samples was measured using the Agilent Cary Eclipse (with an Agilent Cary 60 being used to measure the corresponding absorbance of each sample).

Although the comparative method for determining the fluorescence quantum yield takes a longer time than the single point method, the benefit is that it gives more accurate results.

The quantum yield is a good measure of product quality – the higher the quantum yield, the better the quality of the product. From this experiment we can conclude that the Batch 1 sample having a Q value of 0.70 is better quality than the Batch 2 sample which only had a measured Q value of 0.67.

REFERENCES

1. Joseph R. Lakowicz, Principles of Fluorescence Spectroscopy, 3rd Edition, Springer Science, 2006.

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