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Professional paper

THE INFLUENCE OF SOME CHEMICAL AND PHYSICAL PARAMETERS OF WATER SAMPLES ON SPECTRAL DETERMINATIONS OF FLUORESCENT DYES

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The fluorescence intensity of various substances is dependent on the physical and chemical properties (e.g. pH, temperature, sorption, presence of oxidants, etc.) of the respective sample matrix (e.g. water),

We have studied the influence of some of these factors on the fluorescence intensity of Uranine, Rhodamine WT, and Sulphorhodamine, in order to enable their detection in trace levels by putting respective aquatic samples in optimal conditions. The results indicate that pH modifies the fluorescence intensity of Uranine solutions much more than for the fluorescence of Rhodamine WT and Sulphorhodamine water solutions. This process is reversible. Temperature also has influence on the fluorescence intensity, whereas the presence of oxidants in water samples irreversibly destroy the fluorescing molecules. All of these processes manifest diverse effects on different kinds of fluorescent dyes.

Key words: spectrofluorometer; fluorescence; artificial tracer; spectrum; synchronscan; Uranine; Rhodamine WT; Sulphorhodamine G Extra

ВЛИЈАНИЕ НА НЕКОИ ХЕМИСКИ И ФИЗИЧКИ ПАРАМЕТРИ ОД ВОДНИ ПРИМЕРОЦИ ВРЗ СПЕКТРАЛНИТЕ ОДРЕДУВАЊА НА ФЛУОРЕСЦЕНТНИ БОИ

Флуоресцентниот интензитет на различни супстанции зависи од физичко-хемиските својства (на пример: pH, температура, сорпција, присуство на оксиданти и сл.) на матрицата на испитуваниот примерок (во нашиот случај вода).

Во нашите истражувања го проучувавме влијанието на некои од гореспомнатите фактори врз флуоресцентниот интензитет на уранин (Uranine), родамин (Rhodamine WT) и сулфородамин (Sulphorhodamine G Extra), со цел тие да се детектираат на ниво на траги со доведување на соодветните водни примероци во оптимални услови. Се покажа дека вредноста на pH го модифицира флуоресцентниот интензитет на водниот раствор на уранинот многу повеќе во споредба со истиот параметар кај водните раствори на родаминот и сулфородаминот. Овој процес е реверзибилен. Ист карактер има и влијанието на температурата врз флуоресцентните супстанции, додека присуството на оксиданти во водните примероци иреверзибилно ги разградува флуоресцентните молекули. Сите овие процеси демонстрираат различни ефекти на различни флуоресцентни бои.

Клучни зборови: спектрофлуорометар; флуоресценција; вештачки трасери; спектри; синхроскен; уранин; родамин WT; сулфородамин G Extra

INTRODUCTION

The application of the spectrofluorometer techniques in the Institute of Nuclear Physics in Tirana focuses (for the time being) on the study of aquatic media by using fluorescent substances as artificial tracing agents placed within different water systems intentionally and in conformity with objectives of the study. Following their injections, the content of these substances is monitored con-

stantly or according to a given agenda in different parts of the system [1]. The questions these studies are trying to answer are several: where the groundwater is flowing to; where the groundwater is coming from; is there a hydraulic connection between two or more given points of the system; how is the flow of the groundwater within the system under the study, etc.[2].

The fluorescent tracers should be selected properly due to the fact they possess different chemical, physical and optical properties, which can be further modified according to the conditions of the sample matrix they are placed into (e.g., pH, temperature, oxidants presence, etc).

This paper presents results concerning the influence of pH, temperature and oxidants content of the water samples on the fluorescence intensity of commonly used fluorescent tracers, such as Uranine, Rhodamine WT and Sulphorhodamine G Extra. With this data in hand, one can place the tracers in water samples in optimal conditions in order to detect and measure them in very low concentrations.

EXPERIMENTAL

The spectrofluorometer measurements of water samples are based on the fluorescence of the appropriate substances injected into these samples.

For this study we have chosen water samples from the karstic system Mali me Gropa, injected Uranine and Rhodamine WT, as well as water samples from the lacustric system Ohrid-Prespa, injected with Sulphorhodamine G Extra [3]. Water samples from the same areas without the artificial tracers served as "background". The pH, temperature, and oxidants content of water samples were then changed and the fluorescence intensity was measured.

Apparatus

The measurements were carried out in a Perkin-Elmer spectral fluorometer LS 55, at room temperature (~25 ^{o}C) by using 1 cm quartz cells. Standard solutions for every compound were created for calibration of the instrument.

The pH measurements were carried out with a WTW pH 330 pH-meter calibrated with two standard buffer solutions at pH 4.01 ± 0.02 and 7.00 ± 0.02.

Temperatures were measured through the WTW LF 330 conductivity meter, which was initially calibrated with a certificated glass thermometer (± 0.5 ^{o}C).

Methods

The LS 55 instrument was operated through a special software package (FL WinLab) that offers different application programs. We have realized all needed tracers determination in water samples by using the Synchronous Scan and Concentration Applications. The instrument was validated by the means of Raman spectra (Raman Peak Wavelength, Raman Peak Intensity and Raman S/N ratio) in a sealed water cell [4], and the instrument stability was checked by using an Anthracene sample as a reference material for the fluorescence intensity [5]. Parameters (so called methods of measurements) were then set in order to investigate the Uranine content in water samples through synchronous and concentration applications. These parameters were as follows:

Excitation wavelength (nm):	491
Emission wavelength (nm):	512
Excitation slit (nm):	10.0
Emission slit (nm):	10.0
Delta lambda (nm):	21
Scan speed (nm/min):	1200
Integration time (s):	10.00
Emission filter:	open
Solvent:	water
Auto Background Subtract	

The calibration application was used for Uranine instrument calibration.

The measurement method for Rhodamine WT in water samples had the following parameters:

Excitation wavelength (nm):	554
Emission wavelength (nm):	580
Excitation slit (nm):	10.0
Emission slit (nm):	10.0
Delta lambda (nm):	26
Scan speed (nm/min):	1200
Integration time (s):	10.00
Emission filter:	open
Solvent:	water
Auto Background Subtract	

The calibration application was also used to calibrate the instrument for Rhodamine WT measurements.

Another method was created to measure Sulphorhodamine G Extra concentration in water samples having the following set of parameters:

Excitation wavelength (nm):	531
Emission wavelength (nm):	552
Excitation slit (nm):	10.0
Emission slit (nm):	10.0
Delta lambda (nm):	19
Scan speed (nm/min):	1200
Integration time (s):	10.00
Emission filter:	open
Solvent:	water
Auto Background Subtract	

The calibration application was used to calibrate the instrument for Sulphorhodamine G Extra measurements [4].

Synchronous scan measurements of the background samples and water samples were carried out first to see the spectra and to check respective peak wavelengths; then concentration measurements of all water samples were performed.

RESULTS AND DISCUSSION

Uranine is known for its strongest fluorescence. During its crystallization Uranine forms non-fluorescing elongated crystals. Concentrated solutions of Uranine keep a reddish color and do not fluoresce, too. Further dilution brings about a sensible dissociation of Uranine molecules into Sodium cations and very fluorescent anions.

Besides the degree of the dilution (which means, the degree of dissociation) which influences considerably the fluorescence intensity, this intensity is also influenced by the presence of hydrogen ions in the solution. This explains the strong dependence of the Uranine fluorescence on the pH of the solvent [6].

The fluorescence intensity is abruptly decreased in solutions with lower pH values. Increasing the sample acidity increases the presence of bluish-to-greenish Uranine cations which fluoresce very poorly.

The conversion of the Uranine anion into cation is a reversible process – increasing the pH value returns the fluorescence to its previous intensity. This property of the Uranine is largely used in differentiating it in the background of other dye tracers that fluoresce similarly to Uranine [6, 7].

Rhodamin WT (WT – water tracing) is a fluorescent substance commonly used in tracing groundwater and surface waters [6]. The intensity of its fluorescence does not depend on pH values of the flowing medium.

Sulphorhodamine G Extra can be used in the same time and system with Uranine as its spectral properties favor a very good differentiation of these tracers from each other. Its sensibility against pH variations is very low [7].

The influence of pH values on intensity fluorescence

The fluorescence intensity of fluorescent substances in water solutions is influenced in various ways by the pH of the solution. We have studied this phenomenon for Uranine, Rhodamine WT and Sulphorhodamine G Extra.

First, the pH of the samples and the fluorescence intensity of the tracers under normal conditions were measured. Then the pH value was changed by adding 0.05 N HCl solution through a micropipette, in order to create an acidic medium, and 0.05 N EDTA-Na solution, in order to create a basic medium.

After each acid or basic solutions addition, the respective fluorescence of the sample was measured. The outcome of these experiments are shown in Figs. 1, 2 and 3.

The values presented in Fig. 1 clearly show that the Uranine fluorescence intensity is considerably reduced when the pH value of the sample is decreased below 7.87, reaching 3.5 % of its initial value for pH = 1.66. The highest and the most stable values of the fluorescence are exhibited at pH values exceeding 7.87, but they decline very abruptly for pH values lower than 6.23. Hence, in order to obtain correct fluorescence values, it is imperative to make the measurments at basic pH values of the sample matrix. In practice, each time one is going to measure the fluorescence intensity of Uranine in water samples, one has to add to the solution some drops of EDTA- Na 0.05 N in order to change it into a basic medium.

Meanwhile the diagram in Fig. 2 demonstrates that the fluorescence intensity of Rhodamine WT could be reduced utmost by some few tens percent only for very low pH values (less than 3). It doesn't practically change on the range of pH values between 5.5 and 11.5, which means the analyses of water samples for Rhodamine WT content can be carried out directly (without modifying the ionic character of the respective matrix for the best part of most naturaly occurring waters).

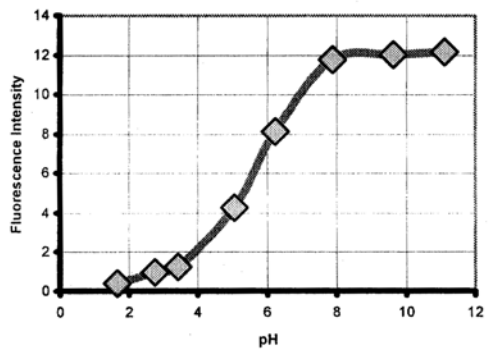

Fig. 1. Influence of pH on intensity of the Uranine fluorescence

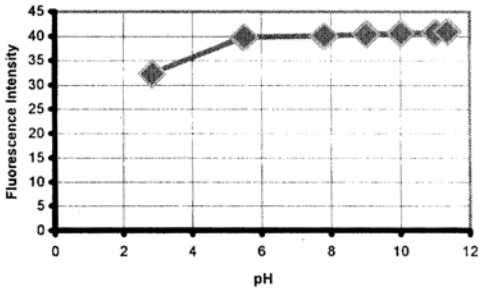

Fig. 2. Influence of pH on intensity of the Rhodamine WT fluorescence

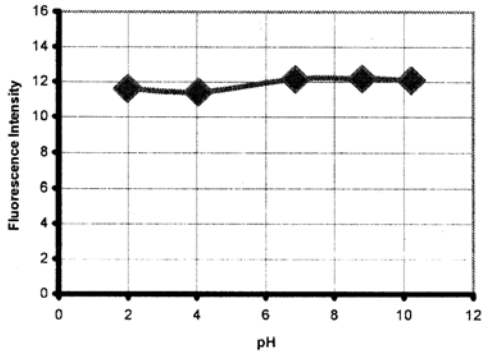

Fig. 3. Influence of pH on intensity of the Sulphorhodamine G Extra fluorescence

Figure 3 shows that the fluorescence intensity of the Sulphorhodamine G Extra remains practically unchanged when varying the pH of the sample. That means the measurements of the water samples for the fluorescence of the Sulphorhodamine G Extra can be carried out directly in the original sample (without having to modify the ionic character of the respective matrix).

The influence of the temperature on fluorescence intensity

When sample temperature is increased the excited molecules are producing more energy in rotational and vibrational movements, which leads to heat production. Therefore the fluorescence declines [6, 7]. The influence of temperature on the fluorescence of different dyes could be different. We have studied this phenomenon for Uranine, Rhodamine WT and Sulphorhodamine G Extra.

The temperature and fluorescence intensity of the tracers in water samples were first measured under normal conditions. Then the samples temperature was changed and the fluorescence intensity was measured again.

The fluorescence intensities dependence on the temperature were measured experimentally for Uranine, Rhodamine WT and Sulphorhodamine G Extra, and are as follows:

Figs. 4 and 6 show that when increasing the temperature, the fluorescence of Uranine and Rhodamine WT is decreased.

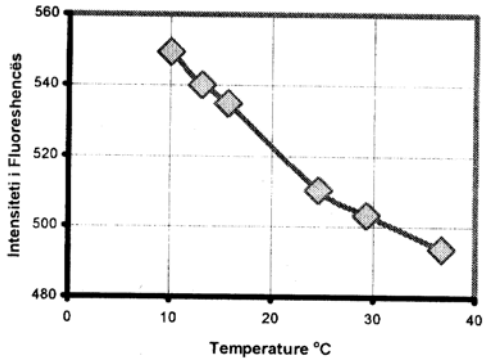

Fig. 4. Influence of temperature on intensity of the Uranine WT fluorescence

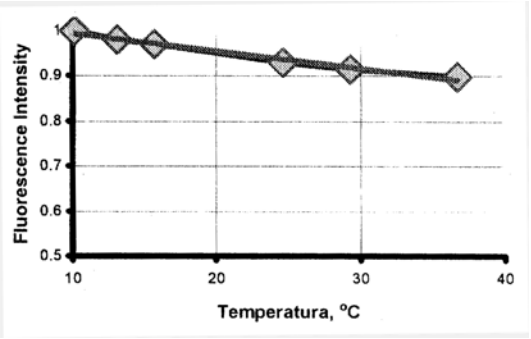

Fig. 5. Influence of temperature on intensity of the Uranine fluorescence (comparative)

The temperature-dependence of the fluorescence is more pronounced for Rhodamine WT. The Uranine fluorescence is lowered by about 20 % of its maximal value if temperature is increased by 26.3 ^{o}C, while the fluorescence intensity of Rhodamine WT is reduced by half of its fluorescence

maximum for the same temperature shift. This behaviour is more clearly presented in Figs. 5 and 7, which are calculated on the basis of concrete experimental data with reference to the respective maxima.

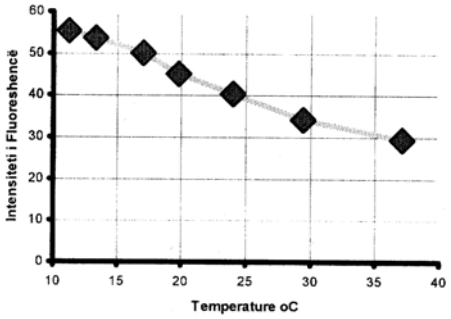

Fig. 6. Influence of temperature on intensity of the Rhodamine WT fluorescence

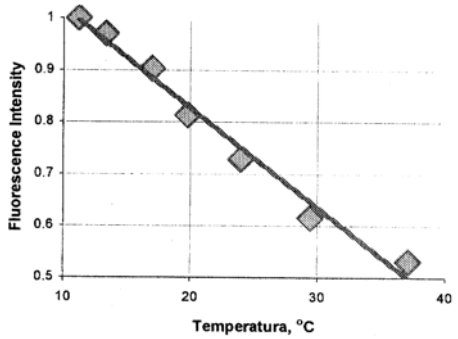

Fig. 7. Influence of temperature on intensity of the Rhodamine WT fluorescence (comparative)

Fig. 8 shows that Sulphorhodamine G Extra behaves somewhat differently in comparison with Uranine and Rhodamine WT. Its fluorescence intensity doesn't change sensibly for temperature values that are commonly encountered when measurements are performed in laboratories.

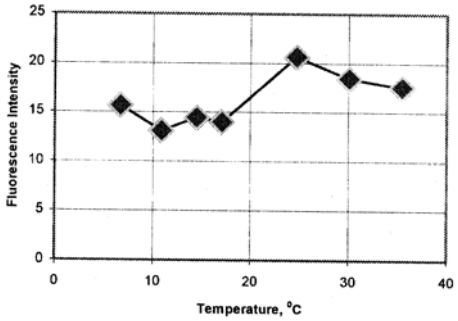

Fig. 8. Influence of temperature on intensity of the Sulphorhodamine G Extra fluorescence

The influence of oxidants on fluorescence intensity

Fluorescent substances can be irreversibly destroyed when reacting with oxidant substances, such as ozone, chloride, etc. [6, 7].

Having this in mind, we have focused our study on investigating the influence of the Hypochlorite of Sodium on the fluorescence of Uranine and Sulphorhodamine G Extra. This substance was chosen because it is used very frequently as disinfectant in water supply systems.

In order to study the dependence of Uranine fluorescence on the presence of oxidants in water samples, fluorescence intensity of Uranine in a sample having no oxidizing agents was measured (Gurrat e Zeza). Then, different portions of oxidant (sodium hypochlorite) were added and the Uranine fluorescence intensities were measured. The results clearly demonstrate the destructive effect of oxidizing substances on the fluorescence of dye tracers in water samples (Figs. 9 and 10).

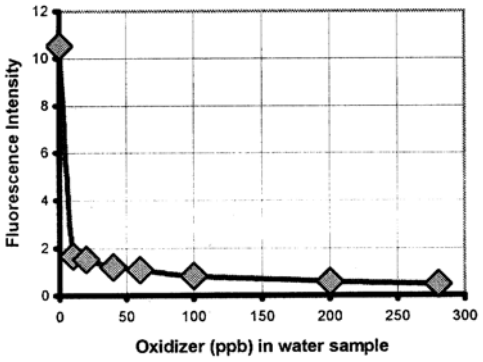

Fig. 9. Decay of the fluorescence of Uranine in aqueous solutions after adding sodium hypochlorite

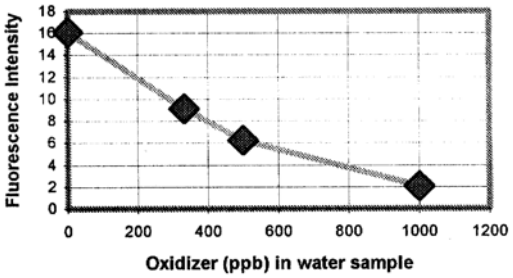

Fig. 10. Decay of the fluorescence of sulphorhodamine G after adding sodium hypochlorite

When the first portion of the oxidizing substance was added to the sample (at 10 ppb) of Uranine, its fluorescence was decreased by 84.1 %

of its initial intensity (Fig. 9). Increasing the concentration of the oxidizing agent to about 200 ppb reduced the fluorescence intensity to 4.4 % of its initial value. The same destroying effect of oxidants was observed on Sulphorhodamine G Extra as well (Fig. 10). That means that the oxidizing agents destroy vigorously and irreversibly the fluorescent capacities of Uranine and Sulphorhodamine G Extra.

Further measurements have shown the fluorescence intensity is reduced in larger proportions when longer time periods of the oxidants presence in the samples are applied. The data in Table 1 are obtained by measuring the fluorescence intensity at different times after having added sodium hypochlorite to the sample. After 15 minutes 54.77 % of the initial fluorescence remains, while after 30 minutes this number is lowered to 33.76 %. That means the time factor should be kept always in view when working with water samples that contain oxidants.

Table 1

The value of the fluorescence intensity in different moments after having added sodium hypochlorite to the sample

Sample	Conc. (ppb)	Intensity	BG	Time (in minutes)
TFSRG	0.100	6.261	0.434	0
TFSRG	0.055	3.429	0.434	15
TFSRG	0.034	2.114	0.434	30

CONCLUSIONS

The highest and most stable values of the fluorescence intensity of Uranine are at pH values higher than 7.87. The intensity is decreased significantly for pH values less than 6.23. This fact makes obligatory the alkalisation of samples and standards to pH > 8 before fluorescence intensity determinations could take place.

The Rhodamine WT and Sulphorhodamine G Extra in water samples could be measured without preliminary treatments of respective samples matrix due to the fact that the fluorescence intensity of these tracers is practically not influenced by the pH values commonly found in natural water samples.

The dependence of the fluorescence on temperature is most pronounced for Rhodamine WT. The Uranine fluorescence is lowered by about 20 % of its maximal value if temperature is increased by 26.3 ^{o}C, while the Rhodamine WT fluorescence intensity is reduced to half of its fluorescence maximum for the same temperature shift.

In this respect the Sulphorhodamine G Extra behaves somewhat differently in comparison with Uranine and Rhodamine WT. Its fluorescence intensity doesn't change sensibly for temperature values commonly encountered in laboratories.

In conclusion, analysis of water samples for fluorescent tracers should take place at temperatures below 30 ^{o}C, otherwise the samples should be preliminarily cooled.

Oxidants destroy strongly and irreversibly the fluorescent capacities of Uranine and Sulphorhodamine G Extra. Their quenching effect on fluorescence is intensified when increasing the concentration of oxidants in water samples as well as the exposure time of fluorescent tracers to such agents.

For this reason, water must not be sampled from water supply network, or one should analyze it first for probable presence of oxidants, and then decide whether to chose sampling points thereof. Otherwise the fluorescence measurements of such water samples could lead to erroneous results.

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