

Cr(VI) in Water: Continuous, on Site Spectrophotometric Determination

Laboratory test preliminary to microfluidic device prototyping

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Abstract—Main parameters of the 1-5 diphenylcarbazide standard method for spectrophotometrical detection of Cr(VI) 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 acid solutions, the optimization of reagents amounts and the radiation of the sample by a single wavelength visible source. The optimized process was verified on a bench prototype.

Keywords—Chrome, diphenylcarbazide, water pollution, water analysis, process optimization

I. Introduction

Chromium is a heavy metal with toxic potential for marine environment. Water contaminations occur by atmospheric pollution as major source. Chromium alloy and metal producing industry, cooling towers, industrial from electropainting and metal finishing industries discharged into the water and runoff from urban areas are the principal sources. In aquatic environment, chromium is found as Cr (III) and Cr (VI) as water soluble complex anions.

Of all the metals, Cr has truly unique toxicological characteristics. Toxicological impact can result both from the action of Cr (VI) itself as an oxidizing agent and from Cr (III) which is capable of complexing with various organic compounds and thus may inhibit several metallo-enzyme systems. Specific studies concluded that Cr (VI) is more toxic than Cr(III) to marine organisms, therefore the study was focused on the former one. [1, 2]

Traces of heavy metals can be detected with high precision in food, water, soil, sludge, by spectrophotometric analysis. UV/vis spectroscopy is in fact an important analytical technique in modern water analysis: many cations and anions in water can be determined with high selectivity and sensitivity, making it competitive with more expensive and sophisticated techniques, like ICP. [3]

The common principle of water analysis 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 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 the water according the well-known Lambert & Beer law. [4]

Diphenylcarbazide (DFC) method is normally used for determining hexavalent chromium in drinking and surface waters, domestic and industrial wastes in the range from 0.1 mg/L to 1.0 mg/L of Cr.[5] Chromates combine with diphenylcarbazide in strong acid solution to produce a red-violet complex with a clear peak of absorbance at about 540 nm. (Fig. 1)

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 was filling this gap. 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 electronical 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.

Of particular interest will be the implementation of this system on a submarine, plumbing huge sea areas with temporized samplings, for a real time monitoring of the seaside pollution.

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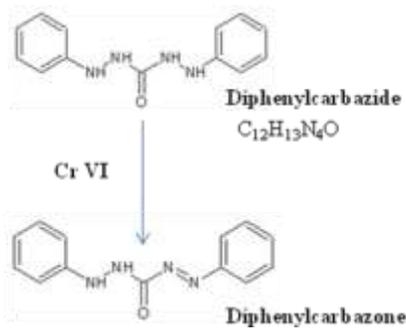


Figure 1. DFC is transparent while in the form complexed by Cr(VI) it assumes a red-violet color.

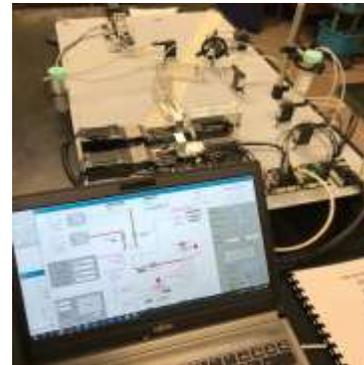


Figure 2. Automatic water sampler designed and built at Politecnico di Torino. An electronic driver was designed and built to control moto-syringes and valves. A software was designed and created to drive the automatic sampler.

II. Experimental

A. Materials and methods

DFC standard method, as reported in literature, was considered as reference. [5]

Analytical grade general reagents were used.

For the calibration curve, 100mL of Cr(VI) solutions were prepared, with concentrations between 0.1 and 1 ppm, starting from the standard Cr(VI) solution at 1000 ppm (Sigma Aldrich), namely: 0.1 – 0.25 – 0.5 – 0.75 – 1 ppm.

A 1-5 Diphenylcarbamide (Sigma Aldrich) solution 5 g/L in acetone was prepared, while sulfuric acid was diluted 1:1 v/v with distilled water.

Each sample for the calibration curve were added with 1 mL of diluted acid and 2 mL of DFC solution; the as prepared samples were mixed and, after a rest period of 10 minutes to develop the final purple color, these were spectrophotometrically analysed by a 6850 spectrophotometer (Janway).

Optical glass cuvettes with an optical path of 10 mm were used while distilled water treated in the same way was assumed as reference. The maximum absorbance peak at 540 nm was considered for the calibration curve.

The process parameters were optimized at laboratory scale and then were tested on a bench prototype. It was composed of an automatic water sampler and an optical analyzer. (Fig. 2) The optical analyzer was composed of a PL520 laser source @520 nm, collimated with an ACL 12708U aspherical lens, a multichambered cuvette designed and built at Politecnico di Torino and a silicon photodiode BPW21R as detector. (Fig. 3)

B. Results and Discussion

The standard method was first tested to assure the accordance with Lambert & Beer law. It was confirmed by the linearity of the calibration curve, in the concentration range 0.1 -1 ppm, with an $R^2=1$. (Fig. 4) Collected spectra were clear and regular, without interferences, with the peak of the curve between 540 and 544 nm. (Fig. 5)

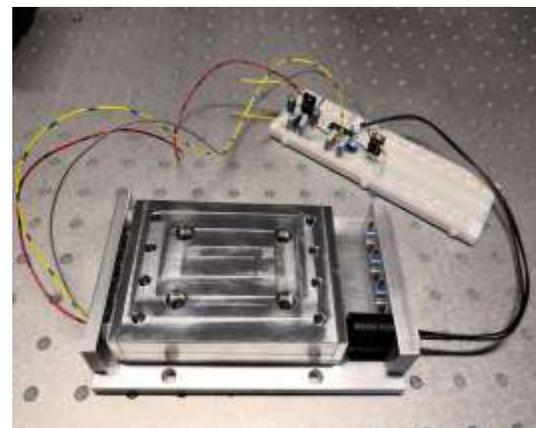


Figure 3. Optical analyser designed and built at Politecnico di Torino.

Due to economic and encumbrance reasons, on the portable instrument a laser diode will be preferable than a tungsten source equipped with a monochromator. A source emitting at the fixed wavelength 520 nm was chosen, so it was firstly necessary to test the linearity of the calibration curve considering the absorbance measured at the fixed λ . In Fig. 4 the obtained result is reported and compared with the reference: the slope of the curve was higher at 520 nm, but R^2 was 1 again, complying with the Lambert & Beer law.

The absorbance at $\lambda= 540, 536$ and 532 nm was also considered for the calibration curve and R^2 never fall under 0.999; it means a certain flexibility in the choice of the suitable radiation source.

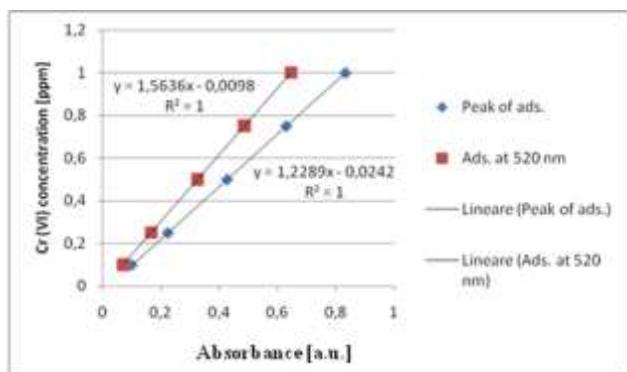


Figure 4. Calibration curves by the standard method, considering the absorbance at the peak of the spectra and at 520 nm.

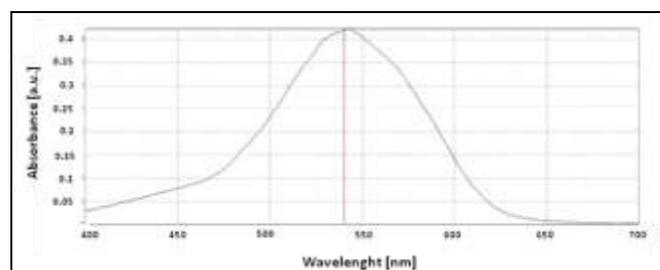


Figure 5. Spectrum of a 0.5 ppm Cr(VI) solution, analyzed by standard DFC method.

Further optimizations were imposed by needs of miniaturization on the prototype: involved volumes should be reduced, highly concentrated acidic media avoided and process time limited. Moreover, while in the lab the cuvettes are carefully washed between following analysis, in a continuous setting it's not so simple; the best rinsing protocol 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 last point, two Cr(VI) solutions 0.25 and 1 ppm 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 two times and drying, rinsing one time with distilled water without drying, not rinsed or rinsing one time with the following sample. The test was carried out comparing the consecutive measurement of samples with the same concentration or testing first the higher concentration, followed by the lower concentrated sample.

TABLE I. RINSING TEST. BEST RESULTS OBTAINED RINSING THE CUVETTE ONE TIME, WITH THE SAME FOLLOWING SAMPLE TO ANALYZE.

TEST	C [ppm]	Abs (λ 540)	Abs (λ 520)
<i>Ref 1</i>	1	0.787	0.663
1	0.25 ->1 ->1	0.790	0.665
<i>Ref 2</i>	0,25	0.209	0.176
2	1 -> 0.25->0.25	0.210	0.178

Results showed that analyzing solutions with the same concentration, any rinsing with water, not followed by the cuvette drying, causes a dilution of the sample with an error on the evaluated concentration of about 1.6-1.8%, that however can be considered negligible. Otherwise, without rinsing the cuvette, results are in total agreement with the reference. On the other hand, the error rises to 15% without rinsing between samples with different concentrations: residues of the first sample, highly concentrated, stay in fact in the cuvette and, mixed with the second sample, increases its absorbance. Any rinsing reduced this error to values near to the previous ones, about 1.7%.

Finally, the test rinsing the cuvette one time, with the same following sample to analyze, gave best results at all, with an error of 1.1% regardless the samples concentrations. (Table I) It was chosen as the best to be implemented on the prototype.

The management of concentrated sulphuric acid can be a problem in a portable instrument: over safety problems due to accidental spill, materials with enough chemical resistance should be considered both for reservoir and microfluidic system. For these reasons, the possibility to use more diluted acid was investigated.

First of all, it was verified that the acidification is necessary because, without the addition of the acid, the sample doesn't color, as expected.

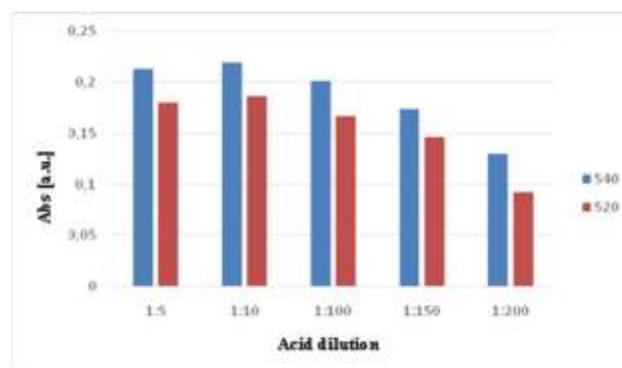


Figure 6. Influence of acid dilution (fixed volume of diluted acid) on absorbance at 540 and 520 nm. Cr(VI) concentration: 0.25 ppm

TABLE II. INFLUENCE OF ACID AMOUNT, REFERRED TO SAMPLE VOLUME, ON ABSORBANCE.

V sample[ml]	V diluted acid [ml]	Acid dilution ratio	Abs (λ 540)	Abs (λ 520)	Acid amount [mL/mL sample]
50	0,5	1:100	0,201	0,167	$1 \cdot 10^{-4}$
50	0,5	1:200	0,130	0,092	$5 \cdot 10^{-5}$
50	1	1:200	0,201	0,169	$1 \cdot 10^{-4}$

Then, samples 0.25 ppm of Cr(VI) were considered as reference and treated according the standard procedure varying the amount of sulfuric acid added: some test were carried out keeping the added volume of 1mL/100mL of sample but adding acid solutions with lower concentrations (pure acid diluted 1:5 – 1:10 – 1:100 – 1:150 – 1:200 v/v); for another series of test the acid dilution was strongly increased at 1:3000 v/v and volumes ranging between 20 and 0.5mL/100mL were added to the samples. In this way, it was possible to assess the influence of both dilution and amount of the acid, evaluating the absorbance of the samples differently treated, but with the same concentration.

Results showed that the absorbance was constant till a dilution 1:100 in the first case (Fig. 6) and a volume of 5 mL in the second case. It means that a more diluted acid can be used instead of 1:1 v/v. For lower amounts of acid added, the absorbance felt, denouncing a lack in the establishment of DFC-Cr(VI) complex. Moreover, the absorbance was measured adding to the 100 mL sample 2 mL or 1 mL of 1:200 v/v acid. In the first case, the amount of acid added was the same as 1 mL of 1:100 v/v acid and in fact the same absorbance value was obtained; in the second case the absorbance was very lower than the previous test, meaning that the amount of acid was not enough. It can be concluded that an amount of 0.01 mL of pure sulphuric acid/100mL of sample is the minimum amount to assure the right acidification of the sample, but it can be introduced in the preferred way in terms of dilution and volume. (Table II) Of course, the dilution of the sample occurring adding an high volume of the diluted acid has to be considered in the calculation of the real Cr(VI) concentration.

The standard method expects the mixing of the sample after the addition of the reagents and a rest time of 10 minutes before the spectrophotometric analysis, to assure the complete complexation of the Cr(VI) ions by the DFC. To reduce the process times and for a process simplification, the necessity of the mixing and the minimum rest time for a satisfactory result were investigated.

A first test was carried out on samples added of all the reagents but not mixed. In this case, after 30 minutes, the complexation was not yet complete; in fact, two phases were clearly visible in the cylinder: at the top the solution was purple, due to the complexation of the Cr(VI) ions by DFC, while at the bottom the sample was completely transparent because it was not reached by DFC. Thus, the mixing of the sample with reagents is absolutely necessary. (Fig. 7)



Figure 7. Samples DFC treated after 30 minutes, without mixing

TABLE III. ABSORBANCES READ ON THE SAME SAMPLE 0.25 PPM Cr(VI), AT DIFFERENT TIMES AFTER MIXING

t [min]	Abs (λ 540)	Abs (λ 520)
5 sec	0,206	0,175
1	0,202	0,171
2	0,198	0,166
3	0,197	0,165
4	0,194	0,163
5	0,198	0,165
6	0,195	0,163
7	0,197	0,165
8	0,192	0,161
9	0,197	0,165
10	0,194	0,162

The rest time, after 5 seconds of vigorous mixing, was optimized monitoring the absorbance of a 0.25 ppm solution during the time. The value was stable after 2 minutes, with a calculated concentration, by the calibration curve, of 0.249 ppm. (Table III) It can be considered a good result, taking in account the operative accuracy in the sample preparation, meaning that the rest time can be strongly decreased from 10 minutes recommended to just 2 or 3 minutes.

A further optimization deals with the DFC solvent. Pure acetone, suggested in the standard method, is in fact chemically aggressive toward many polymers, those with enough resistance being highly performing and expensive. Onchoke and Sasu refer the methanol as suitable DFC solvent for the same purpose [6] so some tests were carried out replacing acetone with both methanol and ethanol. The chemical resistance of polymers to alcohol is better than acetone, so these chemicals, if worked, would enlarge the

choice of the suitable material for both the reservoir of DFC solution and microfluidic device.

Results confirmed the methanol as performing solvent, with any difference between absorbance read on the same sample, that is 0.203 at $\lambda = 540$ nm and 0.171 at $\lambda = 520$ nm, using methanol or acetone DFC solutions.

The dissolution of DFC in acetone is immediate while in methanol a light mixing was required, but then it was stable for several days, without any deposition. On the other hand, DFC solution in ethanol required a vigorous stirring for the complete dissolution and lower values of absorbance was obtained with respect to the acetone solution, so ethanol was no more considered as DFC solvent.

To confirm the methanol as effective solvent for DFC and to test there were no interferences with the complexation process, a calibration curve was built using this solvent. Results are reported in Fig. 8: the slopes of the curves are different with respect to acetone ones, but the R^2 is again higher than 0.99 at both wavelengths, so hereafter methanol replaced acetone in further test.

The concentration of the DFC in methanol was also optimized: 0.25 ppm Cr(VI) samples were treated with DFC solutions at decreasing concentrations, from 0.5 to 0.05g/100mL of methanol. The absorbance was monitored and it was stable till 0.1g/100ml; for lower concentrations, the absorbance decreased of about 10%, due to the lower DFC molecules than required for total Cr ions complexation. (Table IV) It was the limit value, but in the optimized process a concentration of 0.2g/100mL was assumed.

Finally, all the optimized parameters were joined in an optimized method to test the possible synergic effects on the linearity of the calibration curve. It means that 100 mL of the sample were added of 1 mL of sulphuric acid solution 1:30 v/v and 2 mL of DFC solution, 2g/L in methanol. The reagents were manually mixed and after a rest of 3 minutes the sample was spectrophotometrically analyzed considering the absorbance at 520 and 540 nm for the calibration curves. Results are reported in terms of calibration curves in Fig. 9, where a good linearity, comparable with the standard method, was obtained.

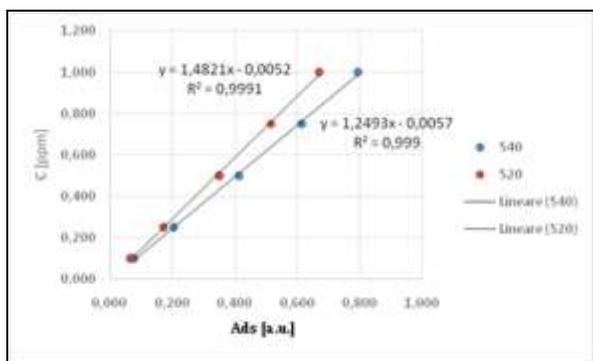


Figure 8. Calibration curve solving DFC in methanol

TABLE IV. INFLUENCE OF THE AMOUNT OF DFC ADDED. CR(VI) CONCENTRATION: 0.25 PPM. DFC VOLUME: 2ML/100ML SAMPLE

Conc. DFC [g/100 mL]	Abs (λ 540)	Abs (λ 520)
0,5 (Ref)	0,204	0,172
0,05	0,185	0,154
0,075	0,19	0,16
0,1	0,207	0,175
0,2	0,213	0,179
0,3	0,206	0,174
0,4	0,211	0,179

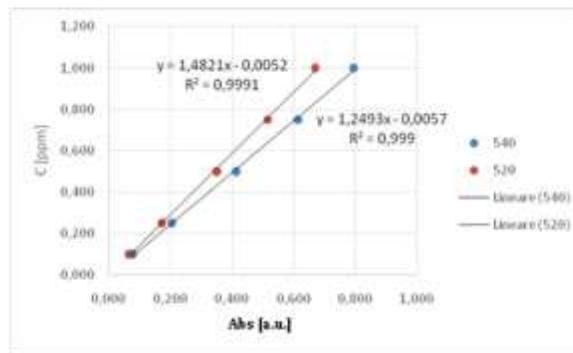


Figure 9. Calibration curve with all optimized parameters

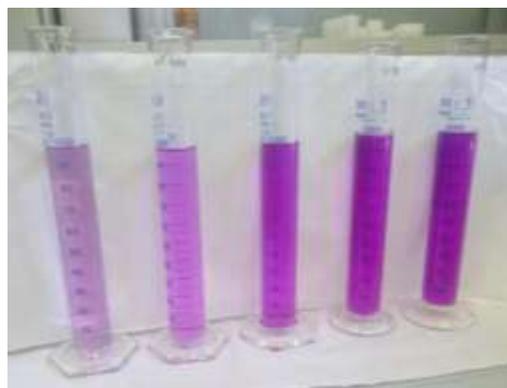


Figure 10. Cr(VI) solutions with increasing metal concentration, treated by the optimized DFC method. From left: 0.1 – 0.25 – 0.5 – 0.75 – 1 ppm

For each parameter (DFC and acid concentrations, volume of added solutions, rest and mixing time) tolerances were determined to set the precision required in the final automatic measuring system; it was found that any of the parameters requires too high precision, but there is a good margin to limit the error of the measures to negligible values. It means that the method is well applicable to the required analysis, at least in the considered Cr(VI) concentration range.

Laboratory experiments ended with the investigations about the stability of the DFC solution during the time and the effect of salt presence in the sample and low temperature of the sample. These are parameters of interest considering the real application of the method. A long lifetime of the DFC solution means longer sessions of online measurements without the need of DFC replacement, while the salt simulates the marine water samples.

The DFC solution stored in the dark, at room temperature, was stable for at least 15 days (5% lower read absorbance). Then the absorbance values, for a reference sample, were lower than those measured with fresh prepared solution, reaching a strong loss of efficiency after 40 days (50% lower read absorbance). It was the same both for acetone and methanol DFC solutions.

The influence of the temperature was tested on samples fridge at 4°C, finding the same results on those maintained at room temperature.

Finally, a concentration of 35g/L of NaCl in the sample was assumed as representative for the Mediterranean Sea. On salted samples any difference was found in the final result, with respect to not salted ones, but a delay in the color development was noted, probably due to a slowdown of DFC molecules in solution, due to interferences with the salt ions. Anyway, the salt did not compete with Cr(VI) in complexation, as proved by the right detected concentration, while the mixing and the rest time of 3 minutes were enough to ensure the complete complexation.

III. Conclusions

Diphenylcarbazide for Chrome detection is a well known method, effective and reliable.

However, an instrument for real time precise and reliable results, using diphenylcarbazide method is not yet present on the market.

In this study we performed an optimization of diphenylcarbazide method, to be suitable for miniaturized and portable devices, for real time monitoring systems.

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

With this in mind, main process parameters were optimized starting from the standard methods reported in literature: acetone was replaced with methanol as DFC solvent, the acid dilution was increased and reagents amounts limited, mixing and rest times were reduced and the considered wavelength for absorbance was moved from the peak to 520 nm. A good agreement of the calibration curve with Lambert & Beer law was obtained in the considered Cr(VI) concentration range, that is 0.1 -1 ppm. (Fig 10)

Laboratory results were finally tested and confirmed on a bench prototype, which simulated a portable device. Bench tests provided compelling evidence that this optimized method allows the creation of portable devices for real-time monitoring of chromium in water environment, with excellent features like minimum threshold and resolution.

However, some limitation are worth noting. Although our method is strong and reliable with samples made in laboratory, other metals and pollution components interferences in real water samples were not tested yet.

Our future works will therefore include real water samples tests and the implementation of a portable device.

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