



Determination of Rhodamine B and Rhodamine 6G dyes in Various Ink Samples by Zero Crossing Method: First Derivative Spectrophotometry

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Abstract

Paper Status

Received : Apr 2020
Accepted : May 2020
Published : Jun 2020

Key Words

Ink Analysis

Dye

Derivative Spectrophotometry

Zero-Crossing Point.

A simple spectrophotometric method has been developed for resolving binary mixtures of Rhodamine B and Rhodamine 6G dyes of red ballpoint pen ink using the first-derivative spectra with measurements at zero-crossing wavelengths. Calibration graphs were linear up to 8 mg L⁻¹ for Rhodamine B and 10 mg L⁻¹ for Rhodamine 6G. This method was successfully applied for the quantitative determination of dyes having overlapping spectra in their bi-component mixtures. The applied derivative method is practical, simple, rapid, inexpensive and suitable for quantitative analysis of bi-component dye solutions in the ballpoint pen ink.

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Citation: Natinael Mekonnen, Mamo Dikamo, Abdurrohman Mengesha, Ali Raza Sansa. "Determination of Rhodamine B and Rhodamine 6G dyes in Various Ink Samples by Zero Crossing Method: First Derivative Spectrophotometry". International Research Journal of Science and Technology, 1 (3), 221-225, 2020.

1. Introduction

Controlling the color or hues of dye solutions is a popular phrase for the industries dealing with colorants, namely textile, paint, ink industries, pharmaceuticals, processed food industries etc. A diversity of shades is regularly produced by mixture of colorants in color industries. One of the most important problems in this process is to find the exact proportions of colorants in order to produce a match for a target shade. Ink quality is closely associated with color and the use of ink colorants has been an age-old practice, enhancing the aesthetical appeal of inks. The appearance of the ink color is one of the deciding factors for market value and demand of a particular company or brand in the writing instruments market [1].

A large variety of analytical methods have been described for quantitative analysis of dye solutions in the field of pharmaceutical science, food science, environmental science and textile industries etc.[1-5], but the dye concentration determination in ink is not reported yet. Among all the analytical methods, UV-visible spectrophotometry is the most simple and common procedure for determination of colorant contents in their mixture. Dyes are highly absorbing species in the visible region and spectrophotometry is frequently used for their determination. However, peak overlapping of dye components is common in the analysis of a mixture. Therefore, the direct absorption measurement (zero-order spectra) is not suitable for resolving multi-component dye mixtures without a separation step [6]. Attempts to resolve complex spectra by means of different approaches have been made [6,7]. Among computer-controlled instruments, derivative techniques and multivariate calibration methods have played a very important role for analysis of the multi-component dye mixtures by UV-Vis spectrophotometry[8,9] In this context, derivative

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spectrophotometry is a very useful technique in which, the rate of absorbance change is measured as a function of wavelength. The process of differentiation [10] generally leads to the enhancement of resolution of overlapping spectra. The principles of derivative spectrophotometry [11] have been applied in several analytical problems for resolving binary and ternary mixtures with overlapping spectra.[12-18].

In this work, the derivative spectrophotometry is the base of a method for simultaneous determination of dyes contents in the red ink formulation of ballpoint pen. The aim of present study is to demonstrate the applicability of the zero-crossing-point first-derivative spectrophotometry for analyzing the bi-component dye mixtures of ink with strongly overlapping spectral bands. In order to minimize possible spectral interferences, centrifugation of methanolic ink solution was used to free the colorants from ink matrix and additives.

2. Materials and Methods

The thin layer chromatographic (TLC) and spectrophotometric studies were done on the sixteen red ballpoint pen of different brand or the different models of the same brand. These red ballpoint pens were purchased from the local stationery shops in New Delhi, India and were marked as PS1 to PS16 for the study. TLC study was performed for screening the dyes present in the red ink of the collected ballpoint pen. The inks which were found to contain a mixture of Rhodamine B (Rh B) and Rhodamine 6G (Rh 6G) dyes were further analyzed by spectrophotometric technique. Standard Rh B, Rh 6G and Eosin Yellow dyes were purchased from Thomas Baker and Loba Chemie and used without further purification. Analytical grade solvents were used for the TLC separation.

For TLC analysis, sufficient quantity of ink was taken out from the pen barrel using a thin capillary. The collected ink was dissolved in 50 μ L of methanol. The TLC was carried out using a pre-coated silica gel (Merck 60 F254) without fluorescent indicator backed with an aluminum sheet.

A ternary mixture, ethyl acetate, ethanol and distilled water in 70:35:30 volume ratios, was used as a developing solvent. TLC analysis was performed by following the literature procedure, as described by K. Tsutsumi[19].

Centrifugation of the ink sample was carried out using a centrifuge instrument of model, Laby, T-24. For centrifugation, desired amount of red ink, e.g., 0.19 g, 0.21 g, and 0.19 g, was taken directly from the pen barrel of the collected ballpoint pen having their corresponding sample numbers of PS 2, PS 9, and PS 11, respectively.

The ink was transferred into a tarson and mixed with 10 mL of methanol. After vigorous shaking and centrifugation for 30 minutes at a speed of 10000 rpm, the supernatant was collected. 20 μ L of supernatant was diluted to 4 mL in methanol. This diluted supernatant solution was used for the spectrophotometric studies.

Spectrophotometric analysis of diluted supernatant solution was carried out using a UV-Visible spectrophotometer of model A Specord 500 Analytik Jena instrument. Standard solutions of Rh B, Rh 6G dyes of their respective concentrations of 0.02, 0.2, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 15.0, and 20.0 mg/L were prepared in methanol. Standard bi-component dye mixtures of different concentrations were prepared using their respective stock solutions of concentration 50 mg/L.

2.1 First Derivative Technique

The absorption spectra of the samples and binary standard mixtures (containing 0.2 to 8.0 mg/L of each dye) were recorded between 300 and 750 nm with a scan rate of 100 nm/s. First derivative spectra were obtained by smoothing (11 experimental points of interval not less than $\Delta\lambda=3$) the measured absorbance data of the binary mixture. The concentration of each dye in different ballpoint pen ink was determined from the calibration plot of the standard dye mixtures.

For calibration in zero-crossing method, the first order derivative spectra of standard binary mixtures were obtained in the wavelength region of 300 – 750 nm. The obtained derivative absorbance spectra were then analyzed at zero-crossing wavelength to make the calibration plot.

3. Results and Discussion

3.1 TL Analysis

TLC method was used for evaluating the dye content in our collected ink samples. The TLC method is based on visual inspection of color bands, relative intensities and R_f values .The TLC of the sixteen ballpoint pen inks showed that the three ballpoint pen inks contain a mixture of both Rh B and Rh 6G. The ballpoint pen ink samples which were found to have a mixture of Rh B and Rh 6G are tabulated in table 1 and the respective TLC chromatogram is shown in Figure 1.

Table1. Pen ink samples which were found to have a mixture of Rh B and Rh 6G

S. No.	Pen Name	Sample No.
1	Cello fine Gripper	PS 2
2	Cello Pin Point	PS 9
3	Cello Gripper	PS 11

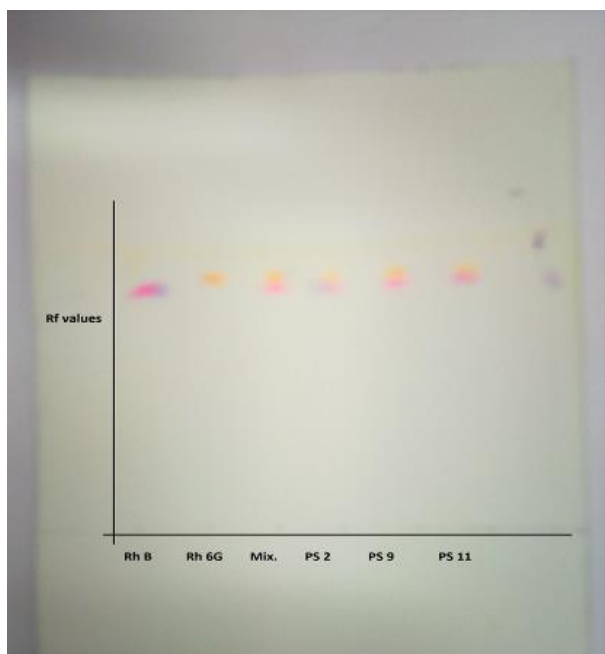


Figure 1. TLC Chromatogram of the Rh B, Rh 6G, and their mixture and of the ink samples PS2, PS9 & PS 11

3.2 UV-Visible Spectrophotometric Analysis

The zero-order spectra of standard Rhodamine B, standard Rhodamine 6G, binary mixture of standard Rhodamine B and 6G solution and one of the ink samples (sample number PS 9) are shown in Figures 2 and 3.

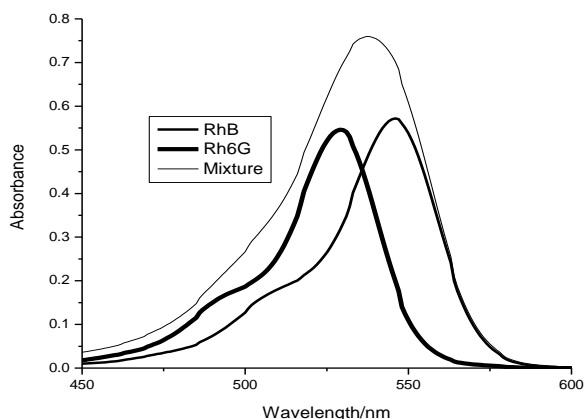


Figure 2. Zero order absorbance spectra of 2.0 mg/L Rh B, 2.0 mg/L Rh 6G, and their binary mixture (Rh B 4.0 mg/L & Rh 6G 2.0 mg/L)

It is evident from these figures that the zero order spectra of the standard dye components overlapped at 534 nm in the mixture. Therefore, simultaneous determination of both dye content in the mixture cannot be determined accurately from the zero order spectra. The determination of two dyes might be possible by means of multivariate analysis. Another possibility to solve this problem is the derivative spectrophotometric method, and the most common practice for this purpose is the use of zero crossing method of first derivative spectra. In practice, by

measuring the derivative values at the wavelength where one of the component has a zero or near zero value, the best linear responses are obtainable, and the calibration graph are less affected by concentration of other component.

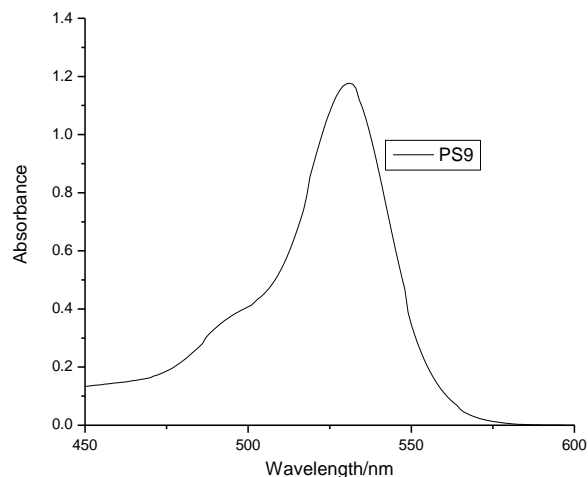


Figure 3. Zero order spectra of ink sample PS 9.

Figure 4 shows the first order derivative spectra of Rhodamine B, Rhodamine 6G and their binary mixture.

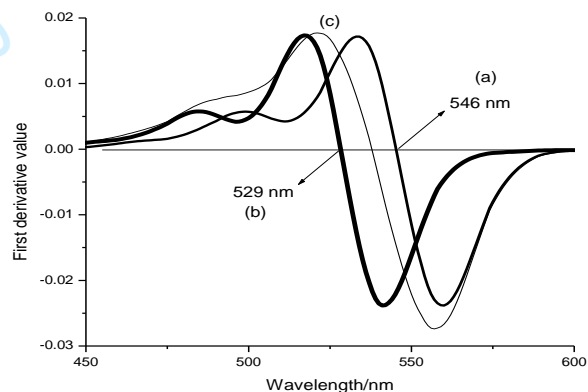


Figure 4. First order derivative spectra of (a) 2.0 mg/L Rh B, (b) 2.0 mg/L Rh 6G, and (c) their binary mixture (Rh B 4.0 mg/L & Rh 6G 2.0 mg/L)

It is clear from Figure 4 that the derivative value at 529 nm is the zero crossing point of Rh 6G, and hence the corresponding absorbance value in the mixture at 529 nm is due to Rhodamine B. Similarly, the derivative value at 546 nm is the zero crossing point for Rh B and the corresponding absorbance value in the mixture at 546 nm is due to Rhodamine 6G. Calibration graphs were linear in the concentration range of 0.02 – 8.0 mg/L for Rhodamine 6G and 0.02 – 10.0 mg/L for Rhodamine B in their binary mixtures (Figures 6 and 7).

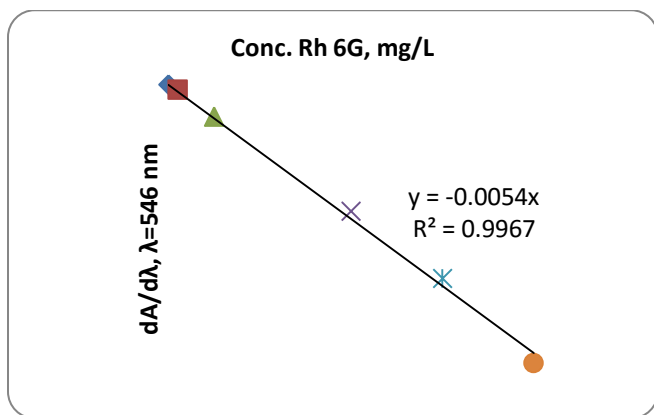


Figure 6. Conformance to Beers Law, for the Dye Rh 6G.

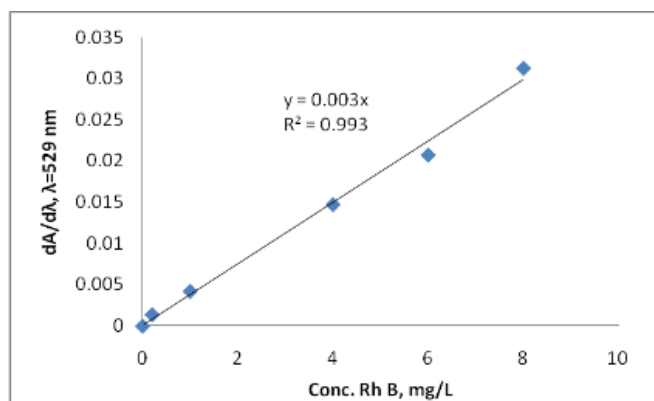


Figure 7. Conformance to Beers Law, for the dye Rh B.

The performance of zero-crossing derivative method in determination of Rh B and Rh 6G concentrations in the synthetic bi-component mixtures of Rh B and Rh 6G was evaluated by relative standard deviation between the actual and calculated concentration of both dyes in the mixtures. Synthetic mixtures were prepared by mixing Rh B and Rh 6G at their varying concentrations. In case of mixtures of varying concentrations of Rh 6G and fixed amount of Rh B, Rh B was used as an interferential. Similarly, Rh B was used as an interferential in the mixtures of varying concentrations of Rh 6G and fixed amount of Rh B. The performance of zero-crossing method in determining Rh B and Rh 6G concentrations from the synthetic samples are presented in Tables 2 and 3.

Table 2. Determination of Rh 6G concentration in the binary mixture using the first derivative method. Rh B of 2.0 mg/L was used as an interferent.

Actual concentration of Rh 6G present in the mixture, mg/L	Concentration of Rh 6G obtained from the first derivative spectra*, mg/L	RSD, %
0.20	0.19	-5.26
1.0	1.0	-
4.0	4.1	-2.43

*Measurements were performed at $\lambda=546$ nm. All values in the table are the mean values of three independent measurements. RSD= Relative Standard Deviation.

Table3. Determination of Rh B concentration in the binary mixture using the first derivative method. Rh 6G of 2.0 mg/L was used as an interferent.

Actual concentration of Rh B present in the mixture, mg/L	Concentration of Rh B obtained from the first derivative spectra*, mg/L	RSD, %
0.20	0.18	+11.11
1.0	1.2	+16.66
4.0	3.90	+2.56

*Measurements were performed at $\lambda=529$ nm. All values in the table are the mean values of three independent measurements. RSD= Relative Standard Deviation.

It is evident from the results that the determination of the Rh B and Rh 6G can be simply and selectively performed in synthetic mixtures of Rh B and Rh 6G in the presence of each other as interferent. Only when Rh B is present in low concentration with a higher concentration of Rh 6G, a significant error in determination of Rh B concentration can be observed.

In case of real ink samples, the concentration of Rh B and Rh 6G components has been determined by the first derivative method. The results show that the concentrations of both dyes are similar in the ink samples of PS 2 and PS 11. This result also supports the fact that both the ink samples were collected from the ball point pen of the same brands. The analytical results obtained are quite acceptable; nominal contents, provided by the manufacturers, were not available.

4. Conclusion

This study introduced zero-crossing point derivative spectrophotometry as a technique for the determination of individual dye concentration from the ink samples containing two dyes with overlapping spectra. The technique allowed the simultaneous determination of Rhodamine B and Rhodamine 6G concentrations in synthetic binary mixtures and real ink samples with satisfactory and acceptable standard deviation. This method is practical, simple, rapid, inexpensive and suitable for quantitative analysis of bi-component dye solutions in the ballpoint pen ink.

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