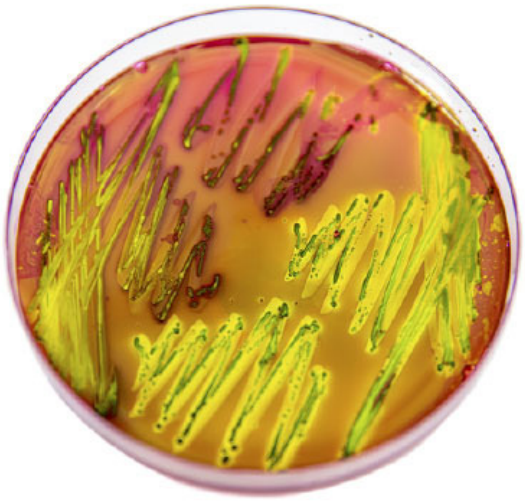
APPLICATION NOTE

MEASURING THE MOLAR ABSORPTION COEFFICIENT OF EOSIN Y AND ROSE BENGAL IN WATER



INTRODUCTION

Among the fluorescent dyes, the group of fluorophores based on xanthene moiety are one of the most popular. Fluorescein and rhodamine are the two most common representatives of this class of fluorescent dyes [1].

With applications in lasers and biological imaging, fluorescein and rhodamine are known for having remarkable photophysical properties such as a high molar absorption coefficient and a near-to-one fluorescence quantum yield [2,3].

Eosin Y and rose bengal are two fundamental xanthene dyes that differ from fluorescein structure by having four halogens atoms instead of hydrogens, see **Figure 2**.

Keywords:

UV-Visible Spectroscopy

DW Light Source

FLEX Spectrometer

Xanthene Dyes

Molar Absorption Coefficient

Figure 1 – Absorbance Configuration.

This change strongly affects their photophysical properties, and for that reason, the most common applications of these dyes span from sensitizers in photodynamic therapy to dyes in food and biological materials [4]. Owing to their anionic nature, both eosin Y and rose bengal are highly susceptible to changes in pH, which also affects their photophysical properties [1,4].

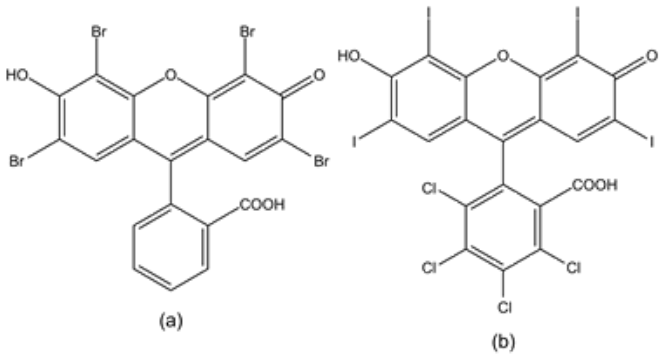


Figure 2 – Eosin Y (a) and rose bengal (b) molecular structures.

From a spectroscopic perspective, food colorants share the property of absorbing ultraviolet and visible (UV-Vis) radiation.

The efficiency of light absorption by an absorbing specie is generally characterized by the absorbance that in many cases follows the Beer-Lambert law,

$$A = \log \frac{I_0}{I} = \varepsilon l c, \text{ (Eq. 1)}$$

where I_0 and I are the incident and transmitted light intensities, ε is the molar absorption coefficient (generally expressed in M^{-1} cm^{-1}), l is the absorption path length (in cm) and c is the concentration of the of the molecule in absorbing medium (in M). The molar absorption coefficient expresses the ability of a molecule to absorb light at a certain wavelength and is an important parameter when performing for the first time the photophysical characterization of an absorbing specie. A high molar absorption coefficient (> 10^5 M^{-1} cm^{-1}) indicates a probable transition [5].

In this application note, we combine the Sarspec's DW light source with FLEX spectrometer in a UV/Vis absorption configuration, see **Figure 1**, to determine the molar absorption coefficient of eosin Y and rose bengal in water, at pH 7.0.

MATERIALS & METHODS

Reagents

- Eosin Yellowish ($C_{20}H_6Br_4Na_2O_5$, Mw ≈ 691.86 g/mol, Alfa Aeser, > 90%);
- Rose Bengal ($C_{20}H_2Cl_4I_4Na_2O_5$, Mw ≈ 1017.64 g/mol, Aldrich Chemicals, 95%);

Instruments and Accessories:

Absorbance Configuration

(see **Figure 1**)

- DW light source;
- 400 µm diameter illumination optical fiber;
- Standard cuvette holder set into an absorbance configuration;
- 200 µm diameter collecting optical fiber;
- FLEX STD UV/Vis spectrometer (Slit: 10 µm);
- 10x10 mm absorption cuvettes in UV quartz;

EXPERIMENTAL PROCEDURE

1. Eosin Y and rose bengal stock solution was prepared by weighting an accurate amount of the selected dye (8.0 mg for eosin Y and 6.8 mg for rose bengal) and diluted with distilled water up to the mark, in a 100 mL volumetric flask.

2. Standard solutions were prepared by taking 1, 3, 5, 7, 9, 11, 13 and 15 mL from the stock solution and diluting with distilled water up to the mark, in a 100 mL volumetric flask.

3. The absorption of each standard solution was measured and the baseline correction was carried out using a blank solution of distilled water.

4. The LightScan software was used and the instruments settings selected are given in **Table 1**.

Table 1 – Instrument settings used for experimental diffuse reflectance measurements.

Parameter	Used Settings
Integration time (ms)	50
Average	150
Smoothing	0

RESULTS

The absorbance spectra of eosin Y and rose bengal standard solutions are given in **Figure 3**.

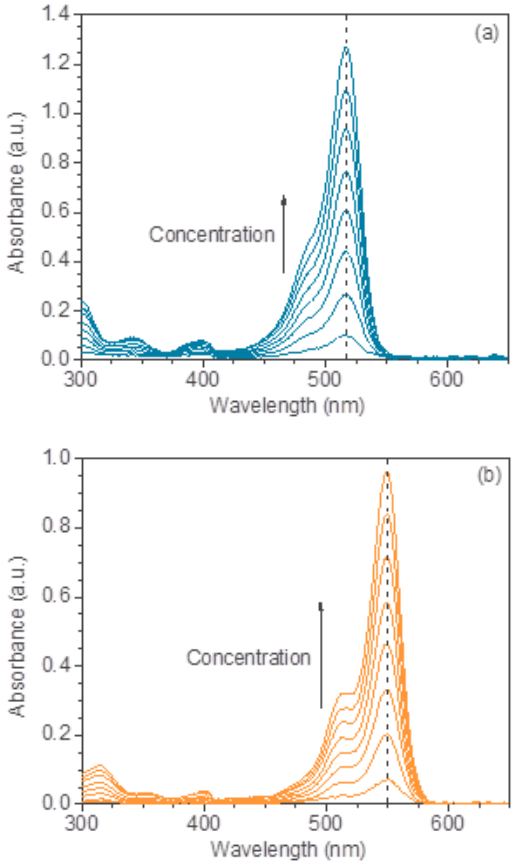


Figure 3 – Absorption spectra of eosin Y (a) and rose bengal (b) standard solutions. The dashed line indicates the maximum absorption wavelength.

According to **equation 1**, the molar absorption coefficient of eosin Y and rose bengal can be obtained from plotting the absorbance maximum (approximately 517 nm for eosin Y and 550 nm for rose bengal) against the concentration, see **Figure 4**.

The line of the best fit shown in **Figure 4** was obtained from the least squares method, a statistical approach in regression analysis that minimizes the sum of the squared residual points from the plot. The molar absorption coefficient values of eosin Y and rose bengal, at 517 and 550 nm, respectively, are obtained from the slope of the best linear fit and are given in **Table 2**.

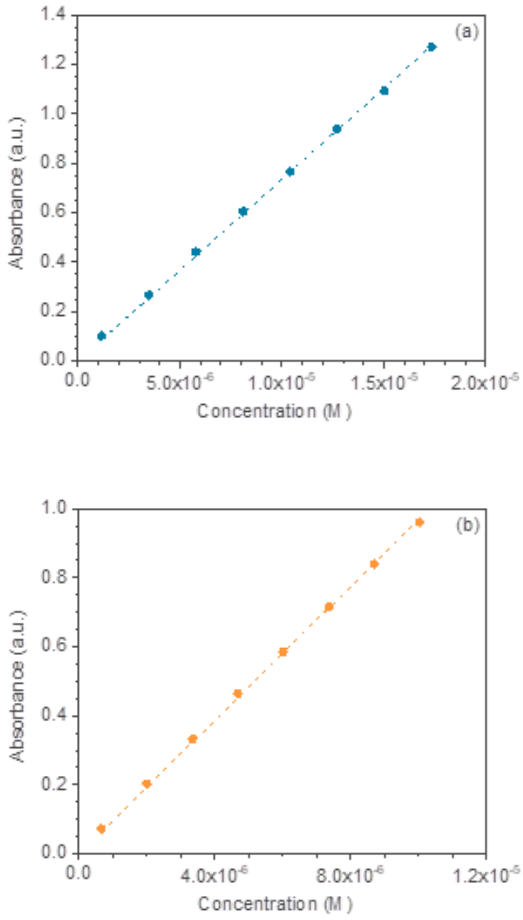


Figure 4 – Absorbance of eosin Y (a) and rose bengal (b) standard solutions against concentration. The dashed line indicates the line of best fit to the experimental data.

Table 2 – Molar absorption coefficient of eosin Y and rose bengal obtained from the slope of the best linear fit, at 517 and 550 nm, respectively.

Compound	Molar absorption coefficient (M^{-1} cm^{-1})	R^2
Eosin Y	73540	0.999
Rose Bengal	96960	0.999

From the data presented in **Table 2**, it is observed that the molar absorption coefficient at the absorbance maximum of eosin Y is around 25 % lower than the molar absorption coefficient at the absorbance maximum of rose bengal.

The plot of the molar absorption coefficient with the wavelength of eosin Y and rose bengal are presented in **Figure 5** and were obtained from the values given in **Table 2**.

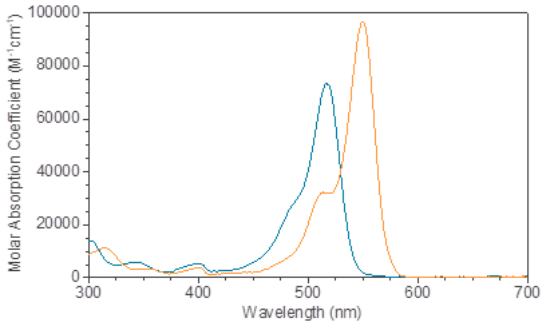


Figure 5 – Molar absorption coefficient spectra of eosin Y (blue line) and rose bengal (orange line).

CONCLUSIONS

The work presented in this application note aims to demonstrate that Sarspec's DW light source and FLEX spectrometer conjugated in an UV-Visible configuration is perfectly suitable to accurately perform a calibration curve with a strong linear relationship and a near-to-one correlation coefficient over a good range of absorption values.

This configuration allows not only to determine the molar absorption coefficient of any group of fluorophores in solution but also to perform quantitative analysis of absorbing species used in various fields such as chemistry, biochemistry, materials science, and chemical engineering.

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