DETERMINATION OF SUNSET YELLOW AND ERYTHROSINE IN BINARY MIXTURE USING PARTIAL LEAST SQUARES (PLS) METHOD

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ABSTRACT: *The research is aimed at the determination of synthetic food colors in commercial saffron sample with overlay spectrums by PLS method. In this method, at the optimum pH the absorption spectra of standard dye and real saffron samples were recorded in the range of 350-700 nanometers. Thus, the concentration of Sunset Yellow and Erythrosine in black system could be determined from the spectra matrices using PLS method. The results showed this method is simple, convenient and dependable. The method has been used successfully to determine Sunset Yellow and Erythrosine in simulated saffron sample with satisfactory results with 2.447 and 5.0 relative errors, respectively.*

Keywords: Sunset Yellow, Erythrosine, Saffron, Chemometric method, PLS method.

1. INTRODUCTION

Synthetic colorants are a very important class of food additives. They are widely used to compensate the loss of natural colors of food, which are destroyed during processing and storage, and to provide the desired colored appearance. Usually synthetic dyes are added to foodstuffs and soft drinks not only to improve appearance, color and texture but also to maintain the natural color during process or storage. Synthetic dyes show several advantages compared with natural dyes such as high stability to light, oxygen and pH, color uniformity, low microbiological contamination and relatively lower production costs. However, many of them may exhibit adverse health effects (allergy, respiratory problems, thyroid tumours, chromosomal damage, urticaria, hyperactivity, abdominal pain, etc.) [1, 2].

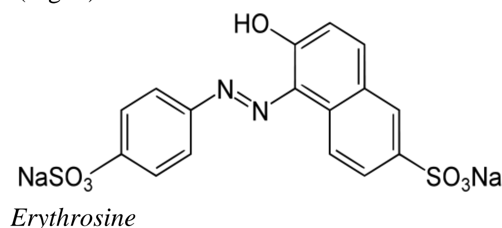
For this reason, safety data, such as the acceptable daily intake, based on toxicological studies on experimental animals and human clinical studies, have been repeatedly determined and evaluated by Food and Agricultural Organization (FAO) and World Health Organization (WHO) [3].

On the other hand, in some cases the use of food dyes is also indicative of foodstuff adulteration such as in their addition to fruit juices. Thus, the use of synthetic dyes is strictly controlled by laws, regulations and acceptable daily intake (ADI) values [4]. These regulations frame the role of the analytical chemist who has to test for the levels of dyes added to food. Some problems found in artificial colors determination are related to the variety of dyes mixtures and the potential interferences present in the commercial samples. Therefore, the analyses have traditionally been focused on separation methods.

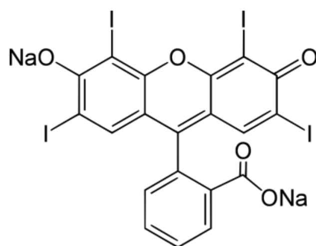
Many analytical techniques have been developed for the identification and determination of various synthetic food colorants, such as thin-layer chromatography [4,5], adsorptive voltammetry [6], and differential pulse polarography [7], derivative spectrometry [8–12] and spectrophotometric methods in combination with chemometrics [13,14], but all of them require time-consuming pretreatment or cannot be applied to complex colorant mixtures. Capillary electrophoresis [15–19] and micellar electrokinetic capillary chromatography [20] have also been used, but they have sensitivity problems as a result of small injection volume. High-performance ion chromatography [21], reversed-phase liquid chromatography [22–24] and ion-pair liquid chromatography [12, 20, 25–30] coupled with UV or diode-array detectors are still the most preferred methods, as they provide unrivalled resolution, sensitivity and selectivity.

However, some disadvantages arise from these methods, such as usage of toxic solvents, spending of time, and the need of sample pretreatments. The direct UV–vis spectrophotometric determination represents a rapid, simple, and cheap method for the determination of these colorants. In spite of this, the direct spectrophotometric measurements show lack of specificity because the spectra are strongly overlapped. In such cases the chemometric techniques become an indispensable tool to overcome these problems. In this sense, Ni and coworkers [31] have carried out a kinetic spectrophotometric analysis of some food colorants in drinks and jellies (previously reacted with a suitable chromogenic reagent) with the aid of several chemometric tools: Iterative target factor analysis (ITTFA), principal component regression (PCR), partial least squares (PLS) and principal component-radial basis function-artificial neural network (PC-RBF-ANN). Also, Lachenmeier and Kessler [32] have compared PLS and multivariate curve resolution (MCR) in the study of artificial food colors by UV–vis, but only reported qualitative results, i.e. they used the multivariate models to parametrically judge the presence or absence of the food colors. The first order calibration methods (such as PLS) need that both unknown samples and standards have the same chemical and physical characteristics, even the eventual interferences.

In the current study, the applicability of the PLS-1 method is exemplified by a direct spectrophotometric method for the determination of two artificial colors –Sunset Yellow (SY) and Erythrosine(ER) in real saffron samples is proposed (Fig. 1).



Erythrosine



Sunset Yellow FCF

Figure 1. Chemical structures of Sunset Yellow and Erythrosine.

2. EXPERIMENTAL

2.1. Reagents and solutions

All solutions were daily prepared. Analytical reagent-grade chemicals and ultra pure deionized water (Barnstead, Dubuque, USA) was used. Sunset Yellow FCF and Erythrosine (1000 mg mL^{-1}) stock solutions (all from Aldrich) were prepared in ultra pure water. Standard solutions and mixtures of dyes were freshly prepared by appropriated dilution of stock solutions with distilled water. Saffron was prepared daily by dissolving 0.01 g of commercial type in water and diluting to 100 mL. All the solutions were protected from light through out the experiments.

Hydrochloric acid solution, concentration 0.10 mol L^{-1}, was prepared with a suitable volume of hydrochloric acid (Merck) dissolved in ultra pure water.

2.2. Apparatus and software

All spectrophotometric measurements were carried out with a UV-VIS spectrophotometer T60 PG (England) double beam spectrophotometer. All measurements were carried out at room temperature using a glass cell (10mm light path). The UV–vis spectra were recorded between 350 and 600 nm, in steps of 1 nm. A Pentium four personal computer was used for controlling the spectrophotometer and collecting the data from its interface. All spectral measurements were performed using a blank solution as a reference. Measurements of pH were made with a Metrohm 827(Switzerland) pH meter using a combined glass electrode. PLS program was modeled using ParLeS v3.1 software.

2.3. Procedure

Standard solution of Saffron, Sunset Yellow and Erythrosine with the concentration 0.1 gL^{-1}, 10 ppm and 5 ppm were prepared from their stock's respectively, and then the titration was performed by HCl & NaOH 2M in the pH range 1-12 and 1 interval.

3. RESULTS AND DISCUSSION

3.1. Effect of pH

Standard solution of Saffron, Sunset Yellow and Erythrosine with the concentration 0.1 gL^{-1}, 10 ppm and 5 ppm were prepared from their stock's respectively, and then the titration was performed by HCl and NaOH 2M in the pH range 1-12 and 1 interval (Fig. 2-4).

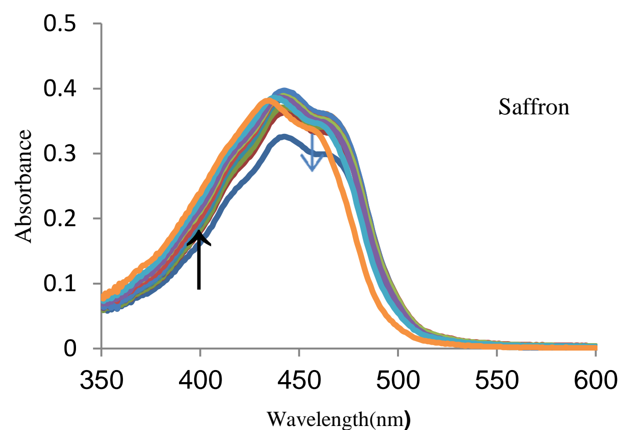


Figure 2. Absorbance curve changes of saffron (0.1 gL^{-1}) in the pH range 1-12 with the 1 interval.

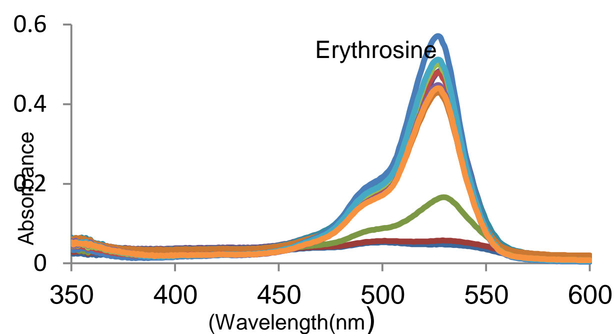


Figure 3. Absorbance curve changes of sunset yellow (10 ppm) in the pH range 1-12 with the 1 interval.

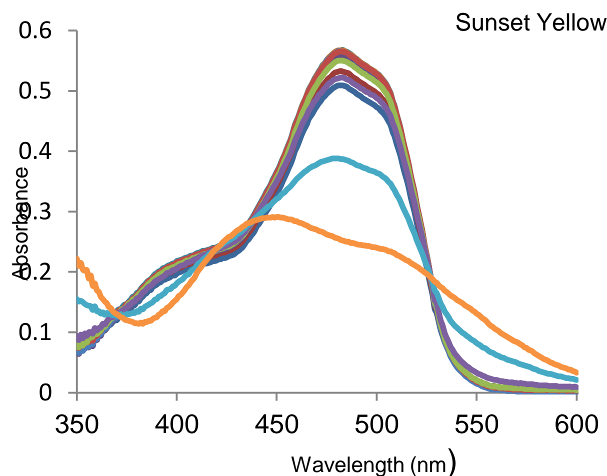


Figure 4. Absorbance curve changes of Erythrosine (5ppm) in the pH range 1-12 with the 1 interval.

Optimum pH was obtained by plotting the maximum absorbance against the pH (Fig. 5). According to figure 3 and 4, Sunset Yellow in 481nm and Erythrosine in 527nm shows the maximum absorbance. Figure 6 shows overlain spectra of Sunset Yellow, Erythrosine and saffron in the optimum pH (pH=7).

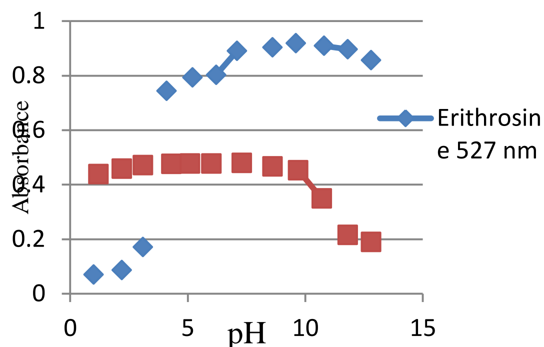


Figure 5. Effect of pH on maximum absorbance of Sunset Yellow and Erythrosine.

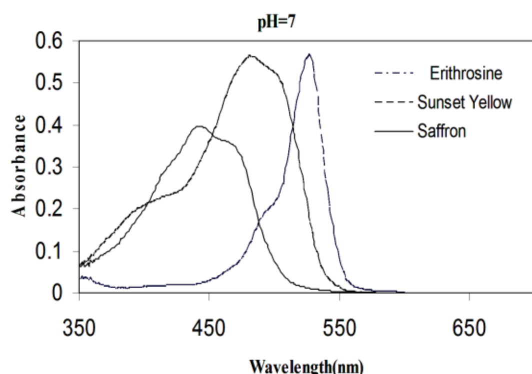


Figure 6. Overlain spectra of Sunset Yellow, Erythrosine and Saffron in the optimum pH (pH=7).

3.2. Individual calibration curves

To verify the governing beer's law, individual calibration graphs were obtained at the analytical wavelength of Sunset Yellow in 481nm and Erythrosine in 527nm for different concentrations of Sunset Yellow and Erythrosine, under conditions discussed in previous sections. The individual linear calibration models and their relative parameters were established and summarizes the results for the analysis of each component in table 1. The correlation coefficients obtained 0.9995 and 0.9984 and linearity over the concentration range of 1.0-27.0 mg mL^{-1} and 0.4- 10.0 mg mL^{-1} for Sunset Yellow and Erythrosine, respectively.

3.2. Multivariate method

Since not all wavelengths in the spectra carry the same quality of information and in order to select each analyte's most appropriate spectral working regionand the number of factors to be used in PLS-1 method, a minimum PRESS (Prediction Residual Error Sum of Squares), search guided by a moving window of variable size was employed [33]. PRESS calculated assign Eq. 1, is a measure of the quality of fitness of the predicted concentration results (C_{pred}) to the data (C_{act}).

$$PRESS = \sum (C_{pred} - C_{act})^2 \qquad (1)$$

The predicted concentrations of the components in each sample were compared with the actual concentrations in the training samples and the root mean square error of cross validation (RMSE) was calculated for each method as follows (Eq. 2):

$$RMSE = \sqrt{PRESS / n} \qquad (2)$$

Where n is the number of training samples.

RMSE indicates both the precision and accuracy of predictions. It plays the same role of standard deviation in indicating the spread of the concentration errors [34]. Appropriate selection of the number of factors to be used to construct the model is the key to achieve correct quantitation in PLS-1 calibration. The most usual procedure for this purpose involves choosing the number of factors that result in the minimum RMSE. The method developed by Haaland and Thomas [35] was used for selecting the optimum number of factors, which involves selecting that model including the smallest number of factors that result in an insignificant difference between the corresponding RMSE and the minimum RMSE. The selection of the optimum number of factors was a very important pre-construction step because if the number of factors retained was more than required more noise would be added to the data. On the other hand, if the number retained was too small meaningful data that could be necessary for the calibration might be discarded. Table 3shows the variation of the RMSE as a function of the number of factors for the determination of each compound by PLS-1 method. A number of factors of 3 were found to be optimum for Sunset Yellow and Erythrosine by the PLS-1 method as in Figure 7.

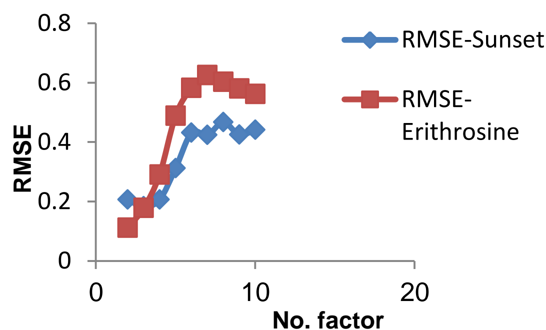


Figure 7. RMSE versus latent variable for a calibration set of Sunset Yellow and Erythrosine using PLS-1 model.

Table 1. Statistical parameters for PLS-1 analysis of Sunset Yellow and Erythrosine in saffron solution.

Parameter	Sunset Yellow	Erythrosine
Optimum spectral range (nm)	350-700	350-700
Linear concentration range (mg mL^{-1})	1-27	0.4-10
Number of PLS Factors	3	3
RMSE	0.137	0.215
RSE (%)	2.00	3.83
r^2	0.9995	0.9984

$$RMSD = \left[(1/N)\sum (C_{pred} - C_{act})^2\right]^{2/Z}$$

$$RSE\% = (100/C_{mean})\left[(1/N)\sum (C_{pred} - C_{act})^2\right]^{2/Z}$$

$$r^2 = 1 - \left[\sum (C_{pred} - Cct)^2 / \sum (C_{act} - C_{mean})\right]^{Z}$$

where C_{mean} is the average component concentration in the N calibration mixtures.

The squares of the correlation coefficients (r^2), which indicate the quality of the straight lines that fit the data, were 0.999 and 0.998 for Sunset Yellow and Erythrosine respectively. Also, the relative error of prediction during calibration (RSE) is an indication of the predictive ability of the models, remained around 2 and 3.83 for Sunset Yellow & Erythrosine respectively. In addition, analytical figures of merit obtained for the models also supported their quality.

3.3. Calibration and validation of the analytical set

In order to extract maximum quantitative information about the samples with the use of minimum experimental trials, the orthogonal array design was applied for the construction of the set of calibration samples [36- 39].

Solutions containing dye concentrations in the range of 1-15 mg mL^{-1} and 1-12 mg mL^{-1} for Sunset Yellow and Erythrosine respectively were produced by dilution of the stock solutions with the aqueous saffron solution with the 0.2 absorbance (Table 2 and 3). These levels were selected to allow for a wide distribution of concentrations, which will also cover the range of levels found in real samples. The Visible absorption spectra were recorded over the wavelength range of 350–700 nm and the data points of the spectra were collected every 1 nm.

Table 4 shows the composition of the sixteen calibration samples, which were designed according to a four-level orthogonal array design. The concentration levels for the analytes were: Sunset Yellow, 0.0, 3.0, 10.0 and 15.0 mg mL^{-1}, and Erythrosine, 0.0, 4.0, 7.0 and 10.0 mg mL^{-1}.

The 9 binary synthetic mixtures of analytes shown in Table 5 with the concentration levels for the analytes Sunset Yellow, 2.0, 8.0 and 12.0 mg mL^{-1}, and Erythrosine, 3.0, 5.0 and 8.0 mg mL^{-1} were prepared and used to validate the different chemometrics models.

Table 2. Four-level concentration levels for calibration set.

sample	Concentration ($mgmL^{-1}$)		Absorbance of aqueous saffron
	Sunset Yellow	Erythrosine	
1	0	0	0.2
2	3	4	0.2
3	10	7	0.2
4	15	10	0.2

Table 3. Three-level concentration levels for validate set.

sample	Concentration ($mgmL^{-1}$)		Absorbance of aqueous saffron
	Sunset Yellow	Erythrosine	
1	2	3	0.2
2	8	5	0.2
3	12	8	0.2

Table 4. Calibration set composition for Sunset Yellow and Erythrosine

Standard	Concentration ($mgmL^{-1}$)	
	Sunset Yellow	Erythrosine
C1	0	0
C2	0	4
C3	0	7
C4	0	10
C5	3	0
C6	3	4
C7	3	7
C8	3	10
C9	10	0
C10	10	4
C11	10	7
C12	10	10
C13	15	0
C14	15	4
C15	15	7
C16	15	10

Table 5. Test set composition for Sunset Yellow and Erythrosine.

Sample	Concentration ($mgmL^{-1}$)	
	Sunset Yellow	Erythrosine
T1	2	3
T2	8	3
T3	12	3
T4	2	5
T5	8	5
T6	12	5
T7	2	8
T8	8	8
T9	12	8

. 3.4. Real sample

The proposed spectrophotometric method was applied for the determination of the two analytes in different saffron solution (Table 6). The recoveries, RSEs and RMSEP were carried out using sixteen different synthetic commercial saffron sample solutions.

Table 6. Determination of Sunset Yellow and Erythrosine in commercial saffron samples by the PLS-1 method

Sample		Added (standard addition) (ppm)	Found (total) (ppm)
1	Sunset Yellow	3	2.9
	Erythrosine	3	2.8
2	Sunset Yellow	5	5.1
	Erythrosine	7	6.7
3	Sunset Yellow	9	8.9
	Erythrosine	3	2.8
4	Sunset Yellow	11	11.1
	Erythrosine	8	7.6
5	Sunset Yellow	3	2.9
	Saffron 1	100	----
6	Sunset Yellow	5	4.9
	Saffron 1	100	----
7	Sunset Yellow	9	9.1
	Saffron 2	100	----
8	Sunset Yellow	11	11
	Saffron 2	100	----
9	Erythrosine	3	2.9
	Saffron 1	100	----
10	Erythrosine	7	6.8
	Saffron 1	100	----

11	Erythrosine	3	2.8
	Saffron 2	100	----
12	Erythrosine	8	7.6
	Saffron 2	100	----
13	Sunset Yellow	3	3.1
	Erythrosine	3	2.9
	Saffron 1	100	----
14	Sunset Yellow	5	4.9
	Erythrosine	7	6.2
	Saffron 1	100	----
15	Sunset Yellow	9	9.1
	Erythrosine	3	2.8
	Saffron 2	100	----
16	Sunset Yellow	11	10.5
	Erythrosine	8	8.1
	Saffron 2	100	----

Since the PLS-1method gave good prediction results, it was the method applied for determination of analytes in the real samples. The analytical results obtained by this method are summarized in Table 7.

Recovery (%) values are generally in the range of 97.5–99.8 with only two samples outside the lower threshold

4. CONCLUSIONS

Multivariate PLS and UV-VIS spectrophotometeric methods enable the quantitation of Sunset Yellow and Erythrosine binary mixture with good accuracy and precision, in laboratory synthetic prepared samples forms. Proposed procedure is simple, accurate, economical and rapid. The good recoveries obtained in all cases proved that the proposed method could be applied efficiently for determination of Sunset Yellow and Erythrosine binary mixture with quite satisfactory results and could be easily used in quality control laboratory for their analysis.

Table 7. The analytical results obtained by using PLS-1 method to determination of Sunset Yellow and Erythrosine in commercial saffron samples

RSE% Sunset Yellow	RSE% Erythrosine	RSE%-T	RMSEP Sunset Yellow	RMSEP Erythrosine	%Recovery Sunset Yellow	%Recovery Erythrosine	%Recovery-T
2.447	5.0009	3.478	0.206	0.29	99.867	97.578	98.722

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