UNIVERSAL FLUOROMETER BASED ON FLUORESCENCE QUENCHING

Georg Gutt[1], Sonia Gutt[1], Cristina Hretcanu[1], Ana Leahu[1]

[1]Stefan cel Mare University, Faculty of Food Engineering, 13th University Str., Suceava, Romania.
E-mail: g.gutt@fia.usv.ro

Abstract. *This paper describes a method and portable device meant to determine the concentration of various chemical species in solutions based on the fluorescence quenching phenomenon. The solution brought about includes the possibility of measuring the concentration of more chemical or biological species using the same optoelectronic fluorescent, requiring the change of a fluorofor capsule only when going from a chemical species to another and setting of the chemical species type analyzed. In addition to the quantitative analysis made by means of the apparatus described, assisted by computer and specialized software, it is possible to carry out a spectral scanning fluorescence in all spectral range covered by monochromatic radiation sources of LED type of the device in question. It allows also carrying out simultaneously both qualitative and semi-quantitative analysis of all fluorescent species in the solution tested.*

Keywords:. *fluorescence quenching, quantitative analysis, qualitative and semi-quantitative analysis.*

Introduction

In order to determine the concentration of fluorescent chemical species in liquids two processes are known. The first process, at the same time the oldest (Skog and Larry 1996; Robinson *et al.* 2006; Gutt, S. *et.al.* 2011), consists in determining photo electrically the fluorescence intensity (I_f) of a species based on the difference between the intensity of incident radiation of excitation (I_0) and intensity of the radiation (I) passing through a sample of a given thickness(b) on a different path than that of the incident electromagnetic monochromatic radiation:

$$I_f = K \cdot (I_0 - I) \quad (1)$$

The dependence between the concentration (c) of fluorescent species and fluorescence intensity (I_f) is determined by the Lambert-Beer law expressed in a synthetic form:

$$I_f = K \cdot c \quad (2)$$

where:
K - is a constant depending on fluorescence efficiency, thickness of the layer crossed and nature of the fluorescent species.

When determining the concentration based on the measurement of fluorescence radiation intensity, the restrictions of linearity specific to Lambert-Beer law are valid, in this sense the equation (2) is valid only under conditions when the intensity of incident radiation (I_0) is constant (Skog and Larry 1996: 198-203).

From the constructive point of view, fluorometers are photometric equipment consisting of a monochromatic radiation source, usually a LED which light emission is tuned with the fluorescence wavelength of the species investigated, a place for solution tank, a photodiode placed on a different direction than that of irradiation (usually at 90^0), an electronic amplifier and electronic unit.

The second process, more recent, is based on the measurement of fluorescence quenching phenomenon, where the concentration of a certain chemical species (Quencher) is determined by means of another chemical one (Fluorofor), on the basis of the relationship which correlates time with intensity decrease of fluorescence,

the last one not being damaged by the Quencher's concentration.

This process is a means of determining the concentration of all chemical species that can act as Quenchers. Considering also the fact that this phenomenon is reversible, once the Quencher is being removed, fluorofor's fluorescence returns to its initial base line.

An essential application based on fluorescence quenching to measure concentration is to determine free oxygen in liquids, especially in water, using portable probes.

In this determination the fluorescence quenching time is measured, being related to the concentration of free oxygen (Quencher) by the relation Stern - Volmer (3). For this purpose, we used a pulsed laser diode with the radiation wavelength ranging usually in blue light as excitation source, fluorescence occurring at higher wavelength values, while in the light red field it can easily be measured by photodiodes or diode-array detectors, being possible to determine either the quenching time or phase change between excitation radiation and fluorescence radiation (quenching time can be determined accurately from the phase change).

Experimental

The disadvantage of the existing apparatus for determining concentration, based on fluorescence quenching, is that the analysis of each species requires the use of a complete equipment (probe + electronic unit), (Gutt, S. *et.al.* 2011; Gutt, G. and Gutt, S. 2011; Gutt, G. and Gutt, S. 2010a).

The technical problem is solved by the authors' solutions, namely they have achieved a fluorometer based on fluorescence quenching that can be used both as a portable unit and laboratory structure as well. The equipment offers the possibility to determine concentration under high accuracy conditions of more chemical species analyzed in turn or simultaneously. In the latter case the semi-quantitative analysis is possible only, having an error margin higher than the quantitative analysis of a single species. Instead, the simultaneous analysis enables also a qualitative analysis, a spectral excitation of fluorescence being made by all monochromatic sources of LED type from the device structure and multiplexed readout of the fluorescence quenching time.

To achieve this device, a modular unit is used consisting of a probe and an optoelectronic unit, figure 1, connected by two optical fibers. The probe is of cylindrical type with metal shell and two optical fibers inside, one for transmitting the monochromatic radiation of excitation from source to sample and the other for collecting and transmitting the fluorescence radiation from sample to the electronic data processing unit.

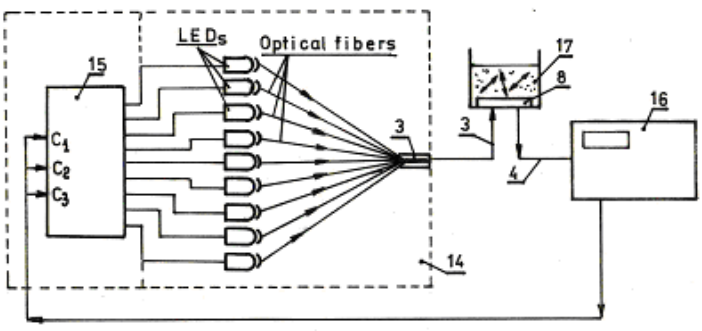


Fig 1. Scheme of universal fluorometer based on the principle of fluorescence quenching.3,4-optical fibers, 8- fluorofor film, 14-multiple monochromatic optical source of radiation, 15-electronic multiplexer, 16-optoelectronics unit, 17-tested solution, C_1, C_2, C_3 - electrical contacts

A key element of the basic structure of fuorometer is the portable radiation source (Gutt, G. and Gutt, S. 2010b), formed in turn from a monochromatic optical source of radiation and multiple electronic multiplexing, both integrated into the structure of fluorometer.

To facilitate the excitation of a large number of fluorescent species the monochromatic source is represented in turn by some compact removable modules, figure 2, each containing a number of the LEDs, emitting monochromatic radiation controlled by multiplexer

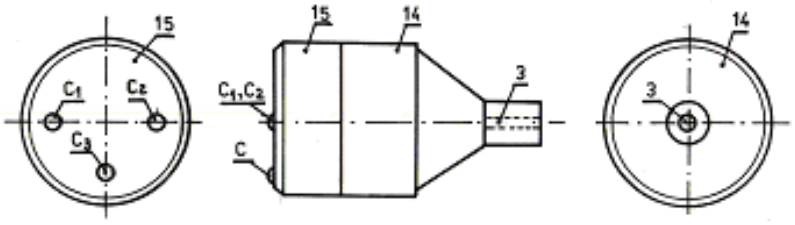
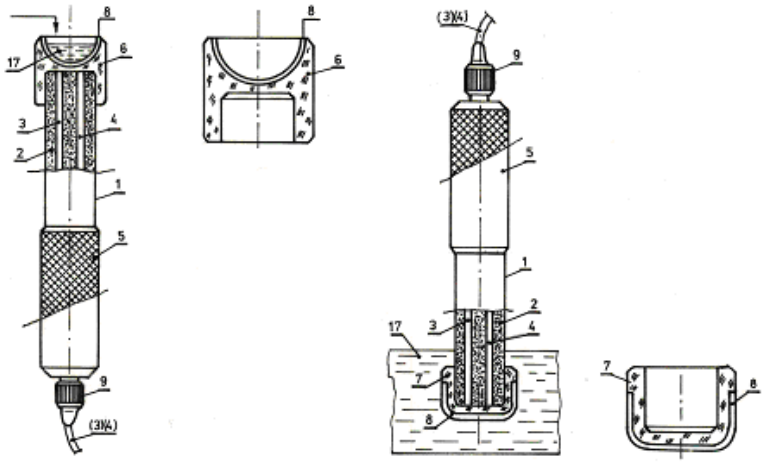


Fig.2. Exterior view of a monochromator module. 3-optical fiber, 14-multiple monochromatic optical source of radiation, 15-electronic multiplexer, C_1, C_2, C_3 - electrical contacts

Each LED within the module structure having the wavelength of emitted radiation tuned with a certain fluorescent chemical species (Quencher). Monochromator modules have LEDs arranged in a crown each LED being aligned to an optical fiber. All optical fibers from a crown of LEDs come together in a single collector optical fiber to irradiate the sample with wavelength specific to the chemical species monitored. When analyzing quantitatively the concentration of a chemical species, the nature of the latter one is set from the device panel, having as effect the LED lighting with the radiation wavelength tuned with the excitation wavelength value of that species. In semi-quantitative and qualitative analysis, multiple monochromatic radiation source works sequentially controlled in multiplexed operating mode, in the sense that the electronic multiplexer controls both the LED lighting in turn as well as reading and management of fluorescence quenching time of each excited species

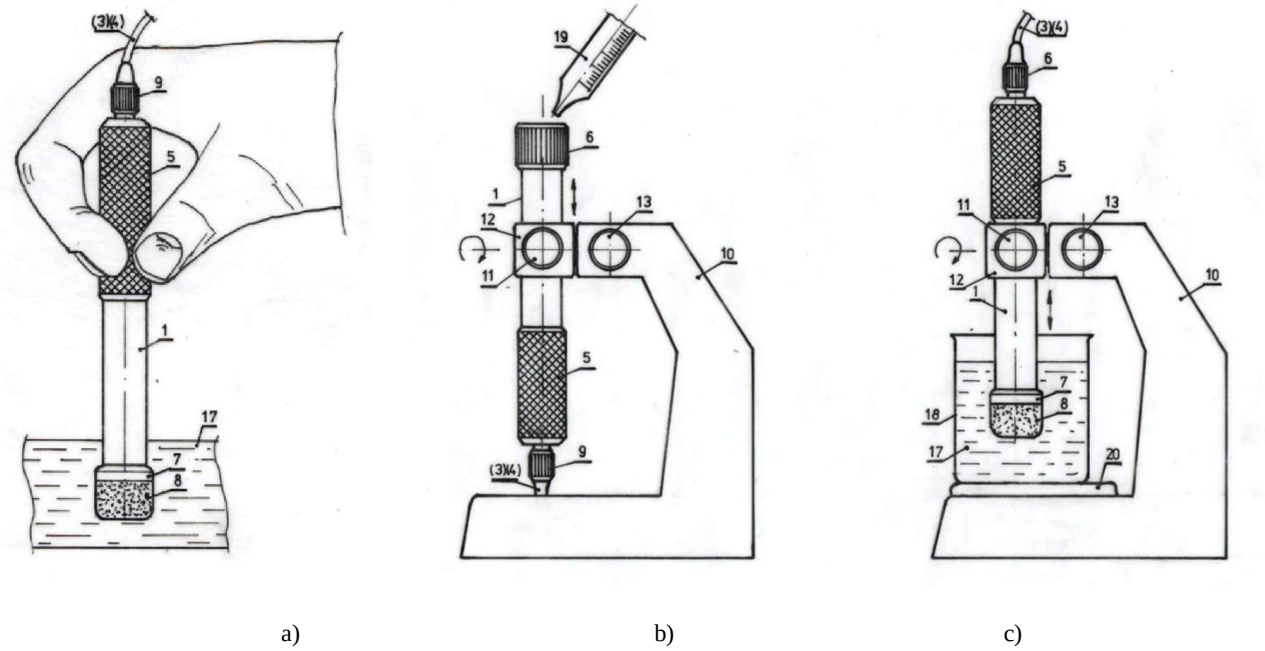


a) b)

Fig 3. Fluorometer probe equipped with hollow capsule (a) and straight capsule (b). 1–probe, 2- polymer material, 3,4 – optical fibers, 5- handle, 6 – hollow capsule , 7 – straight capsule, 8 – fluorofor film, 9 – optical coupler, 17 – analyzed solution.

To increase the universality of the device, a module of LEDS, figure 3, which forms the monochromator unit at one time, can be replaced, simply by pressing hand, by another module which LEDs are tuned with wavelengths of other fluorescent chemical species.

In order to give the possibility of using the same equipment in determining the concentration of more chemical species, different transparent hollow polymer capsules or straight ones are placed on the probe, figure 3, a chemically non-destructive fluorofor being deposited on each capsule in the outer, specific to a certain chemical species which concentration is to be determined by fluorescence quenching.



a) b) c)

Fig 4. Applications of universal fluorometer in laboratory, a) –probe held by hand, b) -probe fixed on a stand, having a hollow capsule mounted and upwards – directed cavity, c)-probe fixed on a stand, having a straight capsule steeped into a flask containing the species to be analyzed. 1-probe, 2-polymer material, 3,4-optical fibers, 5-handle, 6-hollow capsule, 7- straight capsule, 8-fluorofor film, 9-optical coupler, 10-stand, 11,13- screws, 12-rotating head , 17-analyzed solution, 18-glass vessel, 19-glass pipette, 20- thermostatic plate

From the constructive point of view a fluorometer based on fluorescence quenching, in a complete structure for all applications, fig.1, fig. 2, fig.3, fig.4, consists of a cylindrical probe with metal shell 1, a filling polymer material 2, two optical fibers 3 and 4, a handle 5, a hollow capsule type 6, a straight capsule type 7, an adherent film of polymer fluorofor 8, an optical coupler 9, a rack 10, a binding screw 11, a rotating head 12, a rotation-blocking screw 13, a monochromatic multiple radiation source 14, an electronic multiplexer 15, an optoelectronics unit 16, the analyzed solution containing the Quencher 17 (chemical species focused on), a glass vessel used in laboratory work 18, a glass pipette 19 and a thermostatic plate of Peltier type 20.

Results and discussion

The fluorometer presented can operate both under field conditions for in situ analyses and laboratory conditions for serial analyses. Specific dependencies between the intensities of radiation, quenching time and concentration of fluorescence species are given by Stern-Volmer equation valid for dynamic quenching of fluorofor.

$$\frac{I_0}{I} = \frac{\tau_0}{\tau} = 1 + K_{dSV} \cdot c \quad (3)$$

where:

I_0 - intensity of fluorescence of the fluorescent substance in the absence of species that causes fluorescence quenching

I - intensity of fluorescence of the fluorescent substance in the presence of species that causes fluorescence quenching

τ_0 - life time of excited state of fluorofor in Quencher's absence

τ - life time of excited state of fluorofor in Quencher's presence

K_{dSV} - dynamic constant Stern - Volmer

c- concentration of species (Quencher) causing flourescence quenching

When operating under field conditions, the fluorometer probe is held by hand (fig. 4 a), in this way it can be equipped with either a hollow capsule 6, mounted on probe body 1, situation when a precise volume of solution 17 to be analyzed is pipette into the cavity of capsule 6 with a pipette 19, figure 4a, or with a straight capsule, situation when measurements can be made throughout the volume of tested solution, the cylindrical probe 1 being steeped into the solution 17 to be analyzed, figure 4b. It should be specified that the change of capsule type requires manual setting of the electronic unit 16, because each type of probe requires another correction factor. Also, when changing capsules in order to determine another chemical species it is necessary to set properly the chemical species corresponding to the fluorofor deposited on capsule. This setting commands in multiple monochromatic optical radiation source 14 (fig. 1) the LED lighting that emits on the wavelength specific to species and at the same time specific to the fluorofor film from the capsule 6 or 7. When working under laboratory conditions (fig. 4b), the cylindrical probe 1 of fluorometer is easily fixed by the binding screw 11, afterwards depending on the type of application, the rotating head 12 rotates so as the probe head should be either upward or downward (fig. 4c), then this one is screwed by the binding screw to the desired position and the rotation-blocking screw is operated. Setting the probe head 1 upward is meant to series determinations. For this purpose the probe is equipped with the capsule 6 by pushing it on the probe body 1, and then determinations can be made. After each determination, the rotation-blocking screw 13 is being loosened and the probe head is rotated downward, leading to the release of the solution to be analyzed 17 from the capsule cavity 6, then it is being rinsed by bi-distilled water and the probe is brought again to its vertical position for further analysis, the accurate volume of solution analyzed 17 being provided by a glass pipette 19. Setting the probe head downward is meant to the study of kinetic evolution of a certain chemical species under different conditions. For this purpose it is necessary for the straight capsule 7 to be mounted on the body of cylindrical probe 1, afterwards this one is being submerged into the vessel containing the analyzed solution 17, and the concentration evolution of the tested species over time depending on various process parameters is registered by electronic unit 16.

Conclusions

Putting the above described concept into practice has enabled us to carry out an universal portable fluorometric equipment, of low cost, which by minimum auxiliary operations and under high productivity conditions performs the functions of more independent fluorometers, each of these typically specialized on the concentration of a certain chemical species, with no compromise regarding measurement accuracy.

References

Skoog, D.A.; Leary, J.J. 1996. Instrumentelle Analytik, Springer Berlin: 198-203.

Robinson, J.; Skelly Fram, E.M *et.al*. 2006, Undergraduate Instrumental Analysis, sixt edition, Marcel Dekker, New York: 366-376.

Gutt, S.; Gutt, G. *et.al*. 2011. Fluorometru universal bazat pe stingerea de fluorescenta, (in Romanian), [Universal fluorometer based on fluorescence quenching] , Patent proposals Nr. A00163/2011, OSIM Bucuresti.

Gutt, G.; Gutt, S. 2011, *Fluorometru portabil* (in Romanian), [Portable fluorometer], Patent proposals Nr.A00740/2010, OSIM Bucuresti.

Gutt, G.; Gutt, S. 2010. *Fluorometru optoelectronic portabil* (in Romanian), [Optoelectronic portable fluorometer], Patent proposals Nr. A00739/2010, OSIM Bucuresti.

Gutt, G.; Gutt S., *et.al*. 2010. *Monocromator cu fibra optica* (in Romanian), [Monochromator with optical fiber], Patent proposals Nr. A001342/2010, OSIM Bucuresti.