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Continuous, on Site Spectrophotometric Determination of Zinc ions in Water

Laboratory test preliminary to microfluidic device prototyping

Monica Periolatto*, Felice Catania, Luciano Scaltrito, Candido Fabrizio Pirri, Matteo Cocuzza, Sergio Ferrero

***Abstract*—Main parameters of the Zincon® method for spectrophotometrical detection of Zn (II) metal ions in water were optimized for application on a miniaturized and portable device, for continuous monitoring of water pollution. The aim was the reduction of involved volumes, manageable by a microfluidic device, the choice of suitable solvents and buffers, the optimization of reagents amounts and aging times and the irradiation of the sample by a single wavelength visible source. The influence of each parameter was investigated individually with respect to a reference method; finally, all the optimized values were merged and verified on a bench prototype to test any possible mutual effect.**

***Keywords*—Zinc, Zincon, water pollution, water analysis, process optimization**

I. Introduction

Pure zinc is a blue-white, shiny, brittle metal. It has a relatively low melting point (420°C) and conducts electricity fairly well. Compounds of zinc are usually highly colored.

Zinc and its compounds have a variety of uses: in corrosion resistant coatings, in dry-cell batteries, in alloys (mixtures of metals) such as brass and bronze and in the manufacture of paints, plastics, rubber, dyes, wood preservatives and cosmetics. It may enter the aquatic environment through natural or anthropogenic sources, including sewage and industrial discharges.

Releases of zinc can significantly affect local aquatic environments, accumulating in aquatic organisms (but not plants) and poisoning species that then eat them along the food chain. [1]

Zinc is an essential nutrient for humans, but excessive amounts (more than twice the recommended daily intake) can be harmful. Exposure to potentially harmful levels usually occurs through drinking of contaminated water, due to zinc water pipes or accidental industrial releases. Effects include nausea and stomach cramps.

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Long-term exposure to high concentrations may cause "metal fume fever" which affects the lungs and the body's temperature control system.

European Directives covering the control of zinc emissions include those concerned with the evaluation and control of risks posed by certain substances (793/93), the treatment of hazardous wastes (2268/95) and the pollution of the aquatic environment (76/464). At an international level, releases of zinc are controlled through the OSPAR convention on protection of the marine environment of the north-east Atlantic and the Basel convention on the transboundary movement and disposal of hazardous wastes. Zinc is also listed as a priority substance under the Helsinki convention which protects the marine environment of the Baltic sea. [2]

For these reasons, metal speciation in environmental waters is widely considered relevant to characterize the metal toxicity, the mobility between different environmental compartments and for remediation purposes in case of contamination. [3, 4]

The determination of metal species and of the free metal ion concentration in environmental samples is difficult because of the very low concentrations and of the presence of interferences. There is a limited number of methods with the required selectivity and detection limit, namely mass spectrometry techniques, neutron activation analysis, potentiometry with ion selective electrodes or voltammetries.

All these methods are precise and reliable, but they have to be carried out in a lab; this involves a delay with respect to the sampling and, as consequence, the impossibility for a continuous monitoring of productive sites or for a ready identification of pollution sources.

Moreover, none of them is suitable for a miniaturization on a portable device: high energy demanding detectors are required, working in controlled atmosphere; preconcentration steps, flowing on separation media or adsorption columns are necessary in many cases; the main components of each apparatus are very expensive; furthermore only the human operator can guarantee the control of the process in all the steps.

Considering all these drawbacks, authors focused on spectrophotometry as a viable way to reach the goal. The common principle is that the sample is mixed with reagents, which react specifically with the constituent to be analyzed. In most cases a colored compound or complex is formed. The intensity of the color is a measure for the concentration and can be quantified using UV/Vis spectroscopy. Here the attenuation of radiation of a specific wavelength can be

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measured and a relation between absorbance and concentration is made by establishing a calibration curve. The absorbance increases with the concentration of the analyte in water according to the well-known Lambert & Beer law. [5]

A spectrophotometric method for Zinc (II) determination in water, widely reported in literature, is based on the complexation of Zinc ions by Zincon (2-carboxy-2′-hydroxy-5′-sulfoformazylbenzene) one of the most common chromophoric chelating probes for the determination of Zn^{2+} ions. [6, 7] The method is reported as easy, cost effective and precise in a range of zinc (II) concentration between 0.5 to 8 ppm, using analysis cuvettes with an optical path of 10 mm. [8]

Zincon is insoluble in organic solvents but slightly soluble in water, which is one of the advantages leading to the use of this organic reagent as complexing in aqueous solution to avoid the use of expensive and/or hazardous organic solvents.

The analysis is based on the formation, in buffered conditions (pH 9), of the hydro-soluble complex Zn/Zincon with a clear blue color and a peak of absorbance at 618 nm. The stoichiometric ratio of chelation is 1:1; one molecule of Zincon can complex a Zn^{++} ion according the scheme reported in Figure 1. The relatively high molar absorptivity of Zincon, that is 24,200 M^{-1} cm^{-1} at 618 nm, makes it a sensitive complexing agent for zinc (II) determination. [9]

This method is precise and reliable, but it has to be carried out in a lab; it means a delay with respect to the sampling and, as consequence, the impossibility for a continuous monitoring of productive sites or for a ready identification of pollution sources. At the best of our knowledge, an easy and low cost instrument, that can work in continuous, providing in real time precise and reliable results is not yet present on the market but would be of great interest, mainly in the field of environmental protection and control.

The aim of our research, part of a wider project about innovative technologies for offshore platforms safety and environmental monitoring, was filling this gap. [10] The main process parameters of the standard method were here optimized to be moved to a miniaturized and portable device where reagents and samples will be dosed in continuous by a microfluidic electronic system able to manage minimum volumes of liquids. For sample irradiation a laser diode emitting at the required wavelength will be used, instead of a tungsten lamp coupled with a monochromator. Data will be collected and processed by a software providing the metal concentration, alarming in case of legal limit overcoming.

II. Experimental

A. *Materials and methods*

Zincon standard method, as reported in literature, was considered as reference. [11] (Figure 2)

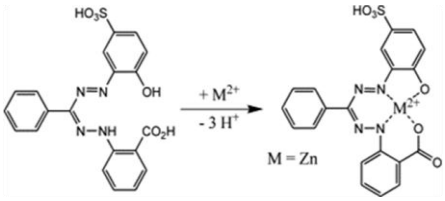


Figure 1. Zincon chemical structure in its free form (left) and metal-chelated (right).

Analytical grade general reagents were used.

For the calibration curve, 20 mL samples of Zn(II) solutions were prepared, with concentrations between 0.5 and 10 ppm, properly diluting the standard Zn(II) solution at 1000 ppm (Sigma Aldrich).

A 1 g/L Zincon (Sigma Aldrich) solution was prepared diluting the proper amount of Zincon in a water solution 2%vol of NaOH 1N (pH 12-13).

The basic buffer (boric acid/potassium chloride/sodium hydroxide, pH 9) was purchased by Sigma Aldrich, while for further test the pH 8.5 buffer was laboratory prepared by solving 4 g NaOH and 6,2 g H_3BO_3 (Sigma Aldrich) in 418 mL of distilled water. The desired pH was controlled by a benchtop pH meter (Hanna Instruments).

Each sample for the calibration curve was added with 3 mL of Zincon solution and buffered by 10 mL of pH 9 buffer. The as prepared samples were added with water until a final volume of 50 mL, mixed and, after a rest period of 20 minutes to develop the final colour, these were spectrophotometrically analysed by a 6850 spectrophotometer (Janway) in the visible range.

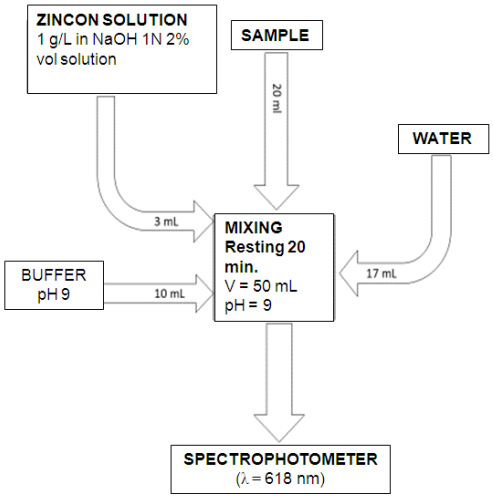


Figure 2. Reference method for Zn(II) analysis by Zincon complexation.

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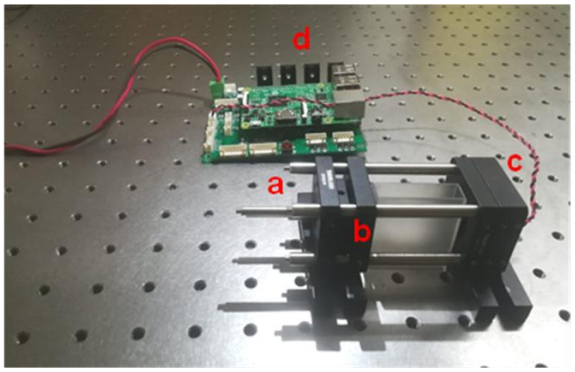


Figure 3. Optical analyzer equipped with a laser source @638 nm. **a.** Laser diode; **b.** Aspherical lens; **c.** Silicon Photodiode; **d.** Proprietary driver.

Optical glass cuvettes with an optical path of 10 mm were used while distilled water was assumed as reference. The maximum absorbance peak at 618 nm was considered for the calibration curve.

The main process parameters were optimized at laboratory scale and then tested on a bench prototype.

The bench prototype consisted of a TO Can laser diode emitting at 638 nm, with a continuous wave output optical power of 40 mW, collimated through an aspherical lens. The optical power was transduced by a silicon photodiode. A proprietary electronic driver was built to drive laser diode current. (Figure 3)

B. Results and Discussion

The standard method was first tested to assure the accordance with Lambert & Beer law. The test was carried out with the same parameters reported in literature. Standard solutions samples (20 mL) ranging from 0.5 to 10 ppm were added of 3 mL of Zincon solution in a water solution 2%vol of NaOH 1N, 10 mL of pH 9 buffer and distilled water, reaching a final volume of 50 mL. After the developing of the final colour (20 minutes), samples were spectrophotometrically analysed collecting the whole spectrum in the visible range.

Distilled water treated with Zincon shows a red-brown color due to the ligand not complexed by metal ions, with an absorption peak in the visible region at about 487 nm, while in presence of Zinc ions, a clear blue coloration of the sample denounced the formation of the complex, with the related absorbance peak at 618 nm.

The second peak was considered for the calibration curve, obtaining a good agreement with Lambert & Beer law, confirming the linearity of the calibration curve in the concentration range 0.5 - 7.5 ppm, with an R^2 very close to 1.

Due to economic and encumbrance reasons, on the portable instrument a laser diode will be preferable than a tungsten source equipped with a monochromator. The absorbance at λ = 600, 620, 618, 625, 637 and 638 nm was measured for the calibration curve, considering the main sources commercially available. Results are reported in table 1 where it is clear that R^2 never fall under 0.999, complying with the Lambert & Beer law; it means a certain flexibility in the choice of the suitable radiation source.

TABLE I. CALIBRATION CURVES: INFLUENCE OF WAVELENGTH.

λ	Linear equation	R^2
Peak 618	y=0.1621x + 0.0289	0.9992
600	y=0.16x + 0.331	0.9992
620	y=0.172x + 0.272	0.9998
625	y=0.124x + 0.0284	0.9991
637	y=0.1099x + 0.0257	0.9991
638	y=0.1083x + 0.0253	0.9991

Further optimizations were imposed by needs of prototype miniaturization : involved volumes should be reduced, highly concentrated acidic or alcaline media avoided and process time limited.

Moreover, the best rinsing protocol of the cuvette was assessed to limit as much as possible the error due to contamination of the sample with the previous one. To avoid mutual effects, each parameter was varied and investigated independently, maintaining all the others fixed to the standard method.

Considering the cuvette rinsing, 1 and 5 ppm Zn (II) solutions were prepared and analyzed, considering the measured absorbance as reference. Then the same solutions were spectrophotometrically tested rinsing the cuvette in different ways between the measures: rinsing with distilled water twice and drying, rinsing once with distilled water without drying, not rinsed or rinsing once with the following sample. The test was carried out comparing the consecutive measurements of samples with the same concentration or testing first the higher concentration, followed by the lower concentrated sample.

Results showed that between samples with different concentrations the error rises to 15 % without rinsing, lowered to 2 %, which can be considered negligible, if cuvette were rinsed by distilled water. Anyway, the test with the cuvette rinsed once, with the same following sample to analyze, gave best results at all, with an error of 1% regardless the samples concentrations. As a consequence it was chosen as the best to be implemented on the prototype.

The presence of concentrated sodium hydroxide solutions, required for Zincon solution preparation, can be a problem in a portable instrument: besides safety issues due to accidental spill, materials with suitable chemical resistance should be considered both for reservoir and microfluidic system. For these reasons, the possibility to use less aggressive alkaline solutions was investigated.

First of all, it was verified that the alkalinisation is necessary because, without the addition of the basic buffer, the sample does not change colour from red to violet, as expected, confirming that the complexation of Zn ions by Zincon can occur only if the pH is basic. (Figure 4)

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Figure 4. Water samples treated by Zincon: 1 ppm Zn (II), Zincon metal-chelated (left) and pure distilled water, Zincon free form (right).

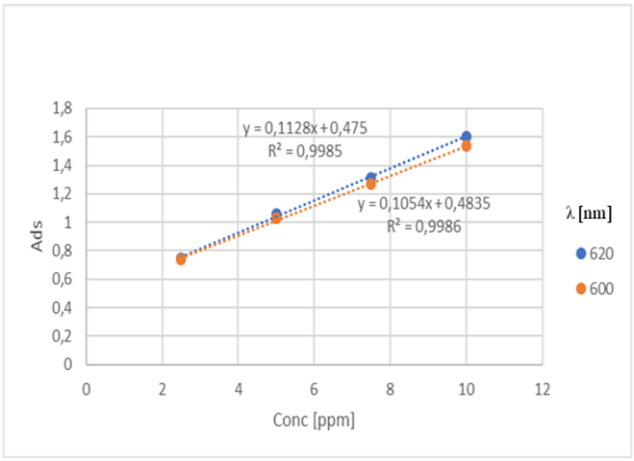


Figure 5. Calibration curves. Buffering of the samples at pH 8.5.

Among the basic pH, tests were carried out varying the pH between 8 to 10. The highest pH was obtained by addition of NaOH 1N solution in the proper amount, while pH 9 or 8.5 were obtained by adding 10 mL of the proper buffer to the sample.

Results confirmed the importance of the pH control during the analysis, as previously reported. At pH 10, in fact, the measurement is not feasible: the change of colour occurs, and the peak related to the Zn/Zincon complex is present on the graph, but the absorbance is not proportional to the ions concentration. On the other hand, if the sample is buffered at pH between 8.5 and 9, a very good calibration curve was obtained, with a reliable slope and an R^2 close to 1. (Figure 5)

The addition of 10 mL of buffer solution at pH 8.5 was considered, at the end, the best solution for the analysis.

A further optimization deals with the Zincon solvent. NaOH 1N solution, suggested in the standard method, is indeed chemically aggressive toward many polymers, those with enough resistance being highly expensive.

Our idea was to replace this solution with the same buffer at pH 8.5 required for the sample treatment.

Tests confirmed that Zincon was dissolved in the buffer even better than in the NaOH solution, and the test carried out with this solution shows results similar to the standard, with a linear calibration curve and an R^2 = 0.9986. It means that the same buffer solution can be used for both Zincon dilution and sample buffering, with advantage in terms of economy, space reduction and process simplification.

Moreover, in this case the colour change from red to violet occurred immediately after the mixing of all the components, remaining stable in time, as confirmed by the absorbance corresponding to the peak at 618 nm, monitored in time. (Table 2) It is probably due to the fast dissolution of Zincon, enhanced by the most appropriate pH and, as consequence, the rapid diffusion in the liquid sample and zinc complexation.

TABLE II. ABSORBANCES READ ON THE SAME SAMPLE 0.5 PPM ZN(II) AT DIFFERENT TIMES AFTER MIXIING

t [min]	Abs (λ 600)	Abs (λ 620)
5 sec	0,116	0,225
1	0,180	0,245
5	0,179	0,242
10	0,178	0,243
15	0,178	0,243
20	0.181	0.244

The result is particularly interesting because it allows a strong time reduction during the test, skipping the 20 minutes resting time required by the standard method for the colour development.

The standard method recommends the dilution of the sample at a final volume of 50 mL before the analysis; it means that the read concentration is 2/5 of the real. Our idea was that the dilution could be avoided, in order to increase the range of detectable concentrations, particularly for low values.

Results show that by such approach the sample with 0.1 ppm concentration is well included in the linearity of the calibration curve (Figure 6), but it is the lowest limit of detection, since lower concentrations, down to 10 ppb, were investigated but the linearity in this case is definitively lost. Samples with concentrations until 10 ppm preserve the linearity of the calibration curve. For this reason, samples will not be diluted in the optimized process, with a useful reduction of involved volumes, the removal of a water reservoir in the final prototype and a consequent simplification of the process.

Best results previously obtained for each investigated parameter can now be merged in an optimized and simplified process. (Figure 7)

A 20 mL sample is added to 10 mL of Zincon solution 0.3 g/L in the pH 8.5 buffer, that is 0.24M both for NaOH and H_3BO_3 in distilled water. The same buffer is indeed used both for Zincon dilution and buffering of the sample, so the fluxes can be merged adding directly the final required volume of buffer containing Zincon in the proper concentration to have the same amount poured (that is 0.3 g/L instead of the starting 1 g/L).

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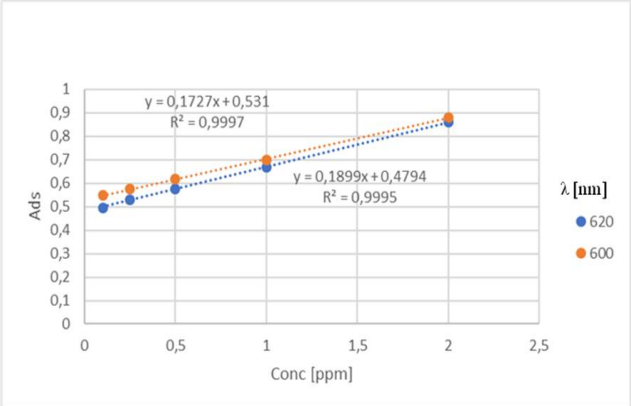


Figure 6. Calibration curves without dilution of the sample, on the final volume of 30 mL. Zinc concentrations between 10 ppb and 2 ppm.

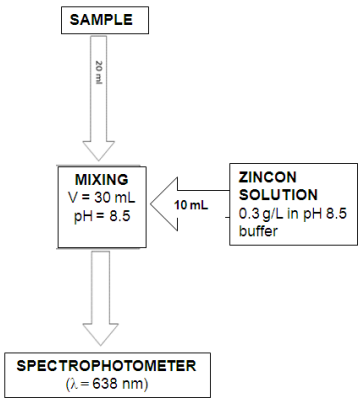


Figure 7. Scheme of the optimized and simplified process.

Components are mixed and then spectrophotometrically analyzed versus distilled water. The absorbance suitable for the evaluation of Zinc concentration can be chosen in a range near the peak at 618 nm, between 600 and 640 nm.

It can be noted that, varying the different parameters, the calibration curves are not exactly the same. By the way, the value of R^2 was always very close to 1 and the slopes of the curves were good for an efficient quotation with low errors. It means that, once fixed the final method, the corresponding calibration curve will be defined in the considered concentration range and it is enough to obtain satisfactory results by the analysis of the unknown samples.

The real analytical application, in the final form of a portable device for continuous monitoring of water, will be surely mainly addressing seawater. This suggested a series of tests on salted water simulating the Mediterranean salinity, to ensure that the presence of salt does not affect the measurement.

For such purpose, distilled water was added with 35 g/L of NaCl; then Zinc ions solution in the proper amount was added to obtain a 10 ppm concentration, and finally diluted for the calibration curve sampling. Samples were analysed by the optimized parameters of the method.

Results show that the calibration curve still follows the Lambert & Beer law, with an $R^2 = 0.999$; moreover the slope of the curve is the same as the one obtained on not salted samples (i.e. 0.17 considering $\lambda = 600$ nm). (Figure 8) Therefore, it can be concluded that the salted water is not an issue for the optimised method.

A further test was inspired again by the final proposed application. The stability of the Zincon solution stored in the device in final application, was tested. Aging tests were carried out analysing samples with a 20 days aged Zincon solution. A longer period was not considered relevant, since a periodical technical maintenance will be required in any case, thus the solution can be replaced with a fresh one.

Results are reported in terms of calibration curves obtained with a Zincon solution aged for 20 days at ambient temperature, in a closed flask stored at dark. The efficiency of the solution was confirmed. (Figure 9)

Final measurements were carried out on the optical analyser based on a laser source at 638 nm, instead of the laboratory spectrophotometer.

Even in this case the calibration curve was evaluated for both higher (Figure 10) and lower (Figure 11) zinc concentrations, applying the above described chemically optimized method.

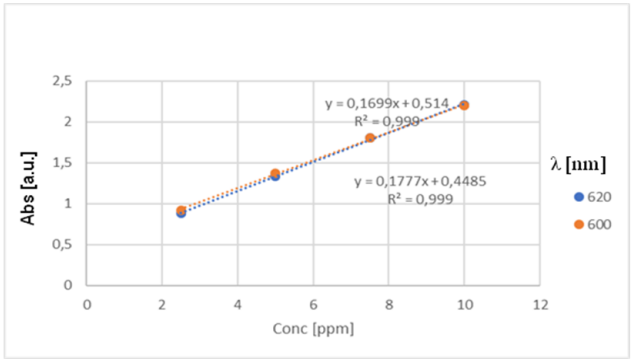


Figure 8. Calibration curves on salted samples (35 g/L NaCl).

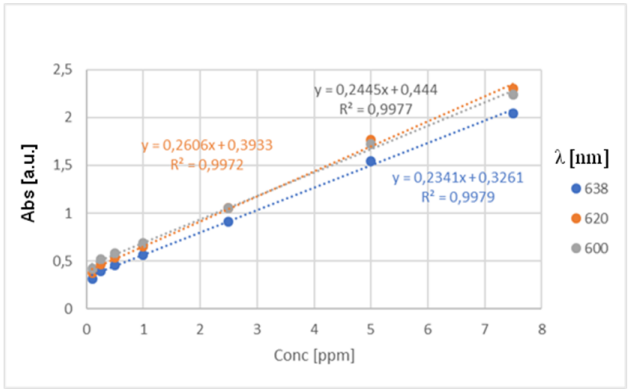


Figure 9. Aging test. Zincon solution 20 days aged.

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Encouraging results were found: the linearity was respected in both cases and in particular, in the range 1-10 ppm the R^2 was very close to 1 (0.9999). It demonstrates that the selected solution is the right choice to be implemented on the portable device, allowing detecting metal ion concentrations until 100 ppb.

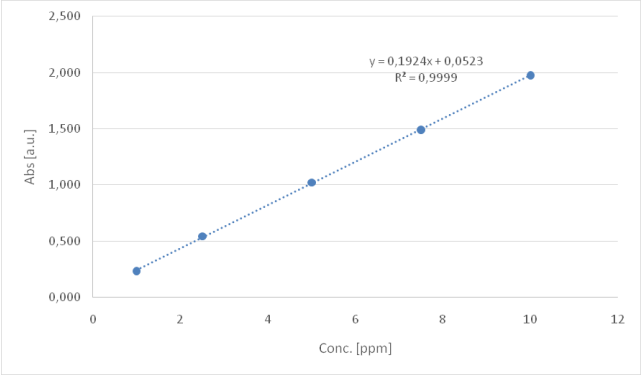


Figure 10. Calibration curve on optical analyzer equipped with a laser source @638 nm. Zinc concentrations between 1 and 10 ppm.

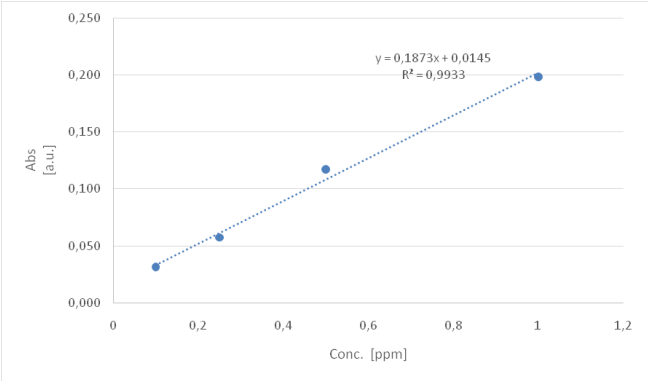


Figure 11. Calibration curve on optical analyzer equipped with a laser source @638 nm. Zinc concentrations between 0.1 and 1 ppm.

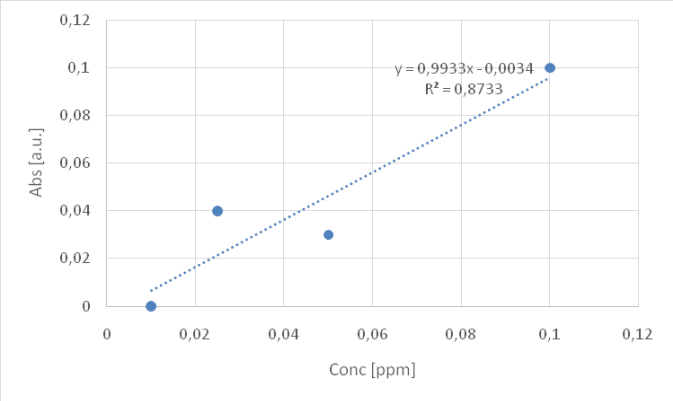


Figure 12. Calibration curve on optical analyzer equipped with a laser source @638 nm. Zinc concentrations between 10 and 100 ppb. Optical path 40 mm.

To decrease the lower limit of detectable zinc concentration, an attempt was performed increasing the optical path, which is the length of the cuvette, to 40 mm instead of the previous 10 mm. Such an improvement allowed extending the lower detection limit to 20 ppb, even if linearity is not so good as before (R^2 = 0.8733), but still of interest. (Figure 11) The possible exploitation of 40 mm cuvette for detection of very low zinc concentrations can so be considered for the final prototype project.

III. Conclusions

Zincon for Zinc ions detection is reported in literature as a well known method, effective and reliable.

However, an instrument for real time precise and reliable results, using Zincon based spectrophotometric method, is not yet present on the market, at best of our knowledge.

In this study the optimization of Zincon method was performed, to be suitable for miniaturized and portable devices, for real time monitoring systems. The ground-breaking idea integrated in this work is the implementation of a system inspired to the latest innovative engineering techniques, with a particular focus on the microfluidic analysis based on Lab-on-Chip technology.

This approach has potential in areas such as environmental pollution monitoring and water metal analysis.

Main process parameters were optimized starting from the standard methods reported in literature: NaOH concentrated solution (pH 12-13) was replaced with a pH 8.5 buffer, less aggressive, as Zincon solvent; the reagents amounts were limited; the required 20 minutes rest time was skipped and the considered wavelength for absorbance was moved from the peak to 638 nm. A good agreement of the calibration curve with Lambert & Beer law was obtained in a wide Zn(II) concentration range, that is 0.1 -10 ppm, probably expandable to 20 ppb increasing the cuvette length and related optical path.

Laboratory results were finally confirmed on a bench prototype, equipped with a single wavelength laser source at 638 nm. Bench tests provided evidence that such optimized method allows the engineering of portable devices for real-time monitoring of Zinc in water environment, with excellent features like minimum threshold and resolution.

However, some limitations are worth to be reported. Although the proposed method is strong and reliable with samples prepared in laboratory, the possible interference due to other metals and pollution components in real water samples was not tested yet.

Future activities will therefore include real water samples tests and the implementation on a portable device. The system will be designed to be embedded, inside a payload to be assembled on an Autonomous Underwater Vehicle (AUV) and guided in the depths of the sea. This vehicle will be able to sail in deep water (down to 300 meters u.s.l.) near any interesting and potentially polluted site, monitoring in real time the presence of zinc ions in seawater.

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