Sustainable and green persulfate-based chemiluminescent method for on-site estimation of chemical oxygen demand in waters

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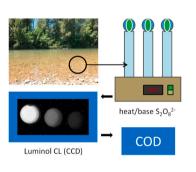
HIGHLIGHTS

- A CL-based method with a CCD camera as detector is used for measuring COD in water.
- Instead of hazardous dichromate as the oxidant, the non-toxic persulfate is used.
- Persulfate is activated through heat and base, enhancing its oxidizing capacity.
- Analysis throughputs are significantly increased compared to the standard method.
- The persulfate method correlates well with those using dichromate or permanganate.

GRAPHICAL ABSTRACT

ARTICLE INFO

ABSTRACT

The standard method for estimating the chemical oxygen demand (COD) of water bodies uses dichromate as the main oxidant, a chemical agent whose use has been restricted in the European Union since 2017. This method is hazardous, time-consuming, and burdensome to adapt to on-site measurements. As an alternative and following the current trends of sustainable and green chemistry, a method using the less toxic reagent sodium persulfate as the oxidizing agent has been developed. In this method an excess of persulfate, activated through heating in an alkaline solution, oxidizes the chemically degradable organic fraction through a 2-step radical mechanism. The remaining persulfate is evaluated by chemiluminescence (CL) using luminol and a portable charge-coupled device (CCD) camera. The method provided quantitative recoveries and a sample throughput of >60 samples h^{-1}. It was validated in river water samples by comparison of COD estimations with the standard dichromate method (R = 0.973, p < 0.05) and with a UV–Vis permanganate-based method (R = 0.9998, p < 0.05), the latter being also used for drinking waters. The proposed method is a sustainable and green alternative to the previous used methods. Overall, the method using activated persulfate is suitable for use as COD quantitation/screening tool in surface waters. Considering that its main components are portable, it can be ultimately adapted for in situ analysis at the point of need.

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1. Introduction

Chemical oxygen demand (COD) is considered one of the most significant water quality indices in environmental projects [1]. In broad terms, the COD parameter refers to the amount of oxygen required to fully oxidize organic materials in water bodies, as estimated by using strong oxidants [2,3] such as dichromate ($E^0 = 1.36$ V), permanganate ($E^0 = 1.51$ V) or cerium(IV) ($E^0 = 1.44$ V) [4] and gives quantitative information about water organic pollution [5]. In this regard, the first COD methods were developed nearly 150 years ago using permanganate [6]. Over time, permanganate has been replaced by dichromate in the standard methods for COD determination [7], the former being limited to relative measurements of organic pollution for checking the quality of potable and surface waters [8,9].

Indeed, the ISO 6060 definition of COD includes dichromate as the oxidizing agent [10]. Recently, REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) EU Regulation (No 348/2013 amendment) has identified potassium (as well as sodium and ammonium) dichromate as a substance of very high concern (SVHC), being included in the list of substances subject to the REACH authorization requirement since September 2017 [11]. Although the ISO 15705:2002 method [12] strongly reduced the amount of toxic chemicals required for COD measurement, there is still a strong request for finding alternatives for dichromate.

Dichromate-based methods [7,10,12] present significant drawbacks: use of hazardous chemicals (hexavalent chromium, mercury, silver and sulfuric acid) at high temperatures, entailing the generation of even more hazardous wastes [13]; cumbersome and time-consuming procedures (reflux time > 2h [14]), especially those that rely on titrimetric detection, resulting in low analysis throughputs and often requiring skilled operators [4]; difficulty in adapting the method to on-line/in-line or on-site measurement following the recent trend in analytical chemistry towards the development of environmentally friendly "point-of-use" methods [15]. In this context, the need for alternative procedures for estimating COD in water bodies has become imperative.

Up to this point, divergent pathways can be followed for finding alternative methods. First, approaches based on a chemical principle different from the "classical" wet digestion can be developed. The oxidation of the organic matter has been carried out by means of photocatalysis [16,17], electrocatalysis [18] or photoelectrocatalysis [19]. The resulting methods, usually referred to as electrochemical methods [8], are absolute and do not require standard compounds. However, they are more suitable for measuring the dissolved COD due to the mandatory requirement for sample filtration, as cell volumes are at the microliter level. Such methods neglect the fact that particles are believed to be the most important COD carrier [8]. In addition, the proper choice of the working electrode can be highly challenging, requiring specific functionalization [19]. More research is needed for them to ultimately become the method of reference. The same can be stated for methods that directly measure COD avoiding an oxidation step, such as machine learning methods [1] and corrected UV absorption spectroscopy [20].

The other approach involves further improving wet digestion methodologies. Although the standard dichromate method is still used [21], numerous efforts [22–26] have been made to address its limitations. However, none of them have yet replaced the dichromate method in COD measurements. Within these efforts, chemiluminescence (CL) detection [17,24,27–29], often coupled to flow analysis, has been exploited to improve sensitivity, dynamic ranges and response times using simple instrumentation [30].

The aim of this work was then to propose a wet CL analytical procedure for COD measurements that addresses all the issues of the dichromate-based methods, while being as accurate and precise [5]. This results in a more environmentally friendly method that meets in a great extent the principles of green chemistry [31] and white analytical chemistry [32,33]. In addition, we intend to work within the boundaries of sustainable chemistry and therefore the trend to search for/establish

well-balanced analytical methods is followed [34,35]. This concept also considers the effects of processing, materials, energy, social and economic impacts [36]. In general, sustainable chemistry should use resources, including energy, at a rate at which they can be replaced naturally, and the generation of waste cannot be faster than the rate of its remediation. There is an intersection area between the two themes mentioned, greenness and sustainability, both included into the broader concept of suitable chemistry [36].

The developed procedure uses persulfate as a "greener" oxidant alternative to dichromate. Persulfate has gained notoriety lately as the reagent of choice for environmental remediation of groundwater and soils, particularly in field applications [37] by means of a process known as in situ chemical oxidation [38]. This oxidizing agent has $E^0 = 2.01$ V, higher than dichromate or permanganate, and oxidizes organic compounds through a radical-mediated mechanism involving species such as hydroxyl ($OH^\bullet$) and sulfate ($SO_4^{\bullet-}$) radicals with even higher standard redox potentials ($E^0 = 2.73$ V and $E^0 = 2.60$ V, respectively [39]) depending on the system conditions (temperature, pH, presence of a catalyst/activator). Persulfate activation can be done via heat, UV light, transition metal catalysts (e.g., iron chelates [40]) and bases [41]. In the present work a combination of heat and a base was employed and the oxidation step by persulfate was performed in alkaline medium using a thermoblock for test tubes as the COD digester. Afterwards, the remaining persulfate was measured by exploiting its CL reaction with luminol, which also took place in alkaline medium employing, a handmade dark box connected to a portable charge-coupled device (CCD)-based camera. Low COD-containing water samples as drinking and surface waters were tested to assess its accuracy and ultimately validate the method.

2. Experimental

2.1. Chemicals and instruments

The details about the reagents used can be found in Section 1 of the Supporting Information (SI). Fluorescein sodium salt (hereafter referred to as fluorescein), D-glucose and potassium hydrogen phthalate (KHP) were used as COD standard substances. Their stock solutions were stored at 4 °C.

For the measurements of the CL signal, a 383L Mono CCD Camera (Atik Cameras, Norwich, United Kingdom) which was thermoelectrically-cooled at 5 °C and two different luminometers were employed: Junior LB 9509 portable tube luminometer (Berthold Technologies, Baden-Württemberg, Germany) and Luminoskan Ascent benchtop microtiter plate reader (Thermo Fisher Scientific, Waltham, MA). The analytical performance of the two portable instruments was compared with each other and also with the more conventional benchtop luminometer. When the latter was used, the analytical signal was measured in the steady-state region, i.e. 30 s after the starting of the CL reaction. Note that the selected luminometers differ in the numerical values range for the measured intensities.

When using the CCD camera, the CL signal was acquired immediately after the starting of the CL reaction for an integration time of 180 s via the Artemis Capture software and analyzed by the freeware ImageJ software v.1.53h (National Institutes of Health, Bethesda, MD). UV–visible spectra were recorded with a Varian Cary 60 Fiber Optic UV–Vis spectrophotometer (spectral bandwidth of 1.5 nm). The analytical signal was recorded between 200 and 1000 nm. A 32-positions thermoblock for test tubes (Opto-Lab, Concordia, MO) was used.

2.2. Water samples

Drinking water (San Benedetto, oxidability <0.5 mg L^{-1}) was purchased from a local store. Surface water was sampled from the Reno River in the Bologna region. Additional surface water samples were provided by ARPA (Agenzia Regionale per la Protezione Ambientale of

Emilia Romagna, Italy). All samples were stored in amber glass containers and kept at 4 °C if they were not immediately processed.

2.3. Wet digestion procedure and CL reaction

The procedure was established from the literature [41–43] by considering the conditions at which the persulfate oxidation capacity of organic substances is enhanced and then, properly optimized. The sample/standard (1 mL) is mixed with 1 mL of a 2.78 mmol L^{-1} potassium persulfate solution containing 0.36 mol L^{-1} NaOH in a 16 × 100 mm glass tube. Then, the tube is covered with a glass ball and heated in the thermoblock at 100 °C for 15 min. After the reaction, the solution is mixed with the CL reagent (2.8 mmol L^{-1} luminol in 0.18 mol L^{-1} NaOH) in the 1:1 (v/v) ratio and the CL signal is measured.

Depending on the instrument used, the mixing procedure is different: for the portable luminometer, 100 μL of CL reagent are manually added to 100 μL of the solution in a test tube; for the benchtop luminometer, 100 μL of solution are transferred in a 96-well plate and the same volume of CL reagent is added by the instrument built-in reagent dispenser; for the CCD camera, 100 μL of solution are transferred in a 96-well plate and 100 μL of the CL reagent is manually added using a multichannel pipette. In any case, the final reacting concentrations for luminol and NaOH were 1.4 mmol L^{-1} and 0.18 mol L^{-1}, respectively.

Different heating procedures were evaluated for COD digestion: the samples were put in Eppendorf vials and then placed in a boiling water bath (procedure A) or a thermoblock set to 100 °C (procedure B). To promote efficient heat transfer the thermoblock holes were filled with mineral oil. For the thermoblock, other sample containers were used such as pierced Eppendorf vials (procedure C) and 16 × 100 mm glass tubes with or without glass balls at the end (procedures D and E, respectively).

2.4. Theoretical COD values, selection of standards and reactions involved

In COD analysis, the concentrations of standards must be converted to COD units (mg O$_2$ L^{-1} or mg L^{-1}). Equations that allow one to calculate COD from its relation to the theoretical oxygen demand (ThOD) are shown in Section 2 of the SI.

ThOD is not necessarily equal to COD [44,45] and can be related to COD by an empirical constant ranging from 0 to 1. The value of *a* depends on the nature of the organic compound [44], and also on the oxidant used [45]. In the present work, the three selected standards belong to different classes of organic compounds, but all have an *a* value close to 1 (0.97, 0.98 and 0.99 for D-glucose, KHP and fluorescein, respectively) when using dichromate. This means that their oxidation is almost complete, thus COD can replace ThOD with a minimal error (see the SI (Section 2)). These compounds were selected as standards for the purpose of comparison with most of the literature methods. Indeed, KHP and D-glucose have been two of the most used standards for COD analysis. However, to date, KHP is still regarded as the conventional COD standard and preferred over others [21,25]. In addition, fluorescein solutions are colored, which represented a useful property in the optimization process since its degradation could be conveniently followed by spectrophotometry.

The proposed mechanism consisting of the two persulfate oxidation reactions [41,42] is fully displayed in Section 3 of the SI.

3. Results and discussion

3.1. Optimization of the involved reactions

As a starting point, the final CL reaction between persulfate and luminol was studied. A benchtop 96-well microplate luminometer was used for performing the preliminary tests. Persulfate can oxidize luminol in basic medium even at room temperature. A key feature of this

reaction lies in its kinetics: CL signals follow a steady-state emission profile or glow-type luminescence as observed in Fig. 1a. This characteristic provides operative advantages over flash-type emissions lasting a few seconds or less, for instance the ease of measurement if the reagents are manually mixed, i.e., in on-site measurements with portable luminometers. For the persulfate concentration range up to 2000 μmol L^{-1}, a luminol concentration of 1.4 mmol L^{-1} at 0.18 mol L^{-1} NaOH was chosen as that which provided a good linear correlation between CL
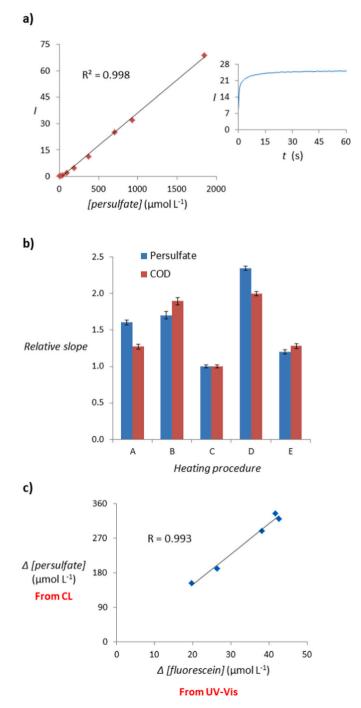

Fig. 1. Optimization of the procedure. a) (Left) correlation (n = 3) between I (CL signal) and persulfate concentration within 0–2000 μmol L^{-1} range at 9 levels of concentrations and (Right) kinetic profile of the CL signal obtained for the persulfate/luminol reaction. b) Comparison of the response for the persulfate and COD calibrations when using different heating procedures (more details in the main text). c) Relationship between the concentration variations for persulfate and fluorescein under the optimized conditions.

signal and concentration of persulfate (Fig. 1a), as well as the highest signal-to-background ratio. Different alkaline pH values higher than 10 were tested, either obtained by NaOH or carbonate-bicarbonate buffer. For comparable pH values (within a range of ±0.1), the slope of the persulfate analytical curve obtained using NaOH was around 1.5 times higher than that obtained using the buffer. Finally, 0.18 mol L^{-1} NaOH was selected, which provided pH > 12, a condition in which the highly oxidative OH$^\bullet$ is the prevailing radical [42].

At room temperature the oxidation of fluorescein by persulfate did not take place, even in the presence of metal ions as catalysts. Since heat activation was instead successful, we first investigated the thermal stability of fluorescein. According to the literature, aqueous solutions of fluorescein are stable up to 100 °C [46]. Indeed, its degradation upon 30 min of heating in a water bath at boiling temperature was negligible (fluorescein concentration decreased by 0.9%). However, when fluorescein was mixed with persulfate in basic medium and heated, the absorbance of the solution decreased rapidly. Heat promotes the formation of sulfate radicals which adds to the basic radical generation resulting in organic degradation (see Section 3 of the SI). In parallel, it was observed that the oxidizing capacity of persulfate solutions in basic medium also decreased under these heating conditions. It has been reported that the reaction temperature is critical for activated persulfate degradations and that its increase leads to a higher rate of formation not only of sulfate radicals but also of potential scavengers, which decreases the analyte oxidation [41,47,48]. Consequently, the more powerful oxidizing radicals generated via heat/base undergo scavenging reactions as temperature and basicity increases, and their capacity for degrading the analyte diminishes. Using the CL luminol reaction to measure the concentration of persulfate, it was assessed that, compared to room temperature, the degradation of persulfate after 15 min of heating at 100 °C was around 30%.

Different sample heating procedures were tested to obtain the best sensitivity, and results are shown in Fig. 1b-c. Fluorescein (60.6 μmol L^{-1}, equivalent to 40 mg L^{-1} of COD) was mixed with persulfate (2.78 mmol L^{-1}) in basic medium and heated at 100 °C in a boiling water bath for different periods of time. A 2.78 mmol L^{-1} persulfate concentration was selected as the initial one considering the dilution of the sample and because it provided a reproducible and intense CL signal whose decrease can be easily monitored. The absorbance of the fluorescein/persulfate solution decreased rapidly with time, and the dye was completely degraded after 15 min, while the solution without persulfate remained unchanged even after 30 min of heating (Figure S1).

The heat-activation procedure (see Section 2.3), able to provide the minimum persulfate inhibition and the maximum fluorescein oxidation efficiency after 15 min of heating, was optimized. To this end, persulfate analytical curves, obtained by monitoring the CL signal in the presence of different amounts of persulfate, were generated. In parallel, COD analytical curves were obtained by monitoring the CL signal in the presence of 2.78 mmol L^{-1} persulfate and different amounts of fluorescein. Heating with the thermoblock provided more accurate results and was easier to handle. As shown in Fig. 1b, for both analytical curves the highest sensitivities were obtained when procedure D was employed; sensitivities were calculated as "relative slopes", that is, the quotient between the slope and the lowest slope value obtained for each calibration. For procedure D, the COD calibration fit was linear (R^2 = 0.992). Generally, it was observed that a closed heating system allowed a more efficient oxidation. Glass balls were optimal because their use did not cause a system overpressure, still allowing vapors condensation and reducing the volume loss of the sample. Precision of measurements (n = 3) was better than 5% in all cases.

Different fluorescein/persulfate mixtures were studied and the residual amounts of fluorescein and persulfate were measured by spectrophotometry and CL, respectively, by interpolation on appropriate analytical curves. In parallel, the corresponding solutions containing only one reagent were also analyzed and the reduction of fluorescein and persulfate concentrations due to the oxidation process was assessed by comparing the results of the two measurements. A positive linear relationship (R = 0.993, P < 0.05) between the decreases (denoted as Δ) in fluorescein and persulfate concentrations was obtained (Fig. 1c) confirming that consumption of persulfate was proportional to the decrease in organic compound concentration.

The suitability of the method was assessed by using D-glucose and KHP as standards; persulfate and organic compound/persulfate standards were processed and analyzed by CL under the optimized conditions. Results for D-glucose, reported in Fig. 2a, show that the maximum CL signal decrease between the persulfate standard containing only the oxidant and the one containing D-glucose (i.e., the greatest CL signal differential ratio) was obtained when the samples were heated for 15 min (initial concentrations of 2.78 mmol L^{-1} and 41.3 mg L^{-1} for persulfate and D-glucose, respectively). A similar behavior was observed for KHP (data not shown). A negligible CL signal was obtained by processing a standard containing only the organic compound (referred to as "blank"), as expected due to the absence of any oxidant.

3.2. Switching from a luminometer to a CCD camera; method calibration

In order to develop an on-site analytical method, portable CL detection systems (i.e., a portable luminometer and a CCD camera) were tested by generating analytical curves for the persulfate-luminol CL reaction using persulfate concentrations ranging from 0 to 800 μmol L^{-1}. The resulting analytical parameters for the different instruments are displayed in Table 1. Limits of detections (LOD) were calculated by using the 3σ-criterion (i.e., the mean signal of the blank plus 3 times its standard deviation). For the CCD camera, the key measuring parameter is the acquisition time (referred to as exposure time) of the CL signals. Thanks to the steady-state kinetics of the CL reaction (see Fig. 1a), it could be possible to use long exposure times, which will result in a better sensitivity. However, for preventing saturation of the CCD sensor the acquisition time was limited to 180 s. As shown in Table 1, values of the same order of magnitude were obtained for LOD, precision, sensitivity, s$_{y/x}$ and linearity. Therefore, the CCD camera can provide a similar analytical performance compared to the luminometers which use a photomultiplier tube detector (PMT). Moreover, the benchtop luminometer and the CCD camera allow performing multiple CL measurements, thus enabling parallel processing of several samples (for the portable one, samples are individually measured). Thus, both multiple processing devices were selected for further experimentation.

Fig. 2b shows the analytical curve obtained by analyzing D-glucose COD standards (COD values up to 200 mg L^{-1}) using the benchtop luminometer. Steady-state CL signal profiles were obtained for all the standards (Figure S2). The plot of CL signals vs COD values shows a reverse correlation that can be fitted to a logarithmic equation (y = (−0.38 ± 0.03)·log x + (1.04 ± 0.04), where y is the normalized CL signal (I$_N$, i.e., the actual CL signal divided by the signal measured in the absence of glucose and x is the COD value; see Table 2) with R^2 = 0.984 (RSD = 3.3%, n = 3). The estimated LOD by using the 3σ-criterion was 3.5 mg L^{-1}. On the other hand, results for the COD calibration using the CCD camera can be found in Fig. 2c. A CL image of the COD standards measured in the 96-well plate is displayed at the bottom of the figure. Through ImageJ analysis, CL signals were calculated and represented as a function of the COD concentration. Again, the signal decreased with COD concentration as in the previous calibration obtaining an equation of y = (−0.380 ± 0.018)·log x - (1.01 ± 0.03), with satisfactory precision (RSD = 3.8%, n = 3) and linearity (R^2 = 0.991). The LOD for the CCD camera method was 1.6 mg L^{-1}. Despite having similar analytical performances (summarized in Table 2), the CCD camera-based method exhibits a lower LOD and allows one to process multiple samples at the same time. A COD analytical curve was also generated using KHP as COD standard (data not shown). Even in this case we obtained a good linear correlation (R^2 = 0.99), but the slope was slightly lower (about 90%) of that of D-glucose (nevertheless, the values were statistically comparable with homogeneous variances confirmed by F and t tests). Note that, in
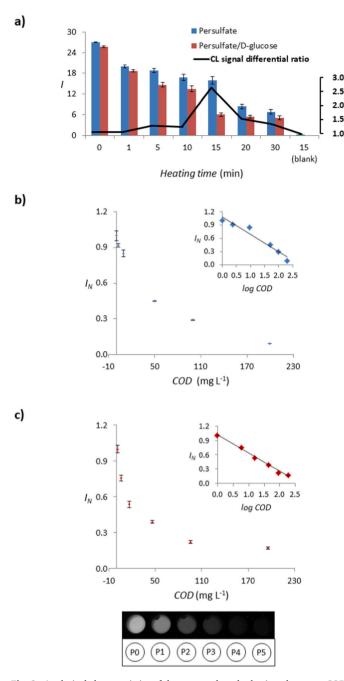
Fig. 2. Analytical characteristics of the proposed method using glucose as COD standard. a) Study of the change in the CL signals for persulfate standards in the absence and in the presence of D-glucose under different heating times (in bold, their corresponding CL signal differential ratio). For b) benchtop luminometer and c) CCD camera: calibration (n = 3) showing I_N (I/I_0) vs COD at 6 levels of concentration; inset: analytical curve in logarithmic form. (Bottom of figure c) the CCD pictures (P0–P5). Note: I_N = normalized CL signal, I_0 = CL signal for the blank.

any case, signals in the I_N vs COD plots tended to a constant value at high organic contents.

A representative scheme of the optimized heat/base-activated persulfate CCD-CL method is presented in Figure S3. Total analysis time is mainly affected by both heating and acquisition times (optimized at 15 min and 180 s respectively), as well as the operator's skills for transferring/adding samples and reagents with the different pipettes. It is fair to state that 32 samples can be processed and measured in less than 25 min, leading to a good throughput (>60 samples h^{-1}).

Table 1

Analytical parameters obtained for each instrument tested in the persulfate calibration in the 0–800 μmol L^{-1} concentration range (the final assay volume was 200 μL, 6 levels of concentration). For a better comparison, I/I_{max} was considered as the analytical signal.

Instrument	LOD[I] (μmol L^{-1})	RSD[II] (%)	$s_{y/x}^{III}$	$y = (a \pm s_a) + (b \pm s_b) \cdot x$		
				$a \pm s_a$	$b \pm s_b$	R^2
Portable Luminometer	45.9	2.1	0.022	−0.045 ± 0.023	0.00138 ± 0.00005	0.998
Benchtop Luminometer	66.8	4.4	0.024	−0.022 ± 0.012	0.00113 ± 0.00003	0.997
CCD	19.5	4.0	0.009	0.036 ± 0.009	0.00144 ± 0.00002	0.9995

[I]Limit of Detection, 3σ-criterion. [II]Relative Standard Deviation, n = 3, intraday. [III]Standard error of the estimate.

Table 2

Analytical parameters obtained for each instrument tested in the COD calibration in the 0–200 mg L^{-1} concentration range (6 levels of concentration). For a better comparison, I/I_0 was considered as the analytical signal. Note: I_0 = intensity for the blank. (Here, $I_0 = I_{max}$).

Instrument	LOD[I] (mg L^{-1})	RSD[II] (%)	$s_{y/x}^{III}$	$y = (a \pm s_a) + (b \pm s_b) \cdot \log x$		
				$a \pm s_a$	$b \pm s_b$	R^2
Benchtop Luminometer[IV]	3.5	3.3	0.06	1.04 ± 0.04	−0.38 ± 0.03	0.984
CCD	1.6	3.8	0.03	1.01 ± 0.03	−0.380 ± 0.018	0.991

[I]Limit of Detection, 3σ-criterion. [II]Relative Standard Deviation, n = 3, intraday. [III]Standard Error of the Estimate, [IV]for the curve parameters, 5 levels of concentration were considered.

3.3. Validation of heat/base-activated persulfate CCD-CL method

LODs for methods to measure COD applied to different aqueous matrices described in the literature range from 0.1 to 900 mg L^{-1} (see Table S1), and the lowest values were obtained by employing sensing devices (i.e., photocatalytic or photoelectric sensors/cells) or CL approaches [27,29,49]. On the other hand, CL methods have also been used for analyzing high COD-containing waters (such as wastewaters [28]), thus proving to be a versatile technique for COD assessment. The low LOD value achieved by the CL method described here (i.e., 1.6 mg L^{-1}) allows one to address scenarios presenting water bodies of different organic matter content, being particularly suitable for the analysis of surface waters (e.g., rivers, streams, lakes, among others). At this point it is important to state that rivers are the most prominent component of water resources [1], which makes them an important subject of study.

COD values reported for rivers are generally in the range of 5–70 mg L^{-1} [17,49–52], although much higher values were reported for a Chinese river [53], whereas for treated and untreated wastewaters COD ranges are 50–250 mg L^{-1} and 50–500 mg L^{-1}, respectively [50]. If a river presents a COD value higher than 50 mg L^{-1} is considered polluted (see Section 8 of the SI). Note that Hejzlar and Kopácek referred to low COD values as those between 1 and 35 mg L^{-1} [54]. For drinking and tap waters, COD values are lower (<1 mg L^{-1} and <5 mg L^{-1}, respectively) [55], although some authors reported a wider range (i.e., 1–10 mg L^{-1}) [54]. Considering this data, our method adjusts well to the COD values expected for surface, tap and drinking waters.

Preliminary experiments showed that in real matrices a significant matrix effect could be present. In some cases, the analysis was straightforward (i.e., results consistent with the standard analytical methods for COD were obtained by simply interpolating the CL signal onto the analytical curve). This was the case for water bodies with a low

content of organic matter. On the other hand, for high COD-containing water samples, the results were not comparable to the reference COD method. This phenomenon might be due to the fact that a higher amount of organic matter (a known binding agent [56]) may be associated with the presence of a higher amount of other compounds and/or particles which could interfere with the measurements. It has been documented that COD determination through the oxidation of organic matter may be interfered with by halides (notably chloride), suspended solids, residual hydrogen peroxide, ammonia, just to name a few [5,8]. In addition, the CL reaction between luminol and oxidants can be affected by different metal ions [27,55]. To avoid these interferences in such samples, an analytical strategy taking into account the possible effect of the sample matrix on the CL signal must be defined. Each scenario was addressed individually.

Water samples with low organic matter content were analyzed. Preliminary tests showed that for river water samples, the COD value was below 10 mg L^{-1}. To evaluate the matrix effect, external calibration (EC) and standard addition calibration (SA) were compared using D-glucose as the COD standard (see Fig. 3). A 4.3% slope variation was obtained, but F and t tests confirmed that the two slopes had homogeneous variances and were statistically comparable (also both EC and SA signal profiles tended to an almost identical constant value at high COD concentrations). Therefore, it can be concluded that for river water samples with such low organic matter content the matrix effect is negligible. As a matter of fact, for low-COD containing samples (i.e., river, drinking and tap waters) a good correlation between the COD values obtained with the CCD-CL method and those measured with an optimized UV–Vis method which uses acidic potassium permanganate (COD$_{Mn}$, as in Ref. [52]) was obtained (Table 3). Moreover, the pictures taken by the CCD camera for river, drinking and tap water samples (without adding standard and fortified with 10 mg L^{-1} of D-glucose) can be found in the SI (Figure S4). The comparison of the results evidenced that the CCD-CL method possesses good accuracy (relative errors <20% and absolute errors < 1 mg L^{-1}), recovery (values between 100 and 110%, indicative of the absence of the matrix effect) and precision (RSD <10%). Drinking water exhibited the highest relative error, a predictable situation for a sample with COD values close to the LODs of the methods. Tap water presented the highest COD value, while the results for river water were in agreement with the preliminary experiments (COD value of 4.9 mg L^{-1}). The estimated values for drinking and tap waters are higher than those reported in Ref. [55], but are within the 1–10 mg L^{-1} range [54], described for these water bodies. In any case, the results for both methods (ours and the one based on UV–Vis spectroscopy) are positively correlated (R = 0.9998, p < 0.05), comparable to those obtained for a similar study (R = 0.996) [17].

Additional river water samples with COD values up to 40 mg L^{-1}, as

Table 3

Analysis of water samples by the heat/base-activated persulfate CCD-CL method. Comparison with a UV–Vis permanganate-based method.

Sample	COD^{I} (mg L^{-1})		Recovery^{I,II} (%)	|Absolute error| (mg L^{-1})	|Relative error| (%)
	CCD-CL	UV–Vis			
drinking water	2.2 ± 0.2	2.7 ± 0.2	105 ± 5	0.5	18.5
tap water	7.5 ± 0.6	7.8 ± 0.7	110 ± 7	0.3	3.8
river^{III}	4.9 ± 0.4	5.2 ± 0.5	100 ± 4	0.3	5.8

^{I}n = 5. ^{II}CCD-CL method. ^{III}Reno river, Bologna.

assessed by ARPA using the dichromate-based ISO 15705 method, were also analyzed. Some samples showed unexpectedly high CL signals, even if fortified with a large amount of COD standard. We tentatively attributed this phenomenon to a catalytic effect on the luminol CL reaction due to impurities contained in the water samples with high COD content. Consequently, the following strategy was adopted to minimize such interference. Each sample was analyzed twice: as is and after fortification with a high amount of COD standard (100 mg L^{-1}). Then, the difference between the CL signals of samples without and with the added COD standard was calculated and used to estimate the COD of the sample by comparison with an analytical curve obtained by applying the same procedure on COD standards prepared in deionized water. This correction, in which the decrease in CL intensity (rather than its absolute value) is the parameter which correlates to COD concentration is based on the assumption that the CL intensity should reach a constant value close to zero (see Figs. 2 and 3) at high COD levels, when no oxidant remained available for the luminol reaction. This decrease in signal intensities, calculated after the previously mentioned correction for each sample, will be referred to as I$_{CCD}$.

Fig. 4 exhibits the comparison of the results for the analysis of those river water samples obtained with the CCD-CL method as described before (COD$_{CCD}$) and the standard method (COD$_{Cr}$). Precision (n = 3) was 5.6%. The pictures taken by the CCD camera for the samples (without adding standard and fortified with 100 mg L^{-1} of KHP) are displayed in Figure S5. There was a significant positive correlation between the calculated I$_{CCD}$ and COD$_{Cr}$ (R = 0.965, P < 0.05), as well as between COD$_{CCD}$ and COD$_{Cr}$ (R = 0.973, P < 0.05). Therefore, using this alternative approach, COD results obtained with the CCD-CL method correlated well to those of reference COD assays. It is worth noting that a similar study for river samples in which a permanganate-based CL method was employed yielded an R = 0.84 for the comparison between relative CL intensity and COD$_{Cr}$ [57] (<0.965, for an equivalent plot); on
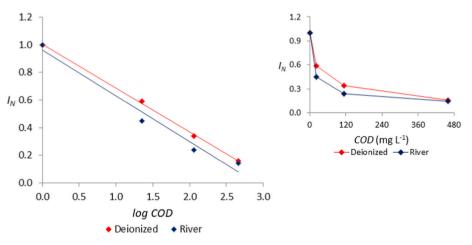

Fig. 3. Validation of the CCD-CL method. Analytical curve (n = 3) in I$_N$ (I/I$_0$) vs log (COD concentration), and (upper-right corner) I$_N$ (I/I$_0$) vs COD representations for external (EC, in deionized water) and standard addition (SA, in river water) calibrations. Note: I$_N$ = normalized CL signal, I$_0$ = CL signal for the blank.
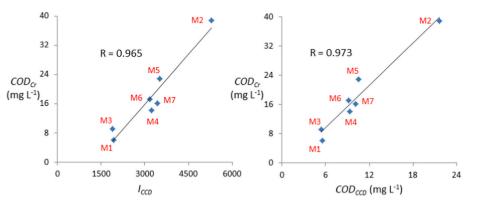
Fig. 4. Analysis of river samples (M1-M7) by the proposed and the standard methods. Comparison plot of COD estimation values for each sample (n = 3) of the dichromate standard method (COD_{Cr}) and: (left) the calculated decrease of the signal intensities of the CCD-CL method (I_{CCD}); (right) the COD estimation values of the CCD-CL method (COD_{CCD}).

the other hand, a photocatalytic sensor-based method obtained an R = 0.97 for the relationship between their COD results and COD_{Cr} [16] (comparable to 0.973) in the study of dam water samples. Thus, the results achieved with the CCD-CL method represent a major improvement, meaning that this tool can be used for COD quantitative determinations. In addition, COD_{Cr} values were 1.6 times higher than COD_{CCD} ones, which in turn were 1.1 times lower than the UV–Vis estimated COD_{Mn}. Again, these observations are in agreement with the literature [53], since variations in COD values are often observed, which can be attributed to a different oxidizing efficiency for each oxidant [57]. The previously described signal correction proved to be effective in minimizing matrix interferences without the need for additional reagents/operational steps.

Finally, some tests were performed to further shorten the analysis time and minimize sample handling. In such a direction, the test tube dimensions were reduced and the possibility of measuring directly in them (i.e., without transferring the samples to the 96-well plate) was explored. Further details can be found in the SI (Section 11). Considering that heating was reduced (from 15 to 10 min) in these final experiments and that the measurements could be made directly in the tubes, multiple samples can be processed (the number only limited by the capacity of the employed devices) in less than 20 min: thus, the method throughput could be significantly improved (~96 samples h^{-1}), differentiating it from the time-consuming standard methodologies and minimizing sample handling.

4. Conclusions

A CL-based method for measuring COD in water using a portable CCD camera as detection device has been successfully developed. Good analytical parameters were obtained in terms of accuracy, precision, LOD and, in particular, sample throughput (>60 samples h^{-1}). For validation, results were compared with the ones obtained by using a UV–Vis-based method and the standard dichromate method, which led to a positive correlation for both scenarios. The assay is fast (total analysis time is less than 20–25 min) and additional tests showed that the dimensions of the proposed system can be further reduced. Despite being better suited for the quantitative analysis of river samples, it can be applied for screening purposes in low COD-containing waters. Compared to the standard method based on dichromate, this method represents a more sustainable and greener option (considering recent assessment tools) not only because it uses the less toxic persulfate, but also because the reagents employed (and hence, the resulting wastes) are lower than in the classical methods since the light signal can be easily measured even in a small volume of <100 μL. In addition, considering the many existing approaches to this matter and the status of dichromate in the European legislation, it is fair to state that the CCD-CL

method employing heat/base-activated persulfate represents a valid, straightforward, faster and more environmentally friendly alternative to the standard method which, despite several drawbacks, is still used and has not been replaced yet. Furthermore, considering that all required instrumentation is portable, in situ analysis of water samples at the point of need can be envisaged.

CRediT authorship contribution statement

R.A. González-Fuenzalida: Investigation, Methodology, Conceptualization, Visualization, Formal analysis, Validation, Funding acquisition, Writing – original draft. **C. Molins-Legua:** Conceptualization, Supervision. **D. Calabria:** Investigation, Methodology. **M. Mirasoli:** Conceptualization, Methodology, Writing – review & editing. **M. Guardigli:** Conceptualization, Methodology, Writing – review & editing. **A. Roda:** Conceptualization, Methodology, Writing – review & editing, Supervision. **P. Campíns-Falcó:** Conceptualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2022.340196.

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