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# From Colour Catcher® to colorimetric sensors: disposable and cheap devices for inorganic ions determination

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*We describe disposable and cheap colorimetric devices obtained by fixing classical dyes on the commercial paper sheet known as "Colour Catcher®" (here named under the acronym CC), the product used to prevent color runs in washing machines cycles. These devices can be used as colorimetric sensors for different analytes of environmental and biological interest since the indicator dye, fixed on the solid material, changes its spectral properties (color and hence UV-vis spectrum) upon contact with the analyte. The relationship between the analyte content and the UV-vis spectrum (or RGB values) change of each sensor is provided using a chemometric tool: the Partial Least Squares regression (PLS). Promising results were obtained when applying these sensors to actual samples, so because of their simple preparation with low-cost reagents, they can be effective for application in environmental and food analysis.*

**Keywords:** colorimetric sensors, disposable devices, UV-vis spectroscopy, PLS, chemometric tool

Metal ions, anions, and biomolecules play a crucial role in biological and environmental processes, so their monitoring is of paramount importance. Classical instrumental techniques, such as high-performance liquid chromatography, inductively coupled plasma atomic emission spectrometry-mass spectrometry, atomic absorption spectrometry, ion-chromatography, and electrochemical methods, are routinely used to detect and quantify analytes in different samples. Nevertheless, these techniques are laborious, time-consuming; they need a pre-treatment step of the samples and sometimes cleanroom facilities for analysis. In the past several years, it has taken hold of optical sensors' employment since they are easy to use, inexpensive, and allow remote and continuous monitoring.

On the other hand, colorimetric sensors hold promise for detecting metal cations, anions, and other toxics thanks to their easy fabrication, rapid analysis, and easy naked-eye sensing, as evinced from several reviews on the fabrication and applications of these devices.[1] Generally, they are prepared by fixing different types of reagents, such as chromogenic, fluorescent, and ionophoric compounds on a substrate, such as synthetic polymers, cellulose-based materials, thin layers metals, textiles, and woven non-woven fabrics [2-3].

In this context, we developed colorimetric devices for metal cations, starting from different supports: chelating resins, triacetylcellulose membrane, cellulose paper, synthetic polymers, or silica gel [4-9].

On the contrary, in the present research, we chose to design colorimetric sensors adopting a simple preparation strategy, standard reagents, and cheap items. In particular, these devices can be obtained by sorption of conventional dyes (dissolved in aqueous solutions), on the Colour Catcher®, a commercial product of the washing powder market (produced in Italy by Grey - Henkel company, and in England by Dylon [10]) generally used in the washing machine to prevent color run problems. It is chosen as excellent and cheap support for the selected dyes thanks to its properties, ease of supply, and low cost (around € 0.2 per sheet of 11 × 25 cm). This research aims to develop very cheap sensors useful for non-experts and in developing countries since it will be possible to get information about the concentrations of the selected analytes just with few reagents and a smartphone.

Among different analytes of environmental and biological interest, we focused on water hardness, three divalent cations (Co(II), Ni(II) and Zn(II)) and sulfides.

Water hardness is of interest to both domestic and industrial users. The amount of calcium(II) and magnesium(II) dissolved in water determines the hardness degree of the water. Hard water produces bothersome daily problems such as rings around toilet WC and tubes and reduced detergents and soaps. Moreover, hard water can clog pipes of households and industrial plants, besides high calcium and magnesium concentrations in drinking water can be harmful to human bodies [11]. There are commercially available sensors for water hardness analysis and the determination of Ca(II) and Mg(II) concentrations, but they are expensive or require experience and special skill to be applied.

Cobalt(II), Nickel(II) and Zinc(II) are essential micronutrients for animals and plants. Nevertheless, large doses (exposure higher than 0.05 mg/m$^3$) of cobalt(II) and salts can be toxic, causing irritants and allergic effects [12]. Ni(II) excess is associated with acute pneumonitis, dermatitis, asthma, cancer of the lung and sinus, and other disorders of the respiratory

and central nervous systems [13]. Therefore, monitoring Co(II) and Ni(II) in environmental, food and industrial samples is needed. The biological role of zinc(II) was recognized in DNA and RNA synthesis, apoptosis, gene expression, or protein structure and function [14]. Therefore, the development of useful zinc(II) sensors has recently been attracting considerable interest. Moreover, the choice fell on Co(II), Ni(II) and Zn(II) as these cations form strong complexes with the PAN dye and give quite different colors compared to that of the uncomplexed ligand.

Since the biological relevance of thiols and sulfides (cysteine, homocysteine, glutathione and hydrogen sulfide), their detection has drawn the scientific community's attention.

Due to the strong nucleophilicity and redox reactivity of the sulfhydryl group, cysteine (Cys), homocysteine (Hcy) and glutathione (GSH) are essential molecules in biological systems. Variations in thiol concentrations can indicate neurodegenerative diseases and cardiovascular disorders [15].

Hydrogen sulfide ($H_2S$) is a toxic gas with a characteristic malodor of dirty eggs. The lungs quickly absorb it once exposed via inhalation. Several occupational epidemiological studies have highlighted that exposure to high concentrations of hydrogen sulfide has severe effects on the respiratory system, resulting in unconsciousness, linked to neurological sequelae and, occasionally, death. $H_2S$ has also been associated with cardiovascular disorders [16]. Hence, it is very important to develop methods for sulfides and thiols determination.

We prepared three different sensors with the following dyes (see the dyes' structures in Table 1):

• Eriochrome Black T (EBT), for water hardness analysis;

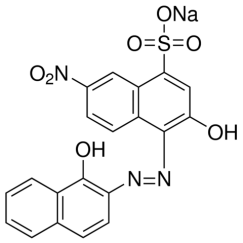
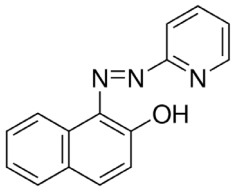
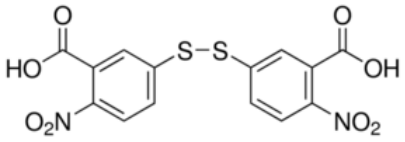
• 1-(2-Pyridylazo)-2-naphthol (PAN), for cobalt(II), nickel(II), and zinc(II) simultaneous determination;

• 5,5′-Dithiobis(2-nitrobenzoic acid) (Ellman's reagent, ELL), for sulfides;

obtaining their respective devices named: EBT-CC, PAN-CC, and ELL-CC.

We registered the sensors' UV-vis spectra and the smartphone pictures' RGB values after equilibration with a single analyte or multianalyte solution to test the devices' sensing properties toward the selected analytes. We then applied the multivariate regression PLS (Partial Least Squares regression) to obtain the relationship between the analyte content and the UV-vis spectrum (or RBG values) change of each colorimetric sensor.

**Table 1**. Structure of the dyes.

| Structure | Name |
|---|---|
|  | Eriochrome Black T (EBT) |
|  | 1-(2-Pyridylazo)-2-naphthol (PAN) |
|  | 5,5′-Dithiobis(2-nitrobenzoic acid) (Ellman's reagent, ELL) |

## Experimental section

*Materials and methods.* All reagents used were of analytical grade. MilliQ water was used throughout. Calcium, Cobalt, Copper, Magnesium, Nickel, Zinc, and standard solutions were obtained by diluting 1000 mg/L Standard for ICP (Sigma-Aldrich, Italy). $Na_2S$ was obtained by Sigma-Aldrich, Italy. Eriochrome Black T (EBT), 1-(2-Pyridylazo)-2-naphthol (PAN), 5,5′-Dithiobis(2-nitrobenzoic acid) (Ellman's reagent, ELL) in powder form, were obtained by Sigma-Aldrich, Italy. Colour Catcher® (hereafter CC) was bought in a common Italian supermarket.

An ICP-OES PerkinElmer Optima 3300 DV (dual view) (Perkin Elmer, Milan, Italy) instrument was used to analyze the cations solutions. The sensors' UV-vis spectra were recorded by a Jasco V-750 spectrophotometer (Jasco Europe SRL, Lecco, Italy), equipped with FLH-740 Film Holder and a homemade, 3D printed holder designed to give the spectrum acquisition quick and easy. This holder was a project of Dr. Simone Marchetti, using the facilities of the laboratory 3D@UniPV (University of Pavia, Italy). The photos were acquired by a smartphone Huawei P20 lite in a lightbox to ensure a constant and reproducible light exposition. The reproducibility referred to differences among photos of the same sensor and was much better with this method than using the camera's flash mode. Our scope needs to evaluate one color that turns into another, and the RGB triplet gives more information than other indices or color spaces. GNU Image Manipulation Program GIMP 2 free software was employed, which allows for the definition of the photo area to be analyzed and returns RGB triplets' average values for each sample. The open-source Chemometric Agile Tool (CAT) program was used for PLS analysis [17].

*Fabrication of CC-based devices.* The immobilization of each dye on the CC was via ion exchange. The obtained materials were denoted hereafter EBT-CC, PAN-CC, and ELL-CC. One original CC sheet was cut into 2 × 2 cm pieces of about 0.03 g. Each device was prepared by contacting a CC sheet's piece with 10 mL of an aqueous solution (or hydroalcoholic solution) of a proper concentration of each dye for the required time to reach equilibrium. After cleaning with MilliQ water, the products obtained were stable for days.

We defined the best pH value for the sorption and the concentration of each dye with preliminary tests. We also performed kinetic experiments to define the suitable timing for obtaining stable and reproducible sensors. Table 2 summarizes the experimental conditions for preparing the three devices.

About the choice of the parameters, for all sensors, the dye concentration selected was the one that provided a sufficient color intensity for appreciating the color change in the presence of the analyte.

In the case of EBT-CC, we performed some tests in different buffer solutions at pH ranging from 10 to 12. The best results, i.e., a more uniform and stable color, were obtained in a $NaHCO_3/Na_2CO_3$ 0.1 M hydroalcoholic solution (25 % ethanol). This same media gave a quicker response: a stable color was obtained in about two hours. Otherwise, in ammonia/ammonium chloride buffer at pH 10 and without ethanol, the color is not stable, and the measurements were completely irreproducible.

For the PAN-CC sensor, we tested both acid and alkaline media. The best results were obtained at pH 12, obtained in hydroalcoholic solution (20 % ethanol) of NaOH 10 mM. In this case, to obtain a stable sensor, the CC has to be equilibrated with the dye solution overnight.

For the ELL-CC preparation, it was critical to define the best operational conditions since the Ellman's reagent is very sensitive: it is scarcely soluble in water, and it degrades at temperatures higher than 30°C and in alkali media. The following protocol for the ELL-CC preparation was developed. The first step is a pre-treatment of the CC sheet with acid for neutralizing the alkaline active site on the solid phase. Then, a portion of the acidified CC is equilibrated with 10 mL of 0.1 mM Ellman's reagent solution, at pH = 3, overnight, at 25 °C, gently stirring on a shaking plate. After rinsing with MilliQ water, the product obtained was stable for days. The pH selected is a compromise value for avoiding decomposition of the Ellman's reagent but enough to have quantitative sorption of the analytes. In these conditions, the time required to obtain a stable color is less than five minutes.

The limit of detection, LOD, and the limit of quantification, LOQ, of each sensor were computed as three times, for LOD, or ten times, for LOQ, the standard deviation of ten blank replicates divided by the slope of the calibration curve. The values obtained were below reported.

**Table 2**. Experimental condition for devices' fabrication: solution pH, dye concentration, media, and the time required to reach the equilibrium ($t_e$) between the CC and each dye.

| Dye | pH | dye concentration, mM | media | $t_e$ |
|---|---|---|---|---|
| EBT | 11.5 | 0.1 | $NaHCO_3/Na_2CO_3$ 0.1M, EtOH 25% | 2 h |
| PAN | 12.0 | 0.05 | NaOH 10 mM, EtOH 20% | 24 h |
| ELL | 3.0 | 0.1 | $HNO_3$ 1 mM | 3 min |

EBT-CC: LOD = 0.8 °F; LOQ = 2.6 °F

PAN-CC: $LOD_{Co}$ = 0.3 μM, $LOQ_{Co}$ = 1.0 μM; $LOD_{Ni}$ = 4.3 μM, $LOQ_{Ni}$ = 1.4 μM; $LOD_{Zn}$ = 8.1 μM; $LOQ_{Zn}$ = 2.7 μM

ELL-CC: LOD = 0.006 mg/L; 0.02 mg/L

Interferences tests were also performed. For EBT-CC, it is well known that the EBT dye forms red complexes with several cations, but since the sensor we prepared should be used for water hardness analysis, the concentration of divalent cations in these media are at least 10-100 times lower than that of Ca(II) and Mg(II). Indeed, in particular, Zn(II) did not interfere in the analysis. PAN-CC complexes with Cd(II), Cu(II) and Mn(II) were also studied, but their color stability is very poor. Moreover, the color of these complexes is completely different from those of the cations under investigation, and the UV-vis spectra do not interfere in the determination of Co(II), Ni(II) and Zn(II). Regarding the ELL-CC, the main cations such as $Na^+$, $K^+$, $Ca^{2+}$ and $Mg^{2+}$, evidently contained in real matrixes, do not interfere in the development of the yellow color of the sensor.

*EBT-CC, PAN-CC, and ELL-AS as sensors: application of the multivariate regression PLS.* The work's core is the development of models for assessing, in unknown samples, the tested analytes' concentration. For reaching the goal, we used the chemometric tool PLS (Partial Least Squares Regression). We elaborate by the PLS, for each sensor, the entire UV-vis spectra of the solid phase after equilibration with the analyte solutions (similar data treatment can be done using the RGB values of the sensor's pictures). We referred to a suitable experimental design to plan the experiments required to create the PLS model (the so-called "training set"). Ref. 18 reports an accurate guideline for designing a series of mono-analyte samples or multicomponent mixtures for calibration. The regression model is first tried with a cross-validated procedure on the training set and then on an external data set (test set). We decide to investigate as external data set some actual samples with a known content of the analytes. The model was considered valid when the prediction error on the concentration was around 10–15%. We performed PLS data treatment with the use of the R-based chemometric software CAT (developed by the Chemometrics group of the Italian Chemical Society) [17].

The limit of detection for each sensor

*Actual samples.* For water hardness and sulfur analysis, we sampled natural waters in Pavia's district (Lombardia, Italy). Before sampling, water was left owing for 20 min and then collected in a 1 L polyethylene bottle. Before analysis, all samples were stored in a fridge at 4 °C.

The following integrators of Co, Ni, and Zn were analyzed as delivered or after dilution with MilliQ water: Catalitic® (Cemon, Italy); Fisiosol5® (Specchiasol, Italy); Gammadyn® (Unda, Belgium); Oligosol® (Labcatal, France).

## Results and discussion

### EBT-CC as a sensor: determination of water hardness

By the EBT-CC sensor, we planned to assess the water hardness in unknown samples.

An interesting PLS model was created considering the UV-Vis spectra of EBT-CC after equilibration with waters with different hardness degrees. The samples required to build the model (training set) were obtained, equilibrating equal portions of EBT-CC with synthetic solutions at different degrees of hardness, spanning very sweet to very hard waters. Figure 1 shows some spectra and the images of the EBT-CC sensor before and after contact with solutions at different hardness degrees.

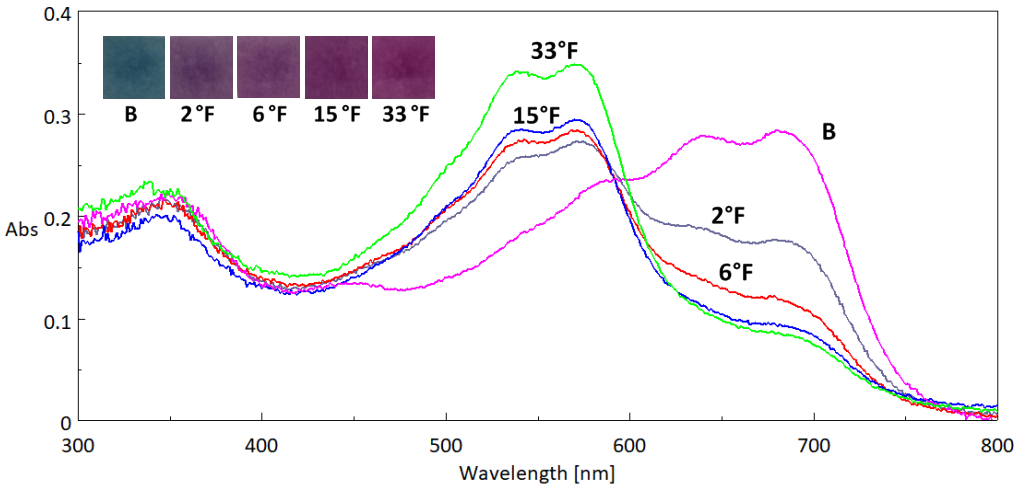



**Figure 1.** UV-vis spectra and images of EBT-CC before (B) and after equilibration with water at 2°F, 6°F, 15°F, and 33 °F.

The pretty good adaptation obtained (see Fig. 2) allows affirming that the PLS model is valid for interpreting experimental data. For evaluating the predictive ability of this model, some real waters were used as test sets. To this end, numerous samples of drinking water are analyzed, taken from the tap. We decided to carry out sampling within the province of Pavia (Lombardia, Italy). The following Figure 3 shows the sampling points; different colors represent the different hardness.

The water hardness values obtained by the EBT-CC sensor have been compared with those achieved by the standard method (official of hardness analysis according to the Higher Institute of Health for water intended for human consumption), i.e., the complexometric titration with EDTA. The results obtained by the tho method agreed pretty well (differences within the 10 %), as shown in Table 3.

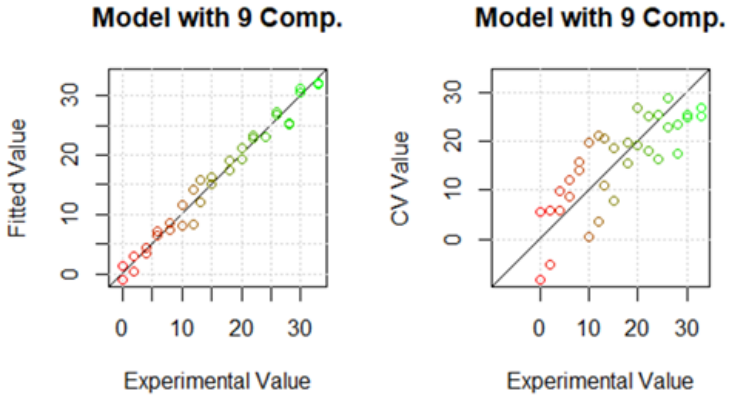



**Figure 2.** Software output for the PLS model, based on 9 latent variables, for the hardness determination by EBT-CC sensor. The different colors refer to two different experiments at low (red) and high (green) hardness degrees. 1) Comparison between the experimental points and those recalculated from the model; 2) the same comparison but in cross-validation conditions.

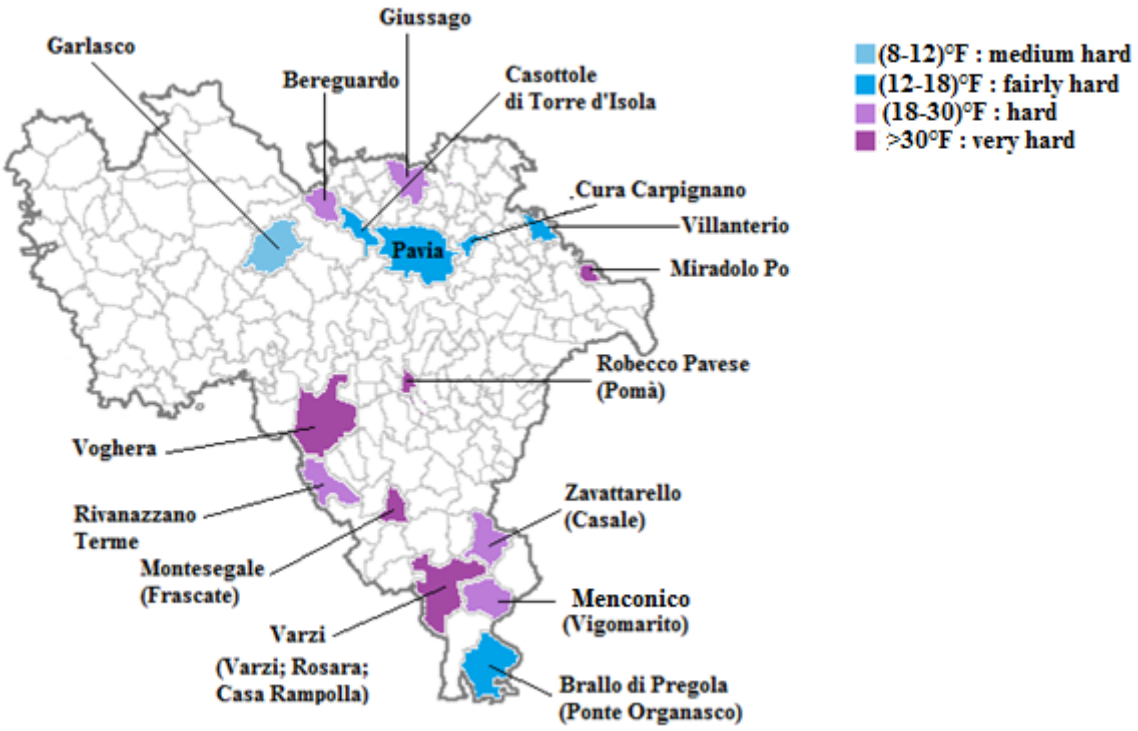



**Figure 3.** Water sampling points within the province of Pavia. The different colors indicate waters with different hardness: light blue zone (8-12) °F; blue zone (12-18) °F; violet zone (18-30) °F; dark purple zone > 30 °F.

**Table 3.** Hardness degree (°F) of natural waters sampled in Pavia district (Italy). EBT-CC sensor and EDTA titration results. The mean value of three replicates for each sample is shown. Numbers between brackets are the standard deviation on the last digit.

| Water sampling points (Pavia, Italy) | EDTA titrations (°F) | PLS (°F) |
|---|---|---|
| Bereguardo | 20.1(5) | 24(3) |
| Casa Rampolla (Bosmenso) | 9.9(5) | 9.6(3) |
| Casale di Zavattarello | 20.1(1) | 16(4) |
| Casottole (Torre d'Isola) | 16.3(3) | 18(2) |
| Cura Carpignano | 13.0(3) | 13.0(4) |
| Garlasco | 9.5(1) | 12(2) |
| Giussago | 25.5(3) | 25.0(7) |
| Miradolo Terme | 33.0(1) | 30(3) |
| Montesegale (Frascate) | 40.7(4) | 38(3) |
| Pavia | 13.3(7) | 14(1) |
| Pomà | 41.0(1) | 40(1) |
| Ponte Organasco | 15.3(5) | 16(1) |
| Rivanazzano Terme | 23.5(2) | 23.0(7) |
| Rosara | 27.2(1) | 26(1) |
| Varzi | 34(1) | 35(1) |
| Vigomarito (Menconico) | 23.3(4) | 22(2) |
| Villanterio | 12.7(4) | 12.2(4) |
| Voghera | 30.3(3) | 31(2) |

*PAN-CC as a sensor: determination of Co(II), Ni(II), and Zn(II) in commercial oligomineral supplements*

By the PAN-CC sensor, we developed a PLS model for simultaneously assessing, in unknown samples, the concentrations of Co(II), Ni(II), and Zn(II).

We registered UV-vis spectra of the solid phase under quantitative sorption conditions (overnight loading of cations, pH = 10, and excess of active sites of the solid phase), and the entire spectrum was used for the PLS analysis. Figure 4 shows the spectra and the PAN-CC images before and after contact with the metal ions solutions.

Since the PLS methodology required planning a suitable "training set," we prepared it by applying an experimental design, mixing standard solutions of the three cations at different concentrations, and covering the experimental domain symmetrically. We chose the experimental design with 5 concentration levels, based on Multilevel Partial Factorial Design (MPFD) [18]. The book of Brerenton [18] reports a proper guideline for designing a series of multicomponent mixtures for calibration.

Table 4 presents the experiments' plan, and Figure 5 the software output for the PLS regression.

**Table 4**. Experimental design: 5 levels for 3 components (Co, Ni, Zn). 25 mL of $NH_3$/$NH_4Cl$ buffer solution at pH =10 and 0.033 g of PAN-CC.

| Variable levels | | Design of Experiments | | | |
|---|---|---|---|---|---|
| n. level | c /μM | | [Co] | [Ni] | [Zn] |
| -2 | 0 | 1 | 0 | 0 | 0 |
| -1 | 3.5 | 2 | 0 | -2 | -1 |
| 0 | 4.5 | 3 | -2 | -1 | -2 |
| 1 | 5.5 | 4 | -1 | -2 | 2 |
| 2 | 7 | 5 | -2 | 2 | 2 |
| | | 6 | 2 | 2 | 0 |
| | | 7 | 2 | 0 | -1 |
| | | 8 | 0 | -1 | 2 |
| | | 9 | -1 | 2 | -1 |
| | | 10 | 2 | -1 | 1 |
| | | 11 | -1 | 1 | 1 |
| | | 12 | 1 | 1 | 0 |
| | | 13 | 1 | 0 | 2 |
| | | 14 | 0 | 2 | 1 |
| | | 15 | 2 | 1 | 2 |
| | | 16 | 1 | 2 | -2 |
| | | 17 | 2 | -2 | -2 |
| | | 18 | -2 | -2 | 0 |
| | | 19 | -2 | 0 | 1 |
| | | 20 | 0 | 1 | -2 |
| | | 21 | 1 | -2 | 1 |
| | | 22 | -2 | 1 | -1 |
| | | 23 | 1 | -1 | -1 |
| | | 24 | -1 | -1 | 0 |
| | | 25 | -1 | 0 | -2 |

The ability of PLS to model the experimental data is manifest from the quite good agreement between the measured and fitted values. The higher dispersion of the data points for Zn(II) is higher than that obtained for the Ni(II) and Co(II), probably due to the significant ease of contamination during the experiments in the presence of a ubiquitous cation as Zn(II), resulting in poorly accurate determinations.

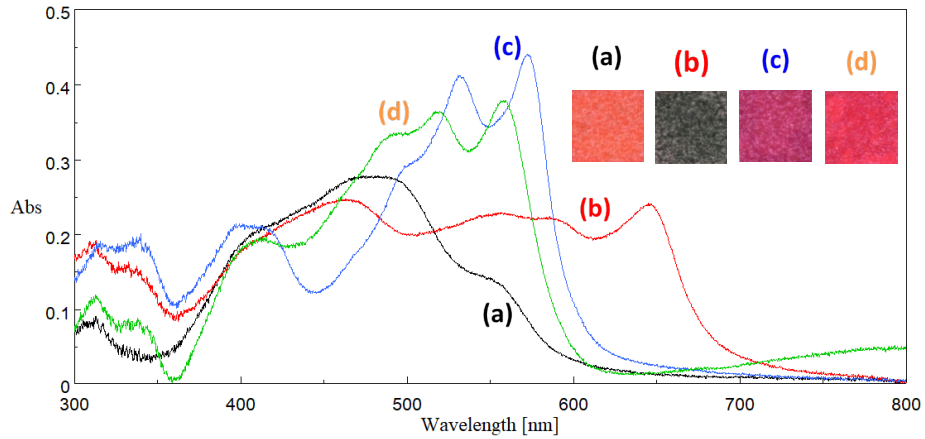



**Figure 4.** UV-vis spectra and images of PAN-CC (a) before and after equilibration with (b) Co(II), (c) Ni(II), and (d) Zn(II) solutions.

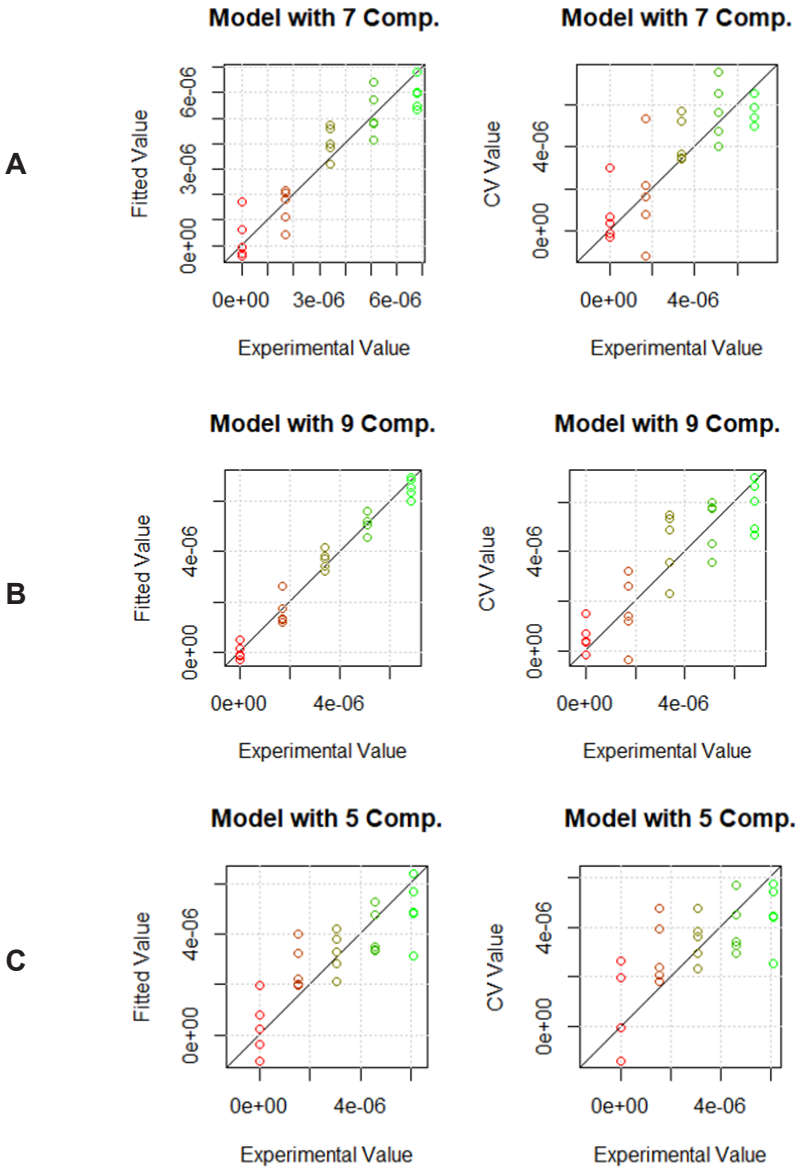



**Figure 5**. Co(II), Ni(II), and Zn(II) on the PAN-CC sensor: software output for the PLS regression model. a) Co(II); b) Ni(II); c) Zn(II). The different colors refer to five replicates at different metal ion concentrations. 1) Experimental points vs. points recalculated from the model; 2) the same comparison but in cross-validation conditions.

The PLS model is first tested with a cross-validated procedure on the training set and then on an external data set, called a test set. Independent samples of the three cations were prepared for this purpose. In particular, we analyzed four integrators of Co(II), Ni(II), and Zn(II). Table 5 summarises the results. By matching the concentrations declared on the packaging with the PLS models' predicted values, the differences are not great, being, in any case, less than 10-15 %. The Co(II) concentration in two integrators (Catalitic and Gammadyn) was not determined since lower than the LOD of the method.

*ELL-CC as a sensor: determination of sulfurs*

As reported above, the ELL-CC is a device for sulfurs obtained by immobilization on the CC support of the Ellman's reagent, i.e., the 5,5'-dithio-bis-(2-nitrobenzoic acid), a versatile compound known for the colorimetric determination of free sulfhydryl groups. When reacting with sulfhydryls in an aqueous solution, Ellman's reagent (DTNB) produces a yellow-colored product (TNB), as shown in the scheme of Figure 6. Accordingly, this reagent can be useful as a receptor in sulfhydryl sensors since its specificity for -SH groups [19-21].

The colorless ELL-CC device turns yellow after the sorption of sulfides and thiols, and the intensity of the color is related to the analyte's concentration. We performed experiments with sodium sulfide, and as aforementioned, we used the partial least square analysis PLS to find the most suitable model able to correlate ELL-CC spectra with the analyte concentrations. Figure 7 shows UV-vis spectra and pictures of the ELL-CC sensors after equilibration in sodium sulfide solutions from 0 to 58 μM.

**Table 5.** Co(II), Ni(II), and Zn(II) concentrations in four commercial integrators using PAN-CC and applying a PLS model on UV-vis spectra. Each integrator was properly diluted before the analysis.

| | $c_{declared}$ (mg/2mL) | $c_{declared}$ (mM) | $c_{PLS}$ (mM) | e% |
|---|---|---|---|---|
| Fisiosol® | | | | |
| Co(II) | 0.0726 | 0.616 | 0.55(1) | -11 |
| Ni(II) | 0.0726 | 0.618 | 0.68(9) | 10 |
| Zn(II) | 0.0674 | 0.515 | 0.48(8) | -7 |
| Oligosol® | | | | |
| Co(II) | 0.0726 | 0.616 | 0.54(8) | -12 |
| Ni(II) | 0.0726 | 0.618 | 0.7(2) | 13 |
| Zn(II) | 0.0674 | 0.515 | 0.45(1) | -13 |
| Catalitic® | | | | |
| Co(II) | 0.062 | 0.526 | n.d. | / |
| Ni(II) | 0.088 | 0.750 | 0.8(7) | 6 |
| Zn(II) | 0.314 | 2.4 | 2.1(4) | -13 |
| Gammadyn® | | | | |
| Co(II) | 0.073 | 0.619 | n.d. | / |
| Ni(II) | 0.073 | 0.622 | 0.6(3) | -3 |
| Zn(II) | 0.067 | 0.512 | 0.5(3) | 1 |

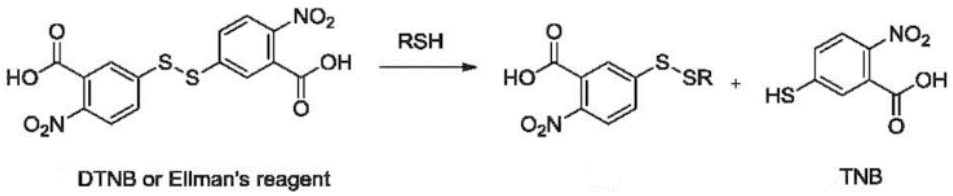



**Figure 6.** The reaction between the Ellman's reagent and a generic thiol (RSH). The yellow color of the TNB product is proportional to the RSH content.

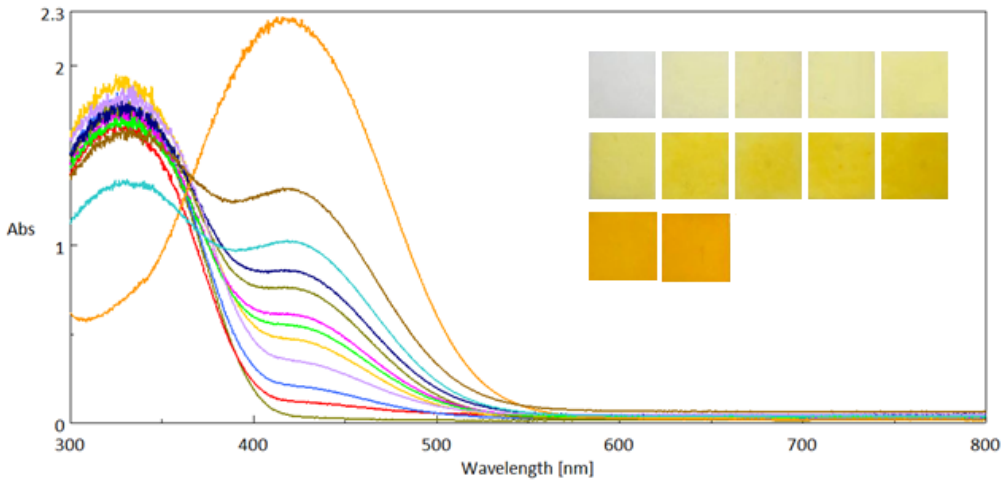



**Figure 7.** UV-vis spectra and images of ELL-CC before and after equilibration in sodium sulfide solutions from 0 to 58 μM.

Figure 8a shows the graph obtained by PLS, which allow obtaining the number of components useful for describing the system (in this case, 2, i.e., the minimum of the curve of the RMSECV graph vs. the number of components; in Figure 8b, the good fitting obtained using these two components. Furthermore, some real samples were used as a test set for evaluating this model's predictive ability. For this purpose, we analyzed several natural waters sampled within the Pavia district. As shown in Table 6, The sulfide concentrations obtained by the ELL-CC device are in quite good agreement with those measured by the iodometric titration, the standard method for sulfide determination in waters.

Given the agreement quite good between the data obtained with the two methods (although the iodometric titration data for samples with sulfide concentration lower than 1 mg/L could be inaccurate), the ELL-CC device is promising to determine sulfides in waters.

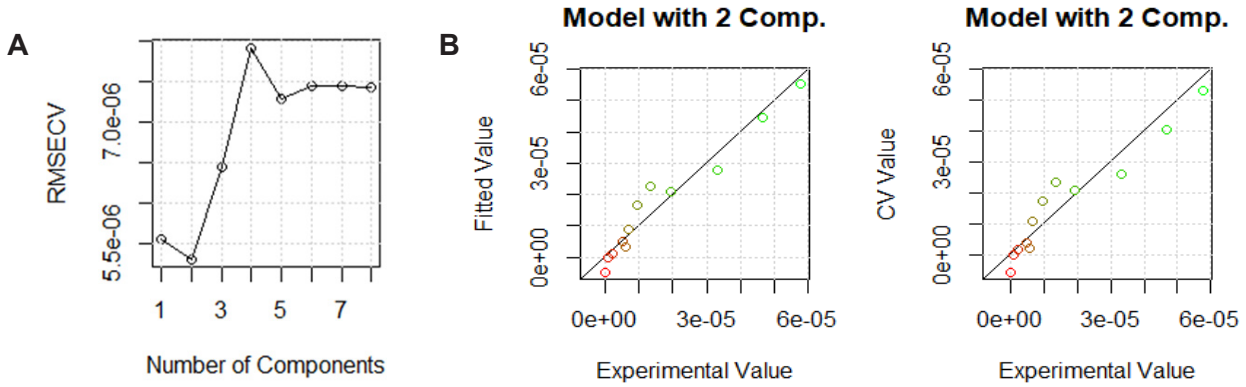



**Figure 8.** Sulfurs on ELL-CC. a) graph for the construction of the PLS model: RMSECV vs. the number of components. b) software output for the PLS regression model.On the left: the experimental points vs. those recalculated by the model. On the right: the experimental points vs. those recalculated by the model in cross-validation conditions.

**Table 6.** Sulfide content in natural waters sampled in Pavia district (Italy). ELL-CC sensor and iodometric titration results.

| Water sampling points (Pavia, Italy) | ELL-CC $S^{2-}$ (mg $L^{-1}$) | titration $S^{2-}$ (mg/$L^{-1}$) |
|---|---|---|
| Retorbido | 21.9 | 24.3 |
| Rivanazzano | 13.0 | 13.1 |
| Gambolò | 4.0 | 3.6 |
| Pavia (lab tap water) | 1.8 | 1.3 |
| Zeccone | 1.8 | 0.6 |
| Confienza | 1.5 | 2.2 |
| Lomello | 1.4 | 2.0 |
| Gravellona | 1.4 | 1.6 |
| Mortara | 1.3 | 2.1 |
| Siziano | 1.2 | 0.3 |
| Bescapè | 1.2 | 1.1 |
| Cura Carpignano | 1.2 | 0.6 |
| Casteggio | 1.1 | 1.0 |
| Tromello | 1.1 | 1.7 |
| S Cristina | 1.1 | 1.2 |
| Mede | 1.0 | 2.0 |
| Casorate | 1.0 | 1.5 |
| Ferrera | 1.0 | 1.8 |
| Cilavegna | 1.0 | 1.6 |
| Palestro | 0.9 | 1.7 |
| Sannazzaro | 0.9 | 2.3 |
| Cassolnovo | 0.9 | 1.7 |
| Varzi | 0.9 | 1.1 |
| Dorno | 0.8 | 1.2 |
| Mornico | 0.8 | 0.6 |
| Pavia (groundwater) | 0.04 | 0.03 |

## Conclusion

This paper describes disposable and cheap colorimetric sensors for different analytes of environmental and biological interest. They were obtained by fixing classical dyes on the "Colour Catcher®" (here named under the acronym CC), the paper sheet used to prevent color runs in washing machine cycles. The devices' fabrication procedure was simple, sustainable, and can be carried out by common people also in regions with limited resources.

Among different analytes of environmental and biological interest, we focused on some divalent cations and sulfides, preparing three different sensors with the following dyes: Eriochrome Black T (EBT), for water hardness determination; 1-(2-Pyridylazo)-2-naphthol (PAN), for Co(II), Ni(II), and Zn(II) simultaneous analysis; 5,5′-Dithiobis(2-nitrobenzoic acid) (Ellman's reagent, ELL), for sulfides.

The naked eye easily observed the color change of the sensors after contact with the analyte's solutions.

For analytical applications of these devices, it is necessary to build a correlation model between the signal (the UV-vis spectrum of the sensor after equilibration with the analyte solutions) and the analyte's concentration. To this end, we decided to apply a chemometric tool: the multivariate regression PLS (Partial Least Squares regression).

The models obtained proved their worth in predicting the analytes' concentrations in aqueous, synthetic, and actual samples. The results obtained are promising, and other experiments are ongoing to improve the colorimetric sensors' performance and create a simple color scale for qualitative analysis.

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## References

1. Liu, B.; Zhuang J.; Wei, G. Recent advances in the design of colorimetric sensors for environmental monitoring. *Environ. Sci. Nano.* **2020,** 7, 2195–2213.

2. Piriya, V.S.A.; Joseph, P.; Daniel, SCGK; Lakshmanan, S.; Kinoshita, T.; Muthusamy, S. Colorimetric sensors for rapid detection of various analytes. *Mater. Sci. Eng*. C **2017**, 78, 1231–1245.

3. Dincer, C.; Bruch, R.; Costa-Rama, E.; et al. Disposable Sensors in Diagnostics, Food, and Environmental Monitoring. *Adv. Mater*. **2019**, 31, e1806739.

4. Alberti, G.; Re, S.; Tivelli, A.M.C.; Biesuz, R. Smart sensory materials for divalent cations: a dithizone immobilized membrane for optical analysis. *Analyst* **2016**, 141, 6140-6148.

5. Biesuz, R.; Emma, G.; Milanese, C.; Dacarro, G.; Taglietti, A.; Nurchi, V. M., Alberti, G. Novel DFO SAM on mesoporous silica for iron sensing. Part I. Synthesis optimization and characterization of the material. *Analyst* **2014**, 139, 3932-3939.

6. Alberti, G.; Quattrini, F.; Colleoni, R.; Nurchi, V.M.; Biesuz, R. Deferoxamine–paper for iron(III) and vanadium(V) sensing. *Chem. Papers* **2015**, 69(8), 1024-1032.

7. Vallejos, S.; Muñoz, A.; García, F. C.; Colleoni, R.; Biesuz, R.; Alberti, G.; García, J. M. Colorimetric detection, quantification and extraction of Fe(III) in water by acrylic polymers with pendant Kojic acid motifs. *Sens. Actuators B-Chem*. **2016**, 233, 120-126.

8. Biesuz, R.; Santos, M.A.; Nurchi, V. M.; Alberti, G. Development of a sensor for trivalent iron: AHP fixed on mesoporous silica. *New J. Chem*. **2018**, 42, 15237-15244

9. Alberti, G.; Zanoni, C.; Magnaghi, L.R.; Santos, M.A.; Nurchi, V.M.; Biesuz, R. DFO@EVOH and 3,4-HP@EVOH: Towards New Polymeric Sorbents for Iron(III). *Chemosensors* **2020**, 8, 111

*10. https://www.dylon.com.my/; https://www.casahenkel.it/brand/grey/grey-acchiappacolore.html* (accessed Dic. 2020).

*11. https://www.who.int/water_sanitation_health/dwq/chemicals/hardness.pdf* (accessed June 2021)

12. Alamgira, S.; Rhamana, M.M.; Basarana, I.; Powell, D.R.; Hossain, M.A. Colorimetric and spectroscopic cobalt(II) sensing by a simple Schiff base. *Polyhedron* **2020**, 187, 114681.

13. Lin, L.; Hu, ST.; Yan, YC.; et al. A highly selective chemosensor for nickel(II) based on fluorescence quenching of a bispyrazole derivative. *Res. Chem. Intermed*. **2017,** 43, 283–295.

14. Yan, M.; Song, Y.; Wong, C.P.; Hardin, K.; Ho, E. Zinc deficiency alters DNA damage response genes in normal human prostate epithelial cells. *J Nutr*. **2008**, 138(4), 667-673.

15. Wang, K.; Peng, H.; Wang, B. Recent Advances in Thiol and Sulfide Reactive Probes. *J Cell. Biochem.* **2014**, 115, 1007-1022.

16. Pandey, S.K.; Kim, K.-H.; Tang, K.-T. A review of sensor-based methods for monitoring hydrogen sulfide. *Trends Anal. Chem.* **2012**, 32, 87-99.

*17. http://www.gruppochemiometria.it/index.php/software/19-download-the-r-basedchemometric-software* (accessed Nov. 2020).

18. Brerenton, R. Multilevel Multifactor Designs for Multivariate Calibration, *Analyst* **1997**, 122, 1521-1522.

19. Ellman, G.L. Tissue sulfhydryl groups, *Arch. Biochem. Biophys*. **1959**, 82, 70-77.

20. Ellman, G.L.; Courtney, K.D.; Andres, V.; Featherstone, R. M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol*. **1961**, 7, 88-95.

21. Eyer, P.; Worek, F.; Kiderlen, D.; Sinko, G.; Stuglin, A.; Simeon-Rudolf, V.; Reiner, E. Molar absorption coefficients for the reduced Ellman reagent: reassessment. *Anal. Biochem*. **2003**, 312(2), 224-227.