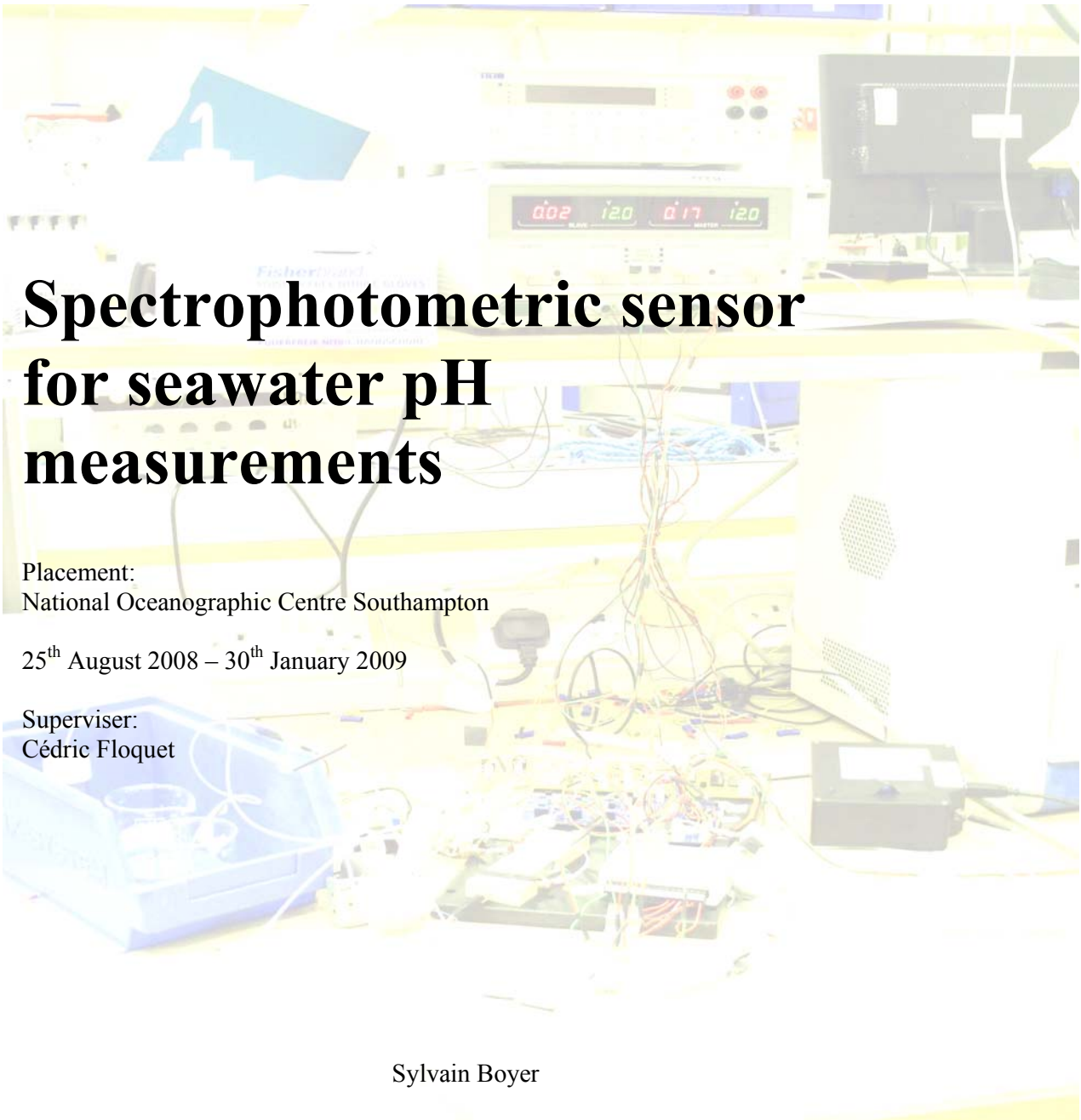
National Oceanography Centre, Southampton
UNIVERSITY OF SOUTHAMPTON AND NATURAL ENVIRONMENT RESEARCH COUNCIL

discovering the unknown



Spectrophotometric sensor for seawater pH measurements

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Abstract

Carbon dioxide is easily dissolved in seawater, leading to a growth of alkalinity. The human activity has then a repercussion on the seawater chemical equilibrium. The unbalanced medium tends to be equilibrated again by acting on the flora and fauna. For more commodities, the pH scale is used rather than CO_2 or pCO_2.

Development of new sensors dedicated to the pH measurement is then necessary to follow the evolutions of seawater. In this report, a new pH sensor is presented, based on the spectrophotometric method. It is a bench-top system which corresponds to the new exigencies: autonomous, high precision and accuracy, long time deployment, small size, low cost…

With every component, software and this present report, reader can understand the principles of the spectrophotometric method, and built the prototype presented.

Sensor – pH – Optics – Chemistry

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Foreword

This report is the outcome of five months of placement at the National Oceanography Centre Southampton (NOCS) developing a pH sensor to measure the seawater alkalinity.

The National Oceanography Centre, Southampton (formerly Southampton Oceanography Centre), is a collaborative Centre owned by the Natural Environment Research Council (NERC) and the University of Southampton. NOCS is based at a purpose-built waterside campus in Southampton, and is home to some 520 research scientists, lecturing support and seagoing staff as well over 700 undergraduate and postgraduate students. In 2005/6 the Centre had a turnover of £35 million.

Within the NOCS, the National Marine Facilities Division (NMFD), incorporates a wide range of services and capabilities to support marine science research within United Kingdoms. The subdivision Underwater System Laboratory – Sensors Development Group (USL – Sensors Development Group) is where the development of the pH sensor takes place. The project has been conducted in quasi-total autonomy and was multidisciplinary: Chemistry, Optics, Electronics, Microfluidics, Computing, Metrology, CAD…

Many thanks to the whole NMFD-USL group, mainly to my supervisor Dr Cédric Floquet, to Dr. Matt Mowlem and to Prof. Hywel Morgan to have trusted me during these five months.

This placement is also a part of the studies followed at the Ecole Nationale d'Ingenieurs du Mans (ENSIM, France), with an expectation of an equivalence of a Master degree.

Why measuring pH in seawater?

I.1 Seawater acidification

With the industrialisation, the emissions of carbon dioxide have increased. Having a high solubility in water, an auto-regulation occurs in the atmosphere by dissolution of the excess of CO_2 in seawater.

When CO_2 dissolves in seawater, it forms carbonic acid, which releases hydrogen and bicarbonate ions into solution. Acidity is a measure of the hydrogen ion concentration in the water, where an increase in hydrogen leads to an increase in acidity (and a *decrease* in the pH scale used to quantify acidity). These hydrogen ions then combine with carbonate ions in the water to form bicarbonate. Carbonate ions are the basic building blocks for the shells of many marine organisms. Thus the formation of bicarbonate through this chemical reaction removes carbonate ions from the water, making them less available for use by organisms. The combination of increased acidity and decreased carbonate concentration has implications for many functions of marine organisms, many of which we do not yet fully understand.

pCO_2 measurement is the direct method to know how much CO_2 has been dissolved, but existing devices present a few non negligible limitations: there are expensive, with low autonomy, rare, bulky and cumbersome and difficult to set up. The majority of potentiometric measurements have proved to be inaccurate as underlined Bellerby [Bellerby et al. 2001].

pH measurement in sea water is an indirect way to measure the Carbon Dioxide dissolved in a compensation phenomena of global warming. Acidity tends to increase of few fractions of unit: 0.1 pH unit since the industrial revolution, according to the UNESCO website[1]. However, pCO_2 can be predicted by pH at on average 1.15% (s=0.23%) [Ohline, S.M. et al. 2007]. The increase in surface ocean pCO_2 due to the atmosphere-ocean transfer of CO_2, results in a reduction of pH by 0.002 units per annum (at constant alkalinity). The required analytical accuracy of seawater pH measurement is therefore in the same order of magnitude, 0.001 pH unit.

I.2 Reasons of pH measurement by spectrophotometric method

The most common method to measure pH in solutions uses electrodes. But such systems have got a number of drawbacks, which made them not efficient in the determination of the pH at 1 mpH. The main drawbacks of the potentiometric approach are the errors associated with the irreproducible liquid junction potentials electrode drift between calibrations due to ion adsorption on membrane surfaces, standing potential as underline Bellerby [Bellerby et al. 1995]. Moreover resolution

[1] http://ioc3.unesco.org/oanet/FAQacidity.html

and accuracy are not adapted to measurements of small variations of acidity, their response time is long.

Measuring pH by measuring the change in absorption of a colored indicator is the method of choice to reach the accuracy required. It is also an absolute method. It is based on the Beer-Lambert's law.

I.3 The pH measurement by absorption

I.3.1 The Beer-Lambert's law

The law states that there is a exponential dependence between the transmission (or transmissivity), T, of light through a substance and the product of the absorption coefficient of the substance ,α, and the distance the light travels through the material, l, called path length, and the concentration c of absorbing species in the substance.

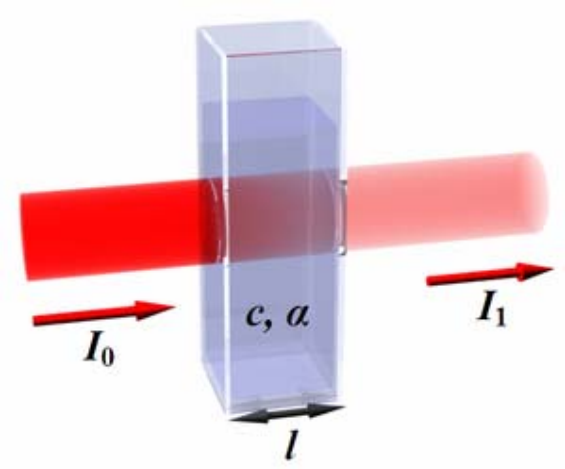


Figure 1: Principe of the absorption measurement (http://en.wikipedia.org/wiki/Beer's_law)

To measure the absorption of such substance α, we enlighten it with the intensity I_0, known, and we measure the light intensity I_1, as shown Figure 1. The Beer's law can be expressed as:

$$(1) \qquad I_1(\lambda) = I_0 . \exp(-\alpha(\lambda).c.l)$$

then we can calculate the transmissivity T_1:

$$(2) \qquad T_1(\lambda) = \frac{I_1(\lambda)}{I_0} = \exp(-\alpha(\lambda).c.l)$$

α depends on the wavelength: λ

This expression can be put on logarithm 10 basis as absorption

$$(3) \qquad A_\lambda = -\log 10\left(\frac{I_1(\lambda)}{I_0}\right) = \varepsilon(\lambda).c.l$$

Where $\varepsilon(\lambda)$ is the molar absorption coefficient.

We should note that this study do not take care about the background. Intensities must be corrected by electric dark corrections.

When a substance contains more than one specie, the global absorption is the sum of each individual absorption:

$$(4) \quad A_\lambda = \sum_{i=1}^{n} A_i^\lambda\left(\varepsilon_i(\lambda), l, c_i\right)$$

In our case, the sample studied is seawater which contains huge number of chemical species. We then use an appropriated coloured indicator: a sulphonphtalein indicator.

I.3.2 The sulphonphtalein indicator: Thymol Blue

The aim of the sensor is to be able to measure pH seawater from 0 to 6000m depth, at the greatest accuracy we can. Considering the range of temperature (from -2 deg C to 30 deg C) and pH unit at such depth, the Bromothymol Blue is the most appropriated dye to use. But for the development of first bench-top system, Thymol Blue (TB) is used. It efficiency is good for surface seawater measurements, and we can expect it will still work at depth.

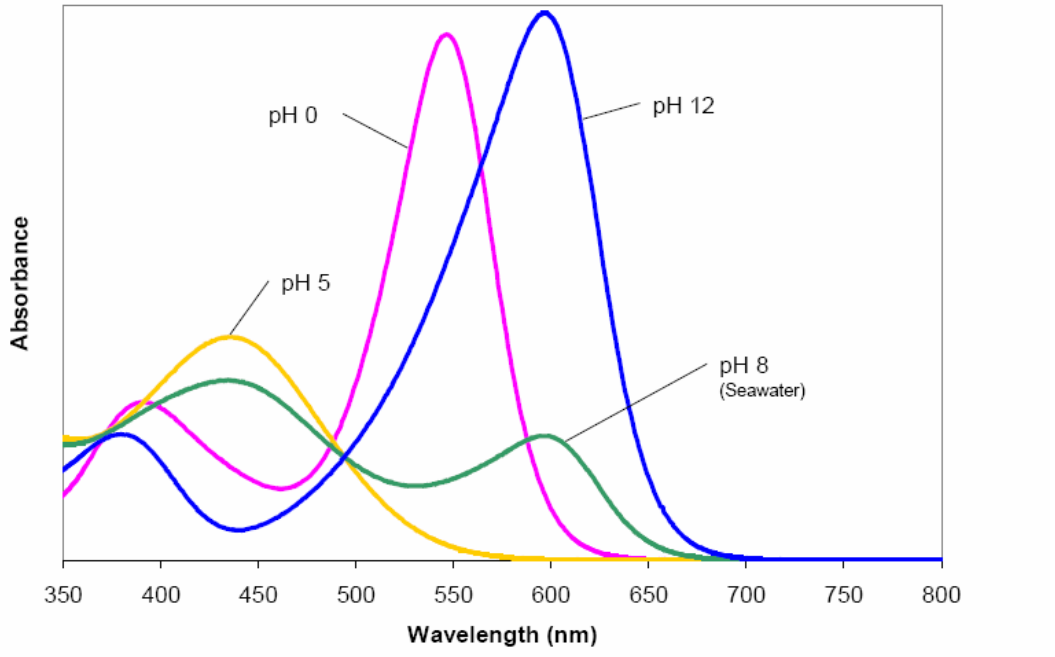


Figure 2: Absorbance of Thymol Blue at various pHs [2]

[2] On Spectrophotometric pH Measurement in Seawater Media, S.LG. Husheer, May 2001 (thesis non published)

When mixed with seawater, the indicator can exist at three different forms: the fully protonated form [H_2I], the monoprotonated form [HI^-] and the unprotonated form [I^{2-}]. Each form can be linked to each other by the reaction equations below:

$$(5)\ H_2I \leftrightarrow HI^- + H^+ \qquad K_1 = \frac{[H_2I]}{[HI^-][H^+]} \qquad K_1' = \frac{[HI^-][H^+]}{[H_2I]}$$

$$(6)\ HI^- \leftrightarrow I^{2-} + H^+ \qquad K_2 = \frac{[HI^-]}{[I^{2-}][H^+]} \qquad K_2' = \frac{[H_2I]}{[HI^-][H^+]}$$

The total concentration of indicator is:

$$(7) \qquad [I_{tot}] = [I^{2-}] + [HI^-] + [H_2I]$$

which can be expressed in function of K_2', K_1' and $[H^+]$:

$$(8) \qquad [I_{tot}] = [I^{2-}] + K_2'[I^{2-}][H^+] + K_1'[HI^-][H^+] = [I^{2-}] + K_2'[I^{2-}][H^+] + K_1'K_2'[I^{2-}][H^+]^2$$

$$(9) \qquad [I_{tot}] = [I^{2-}](1 + K_2'[H^+] + K_1'K_2'[H^+]^2)$$

The pH in seawater attended is around 8.1 pH. The pK_a's [3] for TB are around 1.5 and 8.5 according to Husheer's Thesis (not published). Then neglecting H_2I, (7) and (9) become:

$$(10) \qquad [I_{tot}] = [I^{2-}] + [HI^-] = [I^{2-}](1 + K_2'[H^+])$$

$$(11) \qquad 1 + \frac{[HI^-]}{[I^{2-}]} = 1 + K_2'[H^+]$$

$$(12) \qquad \frac{[HI^-]}{[I^{2-}]} = K_2'[H^+]$$

I.3.3 The Beer-Lambert's law for two species and its application to the pH sensor

Considering that we are working at sea water pH, H_2I is negligible. At the wavelength λ, after having applied dark corrections

$$(13) \qquad A_\lambda = \varepsilon_\lambda^{HI^-}.[HI^-].l + \varepsilon_\lambda^{I^{2-}}.[I^{2-}].l$$

which can be divided by the pathlength:

$$(14) \qquad \frac{A_\lambda}{l} = \varepsilon_\lambda^{HI^-}.[HI^-] + \varepsilon_\lambda^{I^{2-}}.[I^{2-}]$$

[3] $pK_a = 10\log_{10}(K_a)$

Then noting 1 the lowest wavelength, 2 the highest, we can calculate the ratio: $[I^{2-}]/[HI^{-}]$

$$(15) \qquad \frac{[\mathrm{I}^{2-}]}{[\mathrm{HI}^{-}]} = \frac{\frac{A_2}{A_1} - \frac{\varepsilon_2^{HI^-}}{\varepsilon_1^{HI^-}}}{\frac{\varepsilon_2^{I^{2-}}}{\varepsilon_1^{HI^-}} - \frac{A_2}{A_1}\frac{\varepsilon_1^{I^{2-}}}{\varepsilon_1^{HI^-}}}$$

To simplify this ratio, we will define:

- R ratio of absorbances at wavelengths 2 and 1: $R = \frac{A_2}{A_1}$.
- e_1, e_2, e_3, the molar absorption coefficients of indicators :
  - $e_1 = \frac{\varepsilon_2^{HI^-}}{\varepsilon_1^{HI^-}}$
  - $e_2 = \frac{\varepsilon_2^{I^{2-}}}{\varepsilon_1^{HI^-}}$
  - $e_3 = \frac{\varepsilon_1^{I^{2-}}}{\varepsilon_1^{HI^-}}$

Then Eq. (15) becomes:

$$(16) \qquad \frac{[\mathrm{I}^{2-}]}{[\mathrm{HI}^{-}]} = \frac{R - e_1}{e_2 - R.e_3}$$

Eq. (15) is the inverse of (8) then as $\mathrm{K}_2^{'} = \mathrm{K}_2^{-1}$

$$(17) \qquad \frac{K_2}{[\mathrm{H}^+]} = \frac{R - e_1}{e_2 - R.e_3} \Leftrightarrow -\log_{10}([\mathrm{H}^+]) + \log_{10}(K_2) = \log_{10}\left(\frac{R - e_1}{e_2 - R.e_3}\right)$$

Hence:

$$(18) \qquad pH_{\mathrm{T}} = pK_2 + \log_{10}\left(\frac{R - e_1}{e_2 - R.e_3}\right)$$

with:

- $pK_2 \equiv -\log_{10}(K_2)$
- $e_1 = \frac{\varepsilon_2^{HI^-}}{\varepsilon_1^{HI^-}}$
- $e_2 = \frac{\varepsilon_2^{I^{2-}}}{\varepsilon_1^{HI^-}}$
- $e_3 = \frac{\varepsilon_1^{I^{2-}}}{\varepsilon_1^{HI^-}}$

- $R = \frac{A_2}{A_1}$

As underlined by Yao and Byrne, 2007, we have to characterise the Thymol Blue used, knowing the dependence of temperature, pressure and salinity of the constants pK_2 and e_i's. Though this consideration, taking the Zhang & Byrne coefficient are sufficient to have an approximation of results we should have.

The wavelengths to measure the ratio of absorbance are chosen in function of the absorption spectrum given by the mix between the sample and the dye: **Figure 3**. In the way to have the highest resolution, we are choosing the maxima: 435 and 596 nm.

We notice that at 700 nm, Absorption is zero. Then measuring the absorption at this wavelength can be useful and show if the system is drifting or subject to fouling. Absorption measurement can be also disturbed by the gradient of temperature, leading to the formation of liquid lenses and optical disturbances. The phenomenon is known as Schlieren effect[4].

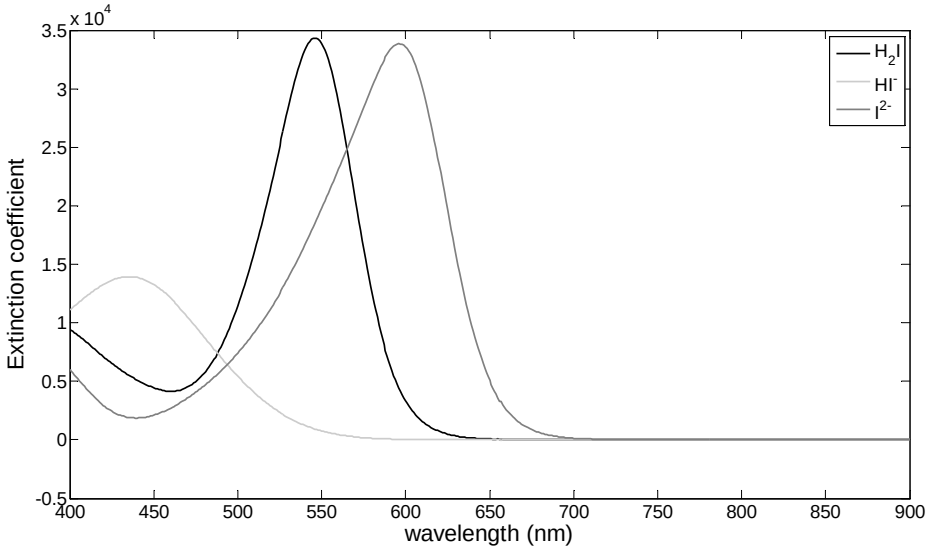


Figure 3: The extinction coefficient spectra for the three species of Thymol Blue, the fully protonated H_2I, the monoprotonated HI^-, and the fully deprotonated I^{2-}

The introduction of indicator into the sample modifies slightly the pH (≤0.005 pH, Clayton and Byrne 1993). This can be found in the Ratio of Absorbances measurement, and we can apply a direct correction on it (Clayton and Byrne 1993, Zhang and Byrne 1996): R(measured) = R(original seawater) + ΔR.

This correction depends on the indicator used, on the temperature, on the composition of the seawater[5] and maybe by the measurement system itself. The determination of

[4] A critical examination of the components of the Schlieren effect in flow analysis, A.C.B Dias, E.P Borges, E.A.G. Zagatto, P.J. Worsfold, Talanta 68 (2006) 1076-1082

[5] Seawater is far from being an homogeneous medium. In our experiments, we are using a synthetic seawater called TRISBuffer, which is a general model including the main ionic species.

the ΔR given by Professor Byrne is based on successive additions of indicator in the studied sample. Because it exist several sulphonphtalein indicators, we will note I the indicator and [I] its molar concentration.

I.3.4 Literature Review of existing systems

Professor Robert H. Byrne has used the Beer-Lambert's law applied to seawater and gave an empirical equation by [Byrne 1987] of the pH. It consists in measuring the absorptions of sea water mixed with a Sulphonphtalein indicator dye (colored reagent) at two wavelengths, as Thymol Blue (TB) that we will use, or green cresol, red cresol.
Then since 1987, a few pH spectrophotometric sensors have been developed in the whole world. Different way to measure, different components : light sources, optical sensor, flow cells, pumps, valve and others have been tried, but always in one way : to have the best accuracy and precision we can, and has possible, accuracy should be better than 0.001 pH unit.
Table 1 and **Table 2** summarise the existing systems.
We can also see two different methods of pH calculation. The first one, proposed by Byrne [Byrne 1987], is to measure the ratio of absorption at two wavelengths. The other one is proposed by Shamus L.G. Husheer[6] [Hunter et al. 2007], named has Full Spectrum Modelling, which is considering the whole spectrum we can have.

[6] See also his Master thesis 2001 non published.

Table 1: Comparative of previous system

PUBLICATION	*Tapp et al. 2000*	*Ohline et al. 2007*	*Bellerby et al. 2002*	*Martz et al. 2003*
Optic sensor	Portable spectrophotometer (Ocean Optics model PS1000)	Ocean Optics USB2000 CCD Spectrophotometer	spectrometers (Ocean Optics model SD1000/2/2) 1 master 1 slave (with light attenuator)	single-beam spectrophotometric detection system Photodiodes placed in the focal plane of the spectrograph (MS10, American) Holographic+converter current voltage
Light source	Ocean Optics LS1-LL tungsten halogen light source	Glass filter to reduce intensity of the tungsten lamp source	tungsten halogen lamp (Ocean Optics, LS-1) + collimating lenses	tungsten lamp (5 V, 0.12 A, Gilway) Technical Lamps
Type of pump	Peristaltic pump 553 mL/h for surface seawater Peristaltic pump 10.3 mL/h for dye	Peristaltic pump Cole–Palmer model 7554-30 18 ml min^{-1} LKB Bromma Microperpex peristaltic pump at a rate of 0.36 mlmin−1	Header tank for seawater, constant pressure Solenoid pump (Biochem Valves, 110TP12)adjusted to 25 uL/stroke	50 uL per pulse solenoid pump (LPLA1210050L, The Lee Co.)
Ratio of dye concentration	Dye dilution tatio of 50 x Final dye concentration : (2e-5M)	Dye dilution tatio of 50 x Dye concentration : 2×10−3 M Final dye concentration : 80 µM in dye = 8e-5 M	On-line measurements : 150uL TB / 55cm3 seawater Perturbation experiments : 55 cm3 seawater for : 50-100-150-200-250 uL of dye	50 uL dye 1.6mL sample(32 pulse of the Leepump) Dye concentration in bag reagent : 2e-2 mol/L
Pathlength's size	1 cm path length	1 cm path length cuvette	44 x 35 x 35 mm➔53.9mm^3, pathlength of 44mm Dryer between collimating lenses and glass	1.72 cm long with a 610 um i.d. ➔5mm^3
System for mixing seawater and dye	Pyrex glass mixing joint	Pyrex glass mixing joint With a bulbous centre that allowed turbulent mixing	Magnetic stirrer	Mixing coil
Type of flow cell intake/outtake system	Thermostated Flowcell 25±0.1°C (Peltier device)	Ocean Optics cuvette holder Thermostated Flowcell at 25±0.05°C (160W heater and a 30W Peltier device)	Intake at the bottom, outtake at the top	-
Temperature control system	Thermostated bath	-	-	-
Temperature sensor	PID controller	-	-	-

Table 2 (continue): Comparative of previous system

PUBLICATION	*Seidel et al. 2008*	*Kaltenbacher et al. 2001*	*Friis 2004*
Optic sensor	silicon photodiodes (S2386-45K, Hamamatsu Corp).	Spectrometer	CCD-spectrophotometer Ocean Optics SD2000
Light source	2.3mm diameter 0.5Wtungsten lamp (4115-B, Gilway Technical Lamp	tungsten lamp	Tungsten lamp + filter (Schott® glass BG 24a/KG 3/BG 40 with a thickness of 1 mm each)+ collimator lenses
Type of pump	Low power 50uL per pulse pump (LPLA1210050L, The Lee Co.)	-	Piston pump with valve selector Peristaltic pump for the debubbler High flow rate : 0.8mL.s^{-1}
Ratio of dye concentration	50uL of mCP at 1e-3mol/L for 1.25mL ?Confused	-	75uL of dye for 50 mL of sample and 75-150-…-975uL of dye by steps of 75 uL for 49mL of sample Dye concentration : 2×10−3 mol.L^{-1} Final dye concentration : 3 and 6 umol.L^{-1} for injection of 75 uL and 150 uL respectively
Pathlength's size	pathlength is 1.1 cm with a 17.7 µl internal volume.	4.47 m	
System for mixing seawater and dye	Static mixer, developed for HPLC, is a 350 µl internal volume convoluted flow path encased in a stainless steel housing (421-0350B, Analytical Scientific Instruments)	?	Thermostated bead-filled coil
Type of flow cell intake/outtake system	z-configuration	Liquid Core Waveguide (flexible pipe)	-
Temperature control system	-	-	Thermostated bead-filled coil
Temperature sensor	-	-	-

Comparing the different systems, we can find common points: they are all using spectrometers to acquire signal, and have as light source tungsten lamp. But at the same time, they are doing their calculations at few bands of frequencies. Tungsten lamps are also expensive and need more power than a Light-Emitting Diode (LED), which will provide only the useful frequency range. (Consumptions: 2.8W to 11.2W for the Ocean Optics LS1-LL tungsten lamp, only 0.24W for a LED)

Pathlengths are various, from 1cm to 4.47m, as well as the ratios TB/sample, due to their link in the Beer-Lambert's law. Having a long pathlength increase the accuracy, but need more light input for the same concentration, comparing to a shorter pathlength. At the contrary, a short pathlength allowing minimisation of the system will be great when using small light intensities. Finally, the dye concentration mixed with the sample has to be optimised.

The mixing methods are different following devices: a coil avoids having a mixer and can save power consuming. The main drawback is that the mixing time can be very long[7].

We can notice that the reference (blank measurement) and measurement are not always done on the same sample. This can lead to wrong pH measurement. On the other hand, the sample rate increases, and then we can have more pH data.

Instead taking only one point to measure the intensity, authors are averaging the signal few pixels around the wavelength chosen. This due to the use of tungsten lamps, but it can also be applied if we have LEDs as light source.

All are taking the coefficients given by Zhang and Byrne 1996: pK2, e_1, e_2, e_3 when using Thymol Blue (Martz et al. 2003 are working with Cresol Red). e_i's are depending on the manufacturer's dye [Yao and Byrne 2007]. Using other provider of chemicals than R.H. Byrne, we will have to characterise our Thymol Blue to avoid imprecision's.

Finally, the temperature control is present only on two prototypes which are bench top systems. We expect to build a low power consuming system which can be easily transferred in an in-situ device. That's why we won't do a thermo-regulation. However, we will plan to simulate an in-situ system by cooling with seawater temperature[8] and use thermistors to know accurately the fluid temperature.

I.3.5 Deployment Condition and instrument requirement

Once built, the sensor can be installed on different structures to measure seawater pH. Ship of opportunity is a solution to measure surface seawater. Generally, ferries are used. The measurements are done around 6-10 meters depth. The time of deployment varies between two days and six weeks. Maintenance is possible, and data can be

[7] The spectrophotometric chemical sensor (SEAS) developed by Kaltenbacher et al. can measures different species in seawater. Some sample rate are given as examples: *"Ferric and ferrous measurements require approximately 1 min. Although nitrite measurements are relatively rapid (3 min) nitrate measurements are relatively slow (~30 min) because of the time required for reduction to nitrite, color development and flushing of the waveguide."*

[8] On shipboard, we have access to seawater tap which can be used to cool the system.

treated in real time. Other way is the use of Conductivity Temperature Depth cast (CTD cast). It can scan the seawater between 0 two 6000m depth. The measurements can last up to 12 hours. Nevertheless, the cast is diving at high speed (1m/s). The sensors must be physically robust to high pressures. pH sensor can take place on submarine vehicles.

pH sensors can also be installed on mooring. It measures seawater properties up to 3000m depth. Argos Float can also be used. For both method, the time deployment can last up to one year, and no maintenance is possible. Data are treated after the period of deployment.

Spectrophotometric pH sensors must have the following properties:

- Small, as places are limited on the most of part of the opportunity location.
- Light and portable.
- Low power consumption.
- Autonomous
- Resistant to biofouling

I.4 Synthetic Seawater: TRIS buffer

In the way to calibrate the system, we are using synthetic seawater as a buffer, which is a mix of ionic components and distilled water. The result gives a sample with characteristics similar to seawater: salinity and pH. In our case, we are using a TRIS buffer which has salinity equal to 35 and pH to 8.2. A description of the chemical species involved in the preparation of the TRIS buffer can be found in the SOP6 protocol (October 2007, version 3.0) and is also described in the Millero and Dickson publications [Dickson and Millero 1987].

II. A new pH spectrophotometric sensor

II.1 Bill of requirements

Following the Zhang & Byrne's publication [Zhang et al. 1996], we have developed a new pH sensor based on the spectrophotometry, for a ship of opportunity, with aim to convert it to an in-situ sensor. Every component has been chosen to build an accurate and cost effective system. The phenomenon of the Taylor dispersion has been taken into account.

At the start of this project, the specifications were:

- Accuracy better than 0.001 pH
- Precision better than 0.0005 pH
- Sampling rate higher than 0.1 Hz

Wanting a cost effective system with low power consumption, we have used LEDs as light sources. We are using a spectrometer, but this one – Hamamatsu RC-VIS C9407 is already potted, and seems to be resistant to high pressures.

In the way to be able to measure the pH on the same sample where we are doing the reference, we decided to build a system in closed loop. With this technique, we can also measure the ΔR, correction to add to the ratio of absorbances R. The software has been computed in MATLAB.

This system can also be used to make the determinations of TB's coefficient, as explained later in paragraph III.2. Therefore, having two systems in parallel, we should be able to know pK_2 and e_i's as the same time we have the R measurement, then an accurate pH.

Once built, the system should be compared with a CTD sensor from IDRONAUT, which is measuring pH by potentiometric method. In theory, the spectrophotometric measurement technique is far better than the pH electrode measurement on the CTD. Comparing both methods will give the confirmation that our system is in the same range of pH and will show the improvement of our technique.

II.2 Prototype

The global schematic is presented **Figure 4**. The prototype is constituted by the fluidic part, the light source, a spectrometer, thermistors, electronic boards and a computer.

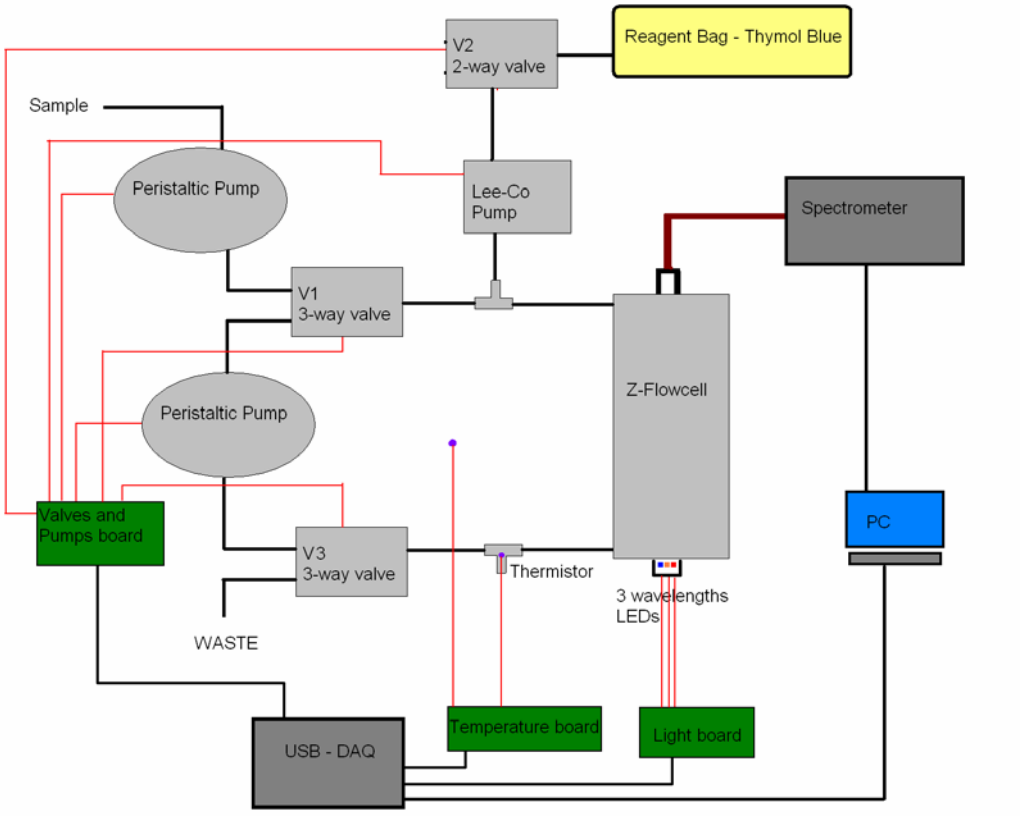


Figure 4: Schematic of the pH spectrophotometric sensor

The prototype has to be the smallest as possible to provide a homogenisation of the temperature. The flow components and thermistors are attached on a frame, and the mount takes place in a primary box. The interstices are filled of oil to insure thermal

properties for thermoregulation. This primary box is enclosed in a second, and the interstices are filled with surface seawater provided by a tap on board of the ship. We expect to have a limitation of the temperature change, mainly caused to valves. The electronic boards, the peristaltic pumps and the spectrometers are out of the boxes. We have to output tubes for them, as electric wires and the optic fibre for the spectrometer. **Figure 5** and **Figure 6** show approximately how the system is assembled and packed.

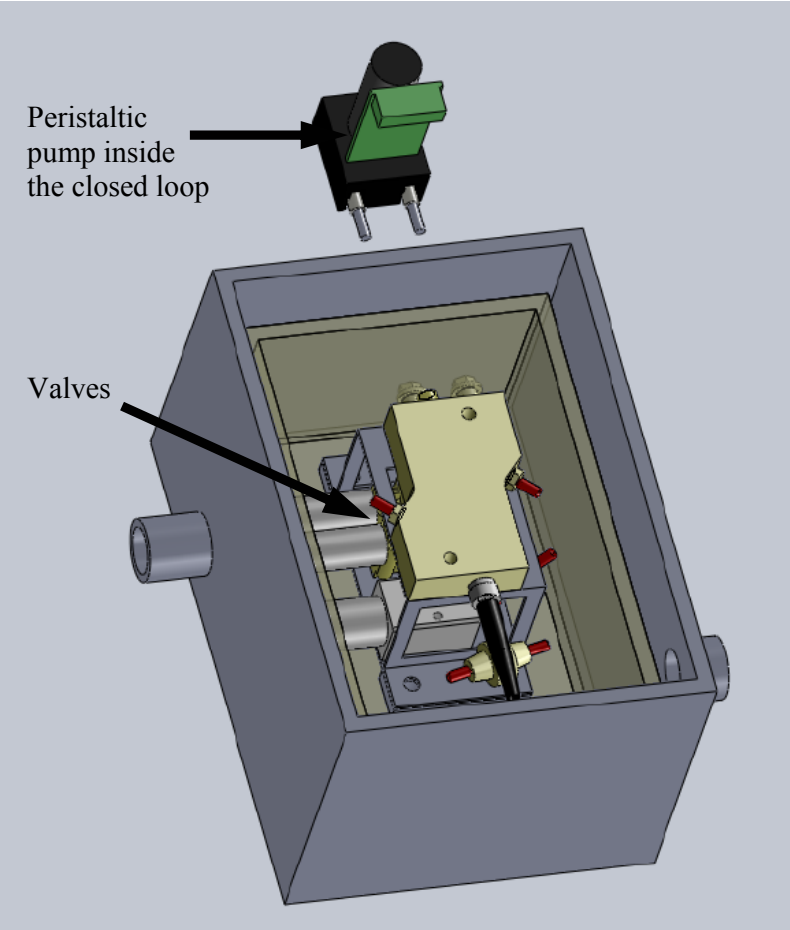


Figure 5: view of the final system planned (without tubes, spectrometer and the Williamson peristaltic pump). Note that on this figure, the 2-way valve has been replaced by a check valve

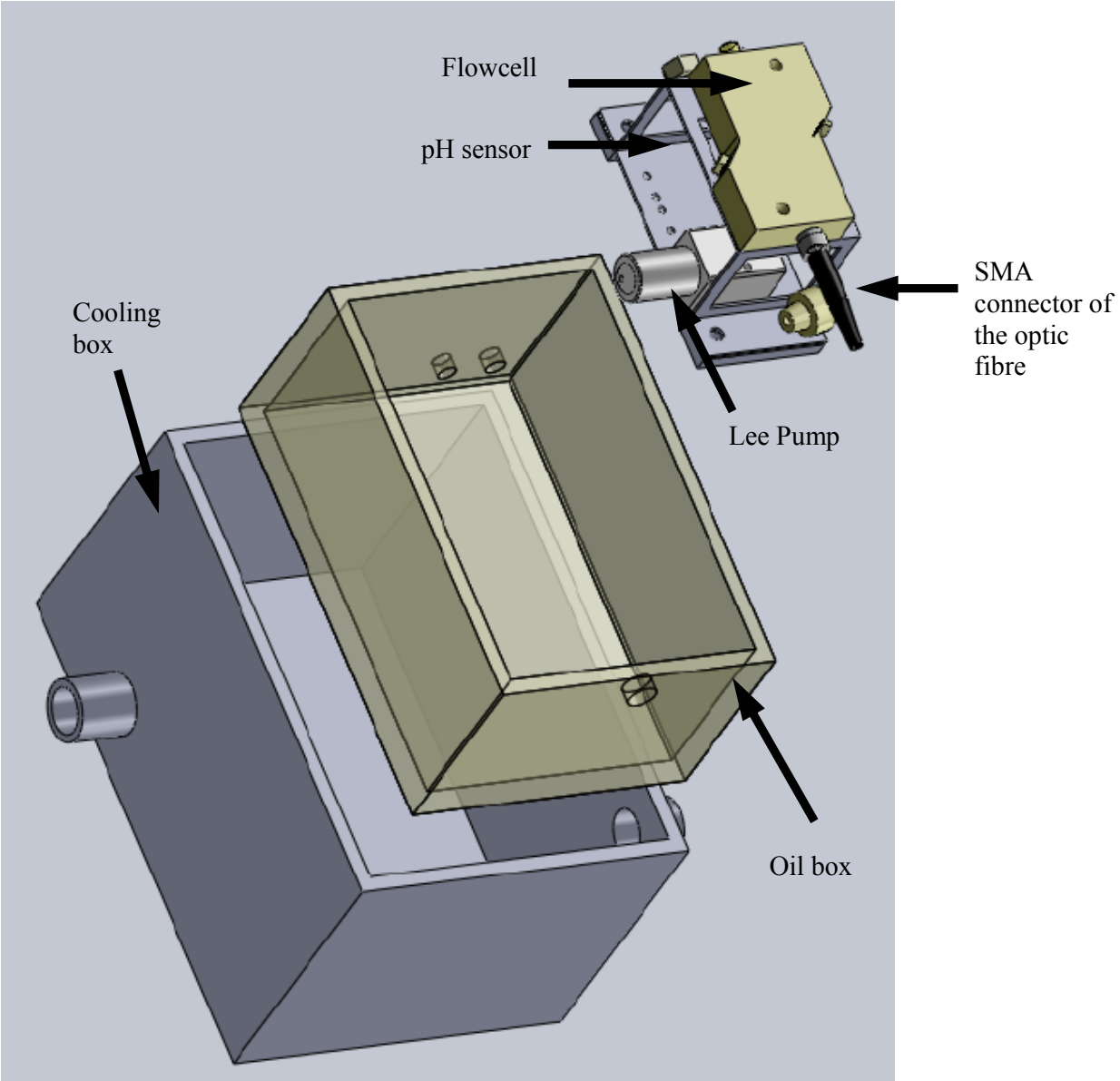


Figure 6: exploded view of the system packed in the two boxes

II.2.1 Fluidic part

II.2.1.1 Taylor-Aris dispersion

As we can see on **Table 1**, Ohline et al. 2007 and Martz et al. 2003 are using the effects of Fluidics and Microfluidics to mix their sample with the dye. We wanted to keep this way, and allowed to have no active mixer on the prototype. As result we are using the Taylor-Aris dispersion: to spread dye along tubes mixing it with sample by means the fluid velocity as shown **Figure 7**. To use it we have to satisfy different conditions

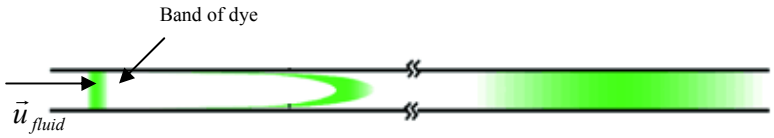


Figure 7: Band of dye spread by the fluid velocity (picture taken from Chaotic Mixer for Microchannels, Strooks et al. 2002)

This configuration can be described with few parameters:

- a the radius of the channel
- l the length of the system
- $V_0 = \left|\vec{u}_{fluid}\right|$ is the fluid velocity (m/s)
- ν the cinematic viscosity of the fluid
- ω the band width of the dye
- D is the diffusivity coefficient of the dye(m^2/s), temperature dependent term

We can define the main numbers used for Fluidics and Microfluidics:

- The Reynolds number : $\mathrm{Re} = \frac{V_0 \times 2a}{\nu}$
- The Péclet number : $Pé = \frac{V_0 l}{D}$

The Taylor's model must satisfy the following condition:

$$1 << Pé << \frac{l}{a}$$

The r-independent axial gradient of the concentration leads to:

$$Pé << 4\frac{l}{a}$$

If the diffusion is neglictabled compare to the dispersion, then

$$\sqrt{48} << Pé$$

Finally, the condition to have a Taylor's dispersion is

$$\sqrt{48} << Pé << 4\frac{l}{a}$$

The time to obtain an homogenous mix between the dye and the sample is given by the followed formula (Theoretical Microfluidics, Henrik Bruus, ed. Oxford Master Series in Condensed Matter Physics):

$$\tau_{Taylor} = \frac{D}{48 \times V_0} \cdot \frac{\omega^2}{a^2}$$

As the diffusivity D is temperature dependent, the characteristic time is various of the temperature. The higher the temperature is the faster the mixing time will be.

II.2.1.2 Description

We have to distinguish three parts in the closed loop. The first one is the common way, starting from the Valve 1 to the Valve 3 through the flow – see the red path **Figure 8**. The valves are 3-way valves from the Lee-Company (LFRX0502850BA). The flow cell is a Z-flow cell from FIALab (FIA-ZSMA-ML-50) which have a 50mm pathlength and an inner tube in PEEK 0.020in x 1/16in.

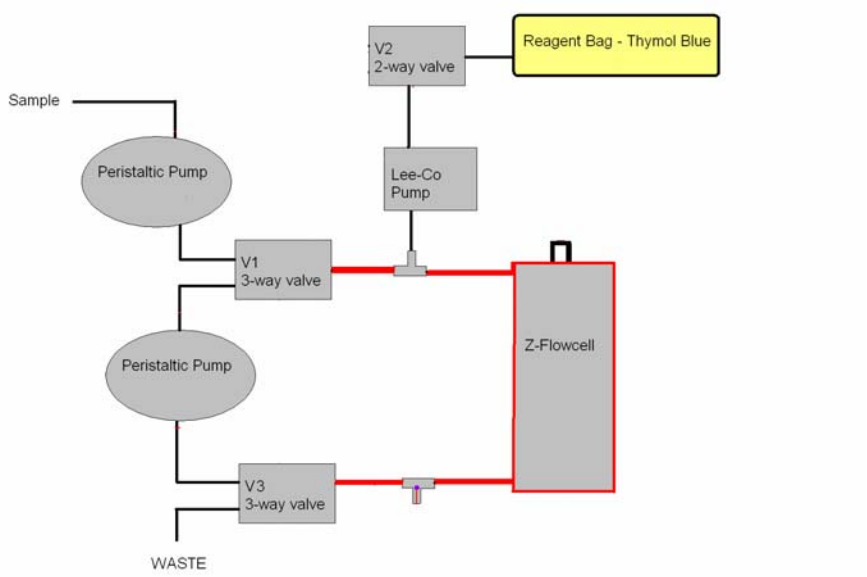


Figure 8: The common way is represented by the red path

The second part is the return way which goes from the Valve 3 to the Valve 1 cell through the peristaltic pump – see the red path **Figure 9**. . The peristaltic pump is a Williamson pump 100 series -12V DC, and its flow rate has been set up at 5mL/min.

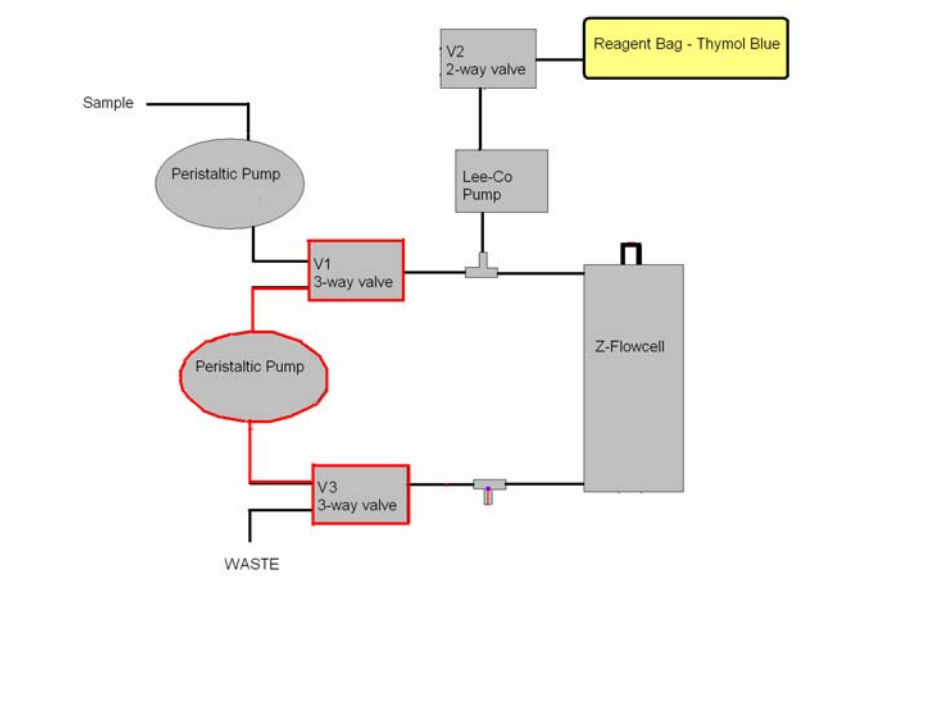


Figure 9: The return way is represented by the red path

The last part is the intake way of dye – see the red path **Figure 10**. It is composed of a reagent bag, a 50uL Lee solenoid pump (LPLX0503100AA), a 2-way valve from the Lee-Company (LFVA1210120H) and a T-connector from the Lee Company.

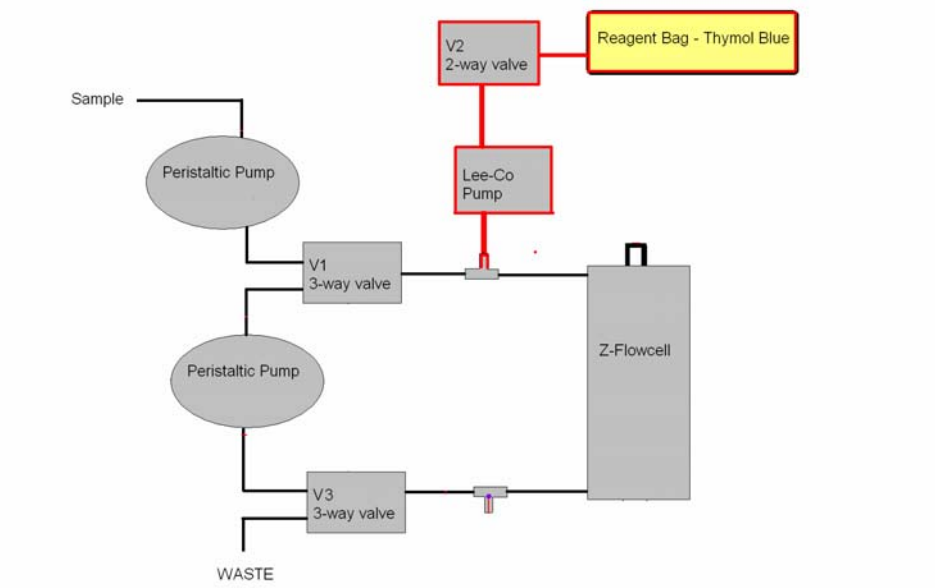


Figure 10: The intake return way is represented by the red path

To intake the sample at a high speed, we are using a peristaltic pump: INSTECH 625/900.143 which flow rate is 1mL/min.

Every component is controlled by digital signal, sent from a PC, through a USB Data acquisition board from National Instrument NI USB-6009[9]. Species are displayed **Table 3**.

Table 3: Species of the USB Data acquisition board USB-6009 from National Instrument

Product	Bus	Analog Inputs[1]	Input Resolution (bits)	Max Sampling Rate (kS/s)	Input Range (V)	Analog Outputs	Output Resolution (bits)	Output Rate (Hz)	Output Range (V)	Digital I/O Lines	32-Bit Counter	Trigger
USB-6009	USB	8 SE/4 DI	14	48	±1 to ±20	2	12	150	0 to 5	12	1	Digital
USB-6008	USB	8 SE/4 DI	12	10	±1 to ±20	2	12	150	0 to 5	12	1	Digital

[1]SE = single ended, DI = differential [2]Software-timed

As underline by Hunter [Tapp et al. 2000], adding a filter where samples are collected, minimises impurities in the fluidic system, as bio-fouling, and improves measurements. To limit heating transfer to the fluid, we have chosen to plug the valves 1 and 3 in manner that when they are not powered, the system is on closed loop[10].

The fluidic part (without peristaltic pumps) is enclosed in two boxes. The first one will be full of oil to assure a uniform repartition of the temperature and avoid rough change. This can happen, when valves are working during flush and intake of dye. This box will be contained in an other one, which will be filled of seawater from a shipboard tap, in the way to do a thermoregulation.

A previous prototype has been studied. **Figure 11** show the fluidic part. Our actual prototype is an evolution of this first plan. This one has only one peristaltic pump localised at a position it can intake the sample and do the mix. However it could be the costless and maybe more robust protocol – only one pump to drive, the problem comes from the flushing time of the closed loop. That is why we have chosen the configuration with two pumps: one for the mix, one to flush fast and have then a higher rate of measurements.

[9] Configuration of input and output of the USB-DAQ can be consult at the **APPENDIX *****

[10] The tube linking the valve 1 and 3 is plugged on the common way. The pipe going from valve 1 to the flow cell is plugged on the Normally Opened way (NO), as well as the pipe going from the peristaltic pump to the valve 3. On Normally Closed way (NC) are plugged the intake and outtake.

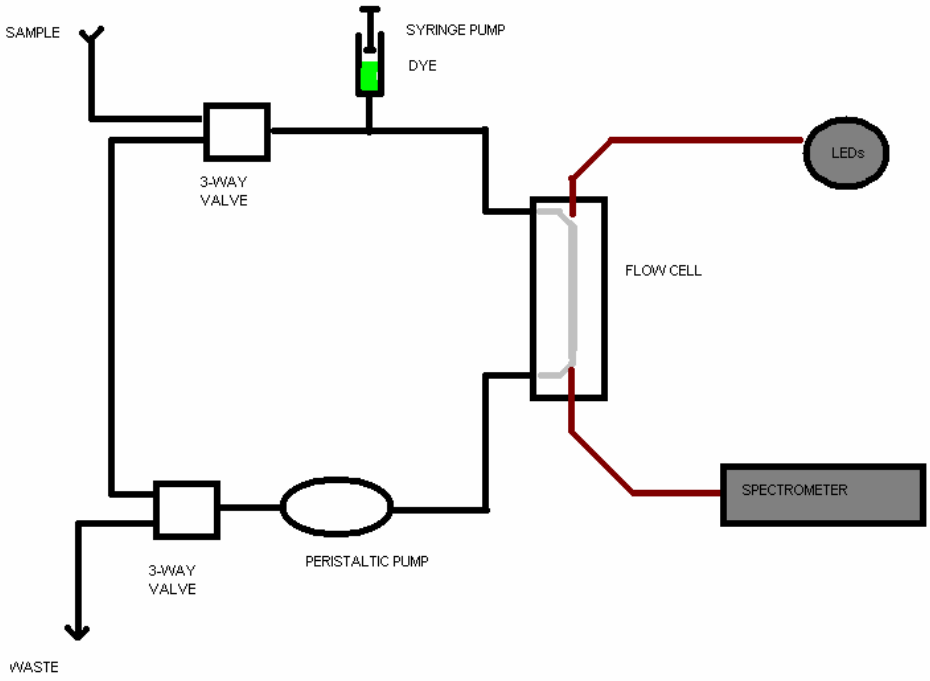


Figure 11: Previous prototype

II.2.1.3 Application of the Taylor-Aris dispersion

For the tubes:

- The Reynolds number is 4.
- The Péclet number is 3450
- $4 \times \frac{l}{a} = 1030$

For the flowcell:

- The Reynolds number is 2.
- The Péclet number is 1840
- $4 \times \frac{l}{a} = 133$

For both cases, the upper boundary condition ($Pé << 4\frac{l}{a}$) is not respected. Nevertheless, experiments show that we can mix a dye with a sample by using our method of mixing (cf. Experiments)

II.2.2 Temperature Sensors

Temperature measurement is done by means of two thermistors. One is potted in a T connector, placed near the flow cell, and it is measuring the fluid temperature. The second is placed outside the fluidic system and it is measuring the ambient temperature. Signals are collected through the USB-DAQ device, on analogic

channels[11]. A third temperature sensor is in the spectrometer, and we can have access to it.

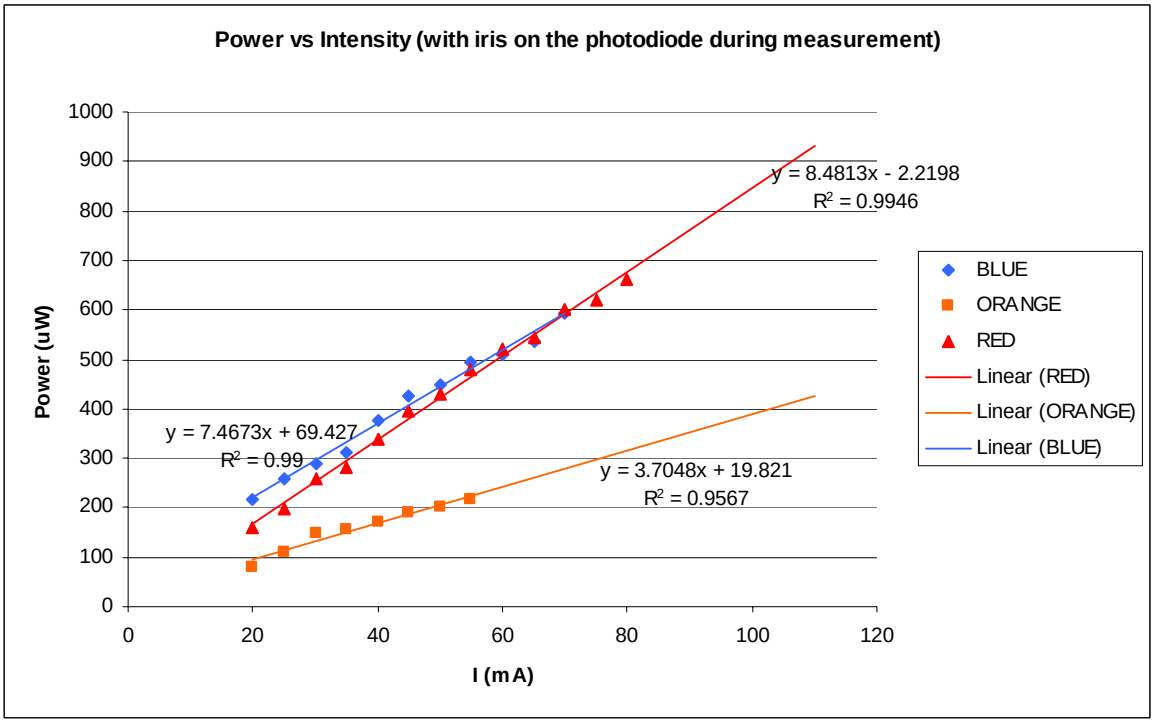
II.2.3 Light sources

Light is provided by a custom built 3-wavelength LEDs by ROITHNER LASERTECHNIK GmbH L435/590/700-30C32. The three wavelengths are able to work together, and none interference has been shown. We can also, in the way to limit the power consumption, commute LEDs and recompose the signals in one spectrum. The command is done via the USB-DAQ device[12]. A SMA connector (SMA 905 MM floating adapter) has been drilled to fit The LED. It is replacing one of the two SMA connectors provided with the flow cell.

Figure 12: 3-wavelength LEDs without the SMA connector

LEDs power has been measured with different intensities on input. Measurements have been done using a photodiode fitted with an iris, and input voltage has been set up to 8V. Results show that LEDs have got a linear response to the input electrical power.

[11] Idem
[12] Idem



With a spectrometer (Hamamatsu spectrometer RC-VIS C9407) we show that a drift exists, mainly for the orange LED: 14nm

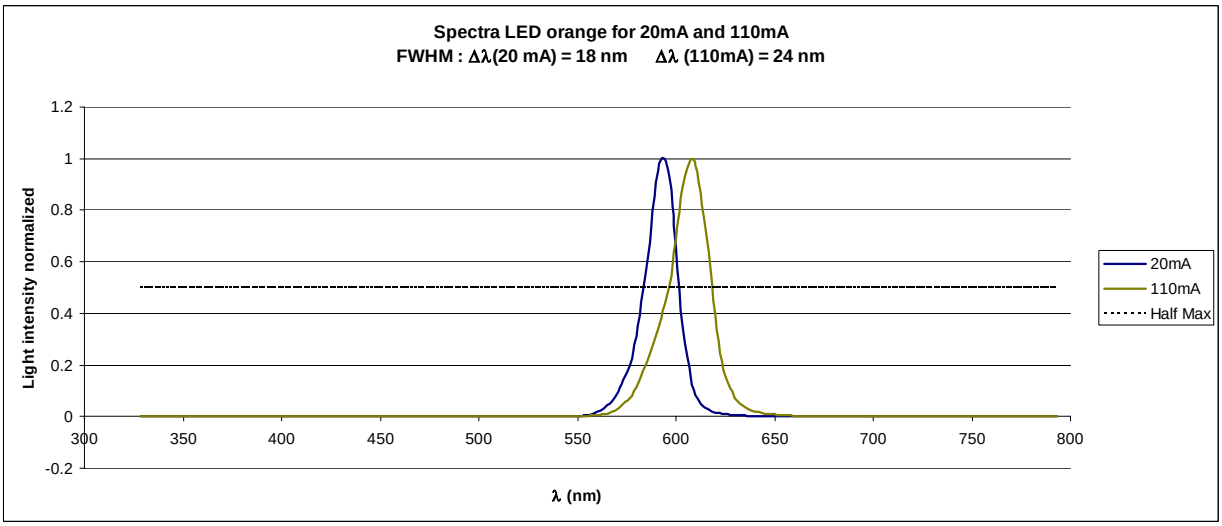


Figure 13: Drift of the orange LED

The three LEDs can be switch on at the same time and their light intensity is the same as when only one LED is switch on. In our methodology (cf. II.2.5 Methodology), we have chosen to record one spectrum by LED and concatenate the three spectra. The computed programs for the spectrometer can be in such way easily adapted when using a photodiode as optical sensor.

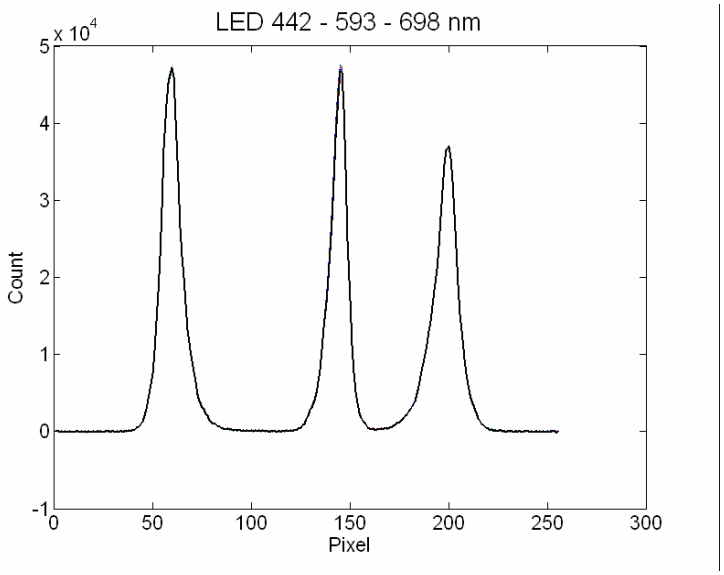


Figure 14: Assembly of the three spectra, each have been corrected for baseline

II.2.4 Optical sensor

We are using an Hamamatsu spectrometer RC pipe and flow cell's inner tube – are 0.062in and fluid connector are MINSTAC fr-VIS C9407, which have 256 pixels. It is linked to the Z-flow cell via a multimode optic cable (a 105 µm Core SMA Connector s Multimode Patch Cable 1m long from THORLAB). Tubing all pipes – excluding peristaltic pump's from the Lee Company.

II.2.5 Methodology

The flush is done in three steps. The first one is flushing the common way, by opening valve 1 and 3and activating the external peristaltic pump. Once done, we are running the system in closed loop a little time using the internal peristaltic pump to evacuate the residue in the return way (valve 1 and 3 switched off). The third step is to flush again the common way. Verification is done to know if there is still a residue. It means that we have to measure a blank and compare to a previous one (This step can be delete if laboratory works have shown that once, twice or more times are sufficient).

A blank is recorded while running the system in closed loop. Then intake dye is done: opening of the valves 2 and 3, and a single stroke on the Lee Pump, closing valves and run the internal peristaltic pump until the mixing time is up. This last has been determined in laboratory. The measurement can be recorded, always running the system in closed loop. In the case we want to measure ΔR, we are doing successively an intake of dye and a measurement acquisition.

II.2.6 Software acquisition

Every acquisition follows the same order:

- Temperature measurement : averages and standard deviations are recorded
- Switch on the **blue** LED (435nm)
- Recording spectrum
- Temperature measurement : averages and standard deviations are recorded

- Temperature measurement : averages and standard deviations are recorded
- Switch on the **orange** LED (595nm)
- Recording spectrum
- Temperature measurement : averages and standard deviations are recorded

- Temperature measurement : averages and standard deviations are recorded
- Switch on the **red** LED (700nm)
- Recording spectrum
- Temperature measurement : averages and standard deviations are recorded

When the acquisition is finished, we obtain a .blk or .mes file switching that we have done a blank or a measurement. These files are processed, and a new file is generated, where date, time pH value and its uncertainty are written. For ΔR measurement, a new folder is created where we can find .dr files.
This last contain on the first line: date and time, the path of the blank. On the following lines are written the ratio of absorbances R, its uncertainty and the path of the .mes file.

By default, ΔR is set up at 0, and no program is calculating it based on the measurements. Further, this value should be replaced by a correction obtained by laboratory experiments.

Table 4: Legend for the acquisition table

Fluid Temperature	
Average	Standard Deviation

Ambient Temperature	
Average	Standard Deviation

Spectrometer Temperature	
Average	Standard Deviation

Pixel value of the spectrometer	
when the blue led is on	
when the orange led is on	
when the red led is on	

Voltage value of the thermistor 1	
Average	Standard Deviation

Voltage value of the thermistor 2	
Average	Standard Deviation

Table 5: Format of the acquisition

11/12/2008 14:54							
28.866088	0.066337	5.901748	0.023454	0	…	0	0
21.296751	0.066893	6.051347	0.0239	0	…	0	0
26.75	0	0	0	0	…	0	0
1	3113	3038	3055	3086	…	3113	3034
2	3063	3003	3003	3049	…	3063	3079
3	3080	3016	3089	3043	…	3099	3088
4	3034	3082	3022	3090	…	3039	3081
5	3089	3095	3057	3033	…	3068	3022
6	3018	3024	3046	3059	…	3070	3096
7	3094	3098	3073	3083	…	3025	3034
8	3097	3030	3014	3090	…	3004	3054
9	3000	3058	3069	3093	…	3018	3090
10	3001	3051	3022	3089	…	3072	3078
20.846643	0.067816	5.894875	0.023963	0	…	0	0
21.657838	0.068179	6.181124	0.024642	0	…	0	0
26.745833	0.015857	0	0	0	…	0	0
20.912666	0.066785	5.918232	0.023655	0	…	0	0
21.679378	0.065779	6.188911	0.023792	0	…	0	0
26.74375	0.019071	0	0	0	…	0	0
1	3113	3031	3020	3034	…	3113	3025
2	3063	3059	3056	3010	…	3063	3099
3	3052	3097	3045	3080	…	3041	3012
4	3034	3090	3067	3027	…	3072	3039
5	3044	3052	3081	3069	…	3049	3054
6	3007	3046	3008	3049	…	3081	3083
7	3076	3017	3079	3068	…	3026	3007
8	3007	3043	3067	3024	…	3050	3011
9	3039	3058	3091	3037	…	3078	3098
10	3099	3080	3028	3013	…	3093	3019
21.071552	0.062878	5.974661	0.024055	0	…	0	0
21.58325	0.065161	6.1598898	0.02313	0	…	0	0
21.75	0	0	0	0	…	0	0
21.06539	0.0675987	5.979586	0..024025	0	…	0	0
21.58325	0.064032	6.124193	0.023498	0	…	0	0
26.74375	0.0197071	0	0	0	…	0	0
1	3113	3067	3049	3002	…	3113	3066
2	3063	3018	3037	3003	…	3063	3054
3	3029	3036	3080	3089	…	3044	3038
4	3076	3049	3036	3091	…	3083	3004
5	3032	3064	3035	3031	…	3064	3089
6	3063	3043	3050	3000	…	3024	3069
7	3004	3091	3020	3084	…	3054	3012
8	3038	3026	3096	3099	…	3092	3020
9	3088	3033	3000	3023	…	3048	3038
10	3018	3048	3084	3085	…	3085	3015
21.212331	0.065197	6.024912	0.023327	0	…	0	0
21.641002	0.069012	6.175041	0.024929	0	…	0	0
26.75	0	0	0	0	…	0	0

II.3 Software implemented

II.3.1 Software resources

The software is running under MATLAB (computed the with 2007-2008 versions). It also need National Instrument driver **NI-DAQmx Run-Time Engine - (Runtime 5)**. The HAMAMATSU spectrometer driver rcu1a.dll must be copied in the folder containing the MATLAB programs.

II.3.2 Spectrometer programs (MEX-Files)

Two programs have been computed specifically for the spectrometer. These have been computed in C++, based on the sample given by HAMAMATSU. The transfer in MATLAB language has been done by compiling these in MEX-File. A C++ compiler must be installed, and MATLAB must be linked to this compiler (for this work, it was Visual C++ 2008).The first program acquires the internal spectrometer temperature. The second one read the 256 values of the spectrometer's pixels.

An error message appears when Integration Time, Gain and Trigger options are set in input from the MATLAB routine, and when the spectra acquisition is done in loop. The issue has been solved by setting up the spectrometer in the MEX-File (when writing the C function):

Integration Time = 10000 ms
Gain = 0
Trigger Edge = 0
Trigger Mode =0

Command to compile the MEX-File:

```
mex -L"C:\\path1\\path2\\path3\\" -lrcu1a Intensity_spectrometer.c
```

where `-lrcu1a` indicates the library file : rcu1a.lib

II.3.3 Calculation of uncertainties

The pH spectrophotometric sensor acquires few data and uses complex formula to calculate the final pH. We need to know uncertainties to show if our results are correct. Then we will use the propagation law of uncertainties:

$$u_X = \sqrt{\sum_i \left(\frac{\partial X}{\partial x_i} u_i \right)^2} \quad \text{with} \quad X = f(x_i)$$

II.3.4 pH Calculation by the software

II.3.4.1 Temperature measurement

Fluidic and ambient temperatures are done via the USB-DAQ board, by a thermistor. It has been set up at the rate 10000 measurement per second. It acquires the voltages of the thermistors at this frequency, during one second, then 10000 values. Equivalent temperatures are calculated from thermistors coefficients which have been determined by calibrations.
At the end of temperature acquisition, the program returns four values: the average temperatures, its standard deviations, the average voltages read at the analogic ports, and its standard deviations.(cf. **APPENDIX VI** Temperature_NI.m.)
Uncertainties on temperatures are calculated when the acquisition file (.blk or .mes) is reopened by the MATLAB program DataProcessing.m (available in **APPENDIX XIII**):

$$u_T^i = \frac{\sigma_T^i}{\sqrt{10000}} \text{ for averaged temperature } \overline{T^i}$$

Fluid temperature (as well ambient and spectrometer's temperatures) are measured before and after spectra acquisitions, for each wavelengths. As the final, we have six values of average temperatures with their uncertainties.

A routine is comparing every ensemble, to know if we can straight calculations. This has not been computed for the moment. Else, we will make them in one average and one uncertainty:

$$\overline{T} = \frac{1}{6}\sum_i T^i \text{ and } u_T = \sqrt{\frac{1}{6}\sum_i u_T^i}$$

These values are returned at the end of program.

II.3.4.2 Ratio of Absorbance R

This calculation is done by the program DataProcessing.m. It is extracting every spectrum, and doing an electric dark correction for the last blank and measurement recorded. After an analysis of Temperatures (still to compute), the three LED spectrum are concatenated to give one single spectra. Small variations around the zero can be neglected comparing the intensities near the LED's wavelengths.

For each scan, Intensities are averaged on the Full Width Half Max (FWMH) of each wavelength. Then Absorptions are calculated as well as the Ratio of Absorbances[13].
R should be in a target interval, defined by laboratory experiments. If the value obtain is outbound, it is replaced by the extreme value, minimum or maximum. By squeezing an aberrant value, we are minimizing the uncertainty, and obtaining a higher precision.

[13] Note that the third wavelength (698 nm) is not used in the program.

The program can still be modified to increase or reduce the number of pixel to average. It appears that using the FWHM procures best results.

II.3.4.3 Uncertainty on *R*

R is the ratio of absorbances defined as $R = \frac{A_2}{A_1} = \frac{\log_{10}\left(\frac{I_2^{ref}}{I_2}\right)}{\log_{10}\left(\frac{I_1^{ref}}{I_1}\right)} = \frac{\ln\left(\frac{I_2^{ref}}{I_2}\right)}{\ln\left(\frac{I_1^{ref}}{I_1}\right)}$

Then we have:

$$u_R = \sqrt{\left(\frac{\partial R}{\partial I_2^{ref}} \cdot u_2^{ref}\right)^2 + \left(\frac{\partial R}{\partial I_2} \cdot u_2\right)^2 + \left(\frac{\partial R}{\partial I_1^{ref}} \cdot u_1^{ref}\right)^2 + \left(\frac{\partial R}{\partial I_1} \cdot u_1\right)^2}$$

$$u_R = \sqrt{\left(D_1 \cdot u_2^{ref}\right)^2 + \left(D_2 \cdot u_2\right)^2 + \left(D_3 \cdot u_1^{ref}\right)^2 + \left(D_4 \cdot u_1\right)^2}$$

$$D_1 = \frac{1}{\ln\left(\frac{I_2^{ref}}{I_2}\right)} \cdot \frac{1}{I_2^{ref}} \qquad D_2 = -\frac{1}{\ln\left(\frac{I_2^{ref}}{I_2}\right)} \cdot \frac{1}{I_2}$$

$$D_3 = -\ln\left(\frac{I_2^{ref}}{I_2}\right) \cdot \frac{1}{I_1^{ref} \cdot \ln^2\left(\frac{I_1^{ref}}{I_1}\right)} \qquad D_4 = \ln\left(\frac{I_2^{ref}}{I_2}\right) \cdot \frac{1}{I_1 \cdot \ln^2\left(\frac{I_1^{ref}}{I_1}\right)}$$

R and U_R are returned at the end of the program DataProcessing.m.

II.3.4.4 Calculation of the pH and its uncertainty

This done by the program pH_calculation.m (cf. **APPENDIX XIV**), which returns *pH* and U_{pH}.

$$pH_T = pK_2 + \log_{10}\left(\frac{R - e_1}{e_2 - R.e_3}\right) = pK_2 + \ln\left(\frac{R - e_1}{e_2 - R.e_3}\right) \cdot \frac{1}{\ln(10)}$$

$$u_{pH} = \sqrt{\left(\frac{\partial pH}{\partial pK_2} \cdot u_{pK_2}\right)^2 + \left(\frac{\partial pH}{\partial R} \cdot u_R\right)^2 + \left(\frac{\partial pH}{\partial e_1} \cdot u_{e_1}\right)^2 + \left(\frac{\partial pH}{\partial e_2} \cdot u_{e_2}\right)^2 + \left(\frac{\partial pH}{\partial e_3} \cdot u_{e_3}\right)^2}$$

$$u_{pH} = \sqrt{\left(D_{pK_2} \cdot u_{pK_2}\right)^2 + \left(D_R \cdot u_R\right)^2 + \left(D_{e_1} \cdot u_{e_1}\right)^2 + \left(D_{e_2} \cdot u_{e_2}\right)^2 + \left(D_{e_3} \cdot u_{e_3}\right)^2}$$

$$D_{pK_2} = 1 \qquad D_R = \frac{1}{\ln(10)} \cdot \frac{e_2 + e_1 \cdot e_2}{(e_2 - R \cdot e_3) \cdot (R - e_1)}$$

$$D_{e_1} = -\frac{1}{\ln(10)} \cdot \frac{1}{R - e_1} \qquad D_{e_2} = -\frac{1}{\ln(10)} \cdot \frac{1}{e_2 - R \cdot e_3} \qquad D_{e_3} = \frac{1}{\ln(10)} \cdot \frac{R}{e_2 - R \cdot e_3}$$

The pK_2 has been calculated following the Zhang & Byrne equation :

$$pK_2 = 4.706\frac{S}{T} + 26.3300 - 7.17218 \cdot \log_{10}(T) - 0.017316 \times S$$

Then

$$u_{pK_2} = \sqrt{\left(\frac{\partial pK_2}{\partial T} \cdot u_T\right)^2 + \left(\frac{\partial pK_2}{\partial S} \cdot u_S\right)^2}$$

$$u_{pK_2} = \sqrt{\left(\left(-4.706\frac{S}{T^2} - 7.17218\frac{1}{T}\right) \cdot u_T\right)^2 + \left(\left(4.706\frac{1}{T} - 0.017316\right) \cdot u_S\right)^2}$$

with u_S and u_T the uncertainties on the salinity and temperature

II.3.4.5 Some values of uncertainties

Hypothesis:

- $T = 25°C$
- $u_T = 0.01$ °C.
- $S = 35$
- $u_S = 0.1$
- $u_{e_1} = u_{e_2} = u_{e_3} = 10^{-6}$
- $R = 1$

$R = 1$ / u_R	0.1	0.01	0.001	0.0001
pH=8.1763 / u_{pH}	0.0464	0.0047	5.53E-04	3.05E-04

III. Experiments, results and discussions

III.1 Verification of the theory by simple flow cell

III.1.1 Experiment

In a 25 mL cylindrical Flowcell (HELLMA 120-OS 10cm pathlength) we mix 24 mL of TRIS Buffer, with Thymol Blue $5x10^{-4}$ mol/kg, added subsequently 100uL by 100 uL. Temperature is recorded with thermocouple calomel and spectra are acquired via a Ocean Optics spectrometer: HR4000CG-UV-NIR. The light sources are the LEDs and Temperature is measured with thermocouples. (The use of thermocouples is not recommended as it interacts with TRIS Buffer)

III.1.2 Calculation of the ratio of absorbances *R* and improvement by averaging

Hypothesis:

- Uncertainty on T is 0.05 °C
- Uncertainty on S is 0.01
- Uncertainty on e_i is 10^{-9}

The pH of the TRIS Buffer can be determined by the temperature dependent equation:

(19) $$pH = -22.5575 + 3477.5496 \cdot \ln(T) - 0.013755 \cdot S + 0.00006165 \cdot S^2 + 6.313\frac{S}{T}$$

At S = 35 and T = 23°C, pH=8.1215.

As shown on **Figure 15,** there is an improvement on the uncertainty on R, by averaging on the signals at each wavelength, on blank and measurements. Knowing that, we have recalculated R, u_R until the Full Width Half Max of each wavelength, for every signals, see **Table 6.** Note that the pH has been calculated without doing a ΔR correction.

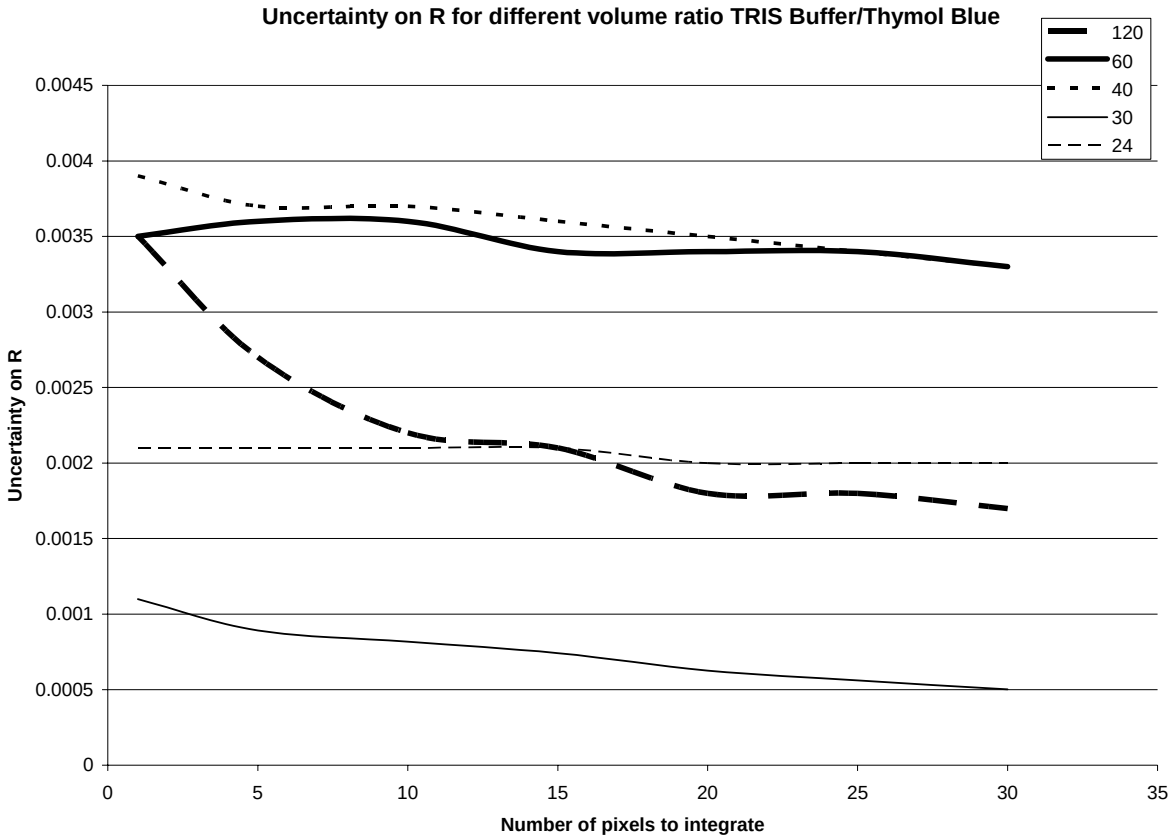


Figure 15: Convergence by integration on more than one pixel, using Ocean Optics spectrometer (HR4000CG-UV-NIR)

ratio TRIS Buffer/TB (approx)	R MATLAB	UNCERTAINCY R	Temperature °C	pH	UNCERTAINTY pH
240	0.8272	0.0232	23.6	8.1054	0.0017
120	0.787	0.0017	23.8	8.0803	0.0016
80	0.7949	0.0014	23.8	8.0848	0.0015
60	0.822	0.0033	23.8	8.1002	0.0023
48	0.8211	0.0018	23.9	8.0985	0.0016
40	0.8611	0.0033	23.9	8.1203	0.0022
34	0.8671	0.0012	24	8.1223	0.0014
30	0.8702	4.94E-04	24	8.1239	0.0013
26	0.8859	8.66E-04	23.9	8.1334	0.0014
24	0.8699	0.002	24	8.1238	0.0017

Table 6: pH calculation by integrating on the FWHM of each wavelength

We also notice the importance of having a ratio TRIS Buffer/TB. Here, 30 volumes for 1 give the best uncertainty on the final pH.

III.2 Test of the prototype

The spectrometer used for developing the sensor is the Ocean Optics HR4000CG-UV-NIR. It has an higher resolution than the HAMAMATSU (2048 pixels against 256), it should give more details and default of the system. The HAMAMATSU spectrometer

will find again its place on the device when results show that we can have a lower resolution.

III.2.1 Mixing time

While developing the software, Thymol Blue has been replaced by a green food dye colorant. It allowed giving some approximations about the Taylor-Aris dispersion. Using water as sample, 10uL of green dye has been injected with a syringe and mixed by running the pump in closed loop. The operation is redone the previous mix is used as sample. The pump used is the INSTECH 625/900.143 which flow rate is 1mL/min.

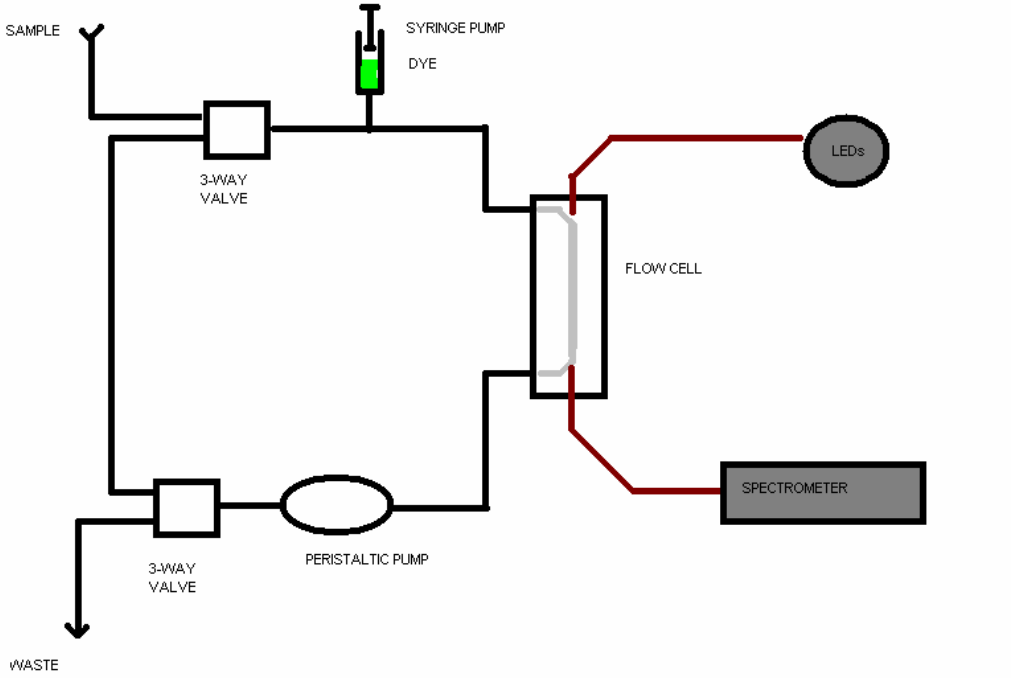


Figure 16: System developed to study the mixing time

Mixing times for four consecutive injections are plotted **Figure 17.**

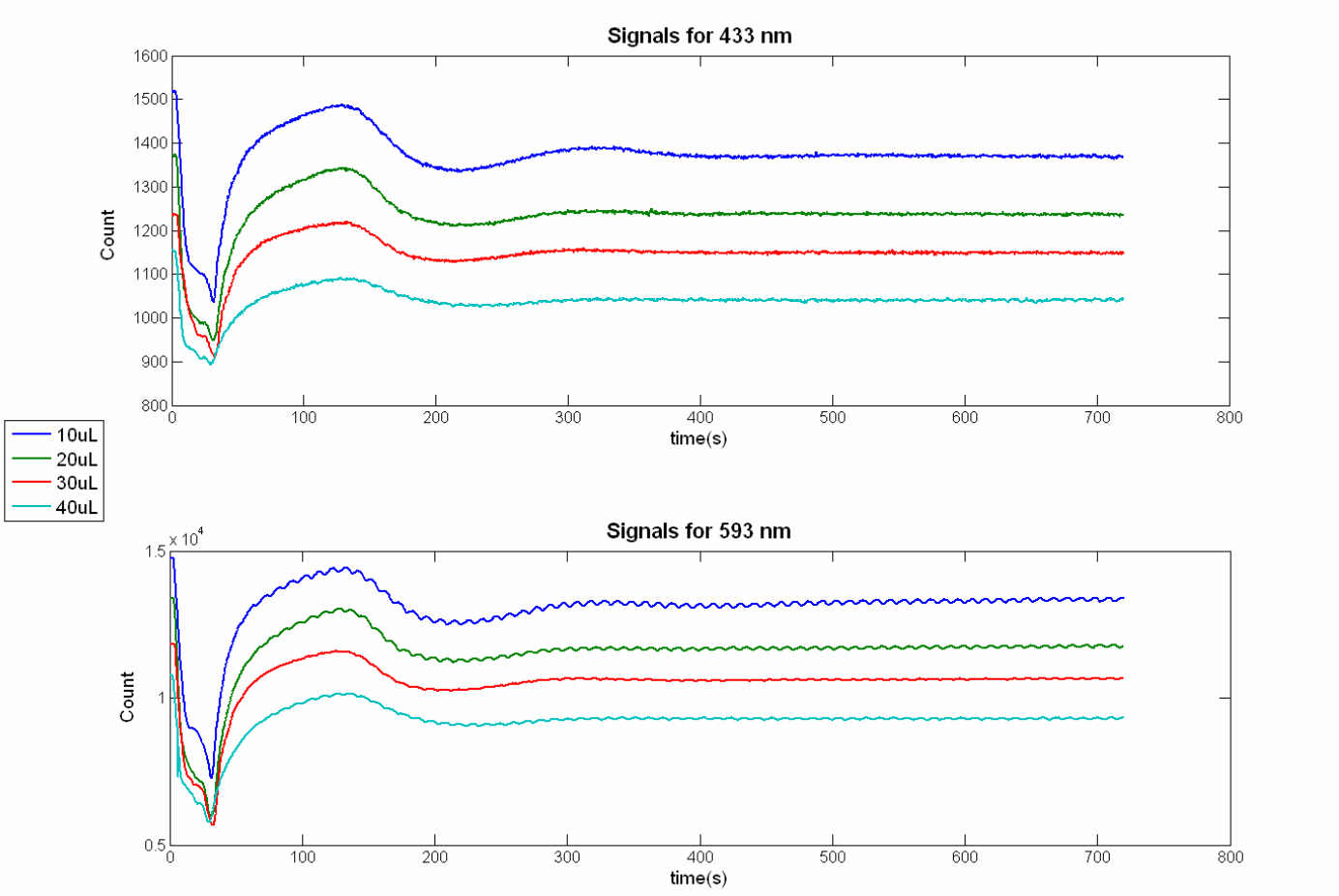


Figure 17: Acquisition of signal when spreading 10uL in an homogenous sample

The band of dye injected is spread and pass few times in the flowcell. This cause the attenuated oscillations at low frequency. We can see that the stabilization is done after 400 seconds. We also note a slightly drift of this curves. This is due to the diffusion in the pipe connected to the syringe. Signals are also noised by a sinusoid which frequency is the rotation speed of the pump.

We can avoid such noise by applying a filter wit a cut frequency below the pump rotation speed. But when using an other pump with higher speed, the noise disappear. The drift stay and the mixing time becomes lower : using the Williamson pump with a flow rate at 5mL/min, the mixing time decrease to 40 seconds. To decrease the mixing time we can decrease the length of the tubes. This becomes hard to conceive as we are limited by their flexibility and by the technique used to plug Minstac connectors.

Our final system is slightly different: The peristaltic pump inside the closed loop is located between the two valves; the syringe is replaced by a Lee pump (which is injecting a precise amount of dye); A second peristaltic pump is place outside the closed loop and ensure the flush of the system. The sample is TRIS-buffer.

In practice, we will add 50uL, in the sample. The diffusivity of the Thymol Blue is different from the green food colorant. Chemical reactions will appears, it is no more a dissolution. The dye concentration will be also different. As a result, we can expect to a very high time of mixing. To confirm it, a try has been run and result is shown **Figure 18**. Here only one injection has been plotted, and we observe the peaks of the

two interesting wavelengths: 435nm and 595 nm. On this figure we can see that the signal is not stabilized after 900 seconds (15 minutes).

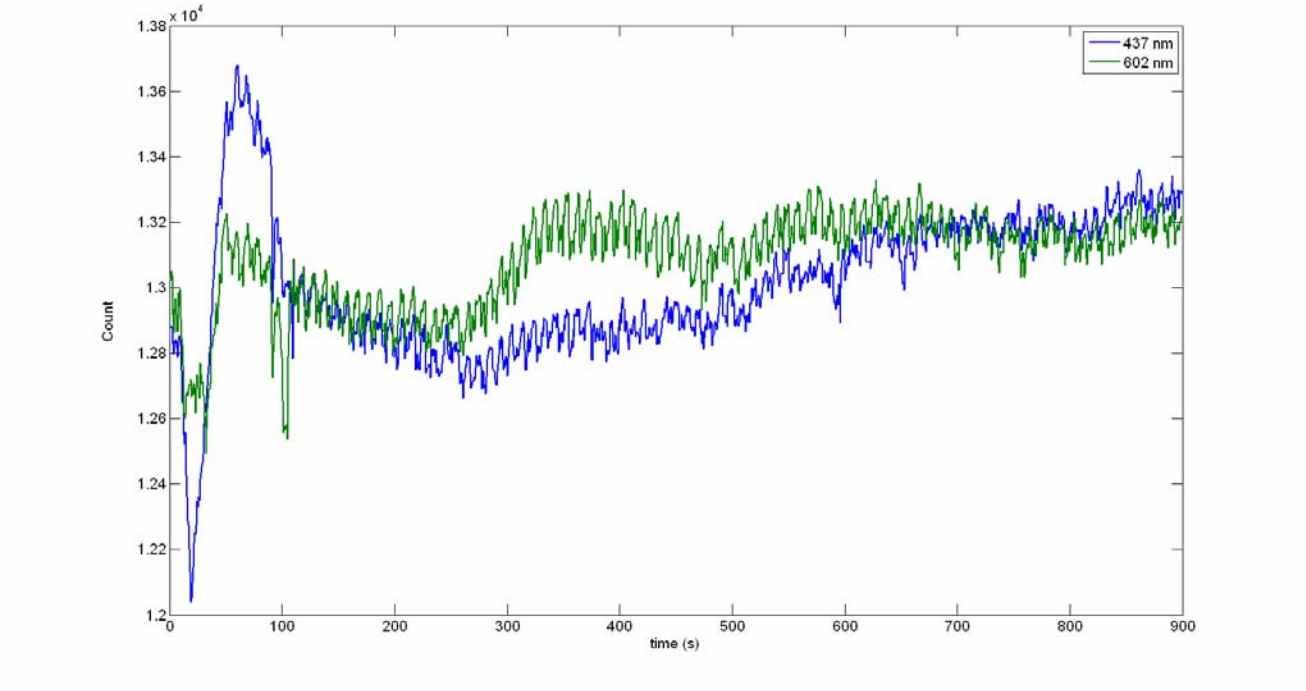


Figure 18: Mixing time measurement on the final system

The mixing time is too long and the quality of the signal is poor. This is due to air bubbles, which are causing a dispersion of the light inside the flowcell. The reason might come from connectors.

Due to this problem, no trustable value of pH has been found with the prototype at the moment. The main issue is the introduction of air bubbles in tube and has to be solved firstly.

IV. Conclusion and further development

By our experiments, we have confirmed that we can have good results on TRIS Buffer using LEDs and a big dimension flowcell. We could have expected better precision and accuracy, using the Z-Flowcell due to no air in tubes. Unfortunately, the development of our pH sensor has been affected by air bubbles trapped in tubes. Other problems appear: electronics failure, computation bugs… Most of practical matters come from connectors.

The main issue to the development of the prototype is the injection and the persistence of air bubbles. The light signal is very sensitive to it and thus the accuracy. Small air bubbles can reduce by half the output signal. This can be improved by using better connectors. We also note that the quantity of air bubbles increase as the flow rate increase. We could then reduce the flow rate, but it will make the system very slow. Reducing the speed of the pumps will make a low mixing time then a low rate in measurements.

As shown Chapter III.1.2 (Calculation of the ratio of absorbances *R* and improvement by averaging), accuracy can be optimized by choosing an appropriate ratio Thymol Blue – Seawater. This can be done by playing on three parameters: The initial concentration of TB (in the reagent bag), the internal volume in closed loop (adding or removing length's tubes), the internal volume of flow components, mainly valves. This last case can be planed only during the reduction of the system's size.

The majority of measurements has been done with the Ocean Optics spectrometer, which include 4000 pixels. Replacing it by the HAMAMATSU (256 pixels) need to review computed programs, and adapt them with the correct relationship number of pixels-wavelength. We also need to be sure that the new resolution – four times less – will not affect the precision and the accuracy on pH measurements.

In the way to decrease the mixing time we suggest to build a system with smaller tubes and components, and limit the numbers of fluidic connectors.

The long term deployment needs to be considered. Some troubles appeared in the software (problem with the MATLAB "wait" function). For long periods we cannot use PC as it is high power consuming. The software can be transcript for microcontrollers. We need to calculate the autonomy of our prototype, based on the volume of the reagent bag, the sampling rate and power consumption. Biofouling by micro-organism can appear and may disturb the pH values.

Once the bench-top system will be optimized, we can start to convert it to an in-situ sensor. With the development of the lab-on-chip systems we can expect to reduce considerably the size of the sensor. The consumption of the sulphonphtalein indicator will also decrease, from microlitre to nanolitre. Size can be gained by using a photodiode instead the spectrometer, which also decrease the weight of the prototype, and making it more robust to shocks. The electronic will be reduced to one single chip. All the system might be potted to resist to high pressures.

V. Personal outcomes

These five months spent at the National Oceanography Centre, was a first experience on a long research project. Starting from basic knowledge on Chemistry, and general skills in Electronics, Optics, Fluidics and Computing, I could develop a prototype of a pH sensor for seawater measurement.

The work started by a long literature review, and I tried to understand the basic principles of pH measurement by spectrophotometric method, as well as its practical limits: determination of Thymol Blue's coefficients, Temperature-Pressure dependence…

It was then good to see how publications are written. I could also note that some of them can be classified as fundamentals (Byrne 1987, Clayton and Byrne 1993, Zhang and Byrne 1996). Other publications were not very accurate by their methodology (Yao and Byrne 2007, Ohline et al. 2007), but show some points to take care when calculating the pH. For the most part, the Thymol Blue's coefficients are taken in Zhang and Byrne 1996, leading probably to wrong results.

I discovered the Microfluidics systems, the principles of the Taylor-Aris dispersion. I could be familiarized with a CTD cast, and work in various domains. My skills in computing in MATLAB language increased: compilation of C/C++ files, Data Acquisition USB card control.

The main matters during the placement were technical: software issues, plug-in not detected, no C/C++ compiler installed on my computer, problem with the license manager, server cuts, lack of tools in the electronic lab when cruise were organized, parts to order in United States…All these minor facts are in fact very time consuming, and have slowed me in my work. Moreover, because they were not dependant on me, it acted on my moral. Other technical issue where: air bubbles and robustness of connectors.

The atmosphere at work was very good, and it was a pleasure for me to work alone on this project. I could work step by step at my speed, without rushes. The communication with other members of the sensors group was friendly, and being understood did not cause problems. My supervisor helped me when I needed. I could ask other peoples when he did not know the answer to my questions, or when he was away. He also gave me some ideas to straight the development of the pH sensor.

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APPENDIX I. Characterisation of the Thymol Blue

Protocol to obtain *Huining Zhang* and *Robert H. Byrne* formulas, depending on temperature and salinity

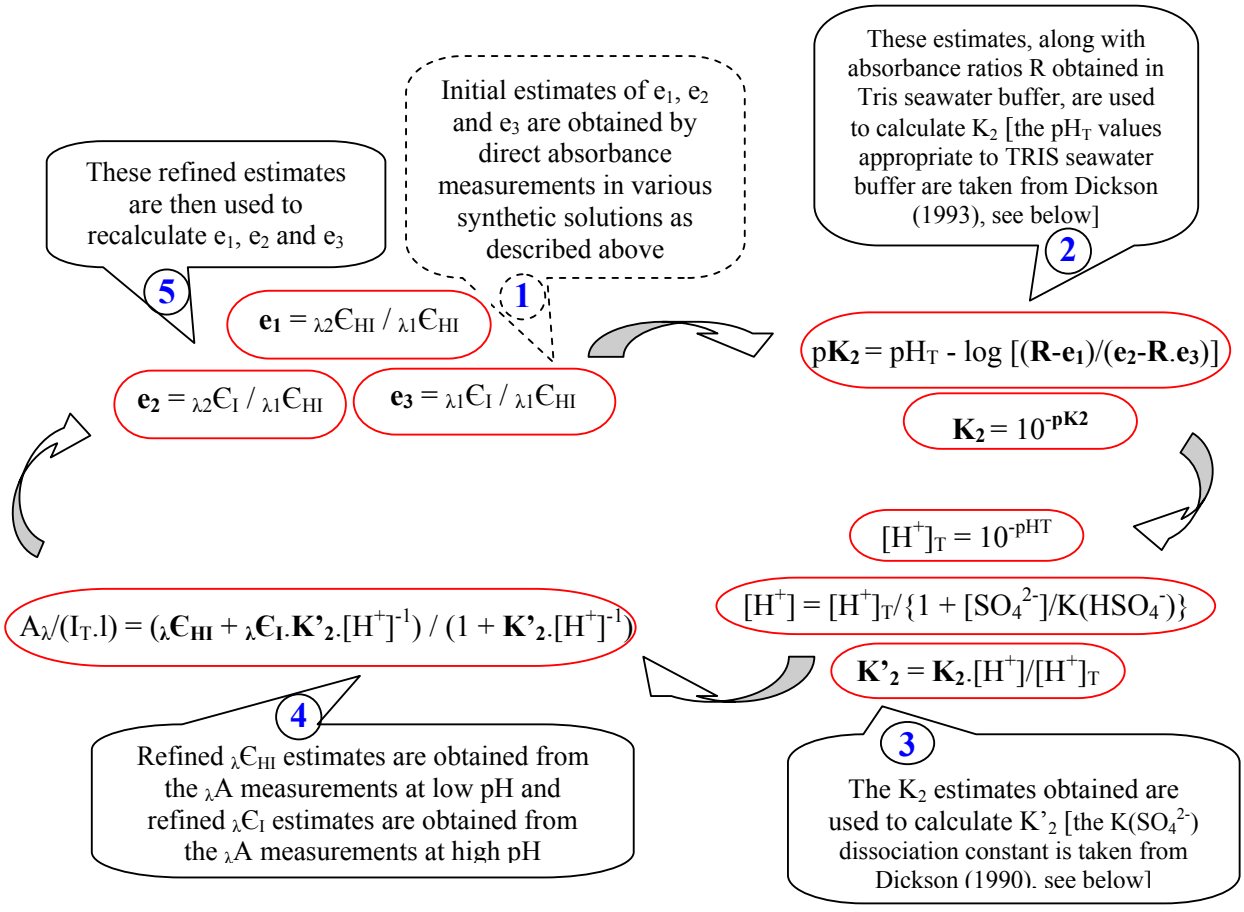
Laboratory method

The determination of Thymol Blue's coefficient is done in laboratory, by an iterative method as described below. The initialization of the loop is the introduction of estimated e_i's by absorption measurements of TB in various synthetic solutions. Experiments are described below.

In order to make very small corrections to the direct measurements of ${}_{\lambda 2}\epsilon_I$, ${}_{\lambda 1}\epsilon_I$, ${}_{\lambda 1}\epsilon_{HI}$ and small corrections to the measurements of ${}_{\lambda 2}\epsilon_{HI}$, we use this formula:

$$A_\lambda/(I_T.l) = ({}_\lambda\epsilon_{HI} + {}_\lambda\epsilon_I.K'_2.[H^+]^{-1}) / (1 + K'_2.[H^+]^{-1})$$

- I_T: total indicator concentration, equal to $[I^{2-}]$ under sufficiently alkaline conditions and to $[HI^-]$ under sufficiently acidic conditions.
- $K'_2 = [I^{2-}][H^+] /[HI^-]$ *Thymol blue dissociation constant expressed in terms of free hydrogen ion concentration*



K_2 and e_i determinations are repeated until no significant changes in either dissociation constants or molar absorption ratio are observed.

1

0.7 mol/L ionic strength solution containing ≈ 0.06 mol/L NaAc + 0.02 mol/L HAc (pH = 5.0)

→ Measure $A_1(HI^-)$ → $\lambda_1\epsilon_{HI} = A_1(HI^-)/[HI^-]l$
1 cm pathlength cell

→ Measure $A_2(HI^-)$ → $\lambda_2\epsilon_{HI} = A_2(HI^-)/[HI^-]l$
10 cm pathlength cell

$\mathbf{e_1} = \lambda_2\epsilon_{HI} / \lambda_1\epsilon_{HI}$

- **Estimation of e_2**: 2 titrations

Basic titration

→ Measure $A_2(I^{2-})$ until absorbance do not increase with further titrant addition

→ $\lambda_2\epsilon_I = A_2(I^{2-})/[I^{2-}]l$

$[I^{2-}]$: alkaline conditions

Acid titration

→ Measure $A_1(HI^-)$ until absorbance do not increase with further titrant addition

→ $\lambda_1\epsilon_{HI} = A_1(HI^-)/[HI^-]l$

$[HI^-]$: acidic conditions

$\mathbf{e_2} = \lambda_2\epsilon_I / \lambda_1\epsilon_{HI}$

- **Estimation of e_3**:

0.02 mol/L NaOH (μ = 0.7 mol/L)

→ Measure $A_1(I^{2-})$ → $\lambda_1\epsilon_I = A_1(I^{2-})/[HI^-]l$
10 cm pathlength cell

0.7 mol/L ionic strength solution containing ≈ 0.06 mol/L NaAc + 0.02 mol/L HAc (μ = 0.7 mol/L)

→ Measure $A_1(HI^-)$ → $\lambda_1\epsilon_{HI} = A_1(HI^-)/[HI^-]l$
5 cm pathlength cell

$\mathbf{e_3} = \lambda_1\epsilon_I / \lambda_1\epsilon_{HI}$

2

We obtain pH_T values for Tris buffer from Dickson equation (1993):

$$pH_T(Tris) = (11{,}997.0 + 3.7669\,S + 0.00178\,S^2)\,T^{-1} - 381.3088 - 0.011634\,S + 67.63163\,Ln(T) - 0.121538\,T - \log(1 - 0.00106\,S)$$

Where T is the temperature in K, and S, the salinity

In units of mol.(kg-H_2O)$^{-1}$
A measure of absorption is done on a TRISBuffer in the way to have a ratio of absorbance.

3

The K(SO_4^{2-}) dissociation constant, in units of mol.(kg-H_2O)$^{-1}$, is taken from Dickson (1990a):

$$\ln K(SO_4^{2-}) = (-13856/T + 324.57 - 47.986.\ln T).\mathbf{I}^{0.5} + (35474/T - 771.54 + 114.723.\ln T).\mathbf{I} - 2698/T.\mathbf{I}^{1.5} + 1776/T.\mathbf{I}^2 - 4276.1/T + 141.328 - 23.093.1nT$$
$$\text{With } \mathbf{I} = 0.7223.S/35.0$$

4

- **<u>At low pH</u>** (acidic conditions): HI^- is predominant ↔ $I_T = [HI^-]$ and ${}_\lambda\epsilon_I \approx 0$.

The equation $A_\lambda/(I_T.l) = ({}_\lambda\epsilon_{HI} + {}_\lambda\epsilon_I.K'_2.[H^+]^{-1}) / (1 + K'_2.[H^+]^{-1})$ becomes:

$A_\lambda/([HI^-].l) = {}_\lambda\epsilon_{HI}/ (1 + K'_2.[H^+]^{-1})$

Thus, we obtain:

$\mathbf{{}_{\lambda 1}\epsilon_{HI} = A_1.(1 + K'_2.[H^+]^{-1})/([HI^-].l)}$ for λ_1

And $\mathbf{{}_{\lambda 2}\epsilon_{HI} = A_2.(1 + K'_2.[H^+]^{-1})/([HI^-].l)}$ for λ_2

- **<u>At high pH</u>** (alkaline conditions): I^{2-} is predominant ↔ $I_T = [I^{2-}]$ and ${}_\lambda\epsilon_{HI} \approx 0$.

The equation ${}_\lambda A/(I_T.l) = ({}_\lambda\epsilon_{HI} + {}_\lambda\epsilon_I.K'_2.[H^+]^{-1}) / (1 + K'_2.[H^+]^{-1})$ becomes:

$A_\lambda/([I^{2-}].l) = ({}_\lambda\epsilon_I.K'_2.[H^+]^{-1}) / (1 + K'_2.[H^+]^{-1})$

Thus, we obtain:

$\mathbf{{}_{\lambda 1}\epsilon_I = A_1.[H^+].(1 + K'_2.[H^+]^{-1})/([I^{2-}].l.\ K'_2)}$ for λ_1

And $\mathbf{{}_{\lambda 2}\epsilon_I = A_2.[H^+].(1 + K'_2.[H^+]^{-1})/([I^{2-}].l.\ K'_2)}$ for λ_2

5

We now have to recalculate e_i's
$\mathbf{e_1} = {}_{\lambda 2}\epsilon_{HI} / {}_{\lambda 1}\epsilon_{HI}$
$\mathbf{e_2} = {}_{\lambda 2}\epsilon_I / {}_{\lambda 1}\epsilon_{HI}$
$\mathbf{e_3} = {}_{\lambda 1}\epsilon_I / {}_{\lambda 1}\epsilon_{HI}$

In **APPENDIX II,** a MATLAB program has been computed : TB_coefficients.m.

This method has the advantage to have a fast convergence, and as shown on **Figure 19** and **Figure 20**, with alpha-numbers input in the program:

- A1 =1
- A2=0.8
- l =0.01 (m)
- T=298 (K)
- S=35
- SO4_2=0.1 mol.L^{-1}
- H_lowpH=10^{-3}
- H_highpH=10^{-10}
- HI=10^{-4}
- I2=10^{-4}
- A_1_lowpH=0.95
- A_2_lowpH=0.75
- A_1_highpH=0.95
- A_2_highpH=0.85

We have input for the initialization:

- e_i=10^{-6} for **Figure 19**
- e_i=10 for **Figure 20**

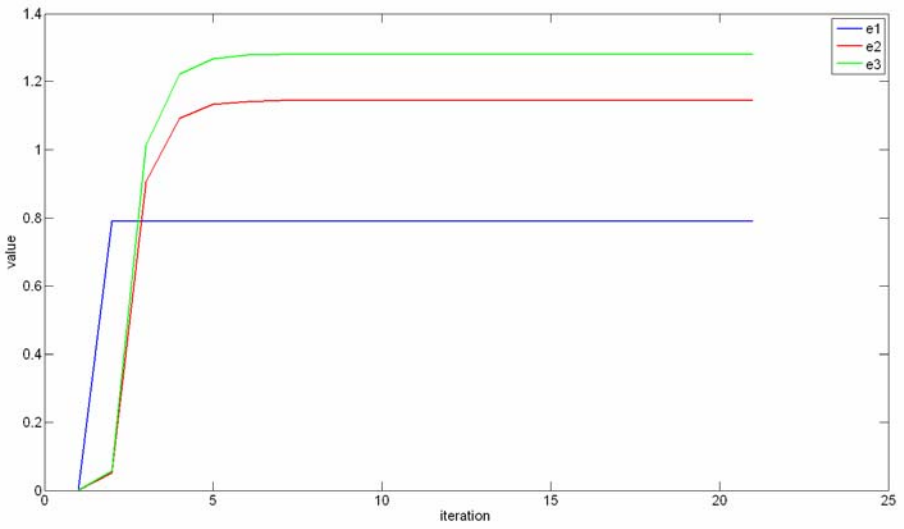


Figure 19: Convergence of the Zhang & Byrne method to refine TB's coefficients

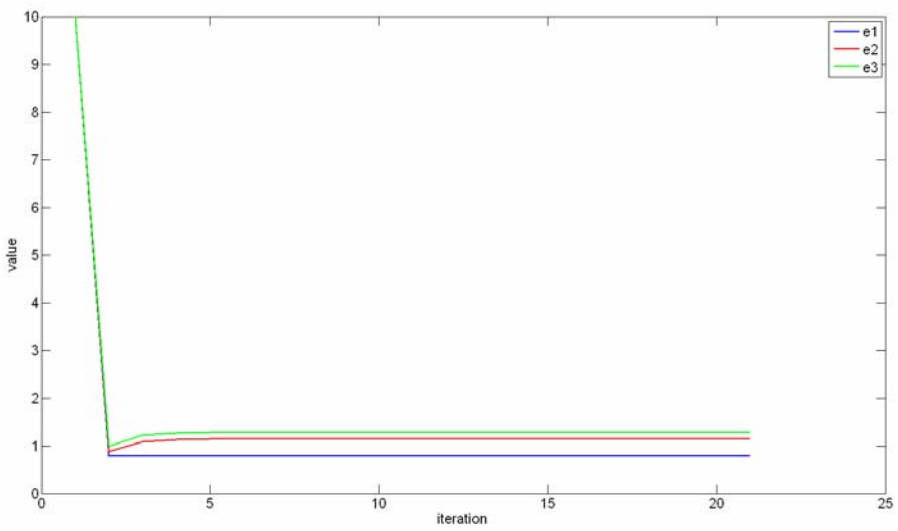


Figure 20: Convergence of the Zhang & Byrne method to refine TB's coefficients

Such implemented method allows having an accurate pK_2 and we can escape to the Zhang & Byrne formula.

The interest of knowing TB's coefficients as the same time of the data acquisitions (in situ refinement)

The main part of publications about pH spectrophotometric sensors is based on the Zhang & Byrne's works, and are using TB's coefficient determined by them. These coefficients are only depending on temperature. Later, Hopkins et al. , 2000 have added a term of pressure to the pK_2 and e_i's. Though it is improving pH measurement, we can be satisfied by this, due to corrections have been studied at constant temperature: 25°C.
Following suggested expressions:

$pK_2 = f(T,S) + g(P)$ with T the temperature in K, S the salinity, P the pressure in

$e_i = g_i(T)$

bars.

Moreover, Hopkins et al. affirm that e_i are not affected by the pressure for depths up to 1 km. We know indeed that temperature and pressure are linked. Then we cannot approve the formula proposed for the pK_2, because we are expecting:

$pK_2 = h(T(P),S)$

$e_i = g_i(T(P))$

Though, it is possible to know in real time TB's coefficient, by measuring the ratio of Absorbances of the test solution as we have done in laboratory (TRISBuffer with Thymol Blue, TRISBuffer's salinity being known), and its temperature. Here the fluid must follow the physical strains –Temperature and pressure– imposed by the system.
Two possibilities can be executed to measure this ratio:

- Doing the same operations as the seawater pH measurement (need valves and Lee Company pump). In this case, a blank can be done every time we want a new R(TRISBuffer)
- We can avoid it by doing single blanks in laboratory for different temperatures. Instead having all the fluidics component, we are using only the flow cell and the peristaltic pump. The fluid inside pipes is the mix Thymol Blue – TRISBuffer, and is pumped when we are measuring its ratio of absorbances.

Then each system can provide *R* and the temperature *T*. In memory, we have approximations of TB's coefficients, and we are using the measured parameters to refine at the medium temperature the TB's coefficients. The MATLAB program, TB_coefficients_realtime.m, availabled in **APPENDIX III,** is a variant of the TB_coefficients.m MATLAB program, but is computed in the way to be implemented in real time; as well we are doing a standard pH measurement.

APPENDIX II. Refefinement of Thymol Blue coefficients: TB_coefficients.m

```
function
[e1,e2,e3]=TB_coefficients(A1,A2,e1,e2,e3,l,T,S,SO4_2,H_lowpH,H_highp
H,HI,I2,A_1_lowpH,A_2_lowpH,A_1_highpH,A_2_highpH)

%iterative method to find e1 e2 e3 (Zhang & Byrne 1996)

%input parameters :
% A1 : absorption at wavelength 1
% A2 : absorption at wavelength 2
% ei : molar absorption ratios of the indicator estimated
% It : total indicator concentration
% l : cell pathlength
% T : temperature of the TRIS buffer
% S : salinity of the TRIS buffer
% mu : density of the TRIS buffer
% SO4_2 : concentration de SO4_2- in the TRIS buffer


%output parameters :
% ei : molar absorption ratios of the indicator obtained after
iterations


% ei : molar absorption ratios of the indicator defined as :
% e1 = E_HI_2 / E_HI_1
% e2 = E_I_2 / E_HI_1
% e3 = E_I_1 / E_HI_1

% R is the ratio of absorbances : R =A2 /A1
% K2 the dissociation constant : K2

% A_1_lowpH: measured absorbance at lamba 1, in a low pH solution
% A_2_lowpH: measured absorbance at lamba 2, in a low pH solution
% A_1_highpH: measured absorbance at lamba 1, in a high pH solution
% A_2_highpH: measured absorbance at lamba 2, in a high pH solution

% H_lowpH: concentration of H+, at low pH
% H_highpH: concentration of H+, at high pH
% I2: concentration of I2- (in high pH solution, alkaline)
% HI: concentration of HI- (in low pH solution, acidic)


      for i=1:20

            % 1st step : Calculate the pHt of the TRIS buffer
(formule Dickson 1993)
            % then the [H+]_TRIS
            pHt_TRIS=(11997.0+3.7669*S+0.00178*S^2)/T -381.3088-
0.011634*S+67.63163*log(T)-0.121538*T-log10(1-0.00106*S);
            Ht_TRIS=10^(-pHt_TRIS);

            % 2nd step : Calculate the pK2 then K2

                % R_TRIS : mesured in a TRIS buffer
                R_TRIS=A2/A1;

            pK2 = pHt_TRIS - log10((R_TRIS-e1)/(e2-R_TRIS*e3));
```

```
            K2 = 10^(-pK2);


            % 3rd step : Calculate the K(SO4 2-) (formule Dickson
1990)

            I = 0.7223* S/35.0;
            log_K_HSO4 = (-13856/T + 324.57 - 47.986 *log(T))* I^0.5
+ (35474/T - 771.54+ 114.723 *log(T)) *I - 2698/T*I^1.5 + 1776/T*I^2
- 4276.1/T + 141.328- 23.093*log(T);

            K_HSO4 =exp(log_K_HSO4);

            % 4th step : Calculate the concentration [H+] : H
            H=Ht_TRIS/(1+SO4_2/K_HSO4);


            % 5th step : Calculate the concentration K2_prime
            K2_prime = K2* H /Ht_TRIS;


            % (Zhang & Byrne 1996, eq. 8) A_lambda / (It*l) =
(E_HI_lambda + E_I_lambda * K2 /[H+] ) / (1 + K2 / [H+])

                % 1st step: at low pH: E_I --> 0 and IT=HI-
                        E_HI_1=
A_1_lowpH*(1+K2_prime/H_lowpH)/(HI*l);
                        E_HI_2=
A_2_lowpH*(1+K2_prime/H_lowpH)/(HI*l);

                % 2nd step: at high pH: E_HI --> 0 and IT=I2-
                        E_I_1=
A_1_highpH*(1+K2_prime/H_highpH)/(I2*l*K2_prime)*H_highpH;
                        E_I_2=
A_2_highpH*(1+K2_prime/H_highpH)/(I2*l*K2_prime)*H_highpH;

                %3rd step : recalculate the ei
                        e1 = E_HI_2 / E_HI_1
                        e2 = E_I_2 / E_HI_1
                        e3 = E_I_1 / E_HI_1

        end
end
```

APPENDIX III. Implemented refinement of Thymol Blue Coefficients: TB_coefficients_realtime.m

```
function [re1,re2,re3,rpK2]=TB_coefficients_realtime(R_TRIS,T,S)
%function [re1,re2,re3,rpK2]=TB_coefficients_realtime(A1,A2,T,S)
%iterative method to find e1 e2 e3 (Zhang & Byrne 1996)

%input parameters :
% A1 : absorption at wavelength 1
% A2 : absorption at wavelength 2
% ei : molar absorption ratios of the indicator estimated
e1=0.0000001;
e2=0.0000001;
e3=0.0000001;
% e1=1;
% e2=1;
% e3=1;

% It : total indicator concentration
% l : cell pathlength
l=0.01; %m
% T : temperature of the TRIS buffer
% S : salinity of the TRIS buffer
% mu : density of the TRIS buffer
% SO4_2 : concentration de SO4_2- in the TRIS buffer
SO4_2=0.1; %mol.L-1


%Measurements done for tarring the system

% A_1_lowpH: measured absorbance at lambda 1, in a low pH solution
A_1_lowpH=  0.85;
% A_2_lowpH: measured absorbance at lambda 2, in a low pH solution
A_2_lowpH = 0.7;
% A_1_highpH: measured absorbance at lambda 1, in a high pH solution
A_1_highpH = 0.9;
% A_2_highpH: measured absorbance at lambda 2, in a high pH solution
A_2_highpH = 0.8;

% H_lowpH: concentration of H+, at low pH
H_lowpH=0.001;
% H_highpH: concentration of H+, at high pH
H_highpH=0.0000000001;
% I2: concentration of I2- (in high pH solution, alkaline)
I2= 0.0001;
% HI: concentration of HI- (in low pH solution, acidic)
HI =  0.0001;




%output parameters :
% ei : molar absorption ratios of the indicator obtained after
iterations


% ei : molar absorption ratios of the indicator defined as :
% e1 = E_HI_2 / E_HI_1
% e2 = E_I_2 / E_HI_1
```

```
% e3 = E_I_1 / E_HI_1

% R is the ratio of absorbances : R =A2 /A1
% K2 the dissociation constant : K2
        for i=1:20
% 1st step : Calculate the pHt of the TRIS buffer (formule Dickson
1993)
% then the [H+]_TRIS
            pHt_TRIS=(11997.0+3.7669*S+0.00178*S^2)/T -381.3088-
0.011634*S+67.63163*log(T)-0.121538*T-log10(1-0.00106*S);
            Ht_TRIS=10^(-pHt_TRIS);

            % 2nd step : Calculate the pK2 then K2
                % R_TRIS : mesured in a TRIS buffer
                %R_TRIS=A2/A1;

            pK2 = pHt_TRIS - log10((R_TRIS-e1(i))/(e2(i)-
R_TRIS*e3(i)))
            K2 = 10^(-pK2);

            % 3rd step : Calculate the K(SO4 2-) (formule Dickson
1990)
            %log_K_HSO4 = (-13856/T + 324.57 - 47.986*log(T))*
mu^(1/2) + (35474/T - 771.54 - 47.986*log(T))*mu - 2698*mu/T +
1776*(mu^2)/T - 4276.1/T + 141.328 - 23.093*log(T)

            I = 0.7223* S/35.0;
            log_K_HSO4 = (-13856/T + 324.57 - 47.986 *log(T))* I^0.5
+ (35474/T - 771.54+ 114.723 *log(T)) *I - 2698/T*I^1.5 + 1776/T*I^2
- 4276.1/T + 141.328- 23.093*log(T);

            K_HSO4 =exp(log_K_HSO4);

            % 4th step : Calculate the concentration [H+] : H
            H=Ht_TRIS/(1+SO4_2/K_HSO4);

            % 5th step : Calculate the concentration K2_prime
            K2_prime = K2* H /Ht_TRIS;

            % (Zhang & Byrne 1996, eq. 8) A_lambda / (It*l) =
(E_HI_lambda + E_I_lambda * K2 /[H+] ) / (1 + K2 / [H+])

                % 1st step: at low pH: E_I --> 0 and IT=HI-
                      E_HI_1= A_1_lowpH*(1+K2_prime/H_lowpH)/(HI*l);
                      E_HI_2= A_2_lowpH*(1+K2_prime/H_lowpH)/(HI*l);

                % 2nd step: at high pH: E_HI --> 0 and IT=I2-
    E_I_1= A_1_highpH*(1+K2_prime/H_highpH)/(I2*l*K2_prime)*H_highpH;
    E_I_2= A_2_highpH*(1+K2_prime/H_highpH)/(I2*l*K2_prime)*H_highpH;

                %3rd step : recalculate the ei
                        e1(i+1) = E_HI_2 / E_HI_1;
                        e2(i+1) = E_I_2 / E_HI_1;
                        e3(i+1) = E_I_1 / E_HI_1;
        end

        figure(1)
            plot(real(e1),'b')
            hold on
            plot(real(e2),'r')
            plot(real(e3),'g')
```

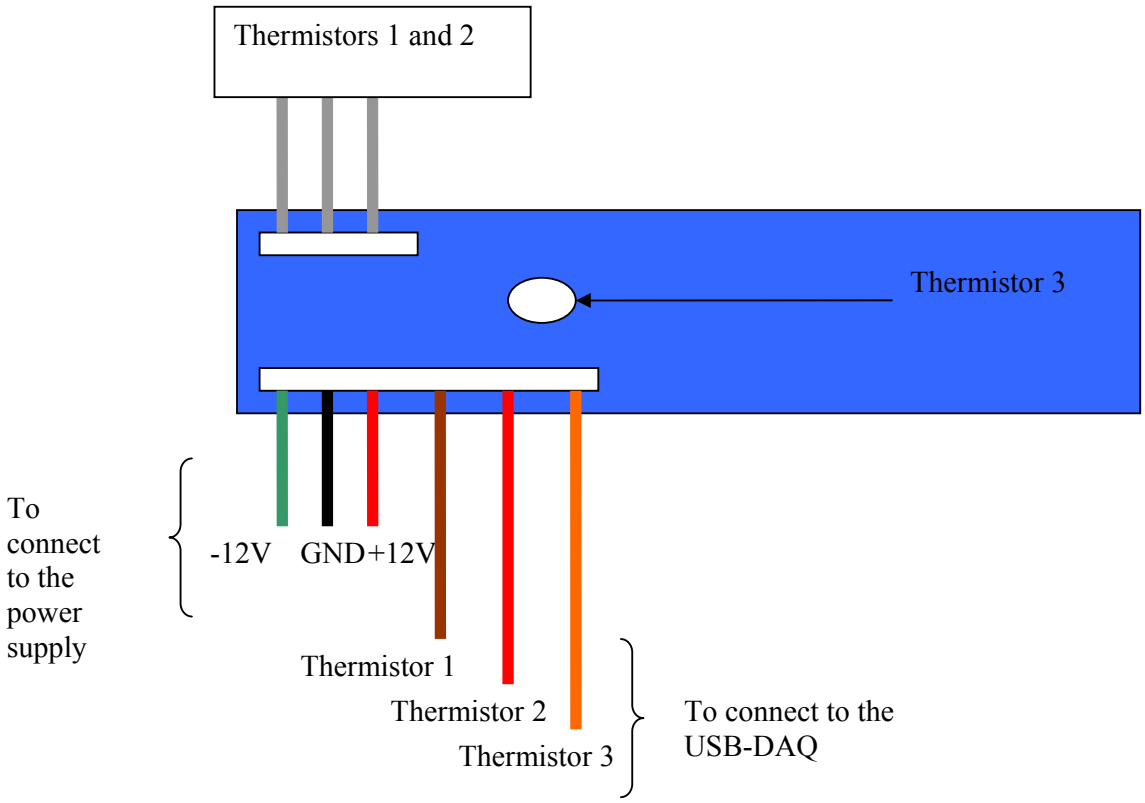
```
        hold off
        xlabel('i')
        ylabel('value')
        legend('e1','e2','e3')

    e1=e1(21);
    e2=e2(21);
    e3=e3(21);
    re1=real(e1)
    re2=real(e2)
    re3=real(e3)
    rpK2=real(pK2)
end
```

APPENDIX IV. Configuration of the National Instrument USB Data Acquisition Board USB-6009

Configuration: Single-Ended

PIN Number	NAME	Connected to
1	GND	Ground
2	AI.0	Thermistor 1
3	AI.4	-
4	GND	
5	AI.1	Thermistor 2
6	AI.5	-
7	GND	
8	AI.2	Thermistor 3
9	AI.6	-
10	GND	
11	AI.3	-
12	AI.7	-
13	GND	
14	AO.0	-
15	AO.1	-
16	GND	
17	P0.0	Internal Peristaltic Pump
18	P0.1	Valve 1
19	P0.2	Valve 3
20	P0.3	Lee Pump
21	P0.4	Valve 2
22	P0.5	External Peristaltic Pump
23	P0.6	-
24	P0.7	-
25	P1.0	Bleu LED
26	P1.1	Orange LED
27	P1.2	Red LED
28	P1.3	-
29	PFI 0	-
30	+2.5 V	-
31	+5 V	-
32	GND	

APPENDIX V. Temperature Board Control

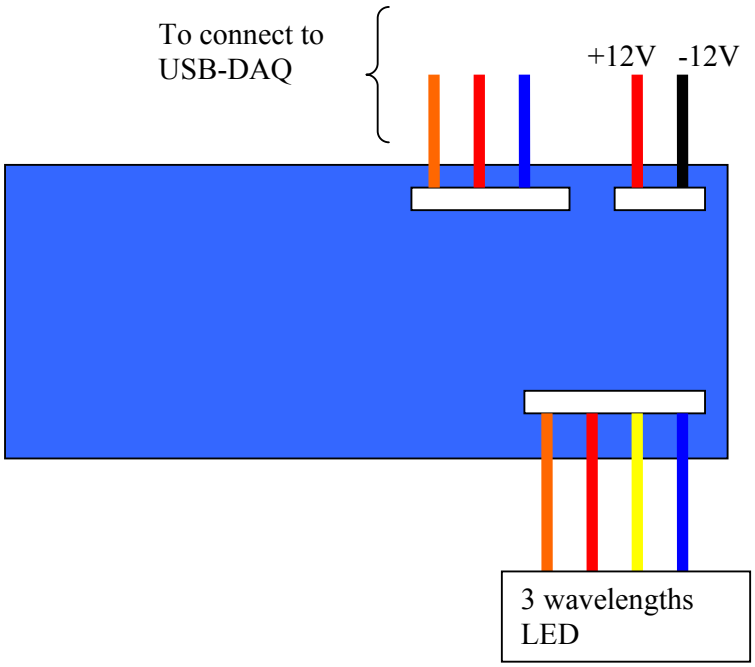


APPENDIX VI. Acquisition of temperatures : Temperature_NI.m

```
function [meanT,sigmaT,meanV,sigmaV]=Temperature_NI
%function Temperature
% program that acquire Voltage from the NI USB-6009
% The DAQ-board is connected to the temperature board sensor
(thermistor)
% Connect the temperature sensor from channel 0 to channel i, without
gap
% between channels!
% It is reading the voltage, calculating the Temperature with
calibration coefficients.
% The outputs are :
% * Average Temperature : meanT
% * Standard Deviation Temperature : sigmaT
% * Average Voltage : meanV
% * Standard Voltage Temperature : sigmaV


%if the device is used by an other program before, it could be cause
a
%troubleshot. Then to solve the problem run  "daqreset"



%SYNTAX as "C function"
%declaration of the board
AI=analoginput('nidaq','Dev1');

duration = 1; %1 second acquisition
%Mode {Differential}|SingleEnded
set(AI,'InputType','SingleEnded');
%Set sampling frequency
set(AI,'SampleRate',10000)
%Getsampling frequency
ActualRate = get(AI,'SampleRate');
%Set Trigger in manual mode
set(AI,'TriggerType','Manual')
%Number of Samples per Trigger(=set Number of scan for average)
set(AI,'SamplesPerTrigger',duration*ActualRate)
%Number of Samples per Trigger (=get the true Number of scan for
average)
blocksize = get(AI,'SamplesPerTrigger');
%Add channels : addchannel(BoardNumber,ChannelNumber)
chan = addchannel(AI,0:1);



%sampling frequency
Fs = ActualRate;

%Acquire data
%Call
start(AI)
%Trigger
trigger(AI)
%Block Windows Commands
```

```
wait(AI,duration+1)
%Get Data
data=getdata(AI);
%End Call
stop(AI)

%Sensor connected to channel 0
%-0.0025x4 + 0.0746x3 - 0.9362x2 + 8.1389x - 6.8611
%calibration coefficient of the transparent heatshrink
a = -0.0025;
b = 0.0746;
c = -0.9362;
d = 8.1389;
e = -6.8611;
T(:,1)=a*data(:,1).^4+b*data(:,1).^3+c*data(:,1).^2+d*data(:,1)+e;

%Sensor connected to channel 1
%-0.0019x4 + 0.058x3 - 0.760x2 + 7.3186x - 5.4356
%calibration coefficient of the transparent heatshrink
a = -0.0019;
b = 0.058;
c = -0.7608;
d = 7.3186;
e = -5.4356;
T(:,2)=a*data(:,2).^4+b*data(:,2).^3+c*data(:,2).^2+d*data(:,2)+e;

meanV=mean(data);
sigmaV=std(data);
meanT=mean(T);
sigmaT=std(T);

clear data
clear T


end
```

APPENDIX VII. LED Board Control



APPENDIX VIII. LED command : LED_NI.m

```
function LED_NI(state)

% Connexions :
% P1.0 : Blue
% P1.1 : Orange
% P1.2 : Red

%Creating Digital I/O
DIO=digitalio('nidaq','Dev1');
%Configure the port in O
addline(DIO,0:3,1,'out');

putvalue(DIO,state)

end
```

APPENDIX IX. Fluidics components command : Pumps_Valve_NI.m

```
function Pumps_Valve_NI(state)

% P0 : INTERNAL PERISTALTIC PUMP
% P1 : VALVE 1
% P2 : VALVE 3
% P3 : LeePump
% P4 : VALVE 2
% P5 : EXTERNAL PERISTALTIC PUMP



 %Creating Digital I/O
    DIO=digitalio('nidaq','Dev1');
    %Configure the port in O
    addline(DIO,0:7,'out');
%output the value
if( state==0 || state==1 || state==38 || state==20 || state==28)
    putvalue(DIO,state)

else
    error('Your Input is FORBIDDEN')
end


end
```

APPENDIX X. Flush.m

```
%completer la condition de melange, interpreter les temps T1, T2, T3
par
%experience et programmation
function Flush
    disp('Flushing')
    T1=1;%time to flush the opened loop: 1s (to change)
    T2=1;%time to flush the residue: 1s (to change)
    T3=1;%time to flush the opened loop the second time: 1s (to
change)
    T4=1;%time to stabilized the: 1s (to change)

   unsufficient_condition=1;

% while(unsufficient_condition)
        AlreadyFlushed=0;


                %PeristalticPump=0
                Pumps_Valve_NI(0)
                %V1=1%V3=1 PeristalticPump=1
                Pumps_Valve_NI(7)
                tic
                while(toc<T1)
                end


                if AlreadyFlushed==0
                    Pumps_Valve_NI(1)
                     tic
                    while(toc<T2)
                    end
                    Pumps_Valve_NI(7)
                     tic
                    while(toc<T3)
                    end
                    AlreadyFlushed=1;
                end


                if AlreadyFlushed==1
                    Pumps_Valve_NI(1)
                     tic
                     while(toc<T4)
                     end
                end

        %unsufficient_condition=???? %to compute
 %end
   Pumps_Valve_NI(0)
    disp('Flush Done')
end
```

APPENDIX XI. IntakeDye.m

```
function IntakeDye

    disp('Intaking Dye')
    Pumps_Valve_NI(0);
    %open valve 3%close valve 3
    %Lee Pump=1 pulse
        T_LeePump=0.5; % Optimal Period for the LeePump

        Pumps_Valve_NI(20);
        tic
        while(toc<T_LeePump)
        end
        Pumps_Valve_NI(28);
        tic
        while(toc<T_LeePump)
        end

    %peristaltic pump=1
    Pumps_Valve_NI(0); %to be sure that the loop is closed before
running the pump
    Pumps_Valve_NI(1);

    %Mixing Time determined by experience
    T_Mixing=1;
   tic
        while(toc<T_Mixing)
        end
    disp('Intake Dye Done')
end
```

APPENDIX XII. DataAcquisition.m

```
function filename=DataAcquisition(type,Nb_scan_Intensity)
    disp('Starting Acquisition')
    %the measurement is done while running the pump
    Pumps_Valve_NI(1)
    %create a file .blk for blank, .mes for measurements
    if type==0
        disp('This is a Blank Measurement')
        s2='.blk';
    elseif type==1
        disp('This is a Measurement')
        s2='.mes';
    end


        s1=datestr(now, 'mmmm-dd-yyyy-HH-MM-SS');
        %change path
        path='C:\Documents and Settings\sylvain\My Documents\SYLVAIN
- PLACEMENT\NOC-placement\program pH sensor\';
        filename=strcat(path,s1,s2);
        f1=fopen(filename,'w');
        date=datestr(now)
        fprintf(f1,'%s',date);

%     LED=0; Blue LED
%     LED=1; Orange LED
%     LED=2; Red LED
for LED=1:3
    LED_NI(2^(LED-1))

    if LED==1
        sLED='BLUE';
    elseif LED==2
        sLED='ORANGE';
    elseif LED==3
        sLED='RED';
    end
   % fprintf(f1,'\n\n%s',sLED);

        %Temperature Acquisition :
        %    T(1)= TFluid= Fluid Temperature (mean)
        %    T(2)=TAmbiant=Ambiant Temperature (mean)
        %    sigmaT(1)=Fluid Temperature (std)
        %    sigmaT(2)=Ambiant Temperature (std)

            [meanT,sigmaT,meanV,sigmaV]=Temperature_NI;

             TFluidBeforeMeasurment=meanT(1);
             TStdFluidBeforeMeasurment=sigmaT(1);
             TAmbiantBeforeMeasurment=meanT(2);
             TStdAmbiantBeforeMeasurment=sigmaT(2);
             VFluidBeforeMeasurment=meanV(1);
             VStdFluidBeforeMeasurment=sigmaV(1);
             VAmbiantBeforeMeasurment=meanV(2);
             VStdAmbiantBeforeMeasurment=sigmaV(2);
            % fprintf(f1,'\nTemperature Before Intensity Acquisition
: ');
             %fprintf(f1,'\n\taverageT\tstdT\t\t\taverageV\tstdT');
```

```
fprintf(f1,'\n\n%f\t%f\t%f\t%f',TFluidBeforeMeasurment,TStdFluidBefor
eMeasurment, VFluidBeforeMeasurment,VStdFluidBeforeMeasurment);
             for i=1:253
                 fprintf(f1,'\t%f',0);
             end

fprintf(f1,'\n%f\t%f\t%f\t%f',TAmbiantBeforeMeasurment,TStdAmbiantBef
oreMeasurment, VAmbiantBeforeMeasurment,VStdAmbiantBeforeMeasurment);
             for i=1:253
                 fprintf(f1,'\t%f',0);
             end

             clear meanT
             clear sigmaT
             clear meanV
             clear sigmaV

            %    TSpectro= Spectrometer's Temperature (mean)
            %     nombre de scans pour acquisition
            Nb_scan_T=30;
                for j=1:Nb_scan_T
                   data(j)=temperature_spectrometer;
                end
            meanT=mean(data);
            sigmaT=std(data);
            TSpectrometerBeforeMeasurment=meanT;
            TStdSpectrometerBeforeMeasurment=sigmaT;


fprintf(f1,'\n%f\t%f\t%f\t%f',TSpectrometerBeforeMeasurment,TStdSpect
rometerBeforeMeasurment,0,0);
             for i=1:253
                  fprintf(f1,'\t%f',0);
              end
             clear meanT
             clear sigmaT

        %Measure Spectrometer
             IntegrationTime=10000;
             Gain=0;
             TriggerEdge=0;
             TriggerMode=0;

             fprintf(f1,'\n');
%              I = rand(10,256);
%
I=Intensity_spectrometer(IntegrationTime,Gain,TriggerEdge,TriggerMode
)
             for i=1:Nb_scan_Intensity
                 index = int2str(i);
                 INDEX=strcat(sLED,' LED ',index,' done');
                 disp(INDEX)
                 IntegrationTime=10000;
                 Gain=0;
                 TriggerEdge=0;
                 TriggerMode=0;

I(i,:)=Intensity_spectrometer(IntegrationTime,Gain,TriggerEdge,Trigge
rMode);
                  tic
                  while(toc<0.1)
```

```
            end
        end

        for i=1:Nb_scan_Intensity
            fprintf(f1,'\n%f',i);
            for j=1:256
                fprintf(f1,'\t%f',I(i,j));
            end
        end

    %Temperature Acquisition :
         [meanT,sigmaT,meanV,sigmaV]=Temperature_NI;

         TFluidAfterMeasurment=meanT(1);
         TAmbiantAfterMeasurment=meanT(2);
         TStdFluidAfterMeasurment=sigmaT(1);
         TStdAmbiantAfterMeasurment=sigmaT(2);
         VFluidAfterMeasurment=meanV(1);
         VAmbiantAfterMeasurment=meanV(2);
         VStdFluidAfterMeasurment=sigmaV(1);
         VStdAmbiantAfterMeasurment=sigmaV(2);


        %  fprintf(f1,'\nTemperature After Intensity Acquisition
: ');

fprintf(f1,'\n\n%f\t%f\t%f\t%f',TFluidAfterMeasurment,TStdFluidAfterM
easurment, VFluidAfterMeasurment,VStdFluidAfterMeasurment);
         for i=1:253
             fprintf(f1,'\t%f',0);
         end

fprintf(f1,'\n%f\t%f\t%f\t%f',TAmbiantAfterMeasurment,TStdAmbiantAfte
rMeasurment, VAmbiantAfterMeasurment,VStdAmbiantAfterMeasurment);
         for i=1:253
             fprintf(f1,'\t%f',0);
         end
         clear meanT
         clear sigmaT
         clear meanV
         clear sigmaV

        %    TSpectro= Spectrometer's Temperature (mean)
         %nombre de scans pour acquisition
         Nb_scan_T=30;
             for j=1:Nb_scan_T
                 data(j)=temperature_spectrometer;
             end

         meanT=mean(data);
         sigmaT=std(data);
         TSpectrometerAfterMeasurment=meanT;
         TStdSpectrometerAfterMeasurment=sigmaT;


fprintf(f1,'\n%f\t%f\t%f\t%f',TSpectrometerAfterMeasurment,TStdSpectr
ometerAfterMeasurment,0,0);
         for i=1:253
              fprintf(f1,'\t%f',0);
          end
         clear meanT
         clear sigmaT
```

```
            LED_NI(0)

end

   %Pour arreter de me peter les burnes quand je teste ce con de
programme
   Pumps_Valve_NI(0)
   disp('Acquisition Done')

 fclose(f1);
end
```

APPENDIX XIII. DataProcessing.m

```
function [R,UR,T,UT]=DataProcessing(B,M,Nb_scan_Intensity)
%Return : Ratio of Absorbtion, Fluid Temperature and their
uncertainties
%load the .blk and .mes
Blank=dlmread(B,'\t',1,0);
Measurement=dlmread(M,'\t',1,0);

%Extract Spectra :
for i=1:Nb_scan_Intensity
    for j=1:256
        BLANK_BLUE(i,j)=Blank(i+3,j+1);
        MEASUREMENT_BLUE(i,j)=Measurement(i+3,j+1);

        BLANK_ORANGE(i,j)=Blank(i+9+Nb_scan_Intensity,j+1);

MEASUREMENT_ORANGE(i,j)=Measurement(i+9+Nb_scan_Intensity,j+1);

        BLANK_RED(i,j)=Blank(i+15+2*Nb_scan_Intensity,j+1);

MEASUREMENT_RED(i,j)=Measurement(i+15+2*Nb_scan_Intensity,j+1);
    end
end

        %read temperature T=[Before After]
        BlankAverageFluidBlue=[Blank(1,1)
Blank(4+Nb_scan_Intensity,1)];
        BlankSigmaFluidBlue=[Blank(1,2)
Blank(4+Nb_scan_Intensity,2)];
        BlankAverageAmbiantBlue=[Blank(2,1)
Blank(5+Nb_scan_Intensity,1)];
        BlankSigmaAmbiantBlue=[Blank(2,2)
Blank(5+Nb_scan_Intensity,2)];
        BlankAverageSpectrometerBlue=[Blank(3,1)
Blank(6+Nb_scan_Intensity,1)];
        BlankSigmaSpectrometerBlue=[Blank(3,2)
Blank(6+Nb_scan_Intensity,2)];

        BlankAverageFluidOrange=[Blank(7+Nb_scan_Intensity,1)
Blank(10+2*Nb_scan_Intensity,1)];
        BlankSigmaFluidOrange=[Blank(7+Nb_scan_Intensity,2)
Blank(10+2*Nb_scan_Intensity,2)];
        BlankAverageAmbiantOrange=[Blank(8+Nb_scan_Intensity,1)
Blank(11+2*Nb_scan_Intensity,1)];
        BlankSigmaAmbiantOrange=[Blank(8+Nb_scan_Intensity,2)
Blank(11+2*Nb_scan_Intensity,2)];
        BlankAverageSpectrometerOrange=[Blank(9+Nb_scan_Intensity,1)
Blank(12+2*Nb_scan_Intensity,1)];
        BlankSigmaSpectrometerOrange=[Blank(9+Nb_scan_Intensity,2)
Blank(12+2*Nb_scan_Intensity,2)];

        BlankAverageFluidRed=[Blank(13+2*Nb_scan_Intensity,1)
Blank(16+3*Nb_scan_Intensity,1)];
        BlankSigmaFluidRed=[Blank(13+2*Nb_scan_Intensity,2)
Blank(16+3*Nb_scan_Intensity,2)];
        BlankAverageAmbiantRed=[Blank(14+2*Nb_scan_Intensity,1)
Blank(17+3*Nb_scan_Intensity,1)];
        BlankSigmaAmbiantRed=[Blank(14+2*Nb_scan_Intensity,2)
Blank(17+3*Nb_scan_Intensity,2)];
```

```
        BlankAverageSpectrometerRed=[Blank(15+2*Nb_scan_Intensity,1)
Blank(18+3*Nb_scan_Intensity,1)];
        BlankSigmaSpectrometerRed=[Blank(15+2*Nb_scan_Intensity,2)
Blank(18+3*Nb_scan_Intensity,2)];
        %read temperature T=[Before After]
        MeasurementAverageFluidBlue=[Measurement(1,1)
Measurement(4+Nb_scan_Intensity,1)];
        MeasurementSigmaFluidBlue=[Measurement(1,2)
Measurement(4+Nb_scan_Intensity,2)];
        MeasurementAverageAmbiantBlue=[Measurement(2,1)
Measurement(5+Nb_scan_Intensity,1)];
        MeasurementSigmaAmbiantBlue=[Measurement(2,2)
Measurement(5+Nb_scan_Intensity,2)];
        MeasurementAverageSpectrometerBlue=[Measurement(3,1)
Measurement(6+Nb_scan_Intensity,1)];
        MeasurementSigmaSpectrometerBlue=[Measurement(3,2)
Measurement(6+Nb_scan_Intensity,2)];


MeasurementAverageFluidOrange=[Measurement(7+Nb_scan_Intensity,1)
Measurement(10+2*Nb_scan_Intensity,1)];

MeasurementSigmaFluidOrange=[Measurement(7+Nb_scan_Intensity,2)
Measurement(10+2*Nb_scan_Intensity,2)];

MeasurementAverageAmbiantOrange=[Measurement(8+Nb_scan_Intensity,1)
Measurement(11+2*Nb_scan_Intensity,1)];

MeasurementSigmaAmbiantOrange=[Measurement(8+Nb_scan_Intensity,2)
Measurement(11+2*Nb_scan_Intensity,2)];

MeasurementAverageSpectrometerOrange=[Measurement(9+Nb_scan_Intensity
,1) Measurement(12+2*Nb_scan_Intensity,1)];

MeasurementSigmaSpectrometerOrange=[Measurement(9+Nb_scan_Intensity,2
) Measurement(12+2*Nb_scan_Intensity,2)];


MeasurementAverageFluidRed=[Measurement(13+2*Nb_scan_Intensity,1)
Measurement(16+3*Nb_scan_Intensity,1)];

MeasurementSigmaFluidRed=[Measurement(13+2*Nb_scan_Intensity,2)
Measurement(16+3*Nb_scan_Intensity,2)];

MeasurementAverageAmbiantRed=[Measurement(14+2*Nb_scan_Intensity,1)
Measurement(17+3*Nb_scan_Intensity,1)];

MeasurementSigmaAmbiantRed=[Measurement(14+2*Nb_scan_Intensity,2)
Measurement(17+3*Nb_scan_Intensity,2)];

MeasurementAverageSpectrometerRed=[Measurement(15+2*Nb_scan_Intensity
,1) Measurement(18+3*Nb_scan_Intensity,1)];

MeasurementSigmaSpectrometerRed=[Measurement(15+2*Nb_scan_Intensity,2
) Measurement(18+3*Nb_scan_Intensity,2)];

        %Compare temperatures Before & After
        %Conclude that if it is acceptable

        %output the mean of temperatures of the fluid (before and
after)
        %during the measurement
```

```
        T=[MeasurementAverageFluidBlue MeasurementAverageFluidOrange
MeasurementAverageFluidRed];
        T=mean(T);
        %uncertainties law propagation

UMeasurementFluidBlue(1)=MeasurementSigmaFluidBlue(1)/sqrt(10000);

UMeasurementFluidBlue(2)=MeasurementSigmaFluidBlue(2)/sqrt(10000);

UMeasurementFluidOrange(1)=MeasurementSigmaFluidOrange(1)/sqrt(10000)
;

UMeasurementFluidOrange(2)=MeasurementSigmaFluidOrange(2)/sqrt(10000)
;

UMeasurementFluidRed(1)=MeasurementSigmaFluidRed(1)/sqrt(10000);

UMeasurementFluidRed(2)=MeasurementSigmaFluidRed(2)/sqrt(10000);



UT=sqrt((UMeasurementFluidBlue(1)^2+UMeasurementFluidBlue(2)^2+UMeasu
rementFluidOrange(1)^2+UMeasurementFluidOrange(2)^2+UMeasurementFluid
Red(1)^2+UMeasurementFluidRed(2)^2)/6);

        %if it is then next step, else, display an error or a warning

        %Average and Standard Deviation the spectra
        for j=1:256
            BLANK_BLUE_average(j)=mean(BLANK_BLUE(:,j));
            BLANK_ORANGE_average(j)=mean(BLANK_ORANGE(:,j));
            BLANK_RED_average(j)=mean(BLANK_RED(:,j));
            MEASUREMENT_BLUE_average(j)=mean(MEASUREMENT_BLUE(:,j));

MEASUREMENT_ORANGE_average(j)=mean(MEASUREMENT_ORANGE(:,j));
            MEASUREMENT_RED_average(j)=mean(MEASUREMENT_RED(:,j));

            BLANK_BLUE_sigma(j)=std(BLANK_BLUE(:,j));
            BLANK_ORANGE_sigma(j)=std(BLANK_ORANGE(:,j));
            BLANK_RED_sigma(j)=std(BLANK_RED(:,j));
            MEASUREMENT_BLUE_sigma(j)=std(MEASUREMENT_BLUE(:,j));
            MEASUREMENT_ORANGE_sigma(j)=std(MEASUREMENT_ORANGE(:,j));
            MEASUREMENT_RED_sigma(j)=std(MEASUREMENT_RED(:,j));
        end

        clear BLANK_BLUE
        clear BLANK_ORANGE
        clear BLANK_RED
        clear MEASUREMENT_BLUE
        clear MEASUREMENT_ORANGE
        clear MEASUREMENT_RED

         %Filter
        FILTER=ones(1,40);
        FILTER_BLUE=[zeros(1,40) FILTER zeros(1,176)];
        FILTER_ORANGE=[zeros(1,122) FILTER zeros(1,94)];
        FILTER_RED=[zeros(1,180) FILTER zeros(1,36)];

        %Dark correction (for each spectrum):
        BLANK_BLUE=BLANK_BLUE_average-
abs(min(BLANK_BLUE_average))*ones(1,256);
```

```
        BLANK_ORANGE=BLANK_ORANGE_average-
abs(min(BLANK_ORANGE_average))*ones(1,256);
        BLANK_RED=BLANK_RED_average-
abs(min(BLANK_RED_average))*ones(1,256);
        MEASUREMENT_BLUE=MEASUREMENT_BLUE_average-
abs(min(MEASUREMENT_BLUE_average))*ones(1,256);
        MEASUREMENT_ORANGE=MEASUREMENT_ORANGE_average-
abs(min(MEASUREMENT_ORANGE_average))*ones(1,256);
        MEASUREMENT_RED=MEASUREMENT_RED_average-
abs(min(MEASUREMENT_RED_average))*ones(1,256);

        n=(1:1:256);
        subplot(2,1,1)
        plot(n,BLANK_BLUE)
        hold on
        plot(n,BLANK_ORANGE)
        plot(n,BLANK_RED)
        hold off

        subplot(2,1,2)
        plot(n,MEASUREMENT_BLUE)
        hold on
        plot(n,MEASUREMENT_ORANGE)
        plot(n,MEASUREMENT_RED)
        hold off

%         BLANK_BLUE=BLANK_BLUE .* FILTER_BLUE
%         BLANK_ORANGE=BLANK_ORANGE .* FILTER_ORANGE
%         BLANK_RED=BLANK_RED .* FILTER_RED
%         subplot(2,1,2)
%         plot(n,BLANK_BLUE)
%         hold on
%         plot(n,BLANK_ORANGE)
%         plot(n,BLANK_RED)
%         hold off

        %Calculate the maximum value for each wavelength and the
uncertainties:
        MAX_BLANK_BLUE=0;
        MAX_BLANK_ORANGE=0;
        MAX_BLANK_RED=0;
        MAX_MEASUREMENT_BLUE=0;
        MAX_MEASUREMENT_ORANGE=0;
        MAX_MEASUREMENT_RED=0;
        for i=1:256
            if BLANK_BLUE(i)>MAX_BLANK_BLUE
                MAX_BLANK_BLUE=BLANK_BLUE(i);

U_BLANK_BLUE=BLANK_BLUE_sigma(i)/sqrt(Nb_scan_Intensity);
            end
            if BLANK_ORANGE(i)>MAX_BLANK_ORANGE
                MAX_BLANK_ORANGE=BLANK_ORANGE(i);

U_BLANK_ORANGE=BLANK_BLUE_sigma(i)/sqrt(Nb_scan_Intensity);
            end
            if BLANK_RED(i)>MAX_BLANK_RED
                MAX_BLANK_RED=BLANK_RED(i);

U_BLANK_RED=BLANK_BLUE_sigma(i)/sqrt(Nb_scan_Intensity);
            end
            if MEASUREMENT_BLUE(i)>MAX_MEASUREMENT_BLUE
                MAX_MEASUREMENT_BLUE=MEASUREMENT_BLUE(i);
```

```
U_MEASUREMENT_BLUE=BLANK_BLUE_sigma(i)/sqrt(Nb_scan_Intensity);
            end
            if MEASUREMENT_ORANGE(i)>MAX_MEASUREMENT_ORANGE
                MAX_MEASUREMENT_ORANGE=MEASUREMENT_ORANGE(i);

U_MEASUREMENT_ORANGE=BLANK_BLUE_sigma(i)/sqrt(Nb_scan_Intensity);
            end
            if MEASUREMENT_RED(i)>MAX_MEASUREMENT_RED
                MAX_MEASUREMENT_RED=MEASUREMENT_RED(i);

U_MEASUREMENT_RED=BLANK_BLUE_sigma(i)/sqrt(Nb_scan_Intensity);
            end
        end


        %coeff Iref2

D1=1/(log(MAX_BLANK_BLUE/MAX_MEASUREMENT_BLUE))*1/MAX_BLANK_ORANGE;
        %coeff I2
        D2=-
1/log(MAX_BLANK_BLUE/MAX_MEASUREMENT_BLUE)*1/MAX_MEASUREMENT_ORANGE;
        %coeff Iref1
        D3=-
log(MAX_BLANK_ORANGE/MAX_MEASUREMENT_ORANGE)*1/(MAX_MEASUREMENT_BLUE*
(log(MAX_BLANK_BLUE/MAX_MEASUREMENT_BLUE))^2);
        %coeff Iref1

D4=log(MAX_BLANK_ORANGE/MAX_MEASUREMENT_ORANGE)*1/((log(MAX_BLANK_BLU
E/MAX_MEASUREMENT_BLUE)^2)*MAX_MEASUREMENT_BLUE);

        A(1)=log10(MAX_BLANK_BLUE/MAX_MEASUREMENT_BLUE);
        A(2)=log10(MAX_BLANK_ORANGE/MAX_MEASUREMENT_ORANGE);
        R=A(2)/A(1);

        UR=sqrt( D1^2 * U_BLANK_ORANGE^2 + D2^2 *
U_MEASUREMENT_ORANGE^2 + D3^2 * U_BLANK_BLUE^2 + D4^2 *
U_MEASUREMENT_BLUE^2 );
end
```

APPENDIX XIV. pH_calculation.m

```
function [pH,UpH]=pH_calculation(R,UR,T,UT)
%formule from Zhang & Byrne
%with coefficients from Zhang & Byrne !!!

%We should input the salinity then we need a salinity sensor
S=35;
US=0.1;
T=273.15+T
e1=-0.00132+1.600e-5*T
e2=7.2326-0.0299717*T+4.600e-5*T^2
e3=0.0223+0.0003917*T

a=4.706;
b=26.3300;
c=-7.17218;
d=-0.017316;

pK2=a*S/T+b+c*log10(T)+d*S
UpK2 = sqrt(((-a*S/(T)^2+c/T)*UT )^2+((a/T+d)*(US))^2)

U_e1=1e-6;
U_e2=1e-6;
U_e3=1e-6;

pH=pK2+log10((R-e1)/(e2-R*e3));

D_pK2=1;
D_R=(1/log(10))*((e2+e1*e2)/((e2-R*e3)*(R-e1)));
D_e1=(1/log(10))*(-1/(R-e1));
D_e2=(1/log(10))*(-1/(e2-R*e3));
D_e3=(1/log(10))*(R/(e2-R*e3));

UpH=sqrt( (D_pK2 * UpK2)^2 + (D_R * UR)^2 + (D_e1 * U_e1)^2 + (D_e2 *
U_e2)^2  +(D_e3 * U_e3)^2 );
end
```

APPENDIX XV. DRMeasurement.m

```
function filename=DRMeasurement(B,Nb_scan_Intensity)

    %create New Folder : DR-date-time
        s1=datestr(now, 'mmmm-dd-yyyy-HH-MM-SS');
        s2 ='.dr';
        path='C:\Documents and Settings\sylvain\My Documents\SYLVAIN
- PLACEMENT\NOC-placement\program pH sensor\';
        path2='DeltaR Measurement\';

        dir=strcat(path,path2);
        mkdir(dir);
        filename=strcat(path,path2,s1,s2);
        f1=fopen(filename,'w');
        date=datestr(now)
        fprintf(f1,'%s\tBlank : %s',date,B);

    %number of repetition of injection of dye
    NMDR=5;
    for n=1:NMDR
         IntakeDye;
         M=DataAcquisition(1,Nb_scan_Intensity);
         [R,UR,T,UT]=DataProcessing(B,M,Nb_scan_Intensity)
         fprintf(f1,'\n%f\t%f\tMeasurement : %s',R,UR,M);
    end

disp('DR Measurement Done')
fclose(f1);
end
```

APPENDIX XVI. main.m

```
function pH=main
%main program
clc
%initialisation :
%all valves closes
%all pump off
%all LED of
Pumps_Valve_NI(0)
LED_NI(0)
%scans for average
Nb_scan_Intensity=10;
NbMes=input('How many measure do you want to do  ?');
Blk=input('Do you want to measure blank(s) ?    [y/n]','s');
% Blk='y';
MDR=input('Do you want to measure {\Delta}R ?    [y/n]','s');
%RIL=input('Do you want to run in Loop the system ?    [y/n]','s');
if Blk=='y'
    DelayBlk=input('How many measures do you want to do between two
blanks ?');
end

%creating a file .pH which will contain every pH measured, with date
path='C:\Documents and Settings\sylvain\My Documents\SYLVAIN -
PLACEMENT\NOC-placement\program pH sensor\';
s1=datestr(now, 'mmmm-dd-yyyy-HH-MM-SS');
s2='.ph';
filename=strcat(path,s1,s2);
f1=fopen(filename,'w');

for n=1:NbMes
    Flush;                                                  %appelle
de la fonction marche
     if Blk=='y'
         if n~=DelayBlk || n==1
         B=BlankMeasurement(Nb_scan_Intensity);
         end
     elseif Blk=='n'
         B=input('Input the path of the blank for data
processing','s')
     end
    M=SingleMeasurement(Nb_scan_Intensity);
    if MDR=='y'
        DR=DRMeasurement(B,Nb_scan_Intensity);
%     else
%         DR=input('what is the correction to bring to the measure of
R (pH modified by the dye injection) :   ')
%     end
%   R=R-DR;
    end
    [R,SigmaR,T,SigmaT]=DataProcessing(B,M,Nb_scan_Intensity);
    [pH,SigmapH]=pH_calculation(R,SigmaR,T,SigmaT)
    date=datestr(now)
    fprintf(f1,'%s\t%f\n',date,pH);
end
fclose(f1);
Pumps_Valve_NI(0)
end
```

```
%function BlankMeasurement
function B=BlankMeasurement(Nb_scan_Intensity)
disp('Starting a Blank Measurement')
    %return the path of the file
     B=DataAcquisition(0,Nb_scan_Intensity);
disp('Blank Measurement Done')
end
```

```
%function Single Measurement
function M=SingleMeasurement(Nb_scan_Intensity)
disp('Starting a Single Measurement')
    %return the path of the file
    IntakeDye;
     M=DataAcquisition(1,Nb_scan_Intensity);
disp('Single Measurement Done')
end
```