



ORIGINAL RESEARCH ARTICLE

MINIMUM LIMIT DETECTION OF RHODAMINE WT AND SRG EXTRA BY SYNCHRONOUS SCAN METHOD

Liljana KOLA¹ and Enver HOXHAJ²

1 Institute of Applied Nuclear Physics, UT, Tirana, Albania. E-mail: kolaliljana@yahoo.it

2 Department of Physics, University of Shkodra, Albania

SYNOPSIS

Key words:

Artificial tracer,
Fluorescence intensity (I_F),
Spectral determination,
Synchronous scan.

Some fluorescent dyes, after been injected as artificial tracer in one water system, have to be measured in all collected water samples in different points of this system. The aim of this study is to detect the minimum limit of these dyes in water samples by spectral measurements. Only spectral determinations of Rhodamine WT and Sulforhodamine G (SRG) Extra in their standard solutions (solvent: distilled water) are described in this paper. Synchronous scan methods were used for the detections and measurement of dyes fluorescence by the means of a Perkin Elmer Luminescence Spectrometer LS 55. According to obtained results, the minimum limit of concentration that can be detected in water samples is respectively: Rhodamine WT 0.0020 ppb and Sulforhodamine G Extra 0.001- 0.002 ppb.

INTRODUCTION

The introduction of fluorescent tracers in hydrology to know and study aquatic systems has led to enormous methodological and instrumental developments (Behrens, 1986). 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.* (Benischke, 1991; Käss, 1994).

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, but when we want to establish **From where?** one certain water body gets its water, several sites must be simultaneously injected with different tracers; 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 (Winter, 2006) etc.

So, 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 (Käss, 1994; Winter, 2006; Talamba et al., 2000).

The synchronous scan and concentration methods were used for the measurement of the different fluorescent dyes in the standard solutions and 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.

In general one uses the concentration methods to determine the minimum limit of elements or compounds. The aim of this study is to detect the minimum limit of some dyes in water samples by spectral measurements. Only spectral determinations of Rhodamine WT and Sulforhodamine G (SRG) Extra in their standard solutions (solvent: distilled water) are shortly described in this paper.

MATERIAL 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 and SRG Extra, too.

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 ± 0.02 and 7.00 ± 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 (Perkin Elmer, 2000). Instrument stability was checked with an Anthracene sample as reference material for fluorescence intensity (Perkin Elmer-boklet, 2000). 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 this purpose enable one to optimize measuring procedures in order to detect and measure tracer contents of trace levels in water samples (Kola & Amataj, 2010; Kola, 2011). These methods are made up of the following parameters: Rhodamine WT- Excitation wavelength (λ_{Ex}) = 554 nm, Emission wavelength (λ_{Em}) = 580 nm; SRG Extra- λ_{Exc} = 531 nm, λ_{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

DETECTION LIMIT OF RHODAMINE WT LIQUID THROUGH THE SPECTRAL DETERMINATION

The detection limit is usually measured by means of the method of concentration. In this paper will be presented the determination of Rhodamine WT detection limit through the spectral determination using synchronous scanning method by the luminiscences spectrometer LS 55. Determination of the detection limit of Rhodamines WT in water samples using synchronous scan method is more accurate than using the concentration method. The peaks of the obtained spectra by synchronous scanning of RWT standards solutions are only contribution of RWT fluorescence intensity (λ_{Ex} = 554 nm). So we can avoid interference of other material found in the water samples (Kola & Lazo, 2010).

To determine the detection limit of Rhodamine WT were prepared Rhodamine WT standard solutions with concentrations: 0.0005, 0.001, 0.002, 0.004, 0.010, 0.020, 0.050 ppb. (solvent: distil water). Measurements of the fluorescence intensity - I_F of the Rhodamine WT standard solutions were made directly in standard solutions, in normal laboratory conditions, without any prior treatment in pH value or temperature. During the optimization of the measurements method it was noted that the pH and temperature does not significantly affect the fluorescence intensity of Rhodamine WT in water samples (Kola, 2011).

First, it was scanned the distilled water (Background) and then all prepared standards beginning from the lower concentrations till the higher ones, to avoid the contamination of the quartz cells, laboratory tools, hands etc.

The obtained results are presented in Table 1 and graphically in Figure 1.

The synchronous scan of the distilled water (BG) gave dark blue spectrum (1) in Figure 2. The spectrum of 0.0005 ppb standard solution overlapped the distilled water spectrum, so it did not appear in the picture to become clearer other spectra.

Spectrum 2 (brown colour) corresponding to 0.001 ppb concentration has a small deviation but we are not sure it is a peak. Spectrum 3 (blue colour) which

belongs to 0.002 ppb Rhodamine WT standard solution has a small peak. The peaks are easily observed for the 0.004 ppb and higher Rhodamine WT standard solutions.

Table. 1: Detection limit of Rhodamine WT with synchronous scan method by LS 55.

Nr.	RWT standard solution (ppb)	I_F of the spectra	I_F of the peak: (I_F of the spectra - I_F of the BG)	Is there any PEAK?
1	0.0000	0.27	-	-
2	0.0005	0.27	-	Not
3	0.001	0.51	0.24	Not
4	0.002	0.57	0.29	Small
5	0.004	0.67	0.40	Yes
6	0.010	0.91	0.64	Yes
7	0.020	1.44	1.17	Yes
8	0.050	2.47	2.20	Yes

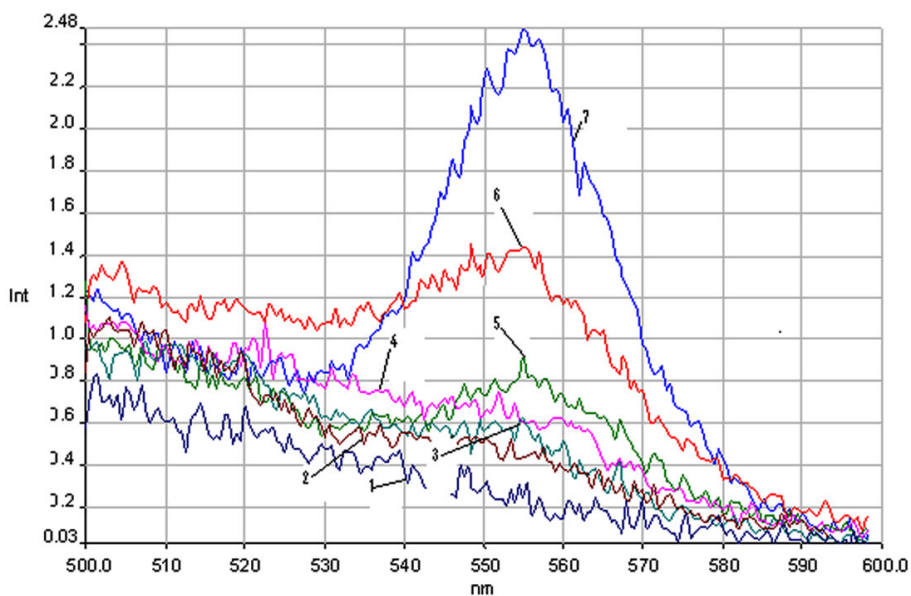


Fig. 1: Detection limit of Rhodamine WT Liq.

- Spectrum 1 (dark blue colour): Distilled water;
- Spectrum 2 (brown colour): Rhodamine WT Liq. Standard solution 0.001 ppb;
- Spectrum 3 (blue colour): Rhodamine WT Liq. Standard solution 0.002 ppb;
- Spectrum 4 (pink colour): Rhodamine WT Liq. Standard solution 0.004 ppb;
- Spectrum 5 (green colour): Rhodamine WT Liq. Standard solution 0.010 ppb;
- Spectrum 6 (red colour): Rhodamine WT Liq. Standard solution 0.020 ppb;
- Spectrum 7 (clear blue colour): Rhodamine WT Liq. Standard solution 0.050 ppb;

Scanning of the solution with higher concentrations was done to observe and compare their peak position with the peak position of the smallest concentrations. In this way we can determine not only the spectrum with significant amount of fluorescence intensity of Rhodamine WT but we can see if the peak has a correct position, too.

So we can conclude that, the detection limit of Rhodamine WT in water samples by synchronous scan, for current conditions of the measurements, can be accepted 0002 ppb concentration (comparable with literature values).

We say for 'current conditions of the measurements' because of the value can vary depending on water quality, natural background and the used instrument for measuring of the fluorescent intensities.

DETECTION LIMIT OF SRG EXTRA THROUGH THE SPECTRAL DETERMINATIONS

To determine the detection limit of Sulphorhodamine G Extra (SRG) through the spectral determination were prepared SRG Extra standard solutions with concentrations: 0.0005 ppb, 0.001 ppb, 0,002 ppb, 0,004 ppb, 0,005 ppb and 0010 ppb SRG (solvent: distil water). Measurements of the fluorescence intensity - I_F of the SRG Extra standard solutions were made using synchronous scan method by luminescence spectrometer LS 55.

First, it was scanned the distilled water (Background) and then all prepared standards beginning from the lower concentrations till the higher ones, to avoid the contamination of the quartz cells, laboratory tools, hands, etc.

The obtained results are presented in Table 2 and graphically in Figure 2.

Table 2: Detection limit of SRG Extra by synchronous scan method.

Nr.	SRG standard solution (ppb)	I_F of the spectra	I_F of the peak: (I_F of the spectra - I_F of the BG)	Is there any PEAK?
1	0.0000	0.45	-	Not
2	0.0005	0.45	0.45	Not
3	0.001	0.65	0.20	? (Small deviation)
4	0.002	0.72	0.27	Yes
5	0.004	0.90	0.45	Yes
6	0.005	1.32	0.87	Yes
7	0.010	1.96	1.51	Yes

The synchronous scan of the distilled water gave blue spectrum (1) in Figure 2. Spectrum 2 (brown colour) belongs to 0.0005 ppb SRG standard solution. It has not any peak. Spectrum 3 (pink colour) with concentration of 0.001 ppb SRG has a

small peak but not so noticeable. The peaks are easily observed for the 0.002 ppb SRG and higher concentrations.

So we can conclude that, the minimum limit of Sulphorhodamine G Extra detection in our LS 55 instrument, for current conditions of the measurements, can be accepted 0001-0002 ppb concentration.

We say for 'current conditions of the measurements' because of the values can vary depending on water quality, natural background and the used instrument for measuring of the fluorescent intensities.

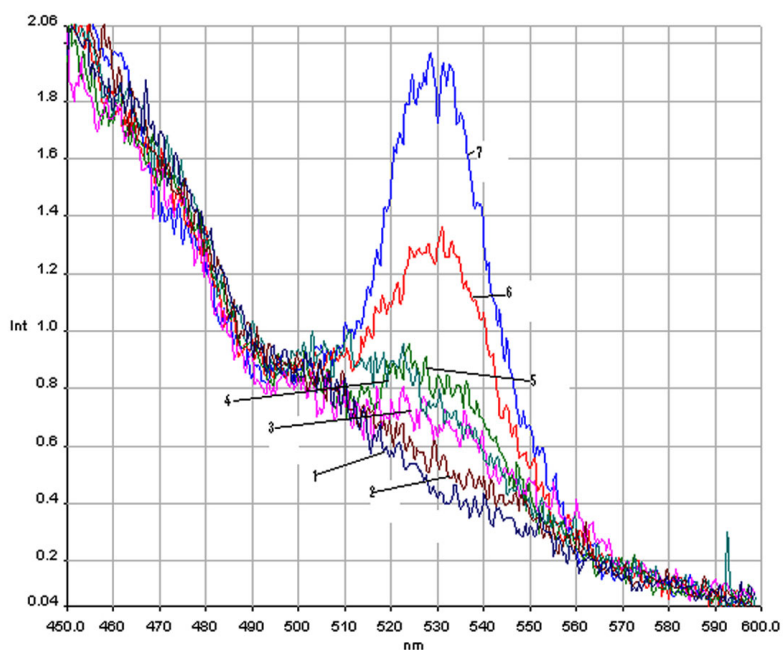


Fig. 2: Detection limit of SRG Extra in water samples.

- Spectrum 1 (dark blue colour): Distilled water
- Spectrum 2 (brown colour): SRG Extra Standard solution 0.0005 ppb;
- Spectrum 3 (pink colour): SRG Extra Standard solution 0.001 ppb;
- Spectrum 4 (blue colour): SRG Extra Standard solution 0.002 ppb;
- Spectrum 5 (green colour): SRG Extra Standard solution 0.004 ppb;
- Spectrum 6 (red colour): SRG Extra Standard solution 0.005 ppb;
- Spectrum 7 (clear blue colour): SRG Extra Standard solution 0.01 ppb.

CONCLUSIONS

Determination of the artificial tracers detection limit in water samples using synchronous scanning method by LS 55 is more accurate than by using the concentration method, because of the possibility to avoid the other interference.

The peak of the obtained spectra by synchronous scanning of RWT standards solutions are only contribution of RWT fluorescence intensity (at $\lambda_{Ex}= 554$ nm). So

we can avoid interference of other material found in the water samples which can emit at different wavelengths.

According to our results, detection limit minimum of Rhodamine WT in water samples by applying synchronous scan method is 0.002 ppb.

Likewise we can say that the peak intensity at $\lambda_{\text{Ex}} = 531$ nm of the obtained spectra by synchronous scanning of SRG Extra standards solutions is contribution of SRG Extra only.

Detection limit minimum of SRG Extra in water samples by applying synchronous scan method is 0001-0002 ppb.

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