

**A New Method for Eosin Preparation and Examination
of its Absorption and Emission Spectra**

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ABSTRACT

Eosin is one of the original dyes of xanthenes derivatives. It is also one of the main derivatives of fluorescein and has brilliant fluorescence. Eosin disodium salt called "Eosin Y" is the most useful form of this compound. In common procedures, eosin is usually obtained by the action of bromine on fluorescein, which has not good yield and purity degree. In this research, it is prepared by the reaction of $\text{Br}^- / \text{BrO}_3^-$ on fluorescein in acidic water- acetone mixture. It has been tried to find optimum conditions, which allow the precipitation of eosin while the other brominated derivatives of fluorescein remain soluble. Elemental analysis, UV-Vis spectrophotometry and FTIR spectrometry are applied to confirm the results of the new method. The absorption and emission spectra have been obtained with using a spectrofluorometer. The total luminescence spectrum is demonstrated as a three-dimensional plot to show the intensity of the luminescence signal at different wavelengths. The results show good agreements with references.

Keywords: Emission; Absorption; Dye; Spectrophotometry

INTRODUCTION

A dye may be defined as a colored substance which applied to the fibers imparts a permanent color and washing or exposing to sunlight does not remove the color. All colored substances are not essentially dyes. Long ago, when there was no industry on synthetic dyes, the fibers were generally dyed by coloring matters obtained from vegetables or in case of cochineal from insects. The revolution began in 1856 when W. H. Perkin discovered aniline purple in the course of research, which was an attempt to synthesize the drug quinine. At present, thousands of synthetic dyes are available. Some dyes having no natural counter parts were developed. New classes of dyes

discovered included xanthenes. Von Baeyer discovered the first xanthene dyes^[1]. He obtained it when he condensed resorcinol and phthalic anhydride to give fluorescein. Caro brominated fluorescein and obtained another useful dye, Eosin. Later on, Orndroff and Hammer tried to purify it chemically. In this research synthesis of eosin is modified and its emission and absorption characteristic of this dye are examined. The pH value of maximum emission at different wavelengths is also defined.

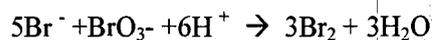
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MATERIALS AND METHODS

In this work, eosin is prepared in two methods. The first method is known in references [2&3] and then it is compared with second one that is a modified synthesis of eosin. The obtained product prepared by the known method, which clearly isn't pure, yields 77.71 %. To purify it, experimental separation by solvents cannot give reliable result, because whenever one of brominated derivatives is dissolved, a large or small amount of another derivative comes to liquid phase. Considering the fact that the major impurity is fluorescein, there is no media in which eosin and fluorescein can be separated perfectly. In subsequent stage, it is decided to make chemical purifying [4&5], which contains several steps: Brominating of a suspension of fluorescein in glacial acetic acid, being poured into a large volume of water, dissolving in dill. sodium hydroxide solution in presence of excess of acetic acid to convert it into diacetate derivative, then being saponified with alcoholic sodium hydroxide solution, acidifying with acetic acid, boiling and washing with a large volume of hot water. Upon the addition of some concentrated hydrochloric acid, flesh - colored crystal of eosin is deposited.

As it seems, these procedures are very boring, long and need to use a large amount of impure eosin or fluorescein to start the process. Also its yield is unbelievable, because some of the

obtained product is lost in different stages of procedures. For this purpose, combination of Br⁻ / BrO₃⁻ is substituted in spite of bromine solution. This can give more time to fluorescein to react. Also its application is easier and safer than bromine solution. According to done studies, these reactants can gradually release bromine in solution [6].



500 mL distilled water is mixed with 10 g KBr and 20 mL concentrated H₂SO₄. After being cooled, 500 ml acetone is added, then 750 mg fluorescein, 160 mg of 0.2 N bromate solution is added and the balloon is shaken strickly for five minutes. The solution is put stagnant down for two hours. After this time, 400 mL H₂O is added and the reaction mixture remained motionless for 3 - 4 hours. The precipitation can be simply gathered, filtrated off and dried at 100 oC. The product is 1.15 g pure eosin, because all derivatives of fluorescein except eosin are soluble in this media. Also this method is better with the view of safety and facilitating the work in laboratory. Elemental analysis is applied to confirm prepared compounds. The results below compare the carbon and hydrogen percents of both two products with reference sample that is Merck with 99% purity degree.

Table I. Results of elemental analysis of eosin produced by original method and Merck reference

Sample No.	Sample No. 1	Sample No. 2	Merck sample	Formula percents
Dry sample (g)	1.463	1.527	2.538	-
Produced CO ₂	1.899	1.942	3.228	-
Produced H ₂ O	0.116	0.119	0.198	-
C%	34.628	34.709	34.687	34.686
H%	0.870	0.851	0.867	0.867

A - t test for carbon

$$\bar{X} = 34.669$$

$$S = 0.057$$

$$t = 0.447$$

B - t test for hydrogen:

$$\bar{X} = 0.861$$

$$S = 0.014$$

$$t = 0.606$$

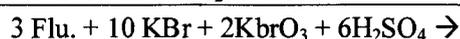
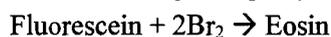
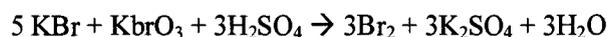


Table 2. Results of elemental analysis of Eosin produced by new method and Merck reference

Sample No	Sample No. 1	Sample No. 2	Merck sample	Formula percents
Dry sample (g)	1.613	2.538	2.538	-
Produced CO ₂	2.052	3.228	3.228	-
Produced H ₂ O	0.126	0.198	0.198	-
C%	34.718	34.687	34.687	34.686
H%	0.868	0.867	0.867	0.867

A-t test for carbon

$$\bar{X} = 34.687$$

$$S = 0.045$$

$$t = 0.707$$

B - t test for hydrogen

$$\bar{X} = 0.865$$

$$S = 0.04$$

$$t = 0.000$$

Comparing the results of the two methods:

A - for carbon percent:

Precision test: $F = 1.604$

Accuracy test: $t = 0.360$

B - for carbon percent:

Precision test: $F = 1.604$

Accuracy test : $t = 0.360$

B - for carbon percent:

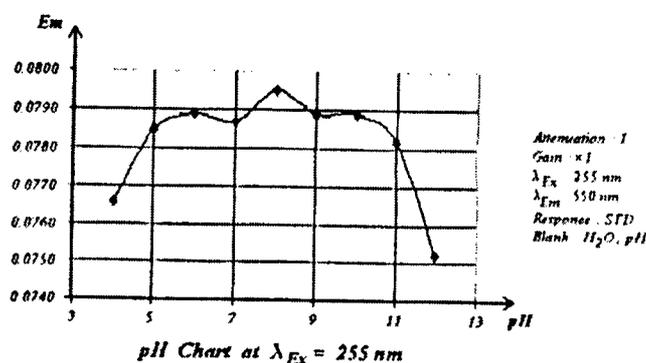
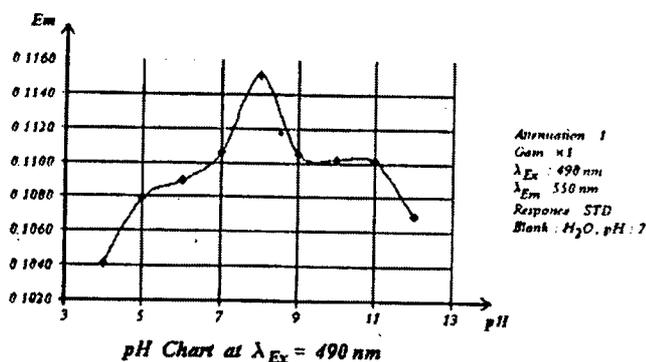
Precision test: $F = 12.250$

Accuracy test: $t = 0.182$

According to elemental analysis and statistical data, there is no reason for both methods to have systematic errors. Also there are no significant differences between the average and variance of these methods.

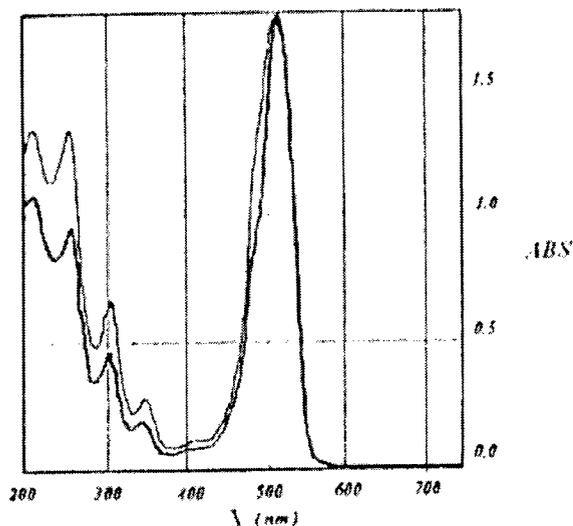
The plots in the figure below show how the emission intensity depends on pH. The best pH for sample preparation is defined in maximum emission.

The spectrum of emission intensity vs. wavelengths at different pH also shows the best pH for sample preparation. The concentration of eosin solution, which is freshly prepared, is 2×10^{-2} g/L.



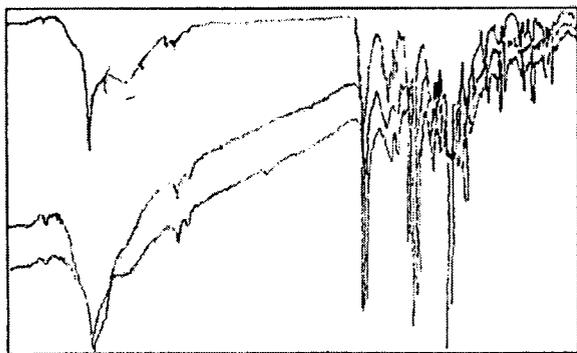
These curves show the best pH to be in about eight. This result can be proved by pH A scale in which eosin has dianion form in this pH and this electric charge in the rigid structure of eosin can help it to emit.

UV-Vis spectrophotometry and FTIR spectrometry are applied to identify the structure. Note that in all stages samples are freshly prepared. All of UV-Vis spectra are drawn by PHILLIPS PU 8700 with bandwidth = 2 nm and scan speed = 1000 nm / min. A weak band in $\lambda > 300$ nm, usually seen in 250 - 360 nm, can be related to $\pi \rightarrow \pi^*$ transmittance that has been seen because of existing of atoms with loan pairs in molecule. Two medium bands in $\lambda > 200$ nm often show an aromatic system available in eosin structure. A strong band in $\lambda < 250$ nm may be defined in all molecules having π system introducing $\pi \rightarrow \pi^*$ transmittance. Another strong peak in visible area (in $\lambda = 515$ nm for eosin) proves colored structure for eosin, which has a polycyclic aromatic chromophore. According to the spectrum, there aren't too many differences in number and locality of peaks between prepared sample with new method and reference sample which was Merck (P.D. = 99%).



Comparison of UV-Vis spectra of sample to Merck reference (99%)

PHILLIPS PU 9800 draws FTIR spectra, applied to characterize the structure of synthesis product. Important functional groups consist of a strong band of carbonyl group (1600 - 1800 cm⁻¹), then -OH group, a wide peak in 2400 - 3600 cm⁻¹ confirmed by C-O near 1100 - 1300 cm⁻¹. Medium or strong bands in 1400 - 1600 cm⁻¹ show an aromatic system proved by a band of C-H at the left side of 3000 cm⁻¹. Medium bands in about 690 cm⁻¹ also show C-Br groups. The major difference in sample and reference spectrum is related to small area around 3200 cm⁻¹. It can be related to the absorbed moisture by reference sample in case where all of produced samples were dried in oven or acetone.

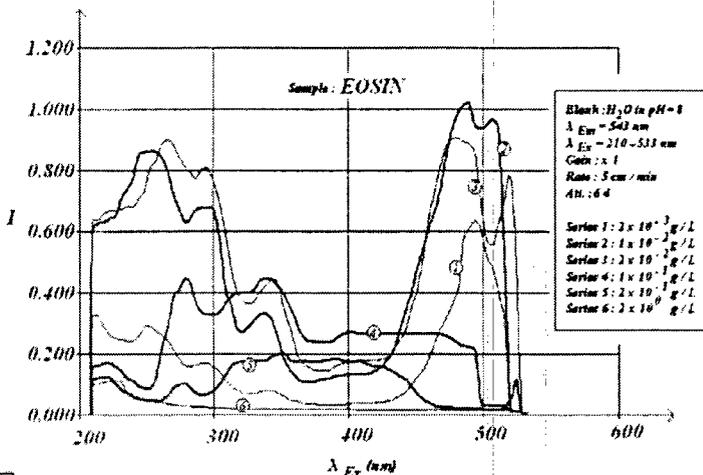


Comparison of FTIR spectra of Merck reference to samples dried by oven or acetone

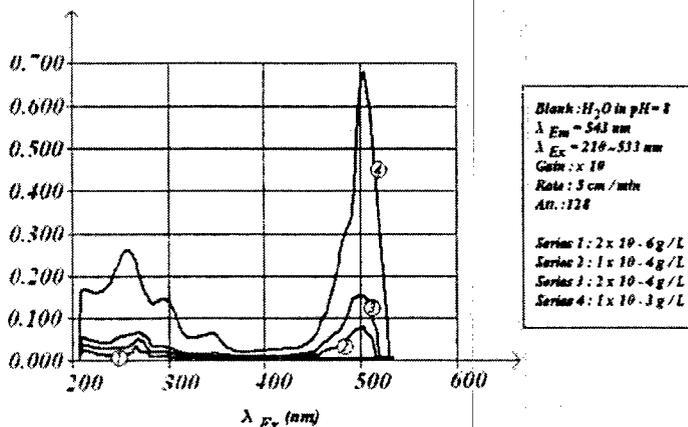
- 1: dried by oven
- 2: dried by acetone

To further study on the emission characteristic of eosin, the absorption, emission and the total luminescence spectra have been studied. For this purpose, ten solutions of eosin

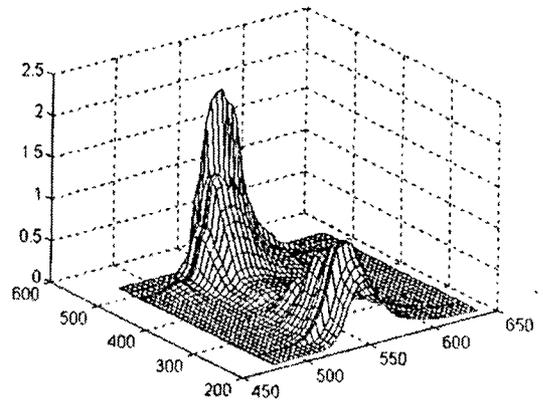
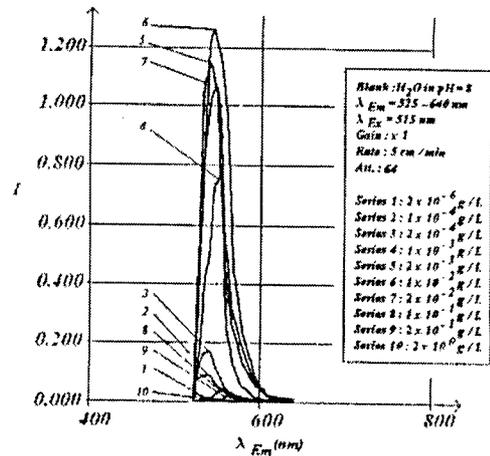
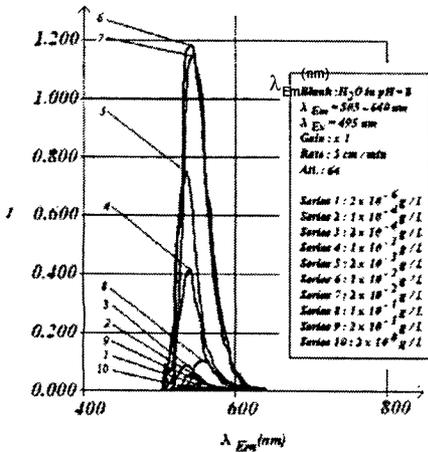
are prepared in concentration of 2×10^{-6} g/L until 2×100 g/L in pH ~ 8. Blank solution was distilled water in the same pH and JASCO MODEL 820 - FP performed all of spectra. In first absorption scan, it is observed that the intensity of emission is getting diminished during 200 - 320 nm. The energetic radiations increase collisions in low wavelengths and self-quenching occurs in lower concentration. Decreasing of emission intensity displaces the maximum of peaks to longer wavelengths. In $\lambda \sim 500$ nm, a piece of emitted radiations are absorbed by unexcited molecules and emission intensity decreases. This effect was so strong in sample no. 4 that the major parts of emitted radiations are absorbed by solution, as its intensity has been in very low level. Emission intensity also decreases to lowest level in sample no. 5&6.



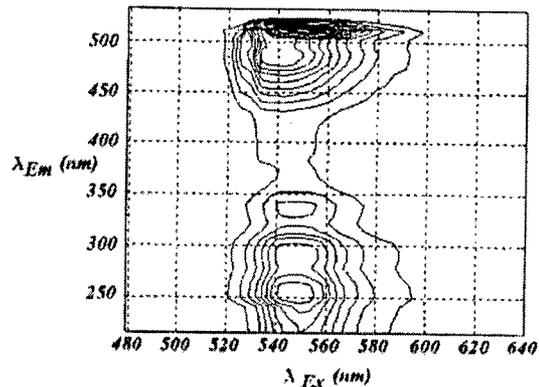
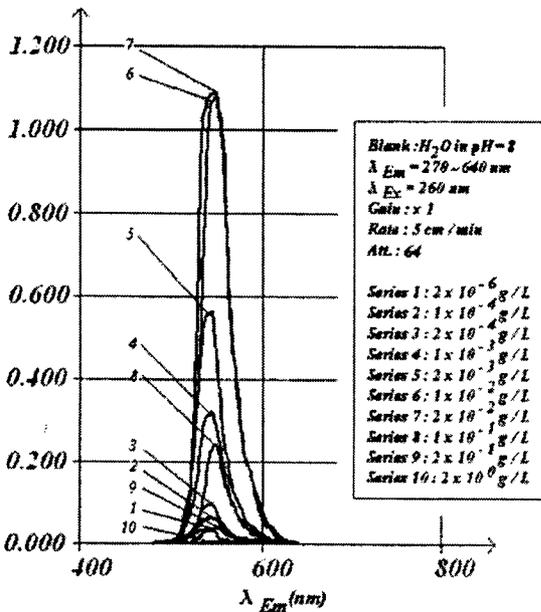
In second absorption scan, similar phenomena have occurred. In this stage, self-absorption and self-quenching haven't been observed, because the concentration is very low. Considering the obtained results, the maximum absorption peaks occur in 214, 260, 495 and 515 nm.



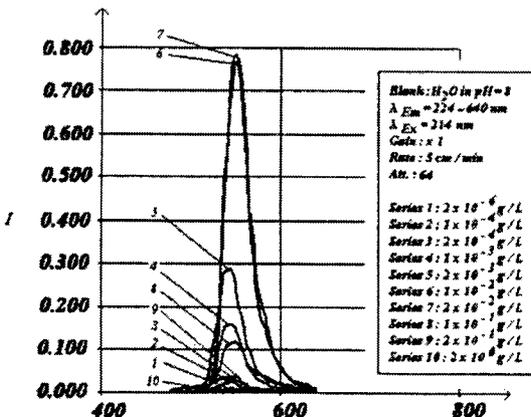
The emission scans were made in the same conditions. In first and second scan, the maximum emission displaces to longer wavelengths according to concentration. The maximum intensity is obtained in 1×10^{-2} g/L and 2×10^{-2} g/L. The maximum emission seems to be in 540-550 nm for a solution of Eosin is pH ~ 8.



Total luminescence spectra of prepared eosin in different absorption and emission wavelengths (Mesh 3D), $C = 1 \times 10^{-2}$ g/L.



Contour map of prepared eosin in different absorption and emission wavelengths, $C = 1 \times 10^{-2}$ g/L.



RESULTS AND CONCLUSION

Considering the statistical data of elemental analysis, the produced eosin (by the new method) is confirmed by comparing C & H percents of reference sample. UV- Vis and FTIR spectra are applied to check the sample structure and well adaptation has been represented. Obtained data of emission spectroscopy show the max absorption at 214 nm and 515 nm. The maximum emission is 214 nm and 515 nm. The maximum emission is

located in 540-550 nm that is equal to reference data for eosin in the same pH. In all stages of this

research, new data have been in a good accordance with references.

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