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# Spectral Separation of Uranine, SRG Extra and Rhodamine WT Fluorescence in Binary Mixtures in Water Samples

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# Interference of some artificial tracers on spectral determinations

## Spektral tayinlere bazı yapay iz sürücülerin giriřimi

Research Article

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### ABSTRACT

The aim of this study is to decide which dyes can be used together as artificial tracers in the same water system study and how to avoid their interference at each other when they are in the same water sample, too. In this paper only spectral separation of fluorescent dyes in binary water sample mixtures and treatments based on pH-variations are described. Concentration and synchronous scan methods were used for the measurement of Rhodamine WT Liquid, SRG Extra, Uranine and Eosin fluorescence by the means of a Luminescence Spectrometer LS 55.

### Key Words

Artificial tracer, fluorescence intensity, synchronous scan.

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### ÖZET

Sunulan çalışmanın amacı; bazı su sistem çalışmalarında hangi boyaların yapay iz sürücü olarak birlikte kullanılabilirliğini ve ayrıca girişim etkilerinden nasıl sakınılacağını belirlemektir. Bu makalede; ikili örnek karışımlarında floresan boyaların sadece spektral ayrımı ve pH değişimine dayalı işlemler tanımlanmıştır. Lüminesans spektrometre LS 55 ile rodamin WT sıvısı, SRG ekstra, uranin ve eosin floresanların ölçümünde derişim ve eşzamanlı tarama yöntemleri kullanılmıştır.

### Anahtar Kelimeler

Yapay iz sürücü, floresan şiddeti, eşzamanlı tarama.

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## INTRODUCTION

The introduction of fluorescent tracers in hydrology to know and study aquatic systems has led to enormous methodological and instrumental developments [1]. The problems they deal with when applied in studying various water systems might be grouped into different groups, according to where is groundwater flowing from, from where it comes, whether exist underground hydraulic connections between different points of the system or not, how is flowing the groundwater in(to) and/or through the system under the study, etc., [2,3].

Related with the aims of the study one can inject one, two or more tracers in the same experiment. Examples: if it needs to be established *Whether?* Is a possible connection between two points it is sufficient to use one injection point and one tracer (for example: Legal proof whether a percolating pollutant from property A impairs property B, bank filtration determination) but when we want to establish *From where?* one certain water body gets its water, several sites must be simultaneously injected with different tracers (for example: Planning water protection zones, establish the catchment area of a spring); Tracing tests using two or more fluorescent dyes in pore-groundwater aquifer or other area needs to be carried out to compare the sorption properties of these dyes, [4] etc.

Water system is labeled through fluorescent dyes as artificial tracers and then their presence is monitored from time to time in various parts of the system under the study [3-5]. Some water samples may contain more than one tracer, which can interfere at each other during their measurements.

The synchronous scan and concentration methods were used for the measurement of the different fluorescent dyes in water samples collected in the different points of the water system. The greatest advantage of the synchronous scan method is the detection of almost all dyes used in hydrology in one spectrum. With this method a better spectral resolution and a diminished Rayleigh and Raman scatter is obtained. But it is not possible to analyze quantitatively a mixture of dyes only by instrumental measurement. According

to the close vicinity of the fluorescence emission maxima spectral overlapping occurs. In practice this happens if two or more tracers are used in the same experiment. To separate the dyes from each other supplementary chemical treatments of the sample are necessary [2, 3, 6].

The present paper reports the results of spectral separation of fluorescent dyes in binary mixtures: SRG Extra & Rhodamine WT, Uranine & Eosin, Rhodamine WT & Eosin, SRG Extra & Eosin and treatments based on pH variation of the water samples. The results assist decision making on which dyes to combine in studying a single water system and on how to detect fluorescence maxima in the water samples [2, 3, 5, 7]. According to our results, SRG Extra and Rhodamine WT cannot be used together at the same time as artificial tracers because of their interference; Eosin and Rhodamine WT do not interfere at each other's fluorescence intensity so they can be used together in the same tracer experiment, but only in underground water studies, etc. Rhodamine WT and Uranine in their binary mixture can be well determined under alkaline conditions of water samples giving maxima of their fluorescence, because the fluorescence peaks of both tracers are far distant from each other in the spectrum and no spectral overlapping occurs. That's why they can be injected in the same artificial tracer experiment in aquatic environments studies [6].

## MATERIALS AND METHODS

Fluorescent intensities of the dyes were detected using a Perkin-Elmer Luminescence Spectrometer LS 55. A special software package (FL WinLab) manages different application programs that the instrument LS 55 offers.

The instrument was previously calibrated with standard solutions by means of the calibration application, for Rhodamine WT, Uranine, Eosin and SRG Extra.

Measurements were carried out at room temperature (~25°C) and with use of 1 cm quartz cells. Standard solutions were produced for each compound prepared for calibration of the

instrument. pH measurements were carried out using a WTW pH 330 pH-meter being initially calibrated by two standard buffer solutions with pH values  $4.01 \pm 0.02$  and  $7.00 \pm 0.02$ .

Function and efficiency of the LS 55 Spectrometer was tested with a special software package (FL WinLab) that offers a range of application programs. Instrument validation was tested by means of Raman spectra (Raman Peak Wavelength, Raman Peak Intensity and Raman S/N ratio) in a sealed water cell [8]. Instrument stability was checked with an Anthracene sample as reference material for fluorescence intensity [9]. All tracer determinations were realized in standard solutions (solvent: water) using the Synchronous Scan and Concentration Applications. Appropriate sets of parameters (so called methods of measurements) were set up in order to investigate dyes content in standard solutions through synchronous and concentration applications.

The methods elaborated to measure dyes content in water samples are made up of the following parameters: Rhodamine WT- Excitation wavelength ( $\lambda_{\text{Exc}}$ ) = 554 nm, Emission wavelength ( $\lambda_{\text{Em}}$ ) = 580 nm; Uranine-  $\lambda_{\text{Exc}}$  = 491 nm,  $\lambda_{\text{Em}}$  = 512 nm; Eosin-  $\lambda_{\text{Exc}}$  = 516 nm,  $\lambda_{\text{Em}}$  = 538 ; SRG Extra-  $\lambda_{\text{Exc}}$  = 531 nm,  $\lambda_{\text{Em}}$  = 552 nm; The other parameters are the same for all the tracers: Ex. slit = 10.0 nm; Em. slit = 10.0 nm;  $\Delta\lambda$  = 21 nm; etc.

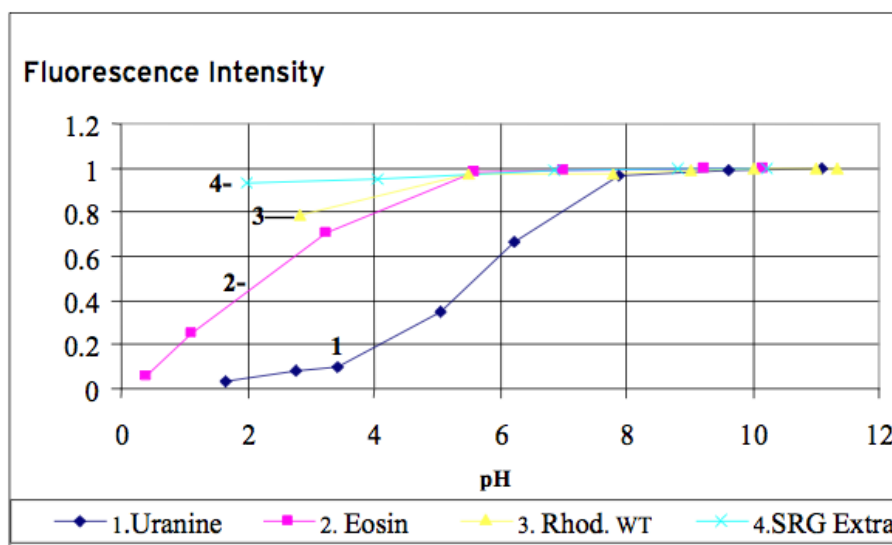
Some “blanks” were previously analyzed in order to assess the natural presence of dyes fluorescence, the so called “background”. Chemical treatment procedure has been applied for both samples and standards.

## RESULTS AND DISCUSSION

In the cases when two fluorescent tracers are injected as artificial tracers in different points of the same water system study, water samples collected after their injection can contain one or both tracers. In these cases both tracers would interfere with each other by overlapping, increasing or decreasing their fluorescence intensity. As a consequence one would obtain erroneous results. We tried to supervise the influences of some tracers at each other and to determine if there is any possibility to detect the maximum of each tracer fluorescence when they both are in the same water sample.

In this paper only separation of fluorescent dyes in such binary mixtures and treatments based on pH-variations of the water sample is described.

Figure 1 shows clearly that the maximum of the fluorescence intensity of Eosin, Rhodamine WT and SRG Extra standard solutions can be detected on  $\text{pH} \geq 5.3$  value [5, 6]. Water samples collected during an artificial tracer experiment in aquatic



**Figure 1.** Influence of pH on some dyes fluorescence intensity.

environments studies (surface and underground water) have in general  $\text{pH} \geq 5.3$ . That's why we can detect and measure the maxima of Eosin, Rhodamine WT and SRG Extra directly in water samples. Except SRG Extra, the fluorescence intensity of Uranine, Eosin and Rhodamine WT decrease in acid medium. We have used these facts in our experiment.

Maximum of Uranine fluorescence intensity in water sample can be detected on  $\text{pH} \approx 8.3$  value, so, only for Uranine we are obligated to add EDTA-Na in every water sample to increase the pH.

Standard solutions in distilled water of Rhodamine WT, SRG Extra, Uranine and Eosin with concentration 1ppb were prepared. Binary mixtures in equal quantity (1:1) of their standard solutions were prepared to supervise practically the behaviour and influence of these dyes at each other when they are both in the same water sample.

### Binary Mixtures of SRG Extra and Rhodamine WT in Water Samples

Fluorescence intensity of SRG Extra and Rhodamine WT Liquid standard solutions and their mixture in equal quantity were measured applying both their respective concentration methods, to compare the results between them. The obtained data were compared with the results by applying synchronous scan method, too. Measurements of the SRG Extra and Rhodamine WT standard solutions were done without preliminary treatment for changing the pH value, because Rhodamine WT gives the maximum of fluorescence at  $\text{pH} > 5.5$  and SRG Extra at  $\text{pH} \sim 7$  (see Figure 1). But Rhodamine WT and SRG Extra

fluorescence intensities in their mixtures were measured before changing and after changing pH values (until  $\text{pH} \leq 2$  and  $\text{pH} \geq 8$ ), too. Obtained results using both the concentration method of SRG Extra and Rhodamine WT are presented in Table 1.

The above results show clearly that the application of the concentration method is not a good way to measure the fluorescence of these mixed dyes in the water samples. We did the same measurements for the other binary mixture under the study and the conclusion was the same. It is necessary to apply the synchronous scan method to detect and measure their fluorescent intensities.

The synchronous scan method was applied to detect SRG Extra and Rhodamine WT in above standard solutions and their mixture, too. Their spectra are presented in Figure 1.

Spectrum 1 (clear blue colour): SRG Extra standard solutions 1 ppb

Spectrum 2 (pink colour): Rhodamine WT standard solutions (1 ppb)

Spectrum 3 (green colour): SRG + Rhodamine WT mixture 1:1

Spectrum 4 (red colour): SRG + Rhodamine WT mixture at  $\text{pH} > 8$

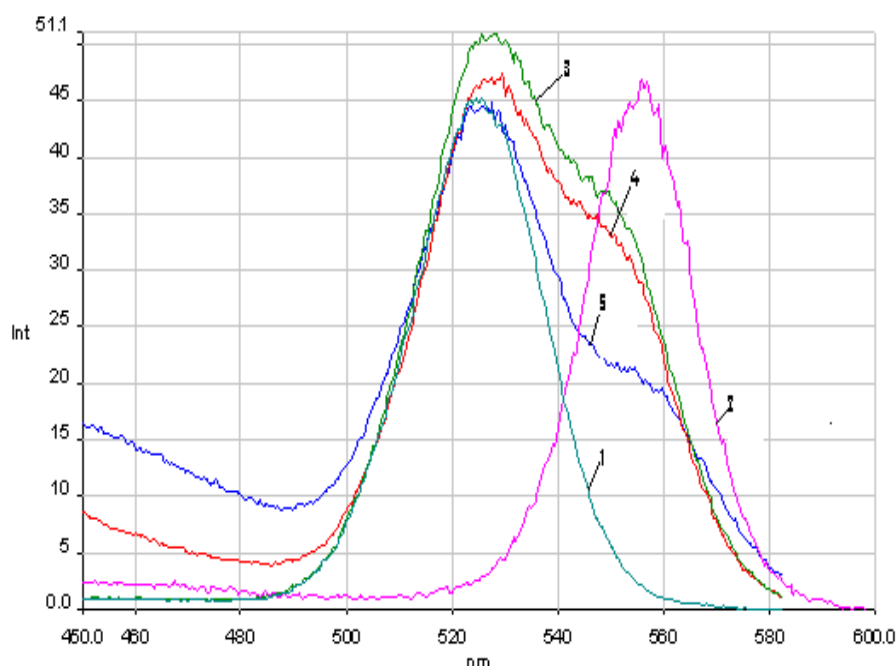
Spectrum 5 (blue colour): SRG + Rhodamine WT the mixture at  $\text{pH} < 2$

Spectrum 1 in Figure 2 shows fluorescence emission of SRG Extra standard solutions 1 ppb which has the peak at  $\lambda_f = 525 \text{ nm}$  with fluorescence intensity  $I_f = 44.86$ .

Spectrum 2 presents fluorescence emission of

**Table 1.** Results obtained by using concentration methods of SRG Extra and Rhodamine WT.

Sample	Fluorescence Intensity (by method of SRG Extra)	Conc. (ppb)	Fluorescence Intensity (by method of Rhodamin WT)	Conc. (ppb)
Stand. sol. 1ppb SRG Extra	77.194	0.871	4.511	0.103
Stand. sol. 1ppb Rhodamine WT	5.795	0.066	47.454	1.09
Mixture: SRG+Rhod.WT (1:1)	82.644	0.933	53.159	1.224
Mixture: SRG+Rhod.WT (1:1) with EDTA-Na	74.893	0.845	50.188	1.156
Mixture: SRG+Rhod.WT (1:1) with HCl	60.202	0.347	24.941	0.574



**Figure 2.** Interference of SRG Extra and Rhodamine WT fluorescence at each other.

the Rhodamine WT standard solutions (1ppb) with the peak at  $\lambda_2 = 551.58$  nm and  $I_F = 45.43$ .

SRG - Rhodamine WT mixture was synchronously scanned without prior pH adjustment of the samples and gave spectrum 3- green colour in Figure 2. This spectrum shows clearly the interference of the tracers at each other because the spectra has only one peak at  $\lambda_1 = 528$  nm with  $I_F = 51$  and a small shoulder at wave length interval  $\lambda_2 = 545-556$  nm and  $I_F = 38.0$  (instead of Rhodamine WT peak). One can see clearly, that the fluorescence peak of SRG was increased because of the contribution of Rhodamine WT fluorescence ( $\Delta I = 51.1- 44.86$ ). The shoulder of spectrum 3 was especially the contribution of Rhodamine WT and the interference of SRG Extra fluorescence ( $\Delta I = 38.0- 31.63$ ).

We tried to avoid their interference at each other through treatments based on pH-variations. Synchronous scan of the SRG - Rhodamine WT mixture after adding EDTA-Na (pH > 8) gave spectrum 4 (red colour) in Figure 2. This spectrum has one peak at  $\lambda_1 = 525$  nm and  $I_F = 46.34$  and one shoulder at wave length interval  $\lambda_2 = 545-556$  nm with  $I_F = 32.22$ . The red spectrum (4) has the same form like green spectrum (3) of SRG - Rhodamine WT mixture. There is not any big difference between

these both spectrums.

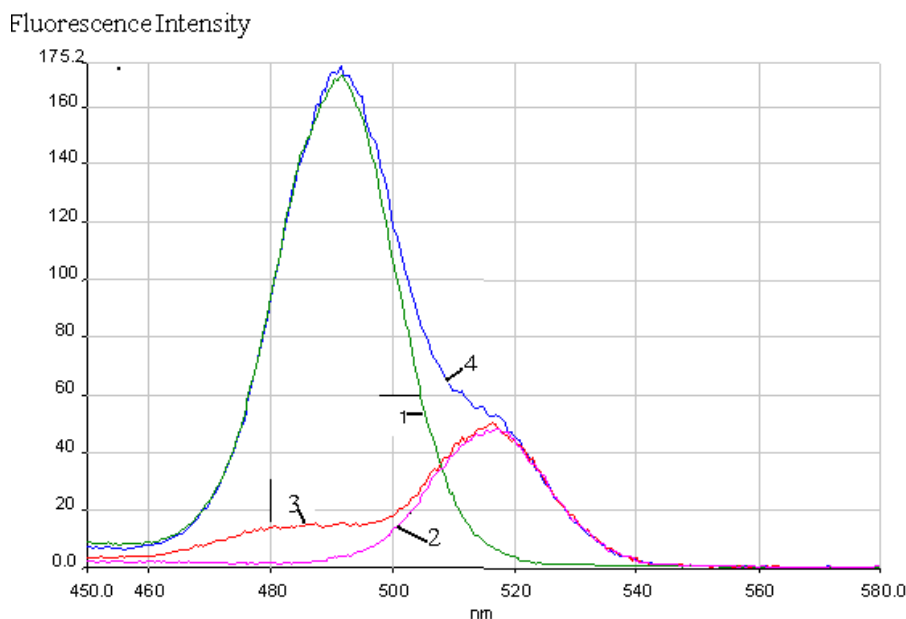
We changed the pH of the mixture at pH < 2. Synchronous scan of these mixture produced spectrum 5 (blue colour) which has the peak at  $\lambda_1 = 525$  nm with  $I_F = 44.18$  and a depressed shoulder at  $\lambda_2 = 550-565$  nm with  $I_F = 19.94$ . The peak of SRG in spectrum 5 is overlapped with the peak of SRG standard solution in spectrum 1 (Figure 2) because of decreasing influence of Rhodamine WT fluorescence at pH < 2 (see Figure 1).

That means that we can separate SRG Extra from Rhodamine WT fluorescence by adding HCl until pH < 2. In this condition we can detect and measure the maximum of SRG fluorescence, but we are not able to separate Rhodamine WT from SRG Extra fluorescence by changing pH value of the samples.

According to our results, it is not suitable to use SRG Extra and Rhodamine WT together, at the same time as artificial tracers in aquatic environments studies, because of their hard interference at each other.

### Binary mixtures of Uranine and Eosin in Water Samples

Fluorescence intensities of Uranine and Eosin



**Fig. 3.** Synchronous scan of a mixture (1:1) of Uranine and Eosin and their standard solutions.

standard solutions and their mixture (1:1) were measured using synchronous scanning method and their spectra are presented in Figure 3. Uranine gives its fluorescence maximum in a basic medium at  $\text{pH} > 8.3$  (see Figure 1), therefore the measurement are made after the treatment of the samples with EDTA- $\text{Na}$   $0.5 \text{ mol/dm}^{-3}$  [4].

Spectrum 1 (green colour): Uranine standard solutions 1 ppb

Spectrum 2 (pink colour): Eosin standard solutions (1 ppb)

Spectrum 3 (red colour): Uranine + Eosin mixture 1:1

Spectrum 4 (blue colour): Uranine + Eosin mixture at  $\text{pH} > 8$ )

Synchronous scan of 1ppb Uranine standard solution gave the green colour spectrum 1, with maximum of the peak at  $\lambda = 491.44 \text{ nm}$  and  $I_F = 163$ . Synchronous scan of 1 ppb Eosin standard solution gave the pink spectrum 2 with peak at  $\lambda = 515.12 \text{ nm}$  and  $I_F = 45$ .

Uranine-Eosin mixture was synchronous scanned without prior treatment with EDTA (spectrum red 3). Fluorescence of Uranine in this spectrum follows the form of a shoulder in the waves length range of 480-497 nm with  $I_F = 15.67$ , because Uranine gives its maximum

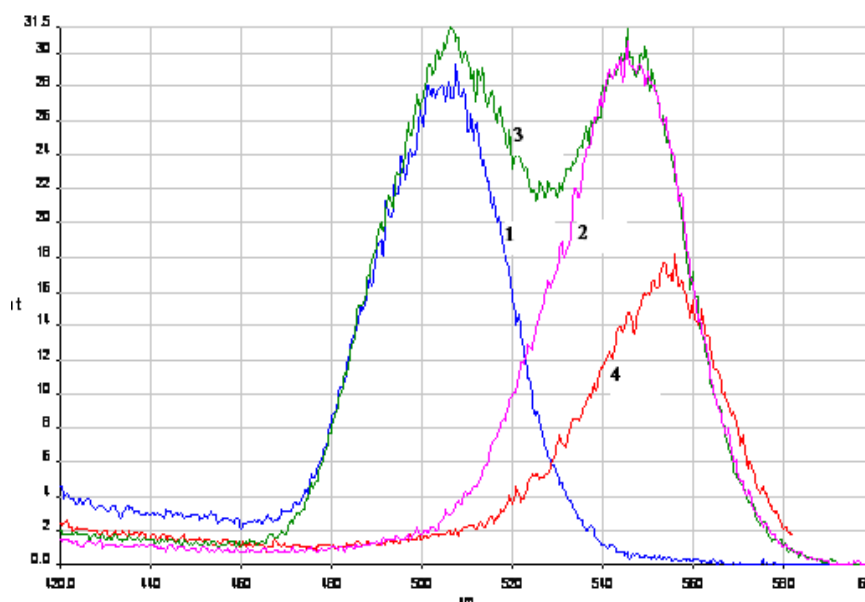
of fluorescence at  $\text{pH} > 8.3$ . Peak at  $\lambda = 515.4 \text{ nm}$  with  $I_F = 48$  is Eosin fluorescence with a little input from Uranine fluorescence. Then, their mixture was treated with EDTA and its synchronous scan gave spectrum 4. First peak at  $\lambda = 491.44$  and  $I_F = 173.64$  presents the full contribution of Uranine fluorescence and a little contribution coming from Eosin. Eosin contribution to this spectra is manifested in the form of a shoulder in the wave length 516.47 nm with  $I_F = 52.37$ . This value is slightly higher than fluorescence intensity value of Eosin obtained from its standard solution due to the interference of Uranine fluorescence. Spectrum of their mixture don't have two separate peaks because of the wave lengths of these fluorescent compounds are very close and their fluorescence interfere to each other.

### Binary Mixtures Rhodamine WT and Eosin in Water Samples

Synchronous scan method was applied to supervise practically the behaviour of dyes in binary mixtures Rhodamine WT- Eosin and Rhodamine WT- Uranine ect.

The spectra of Eosin and Rhodamine WT standard solutions and their mixtures 1:1 are presented in Figure 4.

Spectrum 1 (blue colour): Eosin standard



**Fig. 4.** Spectra of standard solutions of Rhodamine WT and Eosin (conc. = 1 ppb) and their mixture 1:1.

solutions (1 ppb)

Spectrum 2 (pink colour): Rhodamine WT standard solutions 1 ppb

Spectrum 3 (green colour): Rhodamine WT + Eosin mixture 1:1

Spectrum 4 (red colour): Rhodamine WT + Eosin mixture at  $\text{pH} < 2$

Spectrum 1 in Figure 4 presents fluorescence emission of the Eosin standard solutions with a peak at  $\lambda = 506.54 \text{ nm}$  and  $I_f = 28.95 \text{ nm}$ , while Spectrum 2 shows fluorescence emission spectrum of Rhodamine WT standard solutions with a peak at  $\lambda = 545.37 \text{ nm}$  and  $I_f = 30.27$ . Synchronous scan of the Eosin and Rhodamine WT mixture 1:1 is presented in spectrum 3, which has two peaks. One can see clearly that the fluorescence peaks of both tracers are distant from each other with no spectral overlap occurring.

Spectrum 4 of the mixture at  $\text{pH} < 2$  has only one peak, at  $\lambda = 553.95 \text{ nm}$  and  $I_f = 17.59$ , which is provided by decreased Rhodamine WT fluorescence (see Figure 1). Eosin fluorescence was completely quenched in this spectrum.

These results confirm clearly that both tracers Eosin and Rhodamine WT can be well determined without changing the sample pH value.

Eosin and Rhodamine WT can be used

together in the same tracer experiment but only in underground water system studies, because Eosin can be decayed very quickly from UV radiation [4].

### Binary Mixtures SRG Extra and Eosin in Water Samples

According to our results SRG Extra and Eosin cannot be used together at the same time as artificial tracers in aquatic environments studies because of their interference with each other.

Figure 5 depicts the resulting fluorescence emission spectra of SRG Extra and Eosin standard solution 1ppb and their mixture (1:1) by applying the synchronous scan method.

Spectrum 1 (red colour): Eosin standard solution 1 ppb

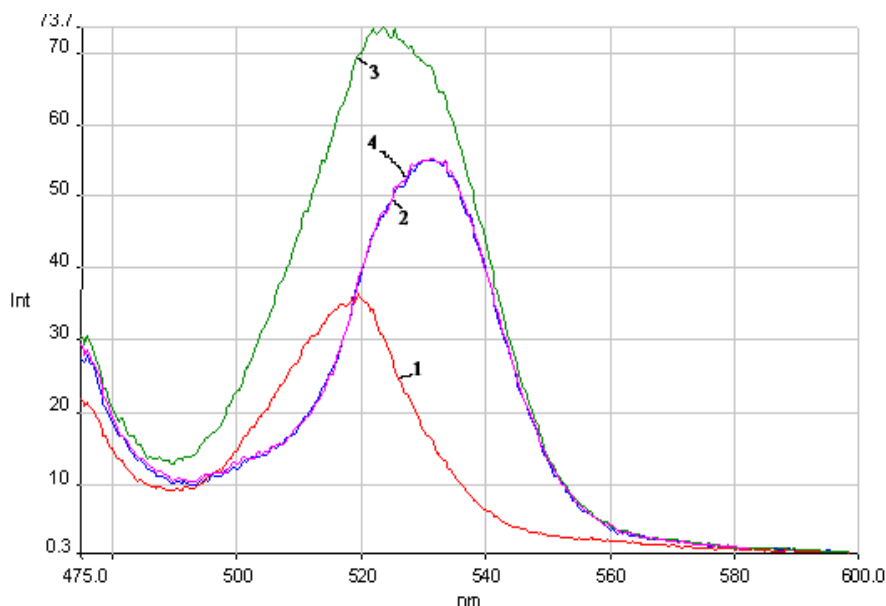
Spectrum 2 (blue colour): SRG Extra standard solution 1 ppb

Spectrum 3 (green colour): SRG-Eosin mixture without prior pH adjustment

Spectrum 4 (pink colour): SRG- Eosin mixture at  $\text{pH} < 2$

Spectrum 1 and 2 are respectively the synchronous scan of Eosin and SRG Extra standard solutions. SRG- Eosin mixture without prior pH adjustment of the samples gave the spectrum 3- green. This spectrum has only one





**Figure 5.** Spectra of SRG Extra and Eosin standard solutions 1 ppb and their 1:1 mixture.

shifted peak, at  $\lambda_f = 525.16$  nm and  $I_f = 70.18$  and a small shoulder at wave length interval  $\lambda = 528-536$  nm. This peak was a contribution of both Eosin and SRG Extra fluorescence that interfere in each other's intensity ( $\Delta I = 70.18-54.44$ ).

Synchronous scan of this mixture at  $\text{pH} < 2$  produced spectrum 4 (pink), at  $\lambda_f = 531.43$  nm with  $I_f = 54.81$ , completely overlapped by SRG Extra standard solution spectrum 2, because the fluorescence of Eosin at  $\text{pH} < 2$  was completely disappeared and SRG Extra was well detected. Total separation of Eosin fluorescence cannot be achieved through manipulation of pH value of the water sample containing both SRG Extra and Eosin.

As a result, Eosin and SRG Extra cannot be used together because of their interference at each other.

## CONCLUSIONS

Measurement of dye's fluorescence as Rhodamine WT Liquid, Uranine, Eosin and SRG Extra in water samples, when they are used together in the same tracer experiment, should be undertaken only by synchronous scanning method (not by the concentration method); or else the results may be incorrect.

SRG Extra and Rhodamine WT interfere at fluorescence measurements when they are present in the same water sample. So, according to our results, it is not suitable to use SRG Extra and Rhodamine WT at the same artificial tracer experiment in aquatic environments studies.

Uranine and Eosin interfere at fluorescence intensity of each other, when they are present in the same water sample. That's why it would be better to avoid the simultaneous use of Uranine and Eosin in the same study.

Using of SRG Extra and Eosin together in the same tracer experiment does not appear suitable because their interference at each other, too.

Rhodamine WT and Uranine are suitable to be used together in the same tracer experiment because they don't interfere at fluorescence intensity of each other in water samples.

Rhodamine WT and Eosin do not interfere with each other's fluorescence intensity when they are present in the same water sample; they can be used together only in underground water system studies because of the high influence of UV radiation at fluorescence intensity of Eosin.

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