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Research Paper

Simultaneous Spectrophotometric Determination of Ponceau-S and Erythrosine in Pharmaceutical, Cosmetic and Food Samples by Using Mean Centering of Ratio Spectra Method

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Abstract: *A new micelle-mediated extraction method for ultra-trace quantities of ponceau-s and erythrosine as a prior step to their simultaneous Spectrophotometric determination has been developed. The mean centering (MC) of ratio spectra method is based on the difference in the absorption spectra for the products of the reaction of ponceau-s and erythrosine with Fe (III) in the presence of Sodium Lauryal Sulphate (SLS) micellar media at pH 5.5. The method allows rapid and accurate determination of ponceau-s and erythrosine. The conditions were optimized for analytical characteristics of the method (e.g. limit of detection, linear range) were obtained. Beer`s law was obeyed in the concentration range of 0.175-6.15 µg mL^{-1} of ponceau-s and 0.304 – 4.56 µg mL^{-1} of erythrosine. The proposed method has successfully been applied for simultaneous determination of ponceau-s and erythrosine in pharmaceutical and food samples.*

Keywords: Erythrosine, Fe (III), Micellar media, Mean centering of ratio spectra, Ponceau-s.

Introduction

Dyes and pigments are widely applied for coloration of food [1], cosmetics and drug products. Color is the first sensory parameter by which food quality and flavor can be judged. The term color additive can be applied to any dye, pigment or other substance made artificially or obtained from plants, vegetable or other natural sources. Color [2] is a vital constituent of food and probably the first characteristic perceived by the human senses. Food colorant is any substance that is added to food or drink to change its color probably, for economic reasons. They are used to supplement and enhance natural colors destroyed during processing or storage and substantially increase the appeal and acceptability of food stuffs.

Ponceau-S dyes are anionic and used in the textile industry[3] for dyeing of all natural fibers, e.g. wool, cotton, silk and synthetics, e.g. polyesters, acrylic and rayon. They are used in a variety of application fields such as in paints, inks, plastics. Ponceau-S is used to prepare a stain for rapid reversible detection of protein bands on nitrocellulose or PVDF membranes, as well as on cellulose membranes. Ponceau S stain is easily reversed with water washes, facilitating subsequent immunological detection.

Erythrosine [4, 5] is a reddish – pink synthetic dye, which is water soluble and most popularly used as a food coloring agent and a host of other applications such as printing inks, a dental plaque disclosing agent. It is also used in drugs and cosmetics, cocktails, tinned fruits, garlic sausage, scotch eggs and snack foods. It is highly toxic, causing various types of allergies, thyroid activities, anemia, and DNA damage behavior. It is also carcinogenic in nature.

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. 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 [6-10], presented a new Spectrophotometric method for the analysis of binary and ternary mixtures, without prior separation steps called “mean centering of ratio spectra” [11-12] method. This

method is based on the successive derivatives of ratio spectra in two steps. The method evaluates and eliminates the blank bias error present in such procedures using mean centering of ratio spectra. This procedure gives more accurate results than by traditional approach using absorbance values against reagent blank.

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.

Material and Methods

Apparatus: A Shimadzu 1800 double beam spectrophotometer loaded with the spectra "TREAT SOFTWARE" and interfaced to a computer in conjunction with HP LaserJet 1010 printer to record the spectra and calculate their derivatives. C_p-901 digital century pH-meter using a combined glass electrode was used for pH measurements. All calculations in the computing process were done in Matlab 7.0 and Microsoft Excel for windows. A simple program was written for this purpose in Matlab 7.0.

Reagents: All reagents used were of anal R grade unless otherwise stated. Double distilled water was used throughout. Stock solution of ponceau-s 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 Fe (III) was prepared by dissolving Ferric Chloride (Loba Chem.) in double distilled water. A buffer solution of pH 5.5 was prepared by mixing 0.2M acetic acid and 0.2M sodium acetate solution and 1.0 % (w/v) Sodium Lauryal Sulphate (SLS) solution was prepared in hot double distilled water.

Individual Calibration: Appropriate volumes of ponceau-s or erythrosine standard solutions in calibration range of (0. and 0.146-4.10 µg/mL for erythrosine, 1.5 mL of 1.0 % SLS, 2.0 mL of buffer solution (pH 5.5), 0.5 mL of 0.1 % Fe (III) solution were added into a 10.0 mL standard flask and volume was made up to the mark with double distilled water. A portion of the solution was transferred into a quartz cell. The absorbance spectrums of the solutions were recorded against standard references prepared under similar conditions and stored in the range of 450-650 nm.

Mean centering of ratio spectra: A calibration graph for ponceau-s is obtained by recording and storing the spectra of standard solutions containing different concentrations of ponceau-s. The stored spectra of the solutions of ponceau-s are divided by standard spectrum of erythrosine according to equation 2. Then mean centering of these vectors with respect to wavelength are obtained according to equation 3. The minimum or maximum of these vectors with respect to wavelength is used for the construction of calibration graph for ponceau-s. For the prediction of concentration of ponceau-s in synthetic binary mixtures and real samples the same procedure was used except that the spectra of the mixture were used instead of the spectra of standard solution of ponceau-s. For samples with unknown matrices standard addition can be used for removing matrix effect. The construction of calibration curves for other active compound and also its prediction step was performed as described for ponceau-s.

Results and Discussion

Mean centering of ratio spectra enables simultaneous determination of species X and Y in a mixture even if their analytical signals overlap extensively and their maxima are coinciding. Absorption spectra of ponceau-s and erythrosine complexes with Fe (III) overlap as shown in Figure 1. Since the spectral bands of the two complexes overlaps. So, simultaneous determination of ponceau-s and erythrosine food colorants is possible only using Binary-Mean centering of ratio spectra.

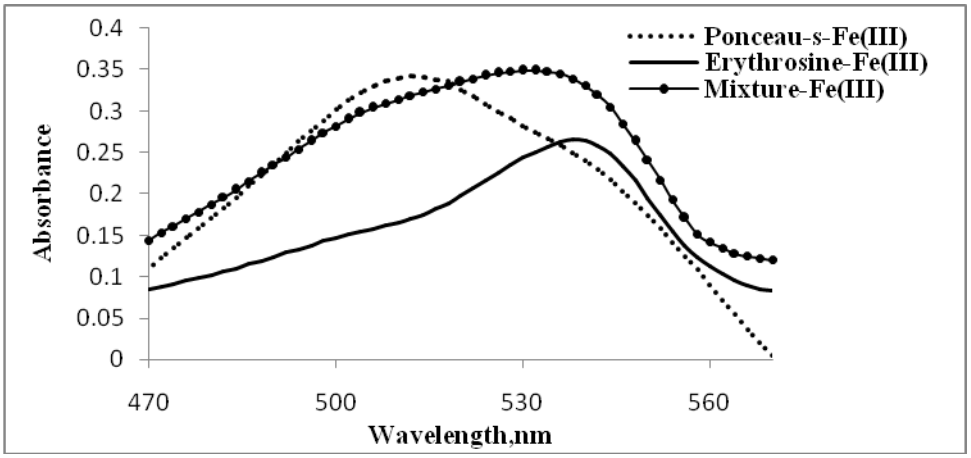

Figure 1: **Absorption spectra of (a) Ponceau-s-Fe (III)-SLS complex (b) Erythrosine-Fe (III)-SLS complex (c) Mixture of Ponceau-s-Fe (III)-SLS and Erythrosine-Fe (III)-SLS complexes**

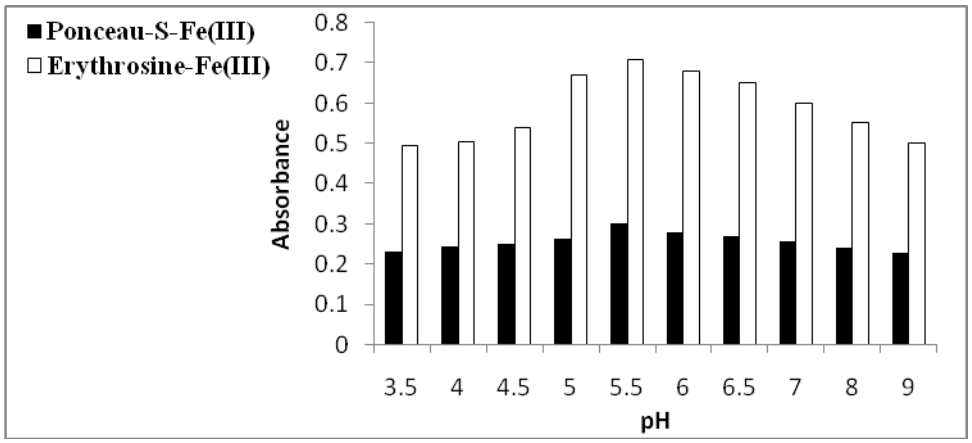

Figure 2: Effect of pH on the absorbance of Ponceau-s-Fe (III)-SLS and Erythrosine-Fe (III)-SLS complexes

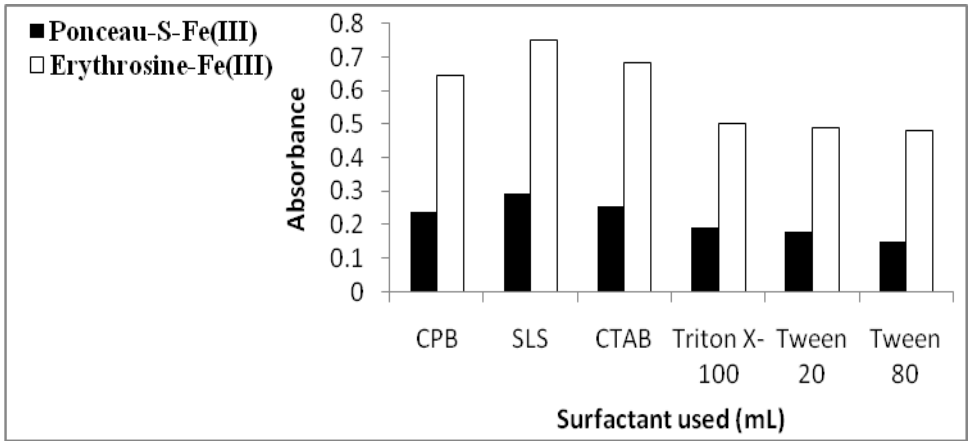

Figure 3: **Effect of different surfactants on complex formation, ponceau-s and erythrosine**

Optimization of variables

Effect of pH: The effect of pH 3.5 – 9.5 on absorbance of Ponceau-s - Fe (III) and Erythrosine – Fe (III) is shown in **Figure 2**. Ponceau-s-Fe (III) complex showed maximum and constant absorbance in the pH range 3.5 – 6.0 and it decreased at higher pH. Absorbance of Erythrosine-Fe (III) complex showed maximum absorbance at pH 5.5, therefore pH 5.5 was selected for simultaneous determination of eosin and erythrosine.

Effect of nature of the surfactant: Various surfactants such as Triton X-100, Tween-20, Tween-80, cetylpyridinium bromide (CPB), sodium Lauryal sulphate (SLS) and Cetyltrimethylammonium bromide (CTAB), were tried as solubilizing agents. Both for Ponceau-s-Fe (III) and Erythrosine-Fe (III) complexes absorbance were

maximum with Sodium Lauryal Sulphate (SLS). So, for simultaneous determination of ponceau-s and erythrosine, 1.5 mL of 1.0% SLS was selected as the working micellizing agent for further studies. Effect of different surfactant is shown in Figure 3.

Effect of reagent concentration: Effect of different amounts of Fe (III) on the absorbance of Ponceau-s-Fe (III) and Erythrosine-Fe (III) complexes was studied. The maximum absorbance in both the cases was observed when 0.5mL of 0.1% Fe (III) was used for individual calibration. Effect of different amounts of 0.1% Fe (III) is shown in Figure 4.

Mean Centering of Ratio Spectra: The absorption spectra of the standard solutions of ponceau-s with different concentrations were recorded in the wavelength range 490.0-550.0nm with 1.0 mm intervals Figure 5 and divided by the normalized spectrum of the erythrosine and the ratio profiles were obtained Figure 6. Mean centering (MC) of the ratio profiles was obtained in the wavelength range 496.0-514.0 nm Figure 7. The concentration of ponceau-s was determined by measuring the amplitude at 496.0 nm corresponding to a maximum wavelength shown in Figure 7. For prediction of concentration of ponceau-s in synthetic binary mixtures and real samples the same procedure was used except that the spectra of the mixture were used instead of the spectra of standard solution of ponceau-s.

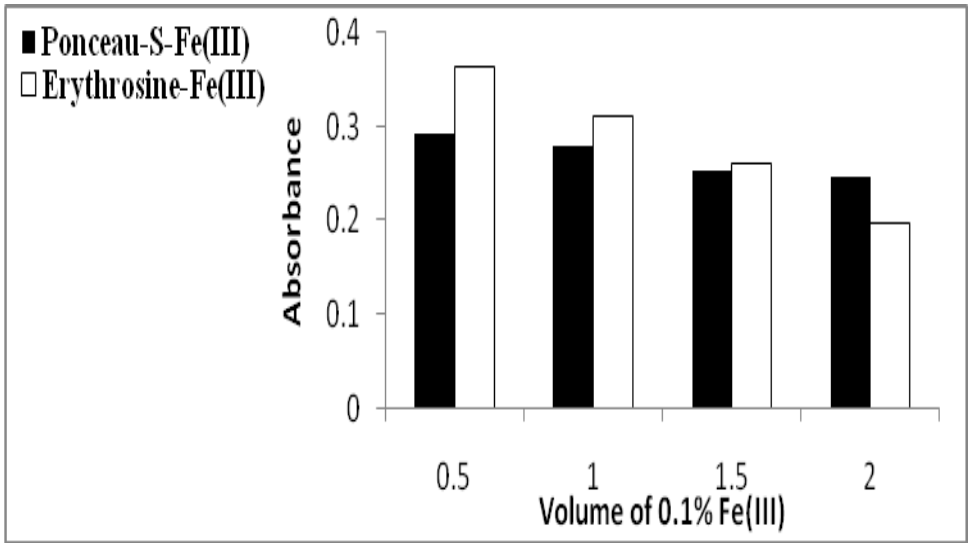

Figure 4: Effect of different amounts of 0.1% Fe (III) on absorbance of Ponceau-s-Fe (III) and Erythrosine-Fe (III) complex

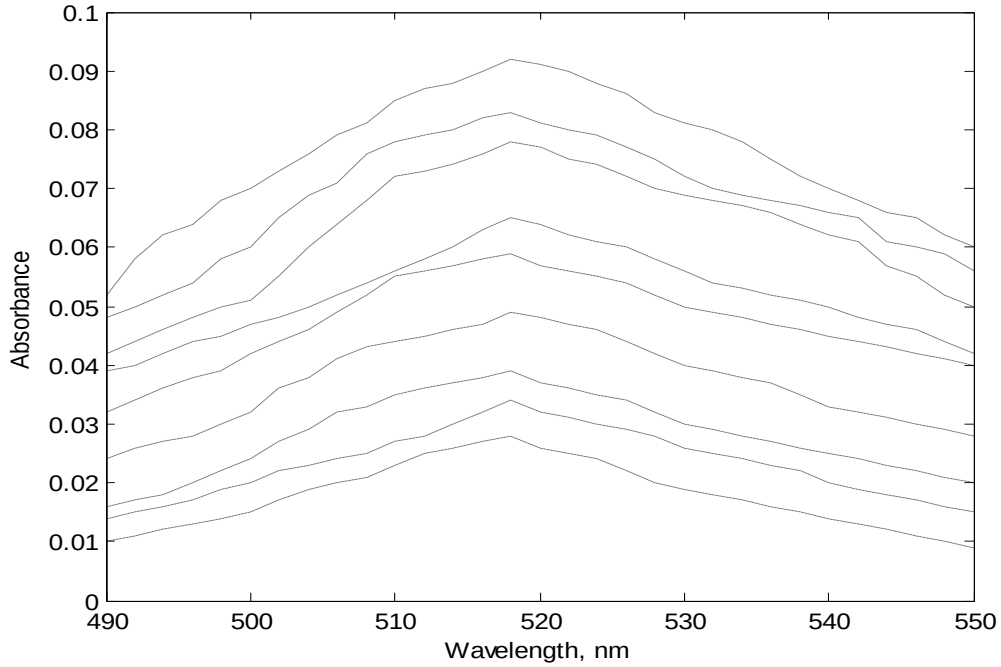

Figure 5: Absorption spectra for the standard solutions of the ponceau-s with different concentrations (0.0249 to 0.374 µg/ mL) in micellar media

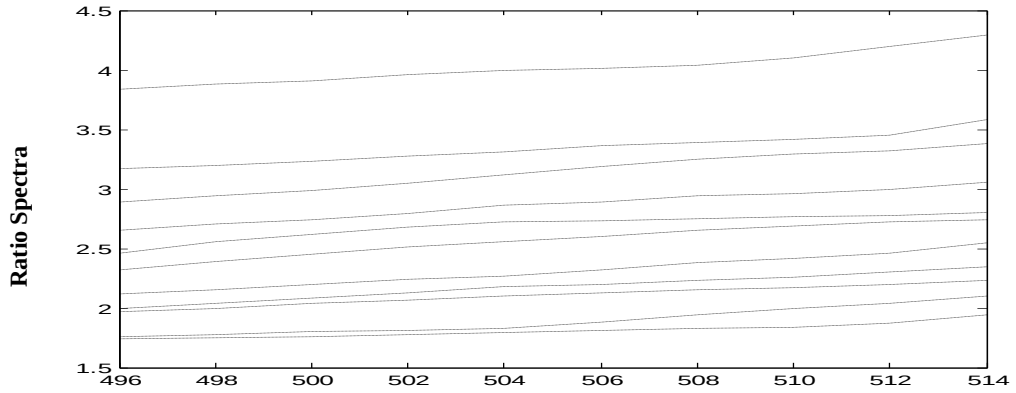

Figure 6: **Ratio Spectra obtained by dividing the Normalized Spectra of the erythrosine in micellar media**

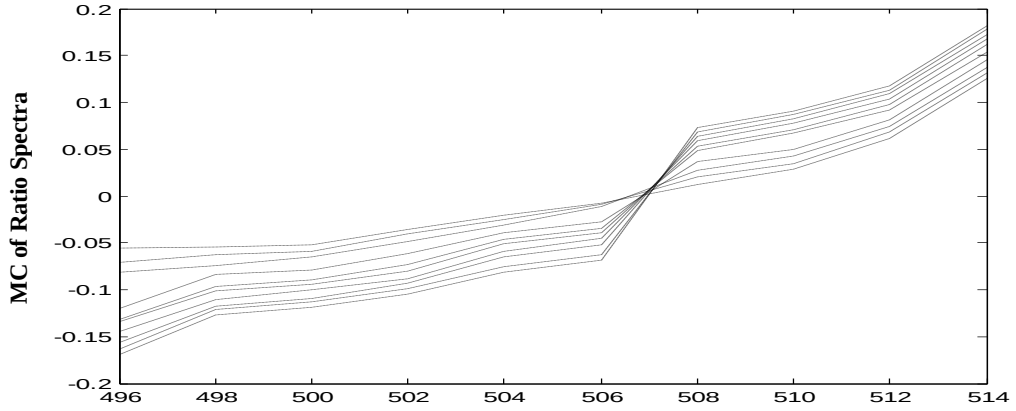

Figure 7: Mean centering of Ratio Spectra of the ponceau-s food colorants in micellar media

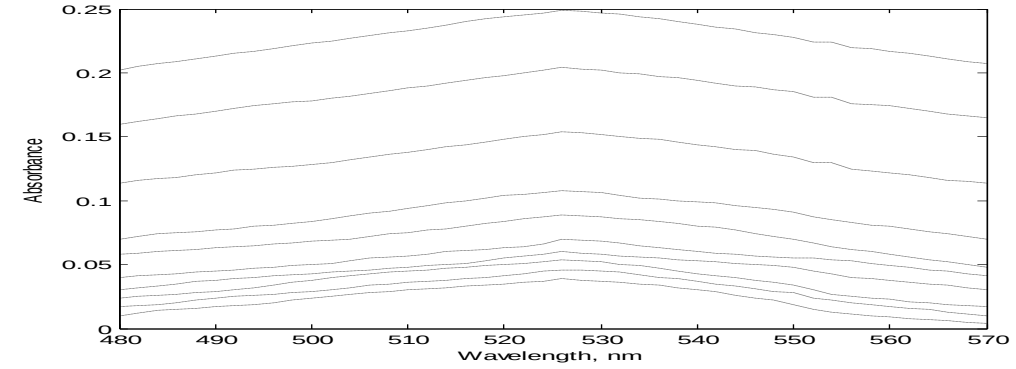

Figure 8: The Absorption spectra for the standard solutions of the erythrosine with different concentrations (0.0527 – 0.615 µg/ mL) in micellar media

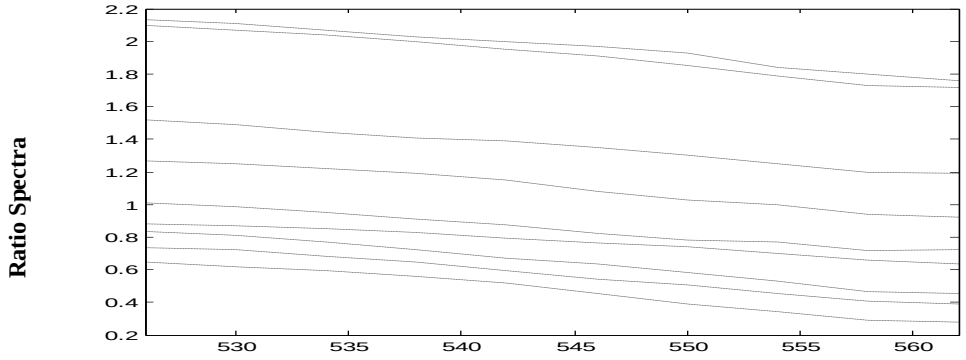

Figure 9: Ratio Spectra obtained by dividing the Normalized spectra of the ponceau-s in micellar media

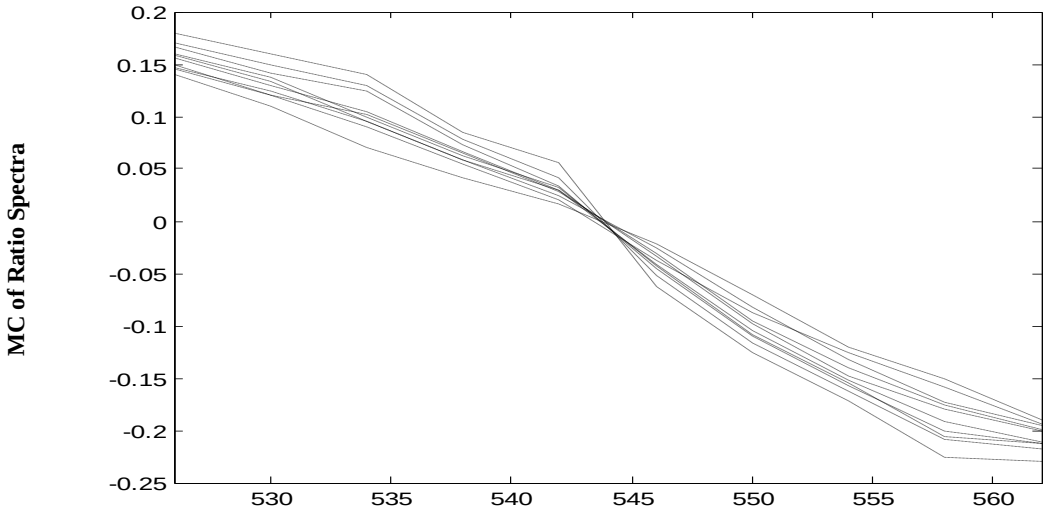

Figure 10: Mean Centering of Ratio Spectra of the erythrosine colorant in micellar media

Table 1
Analytical Characteristics for Analysis of ponceau-s and erythrosine in Binary Mixtures by Mean Centering of Ratio Spectra Method

Analyte	Wavelength (nm)	Calibration equation	r^2	Linear range (µg/ mL)	LOD (µg/ mL)
Ponceau-s	496.0nm	Y=0.001x-0.168	0.455	0.304 – 4.56	1.42
Erythrosine	526.0nm	Y=0.001x+0.008	0.588	0.175 – 6.15	1.52

The absorption spectra of the standard solutions of erythrosine with different concentration were recorded in the wavelength range 480.0-570.0 nm with 1.0 nm intervals Figure 8 and divided by the normalized spectrum of the ponceau-s and the ratio profiles were obtained Figure 9. Mean centering (MC) of the ratio profiles was obtained in the wavelength range of 526.0 – 562.0 nm Figure 10. The concentration of erythrosine was determined by measuring the amplitude at 526.0 nm corresponding to a maximum wavelength shown in Figure 10. For the prediction of concentration of erythrosine in synthetic binary mixtures and real samples the same procedure was used except that the spectra of the mixtures were used instead of the spectra of standard solution of erythrosine.

Analytical Characteristics

In the proposed method mean centering of ratio spectra, Beer`s law was obeyed in the concentration range 0.152 to 4.56 µg/ mL for ponceau-s, and 0.1759 – 5.278 µg/ mL for erythrosine. Table 1 shows the linear regression parameters for calibration data for simultaneous determination of ponceau-s and erythrosine in their binary mixtures. Limit of detection of the method for determination of ponceau-s and erythrosine are also shown in Table 1.

The effect of divisor concentration on the analytical parameters such as detection limit, slope, intercept and correlation coefficient of the calibration graphs was also tested. It was observed that changing the concentration of divisors in their linear calibration range had no significant effect on the analytical parameters. Therefore, a normalized spectrum of each of the ponceau-s and erythrosine was used as divisor profile in the proposed method.

In order to obtain the accuracy and precision of the method, several synthetic mixtures with different concentration ratios of ponceau-s and erythrosine were analyzed using the proposed method. The results are given in Table 2. The prediction error of a single component in the mixtures was calculated as the relative standard error (R.S.E) of the prediction concentration.

$$R.S.E\ (\%) = \sqrt{\frac{\sum_{j=1}^{N}(\hat{C}_j - C_j)^2}{\sum_{j=1}^{N}(C_j)^2}} \times 100$$

Where; N is the number of samples, $_{Cj}$ the concentration of the component in the j_{th} mixture and $\hat{C}_j$ the estimated concentration. The total prediction error of N samples is calculated as follows:

$$R.S.E_t\ (\%) = \sqrt{\frac{\sum_{i=1}^{M}\sum_{j=1}^{N}(\hat{C}_{ij} - C_{ij})^2}{\sum_{i=1}^{M}\sum_{j=1}^{N}(C_{ij})^2}} \times 100$$

Where C_{ij} is the concentration of the component in the j_{th} samples and $\hat{C}_{ij}$ is its estimation. Table 2 also shows the reasonable single and total relative errors for such system.

Table 2
Results for analysis of ponceau-s and erythrosine in Binary mixtures in different concentration Ratios by Mean Centering of Ratio Spectra Method

Taken (µg/ mL)		Found (µg/ mL)		Recovery (%)	
Ponceau-s	**Erythrosine**	**Ponceau-s**	**Erythrosine**	**Ponceau-s**	**Erythrosine**
0.152	0.175	0.150	0.177	98.77	100.1
0.304	0.351	0.305	0.350	100.3	99.7
0.456	0.527	0.458	0.529	100.4	100.3
0.608	0.703	0.606	0.705	99.8	100.2
0.760	0.879	0.762	0.875	100.2	99.5
0.912	1.05	0.914	1.07	100.2	101.9
1.06	1.23	1.04	1.25	98.l	101.6
1.21	1.40	1.20	1.38	99.23	98.6
1.36	1.58	1.37	1.56	100.7	98.7
1.52	1.75	1.54	1.73	101.3	98.9
Mean recovery				100.0	99.9
R.S.E (%) single				1.51	1.43
$R.S.E_t$ (%) total					1.39

Table 3
Determination of ponceau-s and erythrosine in different samples by Mean Centering of ratio spectra

Samples	Ponceau-s (µg /mL) [a]		Erythrosine (µg /mL) [a]	
Lipstick*	3.12	4.10	3.04	4.12
Nail Enamel**	3.44	4.23	3.42	4.25
Fruitsyrup (strawberry)	5.58	4.72	5.67	4.80
Candy***	5.67	4.89	5.65	4.91
Fruitjelly (strawberry)	6.22	5.32	6.34	5.30
Cough syrup****	4.58	5.02	5.98	4.04

Lipstick* - Locally available in market (magenta color), Nail Enamel **- Locally available in market (Magenta color), Candy*** - Locally available in market (Pan Candy, Cough syrup**** - kansali cough syrup locally available in market.

Application

The proposed method was also applied for the determination of ponceau-s and erythrosine in different commercial cosmetic products, pharmaceutical samples, and confectionery sample. One Lipstick, one Nail Enamel, Cough syrup, One Fruit syrup, was selected as appropriate samples.

The slopes of the standard addition curves matched those of the calibration graph, i.e., no matrix effect was discerned in any instance. If the colorants concentrations in the diluted real sample were below the lower limits of the linear ranges of the calibration graphs, good results were obtained by adding constant amounts of both colorants to the sample for analysis. These was usually the case for lipstick, cough syrup, fruit jelly and nail enamel, because these samples were aqueous and must be diluted to give the 10% (v/v) ethanol/water solution to carry out the extraction process. The results are summarized in Table 3.

Conclusion

The important characteristic of this work are:

1. Simultaneous determination of ponceau-s and erythrosine without the use of any expensive instrument is achieved. This reduces the cost of applied method.
2. No extraction step is required as determination has been done in micellar media and hence the use of toxic and carcinogenic organic solvents is avoided. Most of the organic solvents that are being used for extraction are classified as toxic and environmental pollutants and some have been listed as carcinogenic by the US Environmental Protection Agency (EPA).

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