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Spectrophotometric Determination of Fluorgenic Xanthene Dyes

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In this paper, the change of absorbance and their first and second derivatives for the Xanthene dyes as concentration dependence are reported. The effect of change of smoothing on the LOD and other analytical characteristics are investigated.

Key Words: Fluorescein, Rhodamine 6G, Rhodamine B, Xanthene dyes.

INTRODUCTION

Xanthene date from 1871 when Von Bayer synthesized fluorescein by the condensation of two moles of resorcinol with one mole of phthalic anhydride in the presence of concentrated sulfuric acid[1,2].

Xantene dyes are those containing the xanthylium dyes or dibenzo-γ-pyran nucleus as the chromophore with amino or hydroxyl groups *meta* to the oxygen as the usual auxochromes. They are used for direct dyeing of wool and silk and mordant dyeing of cotton. They are important because of their brilliant hues and shades between greenish yellows, to dark violets and blues are obtainable.

Amino derivatives of xanthene dyes are commercially the most important compounds and historically these have been called rhodamines. They are the most important fluorescent orange-red dyes. The rhodamines described thus far are basic rhodamines. Some of rhodamines used as PVC resin, in inkjet printing and in dyeing of silk and wool[1]. Xantenes including fluorescein, rhodamine B, rhodamine 6G have been used as laser dyes[3]. Aromatic fluorescer compounds such as rhodamine 6G can accept energy and changes of bacterial bioluminescence[4].

EXPERIMENTAL

The UV spectra was obtained by using UV-vis spectrophotometer instrument (Cecil 5501). The condition of instrument during obtaining the peaks was as the bellows: Start λ: 350.0 nm; Finish λ: 700.0 nm; Scan speed: 2.0 nm/s; Threshold: 5%; Cell length: 5 mm.

The UV-vis spectra, the first and the second derivatives by the different smoothing (1, 3, 5 and 7) were obtained and the related signal to noise ratio was calculated.

All chemical reagents were purchased from Fluka (Buchs, Switzerland). The dye pigments were prepared in ethanol. These were rhodamine B, rhodamine 6G and fluorescein. The concentration of reagents was as 0.00005 to 0.01.

RESULTS AND DISCUSSION

The spectrophotometric determination of some xantene dyes are investigated in recent years[5-8]. The relationship between absorption, first and second derivatives of absorption versus concentration were plotted and the statistics data such as intercept (α), slope (β), regression coefficient (r), the standard deviation of regression (S_r), the standard deviation of intercept (S_α), the standard deviation of slope (S_β) and limit of detection (LOD) in molarity, by using the following formula are reported[7,9].

$$A = \alpha + \beta C_S \quad (1)$$

$$dA/d\lambda = (1.Der) = \alpha' + \beta' C_S \quad (2)$$

$$d^2A/d\lambda^2 = (2.Der) = \alpha'' + \beta'' C_s \quad (3)$$

$$S_{xx} = \sum^2 x_i - (\sum x_i)^2/n \quad (4)$$

$$S_{yy} = \sum^2 y_i - (\sum y_i)^2/n \quad (5)$$

$$S_r = \sqrt{(S_{yy}-\beta^2 S_{xx})/n-2} \quad (6)$$

$$S_\beta = \sqrt{(S_r)^2/S_{xx}} \quad (7)$$

$$r = S_{xy}/\sqrt{S_{xx}.S_{yy}} \quad (8)$$

$$LOD = 3S_r/\beta \quad (9)$$

The statistical data for each dye are tabulated in Tables 1-3. According to the data in Tables 1-3 the amount of absorbance for fluorescein is lower than each others .This situations depends on to the large extinction coefficient (ε) of fluorescein and therefore this term produce large amount of slope of regression linear plot. In related to importance of use of xanthene dyes in industry, chemical and clinical laboratory, textile, waste water treatment and anti-freeze products we obtained procedure for investigation of determination of these dye stuffs.

The absorbance, first and second derivative (smoothing 7) spectra of fluorescein are shown in Figs. 1-3, respectively.

TABLE-1

THE LINEAR REGRESSION TERMS OF RHODAMINE B DETERMINATION

RB	$\alpha(\pm tS_{\alpha})$	S_{α}	$\beta(\pm tS_{\beta})$	S_{β}	r	S_r	LOD
1-Der. Smoot1	0.0172 ($\pm 8.98 \times 10^{-6}$)	3.2×10^{-6}	960.410 (± 2.06)	0.743	0.8544	3.1×10^{-5}	9.7×10^{-8}
1-Der. Smoot3	0.1026 ($\pm 1.50 \times 10^{-6}$)	5.4×10^{-7}	962.980 (± 2.01)	0.724	0.8650	4.7×10^{-4}	1.4×10^{-6}
1-Der. Smoot5	0.0998 ($\pm 7.78 \times 10^{-6}$)	2.8×10^{-6}	964.530 (± 2.02)	0.730	0.9712	4.8×10^{-5}	1.5×10^{-6}
2-Der. Smoot1	0.0024 ($\pm 8.34 \times 10^{-5}$)	3.0×10^{-5}	45.098 (± 0.086)	0.031	0.9933	2.4×10^{-5}	1.6×10^{-6}
2-Der. Smoot3	0.0011 ($\pm 5.38 \times 10^{-8}$)	2.1×10^{-8}	138.720 (± 0.055)	0.020	0.9939	5.2×10^{-7}	1.3×10^{-8}
2-Der. Smoot5	0.0014 ($\pm 3.05 \times 10^{-8}$)	1.1×10^{-8}	126.489 ($\pm 2.78\times^{-3}$)	0.001	0.9998	6.4×10^{-8}	1.5×10^{-9}
2-Der. Smoot7	0.0012 ($\pm 1.20 \times 10^{-5}$)	4.3×10^{-6}	111.680 (± 0.91)	0.323	0.9996	6.5×10^{-5}	1.7×10^{-6}
ABS	0.5913 ($\pm 2.22 \times 10^{-4}$)	8.0×10^{-5}	3824.390 (± 0.66)	0.241	0.8833	2.6×10^{-8}	2.6×10^{-6}

TABLE-2

THE CHARACTERISTICS OF LINEAR REGRESSION TERMS OF RHODAMINE 6G DETERMINATION

R6G	$\alpha(\pm tS_{\alpha})$	S_{α}	$\beta(\pm tS_{\beta})$	S_{β}	r	S_r	LOD
1-Der. Smoot1	0.6241 ($\pm 6.11 \times 10^{-9}$)	2.2×10^{-9}	1090.16 ($\pm 5.56 \times 10^{-3}$)	0.002	0.9513	4.4×10^{-6}	1.2×10^{-8}
2-Der. Smoot1	0.0083 ($\pm 1.2 \times 10^{-7)}$	4.3×10^{-8}	423.40 ($\pm 2.78 \times 10^{-3}$)	0.001	0.9970	3.0×10^{-4}	2.1×10^{-5}
2-Der. Smoot3	0.0080 ($\pm 6.67 \times 10^{-8}$)	2.4×10^{-8}	423.30 ($\pm 5.6 \times 10^{-3}$)	0.002	0.9336	2.4×10^{-5}	1.7×10^{-6}
2-Der. Smoot5	0.0022 ($\pm 1.8 \times 10^{-6}$)	3.9×10^{-7}	167.71 ($\pm 5.56 \times 10^{-3}$)	0.002	0.8628	3.3×10^{-5}	5.4×10^{-9}
ABS	0.423 ($\pm 2.78 \times 10^{-6}$)	1.0×10^{-6}	2458.00 (± 0.433)	0.156	0.8733	1.0×10^{-5}	1.2×10^{-8}

TABLE-3

THE LINEAR REGRESSION TERMS CHARACTERISTIC OF FLUORESCEIN DETERMINATION

FL	$\alpha(\pm tS_{\alpha})$	S_{α}	$\beta(\pm t\ S_{\beta})$	S_{β}	r	S_r	LOD
2-Der. Smoot1	0.0086($\pm 1.39 \times 10^{-8}$)	5.0×10^{-7}	3706.0 (± 0.86)	0.308	0.9988	2.6×10^{-5}	2.1×10^{-8}
2-Der. Smoot3	0.0006 ($\pm 1.25 \times 10^{-6}$)	4.5×10^{-7}	4429.4 ($\pm 5.65 \times 10^{-3}$)	0.002	0.9996	3.2×10^{-5}	2.2×10^{-6}
2-Der. Smoot5	0.0004 ($\pm 5.28 \times 10^{-8}$)	1.9×10^{-8}	1237.0 (± 0.013)	0.005	0.9869	4.6×10^{-5}	1.1×10^{-8}
2-Der. Smoot7	0.0085 ($\pm 6.39 \times 10^{-7}$)	2.3×10^{-7}	1165.0 (± 0.83)	0.031	0.9910	1.8×10^{-5}	1.8×10^{-7}
ABS	0.1581 ($\pm 1.20 \times 10^{-4}$)	4.3×10^{-5}	2747.3 (± 3.10)	1.117	0.9813	2.0×10^{-5}	2.1×10^{-6}

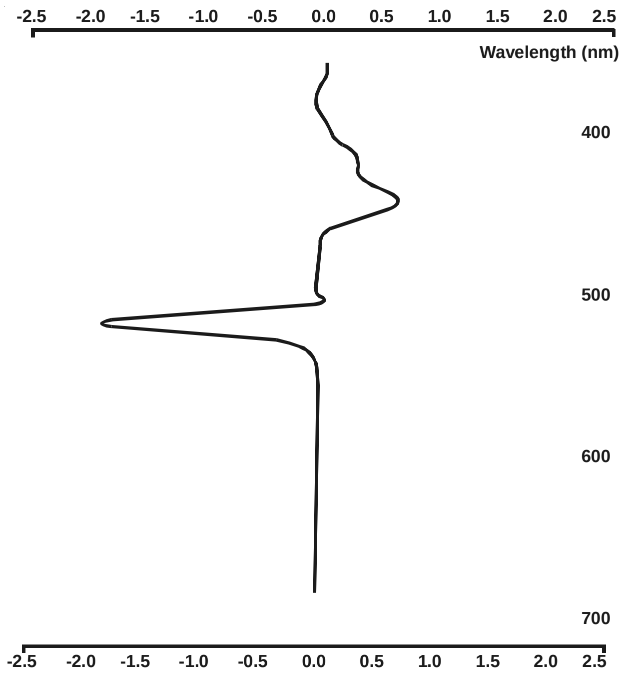

Fig. 1. First derivative of absorption spectra of fluorescein solution

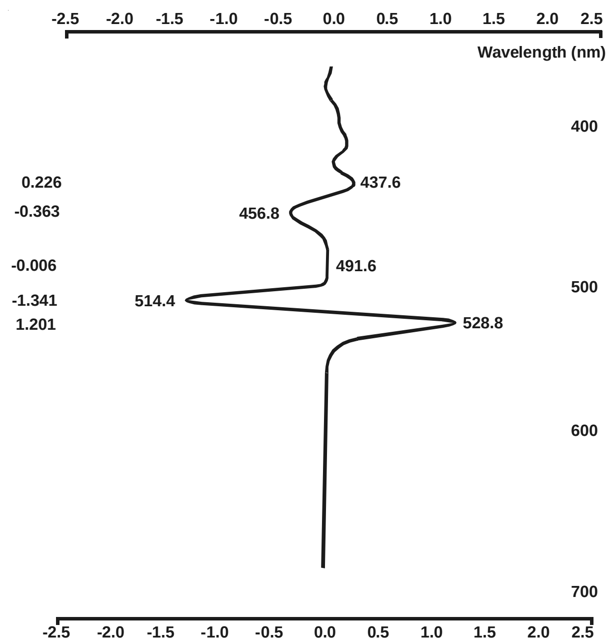

Fig. 2. Second derivative of absorption spectra of fluorescein solution

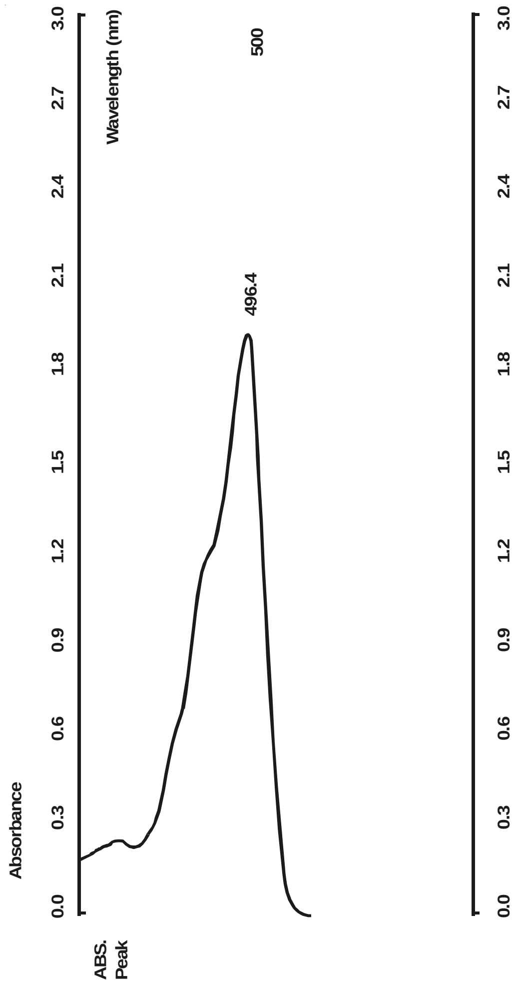

Fig. 3. Absorption spectra of methanolic fluorescein solution

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