Assessment of a colorimetric method for the measurement of low concentrations of peracetic acid and hydrogen peroxide in water

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ABSTRACT

The recent growing interest in peracetic acid (PAA) as disinfectant for wastewater treatment demands reliable and readily-available methods for its measurement. In detail, the monitoring of PAA in wastewater treatment plants requires a simple, accurate, rapid and inexpensive measurement procedure. In the present work, a method for analyzing low concentrations of PAA, adapted from the US EPA colorimetric method for total chlorine, is assessed. This method employs N,N-diethyl-p-phenylelnediamine (DPD) in the presence of an excess of iodide in a phosphate buffer system. Pink colored species are produced proportionally to the concentration of PAA in the sample. Considering that PAA is available commercially as an equilibrium solution of PAA and hydrogen per-oxide (H_2O_2), a measurement method for H_2O_2 is also investigated. This method, as the one for the determi-nation of PAA, is also based on the oxidation of iodide to iodine, with the difference that ammonium molybdate Mo(VI) is added to catalyze the oxidation reaction between H_2O_2 and iodide, quantifying the total peroxides (PAA + H_2O_2). The two methods are suitable for concentration ranges from about 0.1–1.65 mg L^{-1} and from about 0.3–3.3 mg L^{-1}, respectively for PAA and H_2O_2. Moreover, the work elucidates some relevant aspects related to the operational conditions, kinetics and the possible interference of H_2O_2 on PAA measurement.

Keywords:
Peracetic acid Hydrogen peroxide
Spectrophotometry DPD
Ammonium molybdate

1. Introduction

Peracetic acid (PAA) has been used widely as a disinfectant and sanitizing in various industries such as food and beverage processing, brewery, pharmaceutical, pulp and paper, as well as medical applications, water in cooling systems and water process among others [1–3]. In the last decades, PAA has gained increasing attention for wastewater disinfection, establishing itself as a suitable alternative for chlorine-based compounds [3–9] due to some convenient features such as wide spectrum of antimicrobial activity, ease of implementation without the need for expensive capital investment, and absence of toxic, mutagenic or carcinogenic disinfection by-products (DBPs) [10]. The technical synthesis of PAA comprises the reaction of acetic acid (AA) with hydrogen peroxide (H_2O_2) in the presence of a catalyst. Since peroxides are highly unstable, to prevent the spontaneous decomposition of PAA to AA and H_2O_2, PAA commercial solutions are enriched with AA and H_2O_2. Therefore, PAA is not manufactured as a pure compound, instead it exists in a quaternary equilibrium with H_2O_2, AA and water [1,4].

For its application as wastewater disinfectant, as recently reviewed [3], the determination of residual PAA concentrations, often at low values, is essential to assess decay kinetics, disinfection performance, and toxicity of effluents. In particular, regarding the design of the disinfection process, a reliable and accurate method for PAA measurement is needed for estimating the actual PAA dose, due to the rapid decay of PAA in wastewater [11]. Moreover, since PAA coexists with H_2O_2 in disinfection reactors, such analytical method must be highly selective in distinguishing PAA from H_2O_2, in view of the different behavior of PAA and H_2O_2 as disinfectants [12,13] and lifetime of their residues in water, despite the important chemical similarities of the species.

While a wide literature has been published on the determination of each compound alone, several analytical methods were reported to measure PAA and H_2O_2 simultaneously [3,13–21]. These methods are based on the difference in the oxidizing power of both species, and include chromatographic, potentiometric, titrimetric and colorimetric techniques. For some of these methods important operating disadvantages were evidenced, as low sensitivity, complex procedures, expensive instruments and reagents. In addition, in case of wastewater disinfection, the time required to perform the measurement is an important feature, in relation to the instability of PAA diluted solutions. In the following, a brief discussion of the most relevant methods in literature is reported.

Concerning chromatographic methods, Di Furia et al. [15] proposed

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the determination of PAA and H_2O_2 based on the selective oxidation of methyl-p-tolyl sulfide (MTS) to the corresponding sulfoxide species (MTSO) which is then separated by gas chromatography. Alternatively, a method based on the same reaction has been proposed for liquid chromatography by Pinkernell et al. [18]. Although gas and liquid chromatographic techniques offer selectivity and low limits of detection (0.1–10 mg L^{-1}, approximately), expensive instrumentation and reagents are required; in addition, they are not suitable for field applications.

As for potentiometric methods, Awad et al. [20] proposed a technique based on the detection of the transient change of the electrode potential due to the oxidation of iodide by PAA and H_2O_2. The electrical response is obtained within few seconds while maintaining high sensitivity and selectivity down to the micromolar range. Although this method presented good recovery of PAA and H_2O_2 concentrations, it is labor intensive, cumbersome and more suitable for on-line analysis.

Titration methods, such as permanganometry, cerimetry and iodometry titrations [22–25], are among the oldest and most widely used techniques due to the good selectivity between PAA and H_2O_2, but their application is inappropriate for the determination of low concentrations in the order of few mg/L, but rather in as % w/w [26].

Finally, colorimetric methods allow the measurement of the compounds at low concentrations (0.1–10 mg L^{-1} approximately) with good selectivity. A method, adapted from Pütter and Becker [21], was proposed by Wagner et al. [13], based on the oxidation of 2,2′-azino-bis (3-ethylbenzothiazoline)-6-sulfonate (ABTS) and horseradish peroxidase by PAA and H_2O_2. An intensely green radical cation is produced and its spectrophotometric detection is performed at 405 nm. This method is accurate and reliable, although it is quite complex to perform, involving several steps. Nevertheless, its main limitation is the impossibility to distinguish between the two species, providing a total quantification of peroxides. The availability and cost of the reagents as well as the time required to perform the analysis are minor drawbacks of this method. Pinkernell et al. [17] proposed another procedure based on the oxidation of ABTS by peroxides, reporting good results. How-ever, in this case, the time required for the development of the color is in the order of minutes and it is highly dependent on pH. Therefore, limitations in existing analytical methods suggest the need for further investigations.

As for the determination of H_2O_2 as single compound, it has been extensively documented in the past, including amperometric, spectrophotometric, fluorimetric, potentiometric and titrimetic methods [27,28].

A promising colorimetric method allowing the determination of low concentrations of PAA was adapted from the US EPA DPD (N,N-diethyl-p-phenylenediamine) colorimetric method (method #330.5) [29] for the determination of total chlorine; it has been used in numerous previous works related to PAA disinfection [24,30–37]. In detail, the samples containing PAA are treated with an excess of potassium iodide (KI). The PAA oxidizes iodide to iodine, which subsequently oxidizes the DPD to a pink colored species. The PAA concentration can be estimated by means of a linear calibration of PAA concentration vs. absorbance. The oxidation of DPD to its pink product allows detection at a wavelengths of 530 and 550 nm, since the absorption spectra of the pink product has peaks at both wavelengths.

A potential drawback of this method is the lack of selectivity, since both PAA and H_2O_2 can oxidize iodide. Indeed, when PAA concentration is to be selectively determined by the DPD method, catalase is usually added to decompose H_2O_2 in solution [24,33–35,38–40]. However, in many other cases the occurrence of interference related to H_2O_2 is neglected [31,36,37,41–43] in agreement with the results of Liu et al. [44], who reported that H_2O_2 does not disturb the PAA concentration measurement using the DPD method.

Moreover, the literature is also ambiguous about the quenching procedure by catalase: two papers reported that PAA measurement is unaffected by catalase [1,37], whereas the partial decomposition of PAA is highlighted in other two papers [13,45], resulting in an under-estimation of PAA concentration. Moreover, to best knowl-edge of the authors, the determination of the H_2O_2 concentration in equilibrium with PAA by this spectrophotometric method and catalyzed by ammonium molybdate Mo(VI) has not been reported in detail.

The purpose of this work is to assess the DPD method as a technique to selectively determine PAA and an additional technique to determine total peroxides ($PAA + H_2O_2$) in solution at low concentrations, and to discuss the aspects that are still unclear in literature, specifically ad-dressing to its application for wastewater disinfection. Furthermore, the effect of pH on the measurement, kinetic aspects of the reaction as well as possible interferences are addressed.

2. Material and methods

2.1. Reagents

All solutions were prepared using deionized water and all chemicals were of analytical grade, purchased from Sigma Aldrich (USA), except for DPD (N,N-diethyl-p-phenylenediaminesulphate) salt for total chlorine that was provided by Hach Lange (USA). Commercial PAA technical-grade solution (15% w/w of PAA, 23% w/w of H_2O_2 and 16% w/w of acetic acid) was supplied by PeroxyChem (USA).

2.2. Analytical methods

2.2.1. Iodometric titration for PAA and H_2O_2

The concentration of PAA and H_2O_2 in the commercial PAA solution was determined by iodometric titration [23,24]. PAA and H_2O_2 oxidize iodide to iodine in presence of sulfuric acid (H_2SO_4) according to Eqs. 1 and 2, respectively. A starch indicator is added to develop an intense blue starch-iodine complex. Then, iodine is titrated with sodium thiosulfate ($Na_2S_2O_3$) until the disappearance of the color, according to Eq. (3). This iodometric titration is performed in two separate steps to measure PAA and H_2O_2, as detailed in the following.

$$CH_3CO_3H + 2KI + H_2SO_4 \rightarrow CH_3CO_2H + I_2 + K_2SO_4 + H_2O \quad (1)$$

$$H_2O_2 + 2KI + H_2SO_4 \rightarrow I_2 + K_2SO_4 + H_2O \quad (2)$$

$$I_2 + Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2NaI \quad (3)$$

First, in order to determine PAA concentration, an aliquot (1000 μL) of commercial PAA solution is placed into a 250-mL volumetric flask and diluted up to the mark with deionized water. A 25-mL aliquot of the aforementioned solution is placed into a 250-mL Erlenmeyer flask containing 50 mL of deionized water and 10 mL of phosphate buffer solution (0.14 mol L^{-1} of $NaHPO_4{\cdot}12H_2O$, 0.34 mol L^{-1} of KH_2PO_4, 0.003 mol L^{-1} of EDTA). The pH of the buffer solution is adjusted to 5.5 with H_2SO_4. H_2O_2 is quenched by dosing 600 μg of bovine catalase (2900 units mg^{-1}). After 5 min, in which the bovine catalase reacts with H_2O_2, 15 mL of H_2SO_4 (12 N) and 2.5 g of KI are added. The mixture is covered and maintained for 20 min in dark conditions. 50 mL of deionized water are added and the mixture is titrated drop by drop with standardized $Na_2S_2O_3$ (0.1 N) until the solution becomes pale yellow. Afterwards, 2 mL of freshly prepared starch solution are added and the titration is carried out under constant stirring until the disappearance of the color. According to the stoichiometry of the reactions presented in Eqs. 1 and 3, the following relationship can be established to determine the mass of PAA present in the sample titrated:

$$mg\ PAA = A(ml) \times \left[\frac{0.1\ m_{Equiv}\ Na_2S_2O_3}{1\ ml\ Na_2S_2O_3} \frac{1\ mmol\ Na_2S_2O_3}{2\ m_{Equiv}\ Na_2S_2O_3} \frac{1\ mmol\ PAA}{1\ mmol\ Na_2S_2O_3} \frac{76\ mg\ PAA}{1\ mmol\ PAA} \right] \quad (4)$$

Simplifying Eq. (4), the concentration of PAA (mg/g) can be obtained by means of Eq. (5):

$$[PAA] = \frac{A \times [3.8]}{m} \quad (5)$$

where A is the volume of $Na_2S_2O_3$ solution consumed in the titration (in mL), m is the weight of the titrated PAA sample (in g). In this case and according to the procedure, m corresponds to the mass of the sample of commercial solution contained in the 25 mL aliquot placed in the Erlenmeyer flask, which is estimated with the density of the pure commercial solution.

Then, the H_2O_2 fraction in equilibrium with PAA can be determined following the above described procedure without quenching H_2O_2 with bovine catalase. The reaction rate of H_2O_2 with iodide is slower than PAA [19], therefore ammonium molybdate is added as catalyst. The Mo (VI) solution is prepared dissolving 9 g of ammonium heptamolybdate ($(NH_4)_6Mo_7O_{24}$) in 10 mL of 6 N ammonium hydroxide. Then, 24 g of ammonium nitrate are added and the mixture is diluted up to 100 mL. 1 mL of Mo(VI) solution is added subsequently to the KI when performing the titration. Given that this method quantifies all the oxidizing agents in the sample, the aforementioned procedure quantifies both H_2O_2 and PAA. In order to determine the concentration of H_2O_2, the contribution of PAA can be subtracted as follows:

$$Equivalents = \frac{0.1\ m_{Equiv}\ Na_2S_2O_3}{1\ ml\ Na_2S_2O_3} \times (B - A) \quad (6)$$

where A and B are the volumes (in mL) of $Na_2S_2O_3$ solution consumed in the titration procedure in presence and absence of both the Mo(VI) catalyst bovine catalase, respectively. According to the stoichiometry of the reactions presented in Eqs. 1 and 2, the following relationship can be established to determine the milligrams of H_2O_2 present in the sample titrated:

$$mg\ H_2O_2 = Equivalents \times \left[\frac{1\ mmol\ Na_2S_2O_3}{2\ m_{Equiv}\ Na_2S_2O_3} \frac{1\ mmol\ H_2O_2}{1\ mmol\ Na_2S_2O_3} \frac{34\ mg\ H_2O_2}{1\ mmol\ H_2O_2}\right] \quad (7)$$

Simplifying Eq. (7) the concentration of H_2O_2 (mg/g) can be obtained by means of Eq. (8):

$$[H_2O_2] = \frac{Equivalents \times 17}{m} \quad (8)$$

where m is the weight of the titrated sample (in g). In an analogous way as the titration for PAA, m corresponds to the mass of the sample of commercial solution contained in the 25 mL aliquot placed in the Erlenmeyer flask, which is estimated with the density of the pure commercial solution.

2.2.2. Colorimetric determination of low concentrations of PAA

To determine PAA concentration in diluted solution, 50-mL sample is placed in a 250-mL Erlenmeyer flask continuously mixed by a magnetic stirrer. Subsequently, 2 mL of phosphate buffer solution at pH 5.5 and 2 mL of KI (1 M) are added followed by one dose of DPD salt (0.01 g). For practical purposes in this study, a DPD dispenser (DPD Total Chlorine Reagent, Swiftest™ Dispenser, Hach) was used to dose the DPD salt. Alternatively, DPD reagent is also available in individual powder pillows that contain the aforementioned dose. After 10 s, 10 mL of the sample are transferred to a quartz cuvette (optical path 40 mm) and the absorbance at 530 nm is measured by means of a spectrophotometer Hach Lange CADAS 200. The calibration curve is constructed by repeating the measurement procedure for three series of standards (each value replicated three times) covering the PAA concentration range from about 0.1–2 mg L^{-1}, as shown in Fig. 1, being its equation the following:

$$ABS(\lambda = 530\ nm) = (0.552 \pm 0.005) \cdot [PAA] + (0.024 \pm 0.005) \quad (9)$$

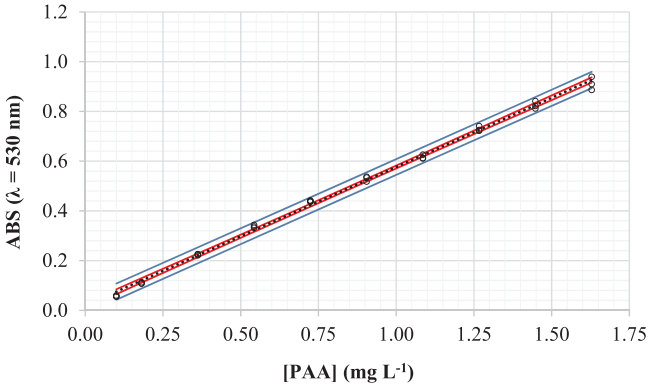

Fig. 1. Calibration curve for PAA measurement by the DPD method. The 95% confidence and prediction intervals are reported in red and blue respectively.

While the 95% confidence interval of parameters is reported in Eq. (4), the R^2 and the predicted R^2 values of the calibration curve were 0.9972 and 0.9967, respectively.

In case of samples with PAA concentration above 2 mg L^{-1}, the sample must be diluted before measurement.

In case of coloration of the sample to be analyzed, it is suggested to measure the absorption spectra of the media. If the colored media absorbs at 530 nm the background interference can be corrected by subtracting its absorbance if it doesn't change during the test. In the present study quartz cuvettes were used. Alternatively, since the assays are in the visible spectral range, either glass or disposable plastic cuvettes may be used giving enough accuracy for this application, being a less expensive alternative.

2.2.3. Determination of low concentrations of H_2O_2

In the methodology adopted to measure H_2O_2, 5 mL of KI (1 M), 0.2 mL of Mo(VI) solution and 0.1 mL of phosphoric acid (H_3PO_4) (20% w/w) are placed in a 100-mL volumetric flask, subsequently, the volume is filled up to 100 mL by adding 94.7 mL of the sample. The volumetric flask is capped and shaken gently. After 6 min of reaction time, the sample is transferred to an Erlenmeyer flask of 250 mL and two doses of DPD are added, subsequently 10 mL of the sample are transferred to a quartz cuvette (optical path 40 mm) and the absorbance is measured by means of a spectrophotometer Hach Lange CADAS 200.

Analogous to the titration to determine PAA and H_2O_2 concentrations, the procedure quantifies all the oxidizing compounds present in the sample. Therefore, the absorbance at 530 nm measures both compounds, PAA and H_2O_2 (total peroxides) in terms total chlorine concentration ($[Cl_2]$), according to the calibration curve shown in Fig. 2, being its equation reported:

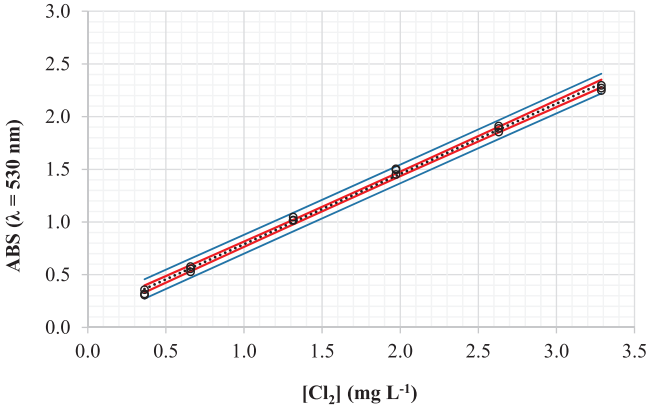

Fig. 2. Calibration curve for PAA and H_2O_2 measurement by the DPD method. The 95% confidence and prediction intervals are reported in red and blue respectively.

$$ABS(\lambda = 530nm) = (0.6706 \pm 0.0091) \times [Cl_2]_{as\ PAA+H2O2} + (0.1125 \pm 0.0183) \quad (10)$$

In order to determine the concentration of H_2O_2, the contribution of PAA determined by the procedure described in Section 2.2.2 has to be subtracted from the total peroxides measured, according Eq. (12), prior to the conversion of the PAA concentration into Cl_2 concentration by means of the molecular weight ratio PAA:Cl_2, as shown in Eq. (11).

$$[Cl_2]_{as\ PAA} = \frac{[PAA]}{1.072} \quad (11)$$

$$[H_2O_2] = 0.480 \times \left([Cl_2]_{as\ PAA+H_2O_2} - [Cl_2]_{as\ PAA}\right) \quad (12)$$

where 0.480 is molecular weight ratio H_2O_2:PAA.

2.3. Effect of pH on the measurement of low concentrations of PAA and H_2O_2

The effect of solution pH on the measurement of low concentrations of PAA was assessed by measuring PAA concentration by the procedure described in Section 2.2.2 on a sample containing 0.36 mg L^{-1} of PAA and 0.47 mg L^{-1} of H_2O_2, using phosphate buffer solutions at different pH (2.5, 4, 5.5, 6.5, 7.5, 9 and 10.5). The phosphate buffer solutions were prepared according to the procedure described in Section 2.1 and the pH was adjusted with H_2SO_4 (pH 2.5, 4 and 5.5) or NaOH (pH 7.5, 9 and 10.5). Each measurement was replicated three times. The effect of pH on the measurement of low concentrations of H_2O_2 was evaluated by analyzing the same sample using the procedure described in Section 2.2.3, repeated in presence and absence of H_3PO_4.

2.4. Effect of H_2O_2 on the measurement of PAA concentration

The interference of H_2O_2 on the determination of PAA concentration was assessed by means of two tests to determine the kinetics of the reaction in presence and absence of H_2O_2 plus a third experiment to assess solely hydrogen peroxide kinetics. In the first case, (a) the method to measure PAA concentration was performed on a sample containing 0.36 mg L^{-1} of PAA in equilibrium with 0.47 mg $L^{-1}$of H_2O_2, and the absorbance was monitored for 120 min at 5-minutes intervals. In addition, the experiment was repeated monitoring the absorbance at 10-second intervals for 6 min. In the second case, (b) the test was performed and monitored as in the first case but an additional step to quench H_2O_2 in the sample was carried out by adding 200 μL of a solution containing 10 mg of bovine catalase (2900 units mg^{-1}) dis-solved in 25 mL of deionized water. In the procedure, the bovine cat-alase solution was added after the phosphate buffer solution at pH 5.5 and before the KI. Finally, a third test (c) was carried out to assess the reaction for H_2O_2 alone in a sample containing 0.47 mg L^{-1} of H_2O_2 using the same procedure and it was monitored for 120 min at 5-minute intervals. In addition, the experiment was repeated monitoring the absorbance at 20-second intervals for 6 min. In addition, blank tests were performed for the measurement methods described in section (d) 2.2.2 and (e) 2.2.3, and performing the same monitoring as for case (a). All the experiments were performed in the dark.

2.5. Effect of Mo(VI) on the oxidation of iodide by H_2O_2

The catalyzed and uncatalyzed oxidation rates of iodide by H_2O_2 were compared in two tests for samples containing 0.47 mg L^{-1} of H_2O_2. For the assessment of the reaction rate of the catalyzed reaction, the procedure to measure H_2O_2 concentration described in Section 2.2.3 was used, and the absorbance was monitored for 180 min at 5-minute intervals. The reaction rate of the uncatalyzed reaction was evaluated using the procedure to measure PAA concentration described in Section 2.2.2, monitoring the absorbance at same time intervals. All the experiments were performed in the dark.

2.6. Method validation

The composition of the equilibrium mixture was determined by the standard iodometric titration describe in Section 2.2.1 (15.6% PAA and 22.3% H_2O_2). This was used to prepare a stock solution containing 0.905 mg L^{-1} of PAA and 1.309 mg L^{-1} of H_2O_2 by placing 0.5 mL of the titrated equilibrium mixture in a volumetric flask of 100 mL and making up to volume with deionized water. Subsequently, this solution was used to dose tap water (prior dechlorination) in order to prepare solutions with six different nominal concentrations of PAA (0.1, 0.25, 0.5, 1, 1.25 and 1.5 mg L^{-1}), which were immediately analyzed for PAA concentration following the procedure described in Section 2.2.2. Thereafter, the same procedure was carried out to measure the associated fractions of H_2O_2 (0.13, 0.324, 0.65, 0.970, 1.23, 1.94 mg L^{-1}) in equilibrium with the aforementioned concentrations of PAA, according to the procedure described in Section 2.2.3. Each experiment was replicated four times.

3. Results and discussion

3.1. Effect of pH on the measurement of low concentrations of PAA and H_2O_2

The parent method to measure total chlorine, hypochlorite ion, hypochlorous acid and chloramines stoichiometrically generates iodine from iodide at pH 4 or lower [29]. The DPD method can be adapted to PAA measurement on the basis of the capacity of PAA to act like chlorine on iodide [41].

In the case of PAA measurement, the maximum peak of absorbance at the same operating conditions is reached at pH comprised between 4 and 6.5 for a PAA concentration of 0.36 mg L^{-1}, as shown in Fig. 3. In this range, the difference in the absorbance values at different pH is not relevant, although the variability of the absorbance values is slightly higher at pH 6.5. For pH values below 4 and above 6.5, an important decrease of the absorbance values is observed, thus it can be inferred that pH values outside the aforementioned range are not optimal for the reaction kinetics, and the reaction is not completed. This pH range agrees with that reported by other authors who implemented the DPD method employing a pH between 5.5 and 6.5 [24,29,31,33]. Moreover, this observation is in agreement as well with the kinetic study carried out by Awad et al. [20] for the potentiometric determination of PAA, in which the first order rate constants for the reaction between iodide and PAA were found to be independent of solution pH in the range 3.5–5.4. Therefore, pH 5.5 was selected as the optimum for the determination of PAA concentration in agreement with other authors and in the view of reducing the variability of the response.

On the other hand, the rate of the reaction of iodide with H_2O_2 is known to be largely dependent on pH, even when the reaction is catalyzed with Mo(VI) [16,20]. The absorbance at 530 nm of a sample

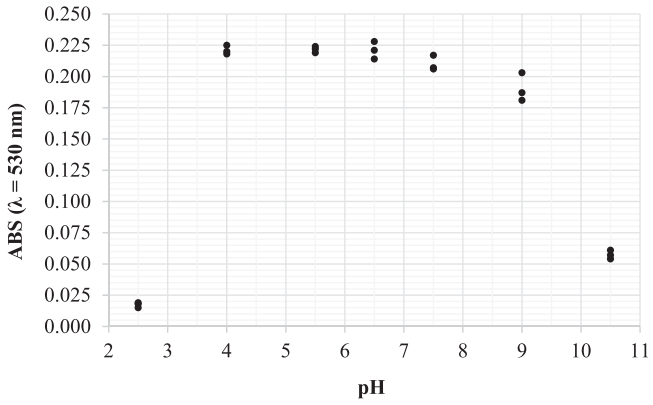

Fig. 3. Effect of pH on the measurement of PAA concentration by the DPD method.

containing 0.36 mg L^{-1} of PAA and 0.47 mg L^{-1} of H_2O_2 treated with H_3PO_4 prior the oxidation of iodide by H_2O_2 catalyzed by Mo(VI) was 1.016, whereas the absorbance was 0.697 without the addition of H_3PO_4. For the sample treated with H_3PO_4, the reaction occurred in acidic media (pH 3.5), while in the other case at more alkaline conditions (pH 6). In agreement with the kinetic study reported by Awad et al. [19], the reaction of iodide and H_2O_2 becomes slower as the pH increases, being the optimal conditions reported for pH 4 for the Mo(VI) catalyzed oxidation of KI by H_2O_2 [46,47].

The procedure for the determination of PAA and hydrogen peroxide cannot be simplified avoiding the addition of a pH buffer (PAA) or an acid (H_2O_2) and the pH values reported in the literature should be strictly observed.

3.2. Effect of H_2O_2 on the measurement of PAA concentration

It is well-documented that the rate of oxidation of iodide by PAA is much higher than that by H_2O_2 [16,20,48]. The uncatalyzed reaction rate of iodide and PAA is reported to be five orders of magnitude higher than that of iodide and H_2O_2 [20]. For instance, the pseudo-first order kinetic rate constant for the uncatalyzed reaction between PAA and iodide calculated by Awad et al. [19] was $4.22{\cdot}10^2\ M^{-1}\ s^{-1}$, being the rate expressed as $r_{uncatalyzed} = 4.22{\cdot}10^2\ [PAA][I^-]$, whereas the rate constant for the uncatalyzed reaction of H_2O_2 and iodide calculated by Cooper and Koubek [47] was $9.5{\cdot}10^{-3}\ M^{-1}\ s^{-1}$, with a rate expressed as $r_{uncatalyzed} = 9.5 \times 10^{-3}\ [H_2O_2][I^-]$. In order to confirm the absence of a detrimental effect by H_2O_2 on the measurement of PAA, the ab-sorbance at 530 nm was monitored over 100 min on a sample con-taining 0.36 mg L^{-1} of PAA and 0.47 mg L^{-1} of H_2O_2 with and without removing the H_2O_2 fraction with bovine catalase, as shown in Fig. 4. Bovine catalase is an oxidoreductase enzyme that selectively catalyzes the decomposition of H_2O_2 in equilibrium with PAA. When H_2O_2 was not quenched (PAA $+H_2O_2$), absorbance values were noticeably higher than the ones obtained when H_2O_2 was quenched (PAA), particularly after 5 min. For the curve PAA $+ H_2O_2$, a plateau of the absorbance values, indicating the completeness of the reaction, was reached after approximately 70 min, whereas for the PAA curve, the levelling of the absorbance was reached after approximately 20 min. In both cases, the absorbance reached a value (~0.220) at t = 0, that remained briefly steady for approximately 30 s, approximately 60 s after the addition of DPD, considering that the spectrophotometrical measurement was carried out approximately 30 s after the addition of the DPD.

A close-up to the first six minutes, is shown in Fig. 5. Indeed, during the first 20 s, the absorbance values are the same for both curves (PAA and PAA $+ H_2O_2$), indicating that the absorbance values can be attributed solely to PAA if they are measured within this time frame.

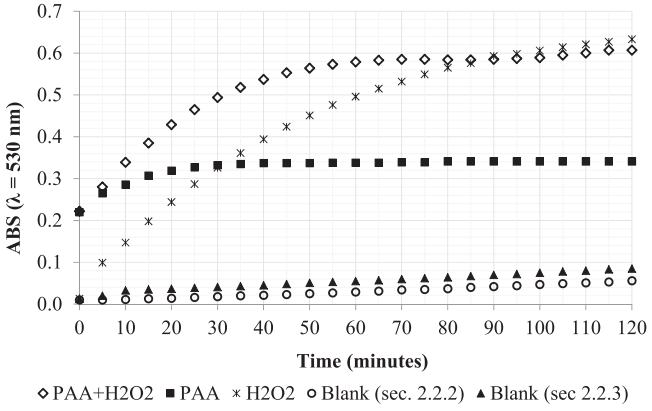

Fig. 4. Absorbance at λ = 530 nm over 100 min (5-minute intervals) for experiments with: (a) 0.36 mg L^{-1} of PAA in equilibrium with 0.47 mg L^{-1} of H_2O_2, (b) 0.36 mg L^{-1} of PAA after the removal of H_2O_2 with bovine catalase, (c) 0.47 mg L^{-1} of H_2O_2, and the blanks described in sections (d) 2.2.2 and (e) 2.2.3.

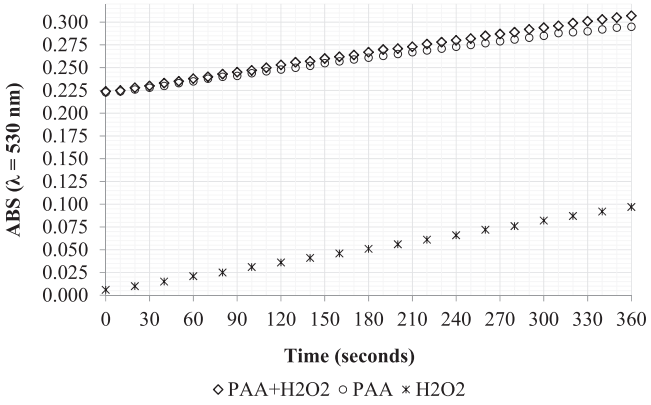

Fig. 5. Absorbance at λ = 530 nm over 360 s (10 and 20-second intervals) for experiments with: (a) 0.36 mg L^{-1} of PAA in equilibrium with 0.47 mg L^{-1} of H_2O_2, (b) 0.36 mg L^{-1} of PAA after the removal of H_2O_2 with bovine catalase, and (c) 0.47 mg L^{-1} of H_2O_2.

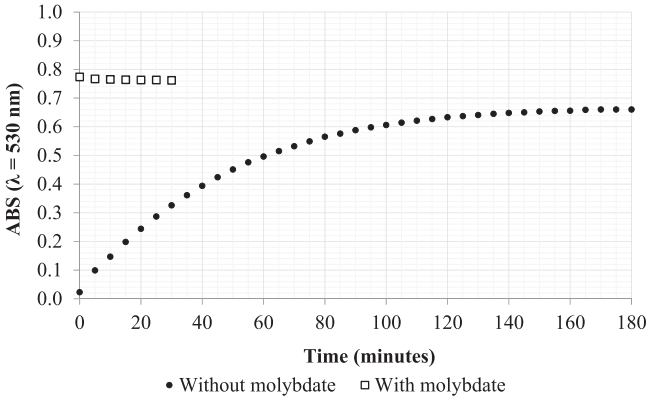

Fig. 6. Absorbance at λ = 530 nm over 180 min (5-minute intervals) for the oxidation of iodide by 0.47 mg L^{-1} of H_2O_2 with and without the dosage of Mo(VI) solution as cata-lyst.

Therefore, no interference of H_2O_2 is expected in the measurement of PAA within about 60 s after adding DPD, in agreement with the memorandum of the US EPA and the patent of Howarth et al. [41,43]. This evidence is confirmed by the curve for the reaction of H_2O_2 alone, in which the absorbance values at the beginning of the experiment are negligible, thus no significant oxidation of iodide by H_2O_2 occurs in the first minute. Furthermore, the trend is very similar to the curve for PAA and H_2O_2, although it is shifted upwards, starting at the corresponding value for the PAA concentration. Regarding the slow-paced growth of the absorbance for the oxidation of iodide by PAA, it can be attributed to the slow decomposition of H_2O_2 by the bovine catalase.

Therefore, the reaction of iodide with H_2O_2 can be considered negligible in the time scale used to carry out the reaction of iodide with PAA, confirming the faster rate of the oxidation of iodide with PAA respect to H_2O_2 [20]. This large difference allows determining PAA concentration without the addition of catalase to quench H_2O_2 as long as the absorbance is read promptly, within about 60 s after the addition of DPD, making time a key parameter to measure accurately PAA concentration. In summary, the ideal procedure to determine PAA concentration without any interference of H_2O_2, entails a standardized measure, without the addition of any quencher for H_2O_2.

3.3. Effect of Mo(VI) on the oxidation of iodide by H_2O_2

The reaction rate of iodide and H_2O_2 can be increased either by the addition of Mo(VI) solution as catalyst or decreasing the solution pH [20], in agreement with the method described for the measurement of H_2O_2 concentration, implementing the dosage of catalyst and the

Table 1
Recovery of PAA, total peroxides and H_2O_2 concentrations in tap water. Recovered concentrations reported as mean ± standard deviation.

PAA dose applied ($mg\,L^{-1}$)	PAA recovered ($mg\,L^{-1}$)	% recovery PAA	Total peroxides applied ($mg\,L^{-1}$ as Cl_2 eq.)	Total peroxides recovered ($mg\,L^{-1}$ as Cl_2 eq.)	% recovery total peroxides	H_2O_2 dose applied ($mg\,L^{-1}$)	H_2O_2 recovered	% recovery H_2O_2
0.1	0.098 ± 0.0017	97.8%	0.363	0.355 ± 0.0021	97.8%	0.130	0.127 ± 0.0027	97.8%
0.25	0.242 ± 0.0031	97.0%	0.908	0.886 ± 0.0059	97.6%	0.324	0.317 ± 0.0067	97.8%
0.5	0.486 ± 0.0044	97.3%	1.816	1.782 ± 0.0099	97.7%	0.648	0.63 ± 0.011	97.8%
0.75	0.730 ± 0.0040	97.3%	2.725	2.65 ± 0.014	97.4%	0.971	0.95 ± 0.015	97.5%
1	0.97 ± 0.014	96.7%	3.633	3.53 ± 0.036	97.1%	1.295	1.26 ± 0.039	97.2%
1.5	1.43 ± 0.032	95.5%	5.449	5.25 ± 0.061	96.3%	1.943	1.88 ± 0.069	96.5%

acidification by H_3PO_4 jointly. When the reaction is catalyzed with Mo (VI), the reaction rate of iodide and H_2O_2 is reduced by two orders of magnitude [16,19,20].

The effect of the Mo(VI) solution on the measurement of H_2O_2 concentration was evaluated following the absorbance values over 180 min for a sample containing 0.47 mg L^{-1} of H_2O_2 in presence and absence of Mo(VI). Experimental results are reported in Fig. 6.

In agreement with the previous results (Fig. 5), no significant increase in the absorbance occurs in the first minutes for the curve of the uncatalyzed reaction. The levelling-off of the absorbance values seems to occur after 180 min, without reaching the same value as the Mo(VI) catalyzed reaction, suggesting that even if the absorbance is stable, the reaction is not completed. For the reaction catalyzed with Mo(VI), the maximum absorbance and, therefore, the completion of the reaction, are reached immediately and maintained for 30 min without significant changes.

Consequently, the oxidation of iodide by H_2O_2 is highly catalyzed by Mo(VI). As already introduced and reported by other authors [16,19,20,47], the rate constant of the Mo(VI) catalyzed reaction is about three orders of magnitude larger than that of the uncatalyzed one. However, the catalytic effect of Mo(VI) on the oxidation of iodide by different peroxides is different depending on the type of peroxide. It has been also reported that the rate of the oxidation of iodide by or-ganic peroxide decreases with increasing the complexity of the organic molecules [49,50].

3.4. Method validation

The results of the measurement of different concentrations of PAA are presented in Table 1. The recovery of PAA ranged between 95.5%and 97.8%, whereas for H_2O_2 the recovery ranged between 96.6% and 97.8%. At lower concentrations, the recoveries tend to be higher and the standard deviations lower. Several authors [24,31,33–43] have applied the DPD method to the determination of low concentrations of PAA, whereas only few of them [24,43] have described the perfor-mance and accuracy of the method. As for the measurement of H_2O_2, to the best knowledge of the authors, the DPD method has not been ap-plied in wastewater disinfection studies to date. Summarizing, both methods display optimal recoveries and high precision, with relative percentage standard deviation below 2% even for the lowest in-vestigated concentration of 0.1 mg L^{-1}.

Comparison of the present figures of merit with literature data may be performed for PAA determination and the study of Cavallini et al.[24]. Lower accuracy and precision is reported in the latter, which may partially be accounted for by the different optical path used in the present work (40 mm) and in the cited paper [24] (10 mm; addition of catalase).

4. Conclusions

A colorimetric method for the measurement of low concentrations of PAA and H_2O_2 in solution was assessed. The method is based on the oxidation of iodide by PAA and H_2O_2, and on the subsequent generation of colored by-products that can be selectively measured by spectrophotometry. Experimental results evidenced that the reaction is independent of pH in the range 4–6.5 for the measurement of PAA, whereas the reaction for the measurement of H_2O_2 catalyzed by Mo(VI) is dependent on the pH, and the optimal pH value for the application of the method is 3.5. Due to the differences in the oxidation rate of both compounds, interferences due to the presence of H_2O_2 when measuring PAA are negligible if the absorbance reading is taken rapidly, within 60 s after the addition of the DPD: the quenching of H_2O_2 with bovine catalase is accordingly not necessary. Therefore, time is a key parameter for getting a reliable measurement. Given the slow oxidation rate of iodide by H_2O_2, the reaction for the measurement of H_2O_2 must be catalyzed by Mo(VI). The optical path should be selected according to the needs of the research; in general, longer optical paths provide higher accuracy and precision whenever low concentrations of the analytes are to be determined.

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