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RESEARCH ARTICLE

Simultaneous Spectrophotometric Determiantion of Eosin and Erythrosine with Cr(VI) Reagent in Micellar Media Using Mean Centering of Ratio Spectra

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Abstract: Spectrophotometric method is described for the simultaneous determination of binary mixtures of eosin and erythrosine in pharmaceutical and food samples, without prior separation steps, using mean centering of ratio spectra. The method is based on the difference in the absorption spectra for the products of the reaction of eosin and erythrosine with Cr(VI) in the presence of CPB micellar media at pH 6.0. The method allows rapid and accurate determination of eosin and erythrosine. The results showed that the method was capable to simultaneous determination of 1.24-6.86 µg/mL and 1.47- 8.06 µg/mL each of eosin and erythrosine. The proposed method was successfully applied to the simultaneous determination of eosin and erythrosine in pharmaceutical and food samples.

Keywords: Eosin, Erythrosine, Cr(VI), CPB, Mean Centering of ratio spectra

Introduction

The chemical behavior of eosin[1-2] and erythrosine[3-5] is very similar. They both belong to same class *i.e.* fluorone dyes. It is used in medicine as an important radioactive tracer. It is also used as staining agent in biological work. It is used in textile dyeing and ink manufacturing. Sodium or potassium salt of this powder is used in biology to stain cells and cytoplasm, collagen and muscle fibers for examination under the microscope. So, it is very important to develop reliable, fast and sensitive methods for the determination of eosin and erythrosine in environmental and biological samples. These dyes cannot be determined simultaneously by the use of ordinary Spectrophotometric methods due to the overlapping of absorption spectra.

The organized molecular assembles such as micelles are used in spectroscopic measurements due to their possible effects on the systems of interest. In the metal-dye complex, at a concentration above critical micelle concentration (CMC) micelles form a ternary complex with advantageous properties, such as hypsochromic and bathochromic shifts that can modify the sensitivity of the method by affecting the interferences and matrix effects[6,7]. The ability of Micellar system to solublize slightly soluble or even insoluble complexes has been used to enhance the analytical merit of developed method.

Recently, Afkhami and Bahram[8-14] presented a new spectrophotometric method for the analysis of binary and ternary mixtures, without prior separation steps which called "mean centering of ratio spectra" method[15]. In this paper, we applied mean centering of ratio spectra for simultaneous determination of binary mixtures of eosin and erythrosine without any preliminary separation steps.

Experimental

The absorbance spectra were recorded on a Shimadzu – 1800 double beam UV-Vis spectrophotometer. Digital century pH-meter CP-901 with a combined glass electrode was used to adjust pH and 1.0 cm quartz cuvettes were utilized for absorbance studies. All calculations in the computing process done in MATLAB 7.0 and Microsoft Excel for windows. A sample program was written for this purpose in MATLAB 7.0.

Reagents

All reagents used were of AnalaR grade unless otherwise stated. Double distilled water was used throughout. Stock solution of eosin and erythrosine (Loba chem.) were prepared in double distilled water. Further dilutions were made as and when required. A 0.1% (w/v) solution of Cr(VI) was prepared by dissolving potassium dichromate (Loba Chem.) in double distilled water. A buffer solution of pH 6.0 was prepared by mixing 0.2 M acetic acid and 0.2 M sodium acetate solution and 1.0% (v/v) CPB solution was prepared in hot double distilled water.

Procedure

Mean centering ratio spectra

Appropriate amounts of eosin and erythrosine sample solution were taken in calibration range and pH 6.0 was adjusted by adding 2.0 mL buffer solution. Then 1.5 mL of 0.1% Cr(VI) and 1.5 mL of 1.0% CPB was added. The stored calibrated spectra of the solution eosin or erythrosine were divided by standard spectrum of erythrosine or eosin respectively. Then mean centering of these vectors with respect to wavelength were obtained. The minimum or maximum of the mean centering of later vectors with respect to wavelength was used for the construction of calibration graph for eosin or erythrosine.

Theory of mean centering of ratio spectra

Consider a mixture of two compounds X and Y. If there is no interaction among the compounds and Beer`s law is obeyed for each compound, it can be written:

$$A_m = \alpha_x C_x + \alpha_Y C_Y \quad (1)$$

Where, A_m = vector of the absorbance of the mixture, α_x and α_y = molar absorptivity vectors of X and Y, C_x and C_Y = concentrations of X and Y.

If equation 1 is divided by α_Y corresponding to the spectrum of a standard solution of Y in the binary mixture, the first ratio spectrum is obtained in the form of equation 2:

$$B = \frac{A_m}{\alpha_Y} = \frac{\alpha_x C_x}{\alpha_Y} + C_Y \quad (2)$$

If the Equation 2 is mean centered (MC), since the mean centering of a constant (C_Y) is zero, equation 3 would be obtained.

$$MC(B) = MC\left[\frac{\alpha_x C_x}{\alpha_Y}\right] \quad (3)$$

Equation 3 permits the determination of concentration of each of the active compounds in the solution (X in this equation) without interfering from the other compounds of the binary system (Y in these equations) and shows that there is a linear relationship between the amount of MC (B) and the concentration of X in the solution. A calibration curve is constructed by plotting MC (B) against concentration of X in the standard solutions of X. For more sensitivity the amount of MC (B) corresponding to maximum or minimum wavelength is measured. Calibration graphs for Y are also constructed as described for X.

Results and Discussion

Absorption spectra

The absorption spectra of eosin and erythrosine complexes with Cr(VI) are shown in Figure 1. As shown in Figure 1, complexes overlap with each other and therefore each compound interferes in the spectrophotometric determination of the others. But simultaneously determination of eosin and erythrosine is possible by using mean centering of ratio profiles.

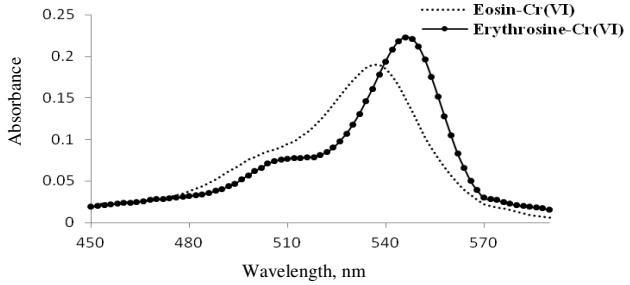

Figure 1. Absorption spectra of complexes of eosin (1.24 µg/mL) and erythrosine (2.19 µg/mL) with Cr(VI) [1.5 mL of 0.1% Cr(VI), 1.5 mL of 1.0% CPB, 2.0 mL buffer solution of pH 6.0]

Optimization of various parameters

Effect of pH

Effect of pH on the absorbance of complexes was studied in the range of 3.5-9.0. Eosin-Cr(VI) complex showed constant absorbance in the pH range 3.5-6.5 and then it decreased at higher pH. Erythrosine –Cr(VI) complexes showed maximum absorbance in pH 6.0. So, for simultaneous determination of eosin and erythrosine with pH 6.0 was selected as an optimum value and was maintained by adding 2.0 mL of acetic acid/ sodium acetate buffer solution. Effect of pH on the absorbance of complexes is shown in Figure 2.

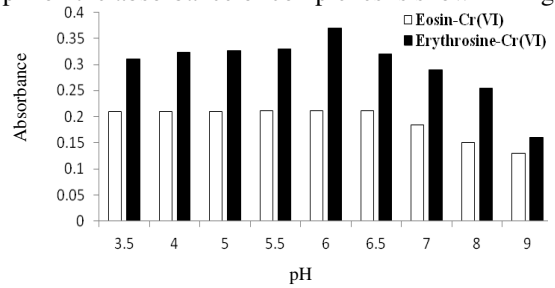

Figure 2. Effect of pH on the absorbance of eosin and erythrosine complexes

Effect of nature of surfactant

Various surfactants such as Triton X-100, Tween-80, Tween-20, cetylpyridinium bromide (CPB), sodium lauryal sulphate (SLS), cetyltrimethylammonium bromide (CTAB) were tried as solubilizing agents. A series of solutions containing fixed amounts of eosin or erythrosine and reagent at optimized conditions of pH were prepared. The complexes were dissolved in different surfactants and the spectra were recorded. Eosin-Cr(VI) complex showed maximum and stable absorbance with cetylpyridinium bromide (CPB) and cetyltrimethylammonium bromide (CTAB) and erythrosine –Cr(VI) complexes showed maximum absorbance with cetylpyridinium bromide (CPB). So, for simultaneous determination of eosin and erythrosine, 1.5 mL of 1.0% CPB was selected as the working micellizing agent for further studies. Effect of different surfactants on the absorbance of dye complexes is shown in Figure 3.

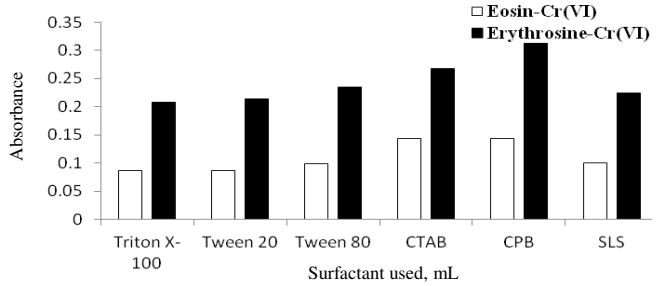

Figure 3. Effect of different surfactants on the absorbance of eosin and erythrosine complexes

Effect of reagent concentration

The effect of different amounts of reagent for the fixed dye ion concentration was studied in detail. The dye solution was adjusted to desired pH value and the complex was solublized in the optimized amount of CPB. The maximum absorbance in both the cases was observed when 1.5 mL of 0.1% Cr(VI) was used for individual calibration as shown in Figure 4.

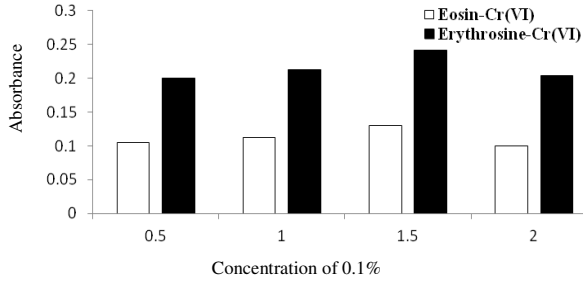

Figure 4. Effect of concentration of Cr(VI) on the absorbance of eosin and erythrosine complexes

Applying mean centering of ratio spectra

The absorption spectra of the standard solutions of eosin-Cr(VI) with different concentrations were recorded in the wavelength range 500.0-550.0 nm with 1.0 nm intervals as shown in Figure 5 and divided by the normalized spectrum of the erythrosine and the ratio profiles of eosin-Cr(VI) were obtained as shown in Figure 6. Mean centering (MC) of the ratio profiles for eosin-Cr(VI) were obtained in the wavelength range 526.0-558.0 nm as shown in Figure 7. The concentration of eosin-Cr(VI) was determined by measuring the amplitude at 526.0 nm corresponding to a maximum wavelength as shown in Figure 7.

The absorption spectra of the standard solutions of erythrosine-Cr(VI) with different concentrations were recorded in the wavelength range 500.0-550.0 nm with 1.0 nm intervals as shown in Figure 8 and divided by the normalized spectrum of the eosin and the ratio profiles of erythrosine-Cr(VI) were obtained as shown in Figure 9. Mean centering (MC) of the ratio profiles for erythrosine-Cr(VI) were obtained in the wavelength range 514.0-532.0 nm as shown in Figure 10. The concentration of erythrosine-Cr(VI) was determined by measuring the amplitude at 514.0 nm corresponding to a maximum wavelength as shown in Figure 10

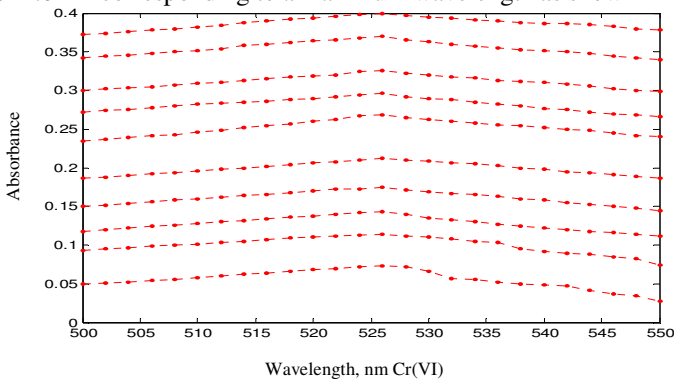

Figure 5. Absorption spectra for the standard solutions of the eosin-Cr(VI) with different concentrations (1.24-6.86 µg/mL)

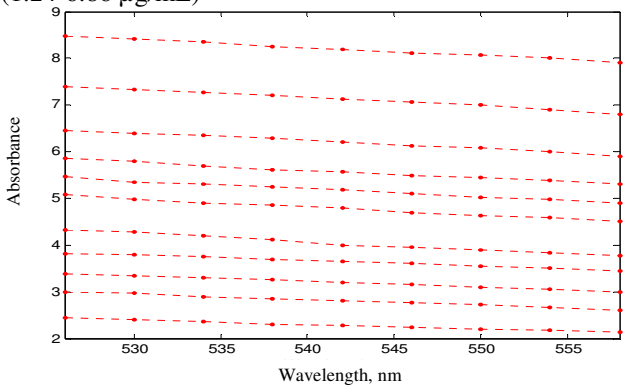

Figure 6. Ratio spectra obtained by dividing the normalized spectra of the erythrosine

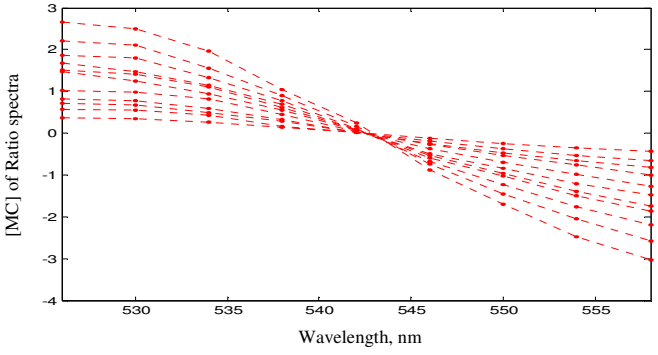

Figure 7. Mean centering of ratio spectra for the eosin-Cr(VI)

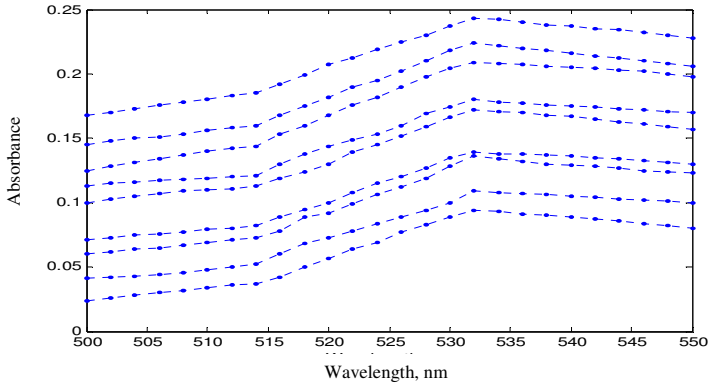

Figure 8. Absorption spectra of the standard solutions of the erythrosine-Cr(VI) with different concentrations (1.47-8.06 µg/mL)

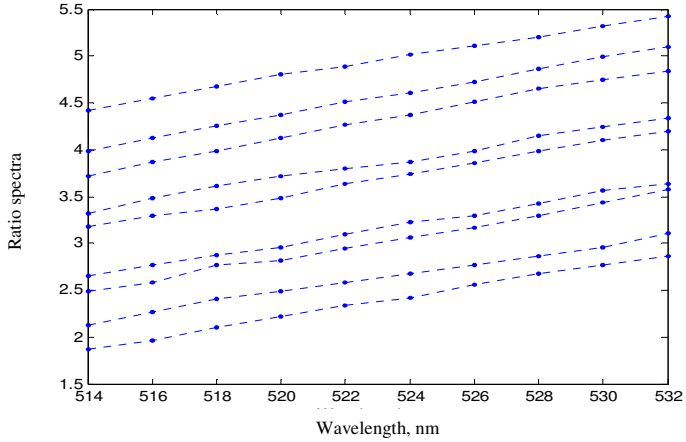

Figure 9. Ratio spectra obtained by dividing the normalized spectra of the erythrosine

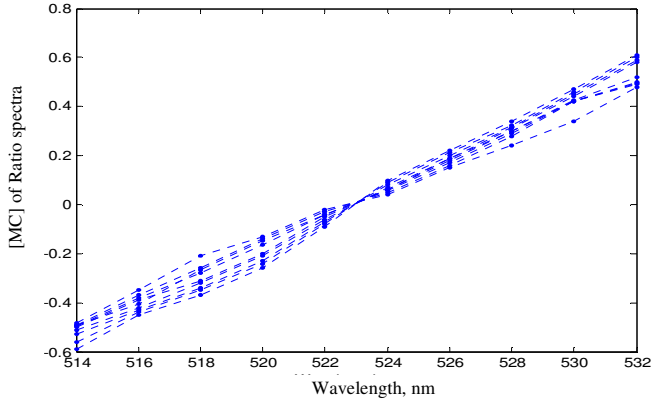

Figure 10. Mean centering of ratio spectra of erythrosine-Cr(VI)

Statistical analysis of results

Mean centering of ratio spectra, Beer`s law was obeyed in the concentration range 1.24-6.86 µg/mL for eosin and 1.47- 8.06 µg/mL for erythrosine. Table 1 shows the linear regression

parameters for calibration data for simultaneous determination of eosin and erythrosine in their binary mixtures. Limit of detection, correlation coefficient of the method for determination of eosin and erythrosine are shown in Table 1.

Table 1. Analytical characteristics for analysis of eosin and erythrosine using Cr(VI) in binary mixtures by mean centering of ratio spectra method

Analyte	Wavelength, nm	Calibration Eq.	R^2	Linear range, µg/mL	LOD, µg/mL
Eosin	526.0	y=0.0513x+0.0139	0.998	1.24-6.86	0.900
Erythrosine	514.0	y=0.0299x+0.026	0.987	1.47-8.07	0.498

Application

The proposed methods were validated by the analysis of eosin and erythrosine in cosmetic samples and confectionary products. An aliquot of the sample solution was analyzed by the procedure described above. The concentrations of these dyes were calculated from the regression equation obtained from calibration curves. The results for the simultaneous determination and certified amount present in the sample are given in Table 2.

Table 2. Results for the application of developed methods to different synthetic mixtures for simultaneous determination of eosin and erythrosine using Cr(VI) reagent

Sample	Added, µg/mL		Found, µg/mL		% Recovery	
	Eosin	Ery	Eosin	Ery	Eosin	Ery
[a]Mascara	0.252	0.672	0.255	0.670	101.2	99.7
[b]Eye Shade	0.275	0.712	0.276	0.713	100.4	100.1
[c]Fruit Cake	0.314	0.624	0.312	0.625	99.4	100.2

[a]Revlon Red color, Batch No. 453R0N, [b]Beauty Red Shade, Batch No. 1222CVF8, [c]Orange flavor, product No

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