Development of a Steady-State Fluorescence Spectroscopy System and a Time-Resolved Fluorescence Spectroscopy System

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Abstract. This document describes thoroughly the construction of two spectroscopic systems: One system for obtaining fluorescence spectra and another system for obtaining the fluorescence lifetime. The first system consists of obtaining the fluorescence spectrum and is constituted by a Czerny-Turner type monochromator, a photomultiplier tube (PMT), a system for modulating the excitation signal and a system for treating and filtering the fluorescent signal constituted by an electromechanical chopper, a lock-in amplifier and a fast response PMT; plus a set of lenses and a samples holder. The fluorescein dissolved in ethanol was used as a calibration standard, and an Nd:YAG laser source was used as a source of excitation, which emits in 532 nm, of continuous wave and stable power in time adjustable by current. On the other hand, a fluorescence time-resolved system was developed. This system consists of obtaining the fluorescence lifetime of different samples under study from a Voltage curve Vs Time obtained from the system and showing the decay behavior of the phenomenon. This has a pulsed laser unit as a source of excitation, emitting at 532 nm, 80 mJ of energy and with a pulse width of 4 ns. For this system, two semiconductor photodiode detectors with fast response have been built; both with a response of temporary uptake above 1 ns, thus providing obtaining fluorescence lifetime up to this value. Fluorescein was also used in this system as a standard sample, but in this case dissolved in a buffer-like solution; accompanied also by a brief study of the change in the average lifespan of fluorescence in function of the pH of the solution. The results obtained show that two automated systems, reproducible, accurate and of very low cost compared to the current ones, have been successfully implemented for the study of steady-state and time-resolved fluorescence spectroscopy.

1. Introduction

Science has found a great motor of progress as different spectroscopic techniques advance, as is the case of fluorescence. Spectrofluorimetry has always been useful and important in fields such as biochemistry, electronics, mineralogy, and so on. [1, 2, 3, 4] This allows from obtaining a clear image of fingerprints from fluorescent compounds such as ninhydrin, to the exploration and detection of small amounts of crude oil in terrestrial perforations due to the fluorescent properties of petroleum. [5, 6] On the other hand, fluorescence lifetime is very useful to show and clarify changes in the size and shape of molecules, interactions between molecules, obtain inter- and intra-molecular distances, kinetic rates of movement and other changes that have
place inside the material under study. [7] Currently, several companies manufacture systems and equipment that allow obtaining fluorescence spectra with high resolution and temporarily resolve the fluorescence of materials with such small lifetimes. The novelty of these systems is that they have a modular presentation in which it allows the user to easily exchange parts and pieces, depending on the characteristics needed to study a specific material (Absorption wavelength, fluorescence lifetime, among others) taking into account the very low cost compared to the existing ones. The operation of the system for obtaining the fluorescence spectrum is based on the radiation absorbed and emitted by a sample of interest. This information is usually represented as a spectrum, which is a graphic representation of the response of the material as a function of the wavelength; In the time-resolved fluorescence system, two responses are studied, one of which serves as a monitoring of the excitation source and as a base signal, and the other receives the response of the material (fluorescence), both illustrated in a curve (V vs. t). For the calibration, the study substance has been chosen in such a way that the intensity of fluorescence that it presents is as high as possible, the excitation wavelength of the source used must be at least absorbed by the material, the wavelength of fluorescence emission as far as possible from the emission wavelength of the excitation source. The fluorescence lifetime of the material must be in the detection range of the detector ($\geq 1$ ns) and also that its results have been previously reported and verified. [8] One of the most influential characteristics for the occurrence of fluorescence emission in a molecule is the existence of conjugated double bonds, especially in those with a high resonance level of stabilization, structures with high rigidity and multi-cyclic structures. [9] The substance chosen for the calibration of the steady-state fluorescence system was fluorescein dissolved in ethanol acting as solvent and base at a concentration of 0.3 mg/L. The molecule is converted to a Di-anion pH $\geq 8$ (basic Ethanol). The molecular structure is illustrated in Figure 9. On the other hand, fluorescein dissolved in a buffer solution with a pH of 6.0 at a concentration of 0.11 mg/L was used to calibrate the time-resolved fluorescence system.

![Molecular structure of fluorescein depending on the pH of the solution.](image)

**Figure 1.** Molecular structure of fluorescein depending on the pH of the solution.

2. Methods and materials

The experimental arrangements and assemblies of both implemented systems are illustrated in Figure 2. The fluorimeter (steady-state fluorescence system) is composed, in the order of processes, by a source of excitation mentioned before. A continuous wave (cw) Nd:YAG laser source emitting at 532 nm, followed by an electromechanical chopper as an adjustable frequency signal modulation system. After the signal has been modulated to the frequency desired by the user, the radiation is collected and focused on the sample (center of the sample holder) by means of a converging lens of 5 cm of focal length. Subsequently, the material is excited when this radiation hits it, and the fluorescence signal is collected and focused by another lens with the same focal distance, towards the monochromator input.

The fluorescence signal is generally weak in intensity, so a synchronous phase detection system has been included, which allows measurements in light environments and with a lot of external interference.
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Figure 2. Experimental arrangement of fluorescence spectra measurement system and fluorescence lifetime measurement system, respectively.

The time-resolved fluorescence system has a pulsed Nd:YAG laser source as an excitation source which emits at 532 nm, 80 mJ of energy and a pulse width of 4 ns. Before the sample is placed a beamsplitter which divides the radiation into two directions, one beam towards the sample and the other beam toward the monitoring detector. Both detectors connected to an oscilloscope.

2.1. Excitation Sources
A continuous wave Nd: YAG laser source emitting at 532 nm is used as a source of excitation for the fluorimeter. The emission spectrum is illustrated in Figure 3 which was obtained using a Czerny-Turner monochromator of half a meter focal length. As mentioned previously, the system is of the modular type, and allows easy change of excitation source, also allowing to use a semiconductor type laser source emitting at 410 nm. The emission spectrum is illustrated in Figure 4. This in order to observe fluorescence in different materials from different sources of excitation, depending on the absorption spectrum of the material to be studied. The time-resolved fluorescence system, as mentioned above, uses as source of excitation a pulsed laser source emitting at 532 nm, 80 mJ of energy and a pulse width of 4 ns.

2.2. Signal Treatment
Since fluorescence spectra are widely studied, it has been very noticeable in the fluorescence signal part of interference (as a superposition of signals) of external signals such as ambient light, those signals generated by the electronic equipment, those generated by the magnetic coupling with some point of the system and many other signals that interfere in the measurement, most of the cases undesirably. [10] In order to reduce the level of the noise signal, a filtering and averaging of the output signal of the system has been carried out (desired signal + noise). It was possible thanks to the inclusion of a lock-in amplifier and an electromechanical chopper which generates the reference signal for the lock-in. This method is based on the frequency domain, because it is a technique to modulate the signal. [10, 11] In the time-resolved fluorescence system, a Tektronix
Figure 3. Emission spectra of Nd-YAG continuous wave laser used.

Figure 4. Emission spectra of semiconductor type continuous wave laser used.

TDS 3032C oscilloscope with a frequency range of up to 300MHz is used as a visualization element, with a scan speed range of 2.5GS/s and two impedance levels, 50Ω and 1MΩ.

From the frequency control system, the reference frequency is taken and configured as a trigger input to the system’s detection and acquisition system. At a higher frequency, the influence of external interference will be less. It is very important to mention that a frequency of 60 Hz or one of its harmonics should be avoided, because it is produced by AC electrical networks, normally.

2.3. Detection and Acquisition System

For the fluorimeter a Czerny-Turner type monochromator (Spectral Products DK480) is used as the dispersing element, with an automated system of possible selection between three different diffraction grids: 300 lines/mm with a wavelength accuracy of 1.2 nm and a resolution of 0.4 nm, 1200 lines/mm with wavelength accuracy of 0.3 nm and a resolution of 0.1 nm and 2400 lines/mm with a wavelength accuracy of 0.2 nm and a resolution of 0.07 nm.

For detection, a photomultiplier tube AD111 Spectral Products PMT is used [12], which is
composed of a pre-amplifier and a high-voltage source for PMT for greater sensitivity. This system has a fast response of 0.75 \( ns \), a resolution of up to microVolts data collection and a constant time, selective and programmable between 1 \( \mu s \) and 10 \( ms \) with a conversion time of 2 \( \mu s \) which makes it possible to obtain a defined spectrum with a pulse scan, taking and storing 10 pulses to average and report just one data.

For the detection and acquisition of data in the time-resolved fluorescence system, two photodetectors were constructed at very low cost but with fast response. One of them is used to monitor the signal coming from the excitation source and the other one to perceive the fluorescence signal. These detectors are reverse polarized to produce a linear response; The photocurrent generated is dependent on the light incident on them. The function of the RC circuit is to filter any noise with high frequency of the supply that can produce a very noisy signal in the output. These output signals are observed on an oscilloscope. The assembly is shown in Figure 5.

![Figure 5. Diagram of the photodetectors built.](image)

2.4. Excited States

The radiation from a light source, at a specific wavelength, can be absorbed by a substance according to the molecular structure, and this process is carried out in a selective manner. This absorption occurs when an incident photon is able to contribute to promote the transition of an electron from one energy level to another energy level with higher energy than the previous one. After some time, these electrons lose the energy gained through a radiation process called photoluminescence to return to its base state. Depending on the nature of the excited state, the luminescent phenomenon can be classified formally into two categories: fluorescence and phosphorescence. [13, 14] One of the most important characteristics to differentiate these phenomena are relaxation times. The fluorescence lifetime is close to 10 \( ns \) (there are many smaller ones), while the phosphorescence lifetime can be approximately a few seconds, even minutes.

The nature of these excited states denotes what process will be finally executed and talking about photoluminescence, can be categorized into two branches: singlet states and triplet states. [14] When the system is in its lowest energy state and is excited by radiation at a certain frequency, there is the possibility of transition to different vibrational levels of the excited states; as you can see in Figure 6. The horizontal line \( S_0 \) represented in Figure 6 is with the energy of the base state of the system (usually being a singlet state) and, like all other states, also has associated vibrational levels. The line \( S_1 \) represents, as \( S_0 \), the energy of the first excited singlet state and \( S_2 \) is associated with the energy of the second singlet excited state; each of them with vibrational levels associated with these states. \( T_1 \) corresponds to the first triplet excited state.

These are represented from the lowest energy level to the highest energy levels, where \( S_0 \) is the minimum energy system. When the system is excited, a process of system evolution occurs to a new excited state represented as \( S_1 \) or \( S_2 \) depending on the nature of the transition. When the system is in the excited state \( S_1 \), after a while the system will evolve back to the state of minimum energy. During this process, the system emits photons with the specific energy equal to the energy difference between the states involved in the transition. This radiation emitted by
Figure 6. Partial diagram of energy levels of a photoluminescent system (Jablonski’s Diagram).

the system during the transition is known as fluorescence, and the time it takes for this emission
to be known as the fluorescence lifetime or decay time. [13]

2.5. Absorption Process

The spectrophotometric methods are based normally and generally on two laws, which combined
form the law known as the **Law of Lambert-Beer**.

**Lambert’s Law** states that the fraction of light absorbed by a transparent medium is
independent of the intensity of the incident light, and in the material, each successive layer
absorbs an equal fraction of the light that passes through it. [15] In this way:

\[ \log \left( \frac{I_0}{I} \right) = k \cdot l \]  

Where \( I_0 \) is the intensity of the incident light, \( I \) is the intensity of transmitted light, \( l \) is the
length by which the light passes through the sample and \( k \) is the absorption coefficient of the
medium.

**Beer’s law** states that the amount of light absorbed is proportional to the number
of molecules of the luminous material through which incident light passes. [15] This can be written
as:

\[ k = \varepsilon \cdot c \]  

Where \( c \) is the concentration of the material and \( \varepsilon \) is the molar absorption coefficient which
is an intrinsic property of the luminescent material.

For the Lambert’s equation (See equation 1):

\[ \log \left( \frac{I_0}{I} \right) = \varepsilon \cdot c \cdot l \]

And:

\[ \log \left( \frac{I_0}{I} \right) = A \]

And writting in transmittance terms,
\[ T = \frac{I}{I_0} \]

Results:

\[ A = \log\left(\frac{1}{T}\right) = \varepsilon \cdot c \cdot l \quad (3) \]

It can be seen that according to the **Lambert-Beer Law**, parameters such as the absorbance or the wavelength of a luminescent system do not only depend on its chemical nature but also on the external environment of the chromophore molecules and the type of links that they possess along with other contributions of the system.

2.6. *Time-Resolved Fluorescence*

Some pure fluorophores show a monoexponential decay when dissolved in a solvent with which they do not interact. [16] A representation of it can be written as:

\[ I(t) = I_0 \exp\left(\frac{-t}{\tau}\right) \quad (4) \]

Where \( I_0 \) is the intensity at zero time and \( \tau \) is the fluorescence lifetime. This time is defined as the time it takes for the phenomenon to decay its intensity to \( \frac{1}{e} \). In the case of biomolecules, it can be written as a multiexponential kinetic decay:

\[ I(t) = \sum_{i=1}^{\alpha_i} \alpha_i \exp\left(\frac{-t}{\tau_i}\right) \quad (5) \]

As mentioned previously, the fluorescence signal (SF) is multiparametric and can be considered as:

\[ SF = f(I, \lambda_{exc}, \lambda_{em}, p, x, t) \]

Where,

- \( I \) is the intensity. Measured by the quantum yield of the system (\( \phi \))
- \( \lambda_{exc} \) is the excitation wavelength.
- \( \lambda_{em} \) is the emission wavelength. Measured by fluorescence spectra.
- \( p \) is the polarization, and is measured by anisotropy.
- \( x \) is the position, and it is an average parameter by fluorescence microscopy.
- \( t \) is the time, measured by the fluorescence lifetime.

3. **Results**

Next, Figure 7 illustrates the previously reported results of the emission and absorption regions of fluorescein dissolved in ethanol (base).

One of the regions of greatest absorption is around 500 nm, and it is also notorious that the sample absorbs radiation from 532 nm. As previously mentioned, fluorescence is independent of the excitation wavelength, therefore it is expected that the region with the highest emission is, like the reported spectrum, at 541.2 nm. The measurements have been made under stable conditions and the most similar to those used to obtain the reported spectrum. Among these, an ambient temperature of 23°C, a base solution of ethanol, at a concentration of 0.3 mg/L, and with an excitation wavelength of 532 nm, stable in time.

Figure 8 shows the fluorescence spectrum obtained with the steady-state fluorescence system.
Figure 7. Fluorescein dissolved in ethanol absorption and emission spectra [8].

Figure 8. Fluorescence emission spectra of fluorescein dissolved in ethanol, measured by the fluorimeter implemented.

The results obtained from the implemented fluorimeter were: It can be observed that a spectrum has been obtained in which the signal-to-noise ratio is quite high, little susceptible to external and environmental interference. The maximum emission value reached was 538.7 nm, obtaining a difference of 2.5 nm, and a percentage error of 0.46%. To achieve a broad study, it was proposed to implement a fluorimeter with different sources of excitation. Below, results obtained with the fluorimeter on other standard samples are presented very briefly.

Other Results
In this section we will illustrate a study of the phenomenon of fluorescence in various materials from different sources of excitation. For this, a 300 mW semiconductor type laser emitting at 410 nm was also used. Figure 4 shows the emission spectrum obtained.

Rhodamine B  Rhodamine B is a chemical compound belonging to the family of rhodamines. It is commonly used as a violet dye that allows monitoring in a liquid to track the rate, flow
direction and transport of the same. Rhodamine is an easily detectable fluorescent substance and widely used as a standard sample for equipment reference or calibration. Rhodamine is soluble in water, acetic acid, ethanol, among others. [17, 18]

![Molecular structure of Rhodamine B dissolved in ethanol.](image1)

**Figure 9.** Molecular structure of Rhodamine B dissolved in ethanol. [17]

![Absorption and emission spectrum of Rhodamine B dissolved in ethanol](image2)

**Figure 10.** Rhodamine B dissolved in ethanol absorption and emission spectrum. [8]

In Figure 10 it can be noticed that the source used for the calibration (semiconductor laser) emits in the region where the absorption of rhodamine B dissolved in ethanol is not maximum but the material absorbs part of the radiation. Figure 11 shows three fluorescence emission curves of rhodamine B dissolved in ethanol for the excitation originated by the emission source at 410 nm using the same configuration and under the same external properties, this as a proof that the system presents reproducibility.

In Figure 10 it is illustrated that the highest point of expected fluorescence emission is at 564 nm. Figure 11 shows an indicator that illustrates the highest point of fluorescence emission obtained from the implemented fluorometer, being 572.4 nm. With a percentage error of 1.5%. The results agree within the margin of error stipulated acceptance with the previously reported results.

### 3.1. Time-Resolved Fluorescence

The time-resolved fluorescence system has the objective of obtaining the fluorescence lifetime of different substances. To do this, we proceeded with the configuration of the assembly previously proposed. Both photodetectors were configured in photoconductive mode. Because the signals
perceived with this system are fast, it is necessary to use a load resistance of 50 Ω, because using a higher resistance would produce parasitic capacitance and shorten the range of perception of the detector.

For the calibration of this system, dissolved fluorescein was used in a buffer solution with a pH of 6.0 at a concentration of 0.11 mg/L. Figure 12 shows the result obtained through the implemented system.

![Figure 11. Emission spectrum of Rhodamine B obtained using the fluorimeter implemented.](image)

![Figure 12. Fluorescein dissolved in a buffer type solution fluorescence decay curve at 0.11 mg/L at pH 6.0.](image)

The curve presented above is a representation of the voltage as a function of time and the exponential behavior and the decay of the phenomenon is noticeable, which is an important characteristic of fluorescence. The fluorescence lifetime is defined as the period of time elapsed in a phenomenon so that the intensity of spontaneous emission decays to its mean value. This is:

If we take the voltage at the highest point of the curve, the point where the curve changes the behavior to decreasing, like $V_0$, then:
\[ V = V_0 \exp\left(-\frac{t}{\tau}\right) \]  \hspace{1cm} (6)

Which is a representation of the exponential decay of the voltage signal perceived by the photodetector. As mentioned before, the fluorescence lifetime is defined as:

\[ \frac{V_0}{2} = V_0 \exp\left(-\frac{t_m}{\tau}\right) \]  \hspace{1cm} (7)

Being \( t_m \) the fluorescence lifetime. This results in:

\[ t_m = \tau \ln 2 \]  \hspace{1cm} (8)

This time \( t_m \) is measured from the curve. The time \( t \) defines the life time or decay time of the fluorescence, and is defined as:

\[ \tau = \frac{t_m}{\ln 2} \]  \hspace{1cm} (9)

In Figure 12 two markers have been placed. These two markers define \( V_0 \) and point \( \frac{V_0}{2} \). These points are separated, according to the time scale by 4.1 ns ± 0.4 ns, which is the uncertainty associated with the scale of the curve obtained with the oscilloscope. This result agrees with the reported results, illustrated in Table 1.

**Fluorescence decay time as a function of pH**

The fluorescence emission intensity \( (I_F) \) is proportional to the radiation intensity of the excitation beam that is absorbed. In this way:

\[ I_F = k(I_0 - I) \]  \hspace{1cm} (10)

Where \( I_0 \) is the intensity of the beam incident on the solution, \( I \) is the intensity of radiation of the light beam after crossing the sample and \( k \) is a constant that depends on the fluorescence quantum yield. In this way, we can rewrite the **Lambert-Beer Law** as:

\[ \frac{I}{I_0} = \exp(-\varepsilon \cdot l \cdot c) \]  \hspace{1cm} (11)

Where \( \varepsilon \cdot l \cdot c \) is the absorbance.

Replacing 11 in 10:

\[ I_F = K \cdot I_0 \cdot (1 - \exp(\varepsilon \cdot l \cdot c)) \]

The term exponential can be developed by means of a **McLaurin’s series** resulting in infinitely small absorbances:

\[ \exp(\varepsilon lc) \approx 1 + \frac{(\varepsilon lc)}{1!} + \frac{(\varepsilon lc)^2}{2!} + \cdots + \frac{(\varepsilon lc)^n}{n!} = \sum_{k=0}^{\infty} \frac{x^k}{k!} \]

Obtaining an expression for fluorescence emission intensity:

\[ I_F \approx K \cdot I_0 \cdot \varepsilon \cdot l \cdot c \]

And it can be seen as:

\[ I_F \propto k \]
The previously described indicates that the fluorescence intensity depends strongly on the quantum yield of the material that in turn depends on factors such as material concentration, pH, temperature, among others. [19] In this way, as the concentration of the material increases, a decrease in fluorescence intensity occurs, resulting in a self-pairing process in which the molecules of the analyte absorb fluorescence produced by other chromophore molecules. [20]

In addition to the result obtained, it was decided to conduct a study of the lifetime fluorescence in function of the pH of the material in order to verify, experimentally from the implemented system, that at a low concentration, they have a radical type relationship. It should also be mentioned that the pH keeps a radical type relation with the quantum yield, which in turn has a linear behavior with the intensity of fluorescence emission. [20]

![Figure 13. Fluorescein fluorescence lifetime as a function of the pH. (pH 3.0, pH 6.0, pH 10.0).](image)

In order to graphically observe the relationship between the fluorescence lifetime and the pH of the sample, three measurement curves of the lifetime parameter obtained were superimposed as a function of the pH in a graph of V vs t. A change in the fluorescence lifetime is observed as the pH increases, as expected, at a low concentration.

Table 1 shows the values from the fluorescence lifetime obtained at different pH.

| Dye       | Solution | pH   | Lifetime Reported (ns) | Lifetime Obtained (ns) |
|-----------|----------|------|------------------------|------------------------|
| Fluorescein| buffer Type | 3.0  | 3.3                    | 3.6 ± 1                |
|           |          | 6.0  | 4.3                    | 4.1 ± 0.4              |
|           |          | 10.0 | 4.65                   | 5.0 ± 0.4              |

4. Conclusions
Two complete and automated experimental arrangements have been presented. The fluorimeter with high precision is designed in such a way that it is possible to study the fluorescence of...
the compounds and reliably obtain a precise result that the system has been calibrated, as well as to be able to study the fluorescence originated from different sources of excitation because the system allows the easy exchange of each one of the elements that compose it (sources of excitation, substances, lenses, frequency control system, etc.). It also concludes and emphasizes the importance in the measurement of fluorescence the strict preparation of the sample, a source of stable excitation and a system of detection and processing of data like the one used, with the capacity of automatic selection of different networks of diffraction depending on their area of higher resolution. The time-resolved fluorescence system allows obtaining fluorescence decay times (\( \tau \) 1 ns) with high resolution using the multichannel method in which the photodetector is in charge of the photon count, perform the histogram and show the transition probability in function of time as a continuum (V vs. t). Both systems allow the easy change of different parameters of the excitation source of the sample, both emission wavelength and pulse width. In addition, a moldable detection system has been constructed, that is, there is the possibility of exchanging the photodetector to obtain different resolutions in different orders of magnitude of time. The characterization of the materials by means of spectroscopic techniques would not be possible without obtaining a system that allows to observe in depth characteristics of the fluorescence and the time in which this phenomenon occurs. On the other hand, it is recommended for an adequate characterization to take into account the concentration, pH and other characteristics that the solution of the sample must have to be analyzed, since, as mentioned, the fluorescence can change significantly when one of these parameters are varied.

References

[1] Udenfriend S 2014 Fluorescence assay in biology and medicine vol 2 (Academic Press)
[2] Cubeddu R, Comelli D, D’Andrea C, Taron P and Valentini G 2002 Journal of Physics D: Applied Physics 35 R61
[3] Rettig W, Strehmel B, Schrader S and Seifert H 2012 Applied fluorescence in chemistry, biology and medicine (Springer Science & Business Media)
[4] Robbins M 1994 Fluorescence: gems and minerals under ultraviolet light (Geoscience Press)
[5] Steffens J, Landulfo E, Courrol L C and Guardani R 2011 Journal of fluorescence 21 859–864
[6] Herod D and Menzel E 1982 Journal of Forensic Science 27 200–204
[7] Becker W, Bergmann A, Haustein E, Petrasek Z, Schwille P, Biskup C U, Anhut T, Rieman I and König K 2005 Fluorescence lifetime images and correlation spectra obtained by multidimensional tcspe Multiphoton Microscopy in the Biomedical Sciences V vol 5700 (International Society for Optics and Photonics) pp 144–152
[8] of the Institute of Analytical Chemistry A S G 2000 Applied sensor group of the institute of analytical chemistry http://www.fluorophores.tugraz.at/ URL http://www.fluorophores.tugraz.at/
[9] González Pérez C, Martín Mateos E J and Almendral Parra M J 2008 Análisis aplicado a la ingeniería química, 2008-09
[10] Smith D C 1992 High frequency measurements and noise in electronic circuits (Springer Science & Business Media)
[11] Plisch M R and Melanson J L 2005 Signal processing system with baseband noise modulation chopper circuit timing to reduce noise uS Patent 6,961,385
[12] Products S 2017 AD111 Photomultiplier Amplifier USB 2.0 User Manual URL http://www.spectralproducts.com/ad111.html
[13] Masters B R 2008 Journal of Biomedical Optics 13 029901
[14] Requena A and Román J Z 2004 Espectroscopía (Pearson Educación)
[15] Swinehart D 1962 Journal of chemical education 39 333
[16] Mack M 1968 Journal of Applied Physics 39 2483–2485
[17] Ramette R and Sandell E 1956 Journal of the American Chemical Society 78 4872–4878
[18] Kubin R F and Fletcher A N 1982 Journal of Luminescence 27 455–462
[19] Kristoffersen A S, Erga S R, Hamre B and Frette Ø 2014 Journal of fluorescence 24 1015–1024
[20] Kurian A, George N A, George S D, Unnikrishnan K, Paul B, Gopinath P, Nampoory V and Vallabhan C 2002 Journal of Optics 31 29–35
[21] Ryder A G, Power S, Glynn T J and Morrison J J 2001 Time-domain measurement of fluorescence lifetime
variation with ph Biomarkers and Biological Spectral Imaging vol 4259 (International Society for Optics and Photonics) pp 102-110
[22] Elmgren H 1980 Journal of Polymer Science: Polymer Letters Edition 18 815–822
[23] Berezin M Y and Achilefu S 2010 Chemical reviews 110 2641–2684
[24] Lloyd W R, Wilson R H, Chang C W, Gillispie G D and Mycek M A 2010 Biomedical optics express 1 574–586