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ОРИГИНАЛЬНЫЕ СТАТЬИ

УДК 543

APPLICATION OF DIFFERENTIAL SPECTRA FROM TWO DIFFERENT ENVIRONMENTS IN SIMULTANEOUS QUANTIFICATION OF NEW FUCHSINE, CRYSTAL VIOLET AND MALACHITE GREEN IN THE PRESENCE OF UNMODELED INTERFERENTS USING FIRST ORDER MULTIVARIATE CALIBRATION METHOD

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This paper proposes the possibility of applying first order calibration methods for unknown sample(s) with unmodeled component(s) using the differential spectra obtained from subtracting of the absorbance spectra of sample that are recorded in two different environments. The main interest is that the method can be performed to quantify systems if the spectra of their unmodeled component(s) are the same in two different phases using first order calibration methods. The procedure demonstrates simultaneous determination of new fuchsine, crystal violet and malachite green in the presence of sunset yellow, tartarazine and amaranth as unmodeled interferences, whose spectra differ in aqueous and nonionic micellar solution of Triton X-100. The obtained satisfactory results indicate the successful applicability of the proposed method in complex samples.

Keywords: differential spectra, unmodeled interferences, PLS, triphenylmethane dyes.

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Triphenylmethane (**TPM**) dyes are an important class of commercial dyes that have potential applications in the textile industry as sensitizers for photoconductivity and in medicine as antibacterial and sterilization agents during blood transfusions [1–6]. Among the TPM dyes, crystal violet (**CV**) and malachite green (**MG**) have been widely used together, as other TPM dyes such as new fuchsine (**NF**) [7, 8].

TPM dyes are often found in wastewaters; some of them have been found to be carcinogenic, genotoxic [7, 9, 10] and tumor promoting [11–13]. About 20% of the dyestuffs produced in the world are discharged into streams without any pretreatment [14]. Thus, many countries have banned the use of MG and CV in aquaculture [15]. However, due to their low cost and high effectiveness, these harmful dyes are still used and will probably continue to be used in the aquiculture in some parts of the world. Several methods have been proposed for their determination such as liquid chromatography–tandem mass spectrometry [16], liquid chromatography–visible spectrophotometry [17], capillary electrophoresis–Raman spectroscopy [18] and magnetic solid phase extraction–spectrometry [19]. These methods developed in literature often require complicated pretreatment procedures; thus, alternative methods with simple pretreatment seem to be crucial for the determination of dyes when they coexist with some interferents. First-order calibration methods such as multiple linear regression, principal component regression, and partial least-squares regression (**PLS**) deal with a vector of variables per sample (e.g., an FT–IR spectrum). These allow the simultaneous quantification of multiple analytes without the need for selectivity in the measured variables for the analytes. The use of these methods provide the so called first-order advantage, the possibility to quantify an analyte in the presence of interferents [20] as long as the interferent compounds present in the calibration samples during the establishment of the calibration model. A drawback of these methods is that all possible interferents in the samples should be qualitatively known in order to be included in the calibration model, which normally leads to the need for a large number of calibration samples. Therefore, developing methods that can eliminate this limitation without the need for repeating calibration stages or referring to second order calibration methods is important. Besides, extensive researches have recently confirmed the ability of surfactants to affect the electronic absorption spectra of solutions of many dyes, viz., triphenylmethane [21, 22]. Surfactants usually affect spectral parameters: the intensity in the absorption bands can be increased or

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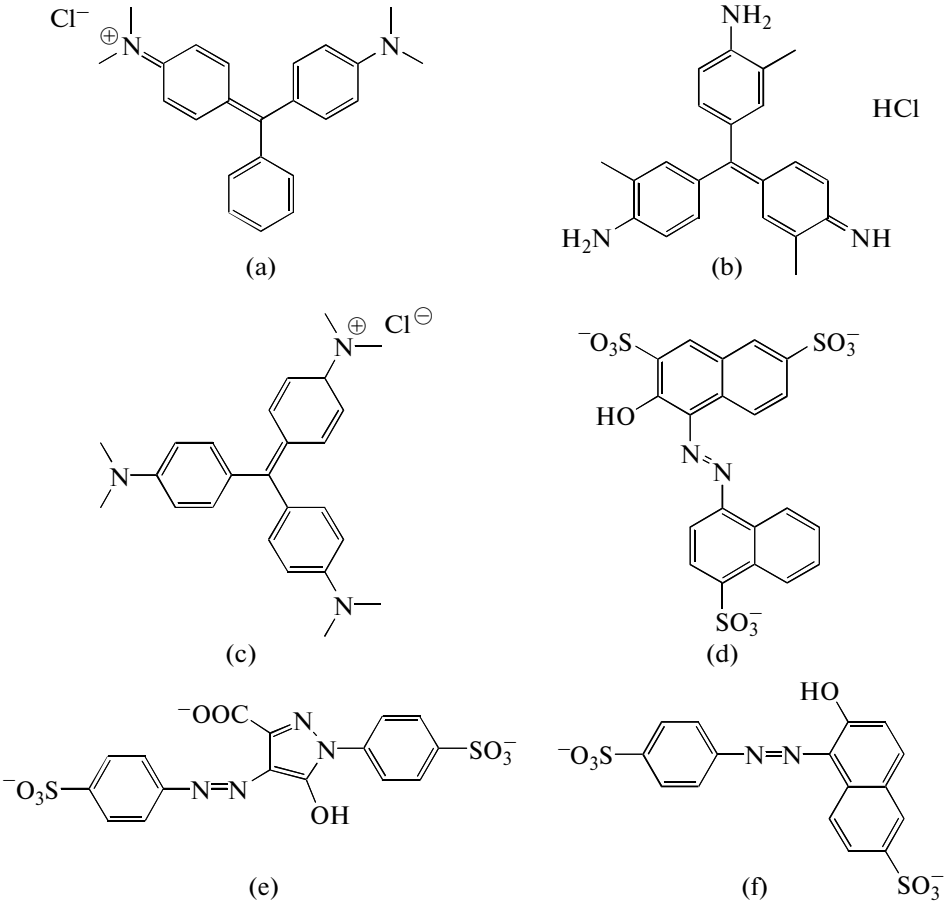
decreased and the absorption maxima of reagents shifted [23–26]. These phenomena depend on type of solutes, pH and surfactants.

The paper illustrates that if the spectra of analytes differ in aqueous and micellar environment while the spectral shapes of interferent(s) are the same in both phases, the differential spectra can eliminate the troublesome unmodeled interferent(s) in the unknown samples. The use of the differential spectra instead of direct spectral data in first order calibration analysis can be applied for determination of analyte(s) in unknown samples with unmodeled interferent(s). The method was evaluated for simultaneous quantification of NF, CV and MG in the presence of sunset yellow, tartarazine and amaranth as unmodeled interferents in unknown samples. Chemical structures of dyes were shown in Scheme.



Structural formula of dyes: MG (a), CV (b), NF (c), amaranth (d), tartarazine (e) and sunset yellow (f).

THEORETICAL BACKGROUND

In this work, it will be assumed that the spectral shapes and molar absorption coefficients of analytes are different in aqueous and micellar environments, but the spectral shapes of interferents are independent in two environments. At first, let us assume that there are two calibration samples and their spectra are measured in aqueous (s_{aw1}, s_{aw2}) and micellar environments (s_{am1}, s_{am2}):

$$s_{am1} = s_{am}c_{a1}, \quad (1)$$

$$s_{aw1} = s_{aw}c_{a1}, \quad (2)$$

$$s_{am2} = s_{am}c_{a2}, \quad (3)$$

$$s_{aw2} = s_{aw}c_{a2}. \quad (4)$$

Here s_{aw} and s_{am} are vectors of molar absorption coefficients in N wavelengths from analyte in aqueous and micellar environments, respectively; c_{a1} and c_{a2} are the concentrations of analytes in two calibration samples; s_{aw1} and s_{aw2} are vectors of analyte's spectra in N wavelengths in two calibration samples in aqueous environment, s_{am1} and s_{am2} are analyte's spectra in two calibration samples in micellar environment. Differential spectra are obtained from the difference between measured spectra in two environments in each sample, s_{ad}:

$$s_{ad1} = s_{am1} - s_{aw1} = c_{a1}(s_{am} - s_{aw}), \quad (5)$$

$$s_{ad2} = s_{am2} - s_{aw2} = c_{a2}(s_{am} - s_{aw}). \quad (6)$$

Then

$$s_{ad1}/s_{ad2} = c_{a1}/c_{a2}. \quad (7)$$

Based on Eq. (7), it is clear that the differential spectrum of analyte is directly proportional to its concentration. It can be expressed by a typical unknown sample including one analyte "a" and an interferent component "i" whose spectra of the samples are recorded in both environments. If there is no interaction between components and they follow Beer's law, it can be written:

$$s_w = s_{aw}c_{ax} + s_{iw}c_{ix}, \quad (8)$$

$$s_m = s_{am}c_{ax} + s_{im}c_{ix}. \quad (9)$$

Here s_{iw} and s_{im} are the pure spectra of interferent components in aqueous and micellar environments, c_{ax} and c_{ix} state the concentration of analyte and interferent component and s_w and s_m are their spectra. Provided that the spectra of interferent component in aqueous and micellar environments are the same, thus:

$$s_{iw} = s_{im}. \quad (10)$$

Hence, subtracting the spectra in two different environments deletes the interferent component share:

$$s_d = s_m - s_w = c_{ax}(s_{am} - s_{aw}), \quad (11)$$

where s_d denotes differential spectrum in two different environments; this differential spectrum is defined as a function of the concentration of analyte.

EXPERIMENTAL

Instrumentation. Absorption spectra in the range of 350–700 nm were recorded on an Agilent 8453 photodiode-array spectrophotometer with spectral resolution of 1 nm and 1 cm path length quartz spectrophotometer cells. The measurements of pH values of solutions was performed on a Metrohm 620 pH-meter. All experiments were carried out at room temperature (25 ± 1°C). The ParLes package38 for implementation of PLS regression algorithm was used. The mathematical program MATLAB 7.6 (R2088a) (Math Works, Cochituate Place, MA) was used for data processing.

Reagents. All chemicals were of analytical-reagent grade. Sodium hydroxide, potassium chloride, MG, CV, NF, sunset yellow, tartarazine and amaranth were commercially obtained from Merck and used without further purification. All solutions were prepared with doubly distilled water. The Stock solutions of 100 mg/L of NF, CV, MG and 1000 mg/L of sunset yellow, tartarazine and amaranth were prepared by 0.1 M acetic acetate buffer at pH 4.00 as solvent and were stored in plastic amber bottles at 5°C protected from light. Working solutions were prepared by appropriate dilution of the stock solutions. Acetic acetate buffer was prepared at pH 4.00 by 1 M NaOH to 1 M acetic acid. A 2.5 M solution of potassium chloride was prepared by dissolving its crystals in doubly distilled water. The nonionic surfactant Triton X-100 (**TX-100**) was a product of Aldrich. The stock solution of 4% (v/v) surfactant was prepared by dissolving 4.00 mL of TX-100 in 10.00 mL doubly distilled water.

Procedure. A calibration set having twenty five calibration samples prepared by mixture of the three analytes according to a 5-level Taguchi orthogonal array design with concentrations distributed in the ranges of (mg/L) 1–3 for CV, 1–3 for MG and 1–5 for NF. In addition, twenty prediction mixture samples including the interested analytes and interferents in the ranges of (mg/L) 2–14 for sunset yellow, 11–50 for tartarazine and 3–19 for amaranth. For fixing ionic strength, it was necessary that all solutions in the experiment be performed in 0.125 M potassium chloride and have the same concentration of pH 4.00 acetic acetate buffer solution (0.1 M). A spectrophotometer was used due to easy method, low-priced analyses and significant savings in time and chemicals in the comparison with HPLC. The absorbance spectrum of 2.0 mL of sample, calibration or prediction sample, in quartz cell was recorded by measuring absorbance against a blank solution in the range of 350–700 nm. Then 100 μL of 4% (v/v) TX-100 was added to 2.0 mL of sample, calibration or prediction sample, in a quartz cell and its spectrum was recorded again. The concentration of TX-100 in the cell, 0.32 mM, was more than its CMC (0.24 mM) [27]. Synthesized textile waste water was prepared from a mixture of dyestuffs (sunset yellow, amaranth, tartarazine, NF, MG and CV) and sodium acetate (1.0 M) in farming waters. 1.00 mL of the sample was diluted to 50.00 mL with water. The concentration of interferents was added by chance considering total absorbance after dilution did not exceed from 1.00.

RESULTS AND DISCUSSION

Differential spectra. From Fig. 1, it can be seen that absorbance spectra of MG, CV and NF as analytes significantly changed with the addition of TX-100. The differential spectra of analytes were obtained from subtracting spectra for each analyte in two environments (Fig. 2). Ranges of concentrations (mg/L) of 1–5 for CV, 1–10 for MG and 1–5 for NF were found to be linear ($n = 10$) in aqueous environment and linear range of 1–5 for CV, 1–10 for MG and 1–5 for NF were found to be linear ($n = 10$) in micellar environment (Table 1).

Absorbance spectra of Sunset yellow, tartarazine and amaranth as interferents showed the same characteristics in two environments (Fig. 3). It can be inferred that there were no interactions between the components and non-ionic surfactant TX-100 due to high charge of the components [28]. As a result, it is clear that the differential spectra of interferents are zero in each wavelength. Previously, the absorbance spectra of the analytes did not change with addition of cationic surfactants like cetylpyridinium chloride and

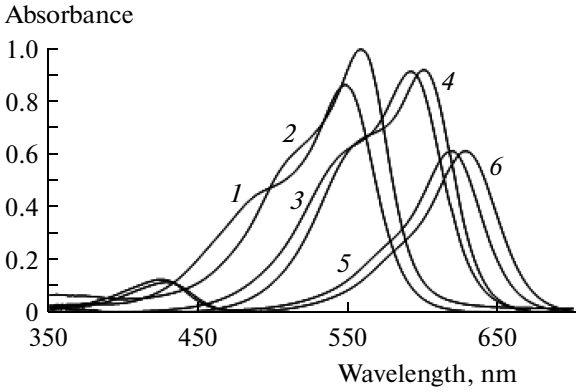


Fig. 1. Absorbance spectra of 4.0 mg/L of analytes: new fuchsine (*1* and *2*), crystal violet (*3* and *4*) and malachite green (*5* and *6*) in aqueous and micellar (0.32 mM of TX-100) environment, respectively.

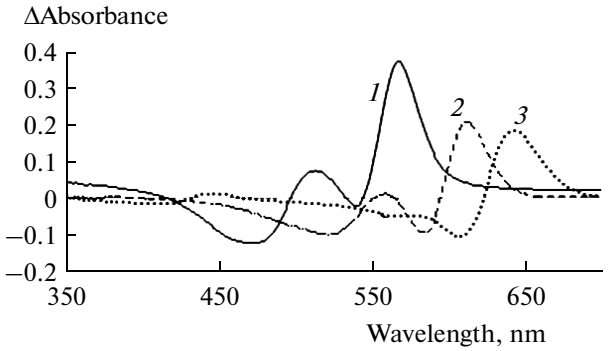


Fig. 2. The differential spectra of analytes: new fuchsine (*1*), crystal violet (*2*) and malachite green (*3*).

cetyltrimethyl ammonium bromide. Following these, the influence of sodium dodecylsulfate (**SDS**) was studied as an anionic surfactant on the absorption spectra of the analytes. Analytes precipitated in the presence of SDS. Subsequently, TX-100 as a nonionic surfactant was examined. TX-100 was selected as a surfactant in this study due to having the special condition of the proposed method.

PLS analysis. PLS1 analysis, built for each analyte by using its concentration vector, was applied to develop a calibration model to progress the relationship between the spectral data of calibration samples and concentrations. PLS1 was performed on obtained differential spectra of calibration samples instead of its directly measured absorbance spectra. To determine the number of factors the cross validation method, leaving out one sample at a time, was applied [29]. The prediction error was calculated for each analyte for the prediction set. The error was expressed as the prediction residual error sum of squares (PRESS). The optimal number of factors obtained was three. According to the theory, the share of interferent components are eliminated using differential spectra in prediction set; consequently, the calibration set represents the prediction set. PLS regression method was performed using differential spectra of calibration and prediction set to quantify MG, CV and NF in twenty prediction samples. The predicted concentrations were highly correlated to actual concentrations. Hence, the applicability of the proposed method can be deduced in removing interfering components. Also, in order to assess the accuracy and prediction ability of the method, the relative standard errors and recovery values parameters were calculated for simultaneous determination of NF, CV and MG in presence of unmodeled interferents. The prediction error of a single component in the mixture was calculated as the relative standard error (**RSE**) of the prediction concentration [30, 31]:

$$\mathrm{RSD}(\%) = \left[\sum_{j=1}^{N}\left(\hat{c}_j - c_j\right)^2 \Big/ \sum_{j=1}^{N} c_j^2\right]^{1/2} \times 100,$$

$$\mathrm{Recovery}(\%) = \left[\left(\sum_{j=1}^{N} \hat{c}_j / c_j\right) \Big/ N\right] \times 100.$$

In these expressions, c_i and $\hat{c}_i$ are, respectively, the known and calculated analyte concentration in sample i, and n is the total number of samples considered in the validation. RSE and recovery are presented in Table 2, the result expressing the accuracy of the method.

In order to assess the capability of the proposed method, a simulated textile waste water sample including 1.00 mL of farming water plus different amount of analytes as standard addition method was used. Then concentrations of analytes were determined by the method again. Figures of merit such as accuracy and precision were calculated by recovery and RSE. From Table 3 it can be inferred the satisfac-

Table 1. Linear ranges of malachite green, crystal violet and new fuchsine

Analyte	Aqueous environment			Micellar environment		
	linear range, mg/L	R^2	λ_m, nm	linear range, mg/L	R^2	λ_m, nm
Crystal violet	1–5	0.9999	591	1–5	0.9996	599
Malachite green	1–10	0.9996	618	1–10	0.9996	524
New fuchsine	1–5	0.9997	547	1–5	0.9998	558

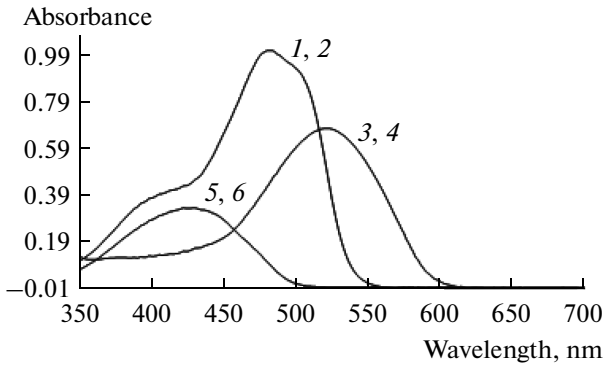


Fig. 3. Absorbance spectra of interferents: 40.0 mg/L of tartarazine (*1* and *2*), 20.0 mg/L of sunset yellow (*3* and *4*) and 20.0 mg/L of amaranth (*5* and *6*) in aqueous and micellar (0.32 mM of TX-100) environment, respectively.

tory results are obtained, which confirms the applicability of the proposed method to complex samples.

* * *

Consequently, the basic principle of the differential spectra is that spectra of analytes differ in two environments while the spectral shapes of interferent(s) are the same in both phases. In this work, PLS approach was used as first order calibration method. However, there is no doubt that other first order calibration approaches can be applied. In general, the main advantage of using the method was the predictive capability of first order calibration approaches used to determine the concentrations of analytes in presence of unmodeled interferences in unknown samples.

Table 2. Statistical parameters of the proposed method for the prediction set

Parameter	Crystal violet	Malachite green	New fuchsine
RSE, %	2.95	2.26	3.42
Recovery, %	99.0	100	95.2

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Table 3. The results (mg/L) of the determination of new fuchsine, malachite green and crystal violet in simulated textile waste water using the proposed method

Added			Calculated total			Recovery, %			RSD, %		
NF	MG	CV	NF	MG	CV	NF	MG	CV	NF	MG	CV
–	–	–	2.00	1.67	1.00	–	–	–	1.85	1.32	1.99
1.00	3.75	–	3.19	5.41	1.01	119.0	99.7	–	1.02	0.84	2.23
2.20	–	2.50	4.20	1.81	3.55	100.0	–	102.0	2.52	1.53	1.79
3.00	1.80	1.25	5.09	3.39	2.33	103.0	95.5	106.4	2.79	1.69	2.05

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