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THE SPECTROPHOTOMETRIC DETERMINATION OF PYRENE DYES IN PHARMACEUTICALS AND COSMETICS

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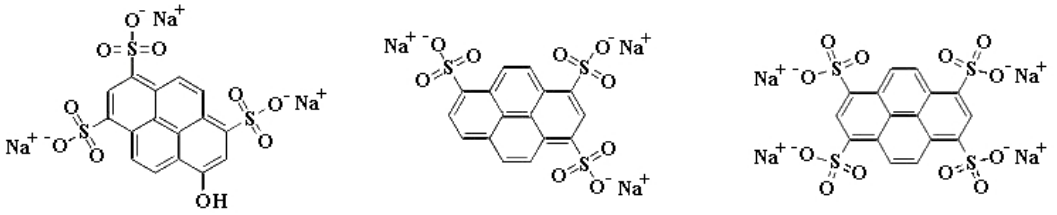
ABSTRACT: The paper introduces a method used for the determination of the component concentrations of a ternary mixture of pyrene dyes. The method uses the zero-order spectra of these dyes in the spectral range 190÷500 nm. It is based on a combination of the determinant method (Cramer's method) with information on the absorption bands of the dyes in the mixture. The method is applied using the slope and intercept values from the linear calibration curves of the pyrene dyes standard solutions: HP3S, P3S and P4S, at three different wavelengths: The three wavelengths: 290nm, 367nm and 375nm, were selected by means of the Kaiser method. When there is a spectral region in which only one of the components has absorption, the concentration can be determined independently of the presence of the other two dyes in the ternary mixture. The method used to determine the concentrations of the other two dyes was the Cramer method or the bivariate spectral calibration method (BSC) that solves a system of two equations with two unknowns.
KEYWORDS: Cramer method, spectrophotometry, pyrene dyes, HP3S, P3S, P4S

INTRODUCTION

The paper describes a method used for the determination of the pyrene dye concentrations in a ternary mixture when there is a spectral region in which only one of the components shows absorption [1]. The method is based on combining the Cramer determinant method or the bivariate spectral calibration method (BSC) with information on the absorption bands of the dyes in mixture.

The dyes used in this paper are: trisodium salt of 8-hydroxi-1,3,6-pyrene trisulphonic acid (Pyranine, HP3S), trisodium pyrene-1,3,6-trisulphonate (P3S) and 1,3,6,8-pyrenetetrasulphonic acid tetrasodium salt (P4S). They are used in a mixture (D&C Green No.8) as additives in pharmaceuticals and cosmetics. The pyrene dyes contain aromatic ring structures so that they are harmful to human health when they are in high concentrations. The controlled use and the accurate analysis of their contents are of great importance. The structures of these dyes are shown in Figure 1.



HP3S P3S P4S

Figure 1. The structures of the pyrene dyes: HP3S, P3S and P4S

The absorbance corresponding to a substance depends on its concentration, the depth of the material and the wavelength. Within a limited range of concentrations, the absorbance depends linearly on the concentration. The slope (sensitivity) ***m*** *and the intercept* ***n*** *of the straight line calibration, depends on the wavelength.*

$$A = \varepsilon \cdot d \cdot c + n = m \cdot c + n \qquad (1)$$

The absorbance of a mixture of the three components, that have concentrations in the domain in which the absorbance of each depends linearly on the concentration, is additive.

Measuring the absorbance at three different wavelengths: λ_1, λ_2, λ_3 allows the determination of the concentration: c_1, c_2, c_3 of the three components in the mixture.

$$\begin{cases} A_X^1 = A_1^1 + A_2^1 + A_3^1 = m_1^1 \cdot c_1 + n_1^1 + m_2^1 \cdot c_2 + n_2^1 + m_3^1 \cdot c_3 + n_3^1 \\ A_X^2 = A_1^2 + A_2^2 + A_3^2 = m_1^2 \cdot c_1 + n_1^2 + m_2^2 \cdot c_2 + n_2^2 + m_3^2 \cdot c_3 + n_3^2 \\ A_X^3 = A_1^3 + A_2^3 + A_3^3 = m_1^3 \cdot c_1 + n_1^3 + m_2^3 \cdot c_2 + n_2^3 + m_3^3 \cdot c_3 + n_3^3 \end{cases} \qquad (2)$$

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In spectroscopy, a multiplicative interference may occur, when the absorbance due to a component may be influenced by the presence of other components [2].

According to Kaiser, an analytical technique, used for the simultaneous determination of several components of a total mixture, is completely selective if it is able to determine the concentrations of the components independently of each other [3,4]. The analytical system able to determine the three components is completely selective if only the main diagonal elements of the matrix absorption coefficients:

$$\begin{bmatrix} \varepsilon_1^1 & \varepsilon_2^1 & \varepsilon_3^1 \\ \varepsilon_1^2 & \varepsilon_2^2 & \varepsilon_3^2 \\ \varepsilon_1^3 & \varepsilon_2^3 & \varepsilon_3^3 \end{bmatrix} \quad (3)$$

are different from zero.

Total selectivity is obtained usually in very limited cases. In this case, the best selectivity is obtained when the determinant of the Kaiser matrix has a maximum value.

When there is a wavelength λ_1 where the absorbance of the ternary mixture is given by one of the components in the mixture, its concentration can be determined by solving relation (1). For this, it is necessary to establish the linear calibration curve A = f (c) of this component. Standard solutions of known concentrations are used, and the absorbances of these solutions are measured. The slope and the intercept of the linear calibration curve are used to determine the concentration of the component.

Then, the concentrations of the other two components are determined by solving the system of two equations with two unknowns at wavelength λ_2 and λ_3:

$$\begin{cases} \left(m_2^2 \cdot c_2 + m_3^2 \cdot c_3\right) = A_X^2 - m_1^2 \cdot c_1 - n_1^2 - n_2^2 - n_3^2 \\ \left(m_2^3 \cdot c_2 + m_3^3 \cdot c_3\right) = A_X^3 - m_1^3 \cdot c_1 - n_1^3 - n_2^3 - n_3^3 \end{cases} \quad (4)$$

EXPERIMENTAL

The standard solutions of known concentrations were obtained by dissolving the three dyes in distilled water in volumetric flasks. The absorption spectra of the standard solutions and the mixtures of dyes solutions thus prepared were recorded, against a blank of de-ionized water, with a JASCO V-550 UV-VIS spectrophotometer, in the spectral range of 190 to 600 nm, with a scan rate of 600 nm/min.

The spectral data, stored in the computer, were used to obtain the linear calibration curves at the wavelengths corresponding to the absorption bands of the dyes. The absorbance values at selected wavelengths were obtained from the registered spectra using the Kaiser method.

The absorbances and the data from the linear calibration curves of the dyes at selected wavelengths by the Kaiser method were used to determine their concentrations in ternary mixtures.

All data processing, regressions and statistical analysis were calculated by using the EXCEL and Matlab 7.0 software.

RESULTS AND DISCUSSION

Figure 2 shows the UV-VIZ absorption spectra of the three pyrene dyes: HP3S, P3S and P4S.

Although the absorption bands are close, due to their similar structures, it is possible to quantitatively analyze the mixture of the three dyes in the spectral range 280 ÷ 450 nm.

Out of the 19 absorption bands of the 3 dyes, 3 wavelengths were selected (one for each dye) in order to apply the Cramer method. The optimal wavelengths, selected by the Kaiser method that use the absolute values of the sensitivities matrix, are: 290nm for HP3S, 367nm for P3S and 375nm for P4S.

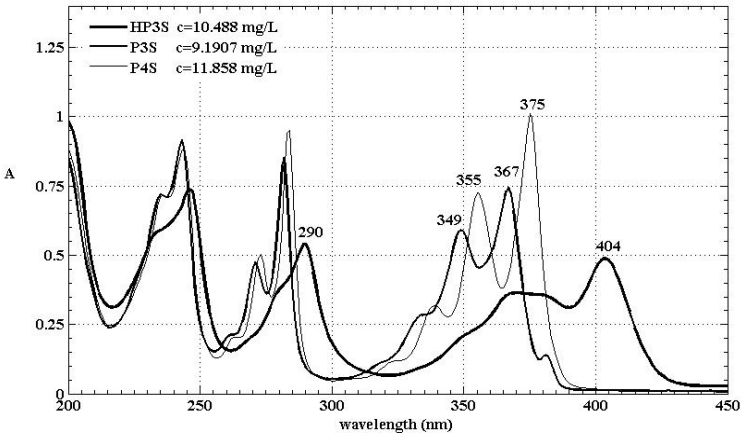

Figure 2. The zero-order UV-VIZ spectra of the pyrene dyes: HP3S, P3S and P4S

At the three selected wavelengths, the linear calibration curves A= f (c) were plotted for each dye. The linear regression equations were calculated, from which we obtained the slope (sensitivity) and intercept. The values of the sensitivities at the three wavelengths are shown in Table 1.

Table 1. The values of the sensitivities

Wavelength (nm)	m_{HP3S}	m_{P3S}	m_{P4S}
290	0.048337	0.008522	0.008944
367	0.032716	0.075295	0.035142
375	0.032947	0.021304	0.077612

The sensitivities for HP3S, P3S and P4S, determined in solutions with a single component, at 3 wavelengths, selected by means of the Kaiser method (m_{HP3S}, m_{P3S} and m_{P3S} = the slope values of the linear calibration curves for HP3S, P3S and P4S)

$$A = \varepsilon \cdot d \cdot c + n = m \cdot c + n$$

The statistical parameters of linear calibration curves for the three dyes are shown in Table 2.

*Table 2. The statistical parameters of the calibration graphs for each dye * [5]*

λ (nm)	Dye	Linearity range (mg/l)	The linear regression equation	Sy	Sm	Sn	LOD	LOQ
290	HP3S	5÷26	A=0.048337c+0.0181 r=0.9994	0.0188	0.0012	0.0212	1.1644	3.8812
367	P3S	5÷40	A=0.075295c+0.054384 r=0.9998	0.0247	0.0010	0.0213	0.9855	3.2849
	P4S	2÷30	A=0.035142c+0.014705 r=0.9999	0.0011	0.0001	0.0009	0.0956	0.3188
375	P3S	5÷40	A=0.021304c+0.02856 r=0.9999	0.0043	0.0002	0.0037	0.6107	2.0357
	P4S	2÷30	A=0.077612c+0.057262 r=0.9997	0.0292	0.0014	0.0227	1.1297	3.7655

** S_y=the standard deviation of the regression line; S_m= the standard deviation of the slope; S_n= the standard deviation of the intercept; the limit of detection LOD=3*S_y/m; the limit of quantitation LOQ=10*S_y/m; m= the slope of the calibration curve*

The correlation coefficient of the regression lines shows very accurate values of the sensitivities for the three dyes.

The analysis of the absorption spectra of the three dyes shows there is a wavelength (290nm) where the absorbance of the mixture is the result of a single dye (HP3S). At this wavelength the absorbances of P3S and P4S dyes are very small, as compared to the absorbance of the HP3S dye. This observation, shown in Figure 3, can be used to determine the HP3S dye concentration in the ternary mixture, independently of the other two dyes.

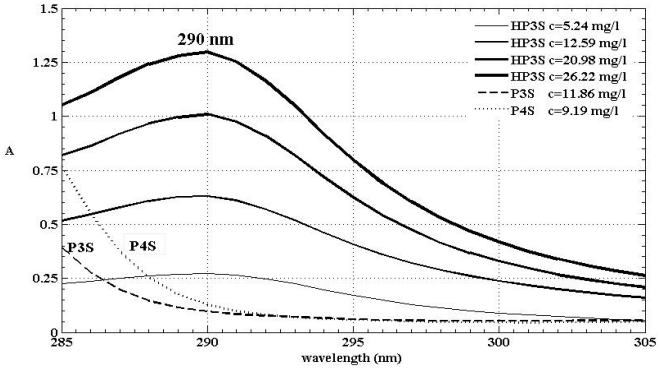

Figure 3. The absorption band at 290nm in the UV-VIZ spectra of the pyrene dyes.

At this wavelength:

$$A_X = A_{P3S} + A_{P4S} + A_{HP3S} \approx A_{HP3S} = \varepsilon_{HP3S} d c_{HP3S} + n_{HP3S} = m_{HP3S} \cdot c_{HP3S} + n_{HP3S} \quad (5)$$

This observation is used to determine the concentration of the HP3S dye in the mixture.

Equation (5) leads to:

$$c_{HP3S} = \frac{A_X - n_{HP3S}}{\varepsilon_{HP3S} \cdot d} = \frac{A_X - n_{HP3S}}{m_{HP3S}} \quad (6)$$

To check the method, five different mixtures of the three dyes: M1, M2, M3, M4 and M5 were prepared. For these samples the UV-VIZ absorption spectra were recorded. Figure 4 shows the UV-VIZ absorption spectrum of the three dyes pyrene mixture M1.

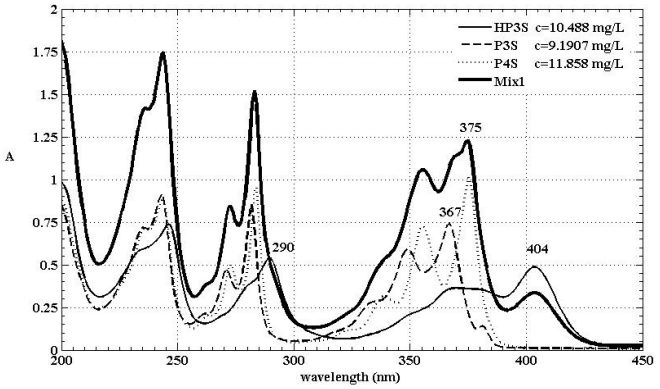

Figure 4. The zero-order spectrum of the HP3S, P3S, P4S pyrene dyes and their mixture

From these spectra, the absorbances measured at the three wavelengths, determined by the Kaiser method, were used. First, the concentration of HP3S dye was determined using the absorbance of the dye mixture measured at a wavelength of 290 nm and the sensitivity of the dye at this wavelength. For the five samples, the HP3S concentrations in the mixture are given in Table 3.

Table 3. The HP3S dye concentrations determined from the absorbances corresponding to the absorption band at 290 nm, according to relation (6), (mg/l)

Sample	m	n	A	A-n	c=(A-n)/m
M1	0.04834	0.01810	0.337	0.319	6.58075
M2			0.499	0.481	9.90201
M3			0.212	0.194	4.02233
M4			0.120	0.102	2.13598
M5			1.221	1.203	23.4862

In order to determine the concentrations of the other two dyes in the mixture: P3S and P4S, the bivariate spectral calibration method was used [6]. For the wavelengths: λ_1 = 367nm and λ_2 = 375nm, selected by the Kaiser method, the system of two equations with two unknowns (c_{P3S} and c_{P4S}) is solved.

For the two dyes, the linear regression equations at the two selected wavelengths, are given in Table 4. The set of equations used for the application of the bivariate spectral calibration method in order to obtain the P3S and P4S dyes concentrations is shown in Table 5. The absorbances for HP3S, measured at the two wavelengths: λ_1 = 367nm and λ_2 = 375nm, are used to solve the system of equations. The values of the concentrations for the three dyes in ternary mixture, obtained by the determinant method are shown in Table 6.

Table 4. The individual linear regression equations at the selected two wavelength set.

λ (nm)	P3S	P4S
367	A=0.075295c+0.054384	A=0.035142c+0.014705
375	A=0.021304c+0.02856	A=0.077612c+0.057262

Table 5. The set of equations used to apply the bivariate spectral calibration method.

λ (nm)	
367	$A = A_{HP3S} + 0.075295c_{P3S} + 0.035142c_{P4S} + 0.069089$
375	$A = A_{HP3S} + 0.021304c_{P3S} + 0.077612c_{P4S} + 0.085822$

Table 6. The concentrations of the HP3S, P3S and P4S dye in the ternary mixture.

Sample	Taken (mg/L)			Found (mg/L)			Recovery (%)		
	HP3S	P3S	P4S	HP3S	P3S	P4S	HP3S	P3S	P4S
M1	6.992	6.12717	9.88171	6.58075	5.8012	10.3395	94.12	94.68	104.63
M2	10.488	6.12717	1.97634	9.90201	6.5182	1.7568	94.41	106.38	88.89
M3	4.1952	15.3179	19.7634	4.02233	14.9117	20.4851	95.88	97.35	103.65
M4	2.0976	7.65896	9.882	2.13598	7.630	10.748	101.83	99.63	108.77
M5	26.22	5.51445	1.1858	23.4862	6.114	1.156	89.57	110.87	97.50
						Mean	95.16	101.78	100.69
						SD	4.41	6.68	7.73
						RSD	4.64%	6.56%	7.68%

SD = the standard deviation, RSD= the relative standard deviation.

The analysis of the results from table 6 shows that for the three dyes, similar values of the corresponding concentration in the mixture were obtained.

CONCLUSIONS

In reference literature, several graphical and chromatographic methods can be found for the determination of the overlapping binary or ternary mixture systems. These approaches require some initial steps which are time consuming and involve higher cost (for HPLC).

In this paper, the determinant method was applied to determine the concentrations of three pyrene dyes: HP3S, P3S and P4S in a ternary mixture. The zero-order UV-VIZ spectra were used.

The method suggested was applied satisfactorily to the determination of HP3S, P3S and P4S in ternary mixture. The results show that the Cramer method is a good alternative to other methods that require a mathematical treatment of the obtained spectra, such as the derivative spectrophotometry or the spectral ratio derivative spectrophotometry.

The method suggested in the paper does not require any graphical and separation procedure, is easy to apply and very cheap.

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