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Hydroxyl Radicals quantification by UV spectrophotometry

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ABSTRACT

Hydroxilation of aromatic compounds is an oxidative process that has been employed as indirect method to quantify the produced hydroxyl radicals during an advanced oxidation process ($^{\bullet}$OH). This is usually complicated by the radical high reactivity and short life time and therefore a scavenging agent such as salicylic acid is commonly employed. This usually implies a rather sophisticated analytical method such as chromatography. In this work, however, a relatively simple and low cost method is proposed to achieve the aforementioned objective. This method is based on UV-Vis spectrophotometry and was employed to quantify the hydroxyl radicals produced during the electrochemical oxidation of water when employing a platinum anode in a galvanostatic type process. Salicylic acid was employed as $^{\bullet}$OH scavenger. The concentration of the hydroxilated resulting products, 2,3-dihidroxybenzoic Acid and 2,5-dihydroxibenzoic Acid, was determined by UV-Vis Spectroscopy and utilized to quantify the hydroxyl radicals. In addition, mineralization was followed by Chemical Oxygen Demmand measurements.

1. Introduction

Recently, Advanced Oxidation Processes (AOP) have been recognized and employed as efficient methods to remove dangerous and recalcitrant environmental pollutants. These processes have in common the production of hydroxyl radicals ($^{\bullet}$OH) to conduct oxidation reactions [1]. Albeit other oxidants, hydroxyl radicals are attractive for effluents purification treatment since their short life time minimizes the undesirable side effects observed with chlorine and ozone, for example [2]. Therefore, it is not surprising that in the last decades the research on organic compounds mineralization has been strongly addressed to producing hydroxyl radicals by several forms, i.e. photocatalysis, electrochemistry, ozonation and photolysis of H_2O_2. At this point, it becomes desirable to quantify the produced hydroxyl radicals as an attempt to understand the difference among each AOP. Regarding this matter, some methods have been developed such as aromatic hydroxylation and this has become one of the most sensitive methods to quantify $^{\bullet}$OH. Phenylalanine, benzoic acid, salicylic acid (SA) and the esterificated form of aspirin have been reported for this purpose. Among these compounds, however, SA has been preferred due to (i) high reaction rate ($5 \times 10^9\ M^{-1}\ s^{-1}$), (ii) its high competitiveness against some other scavengers and (iii) the stability of its reaction products which facilitates the samples treatment and analytical process [3].

Some analytical methods like High Performance Liquid Chromatography (HPLC) have been employed to detect $^{\bullet}$OH by using SA as scavenger in a Fenton process [4]. Other employed analytical methods for the same purpose are capillary zone electrophoresis with amperometric detection (CZE-AD) [5], Electronic Spin Resonance (ESR), employing phenyl-ter-butilnitrone (PBN) as scavenger in a Fenton process [2]. Also dimethyl sulfoxide (DMSO) has been employed as scavenger in a Fenton reaction, photolysis of H_2O_2 and γ irradiation of H_2O [6]. Other employed $^{\bullet}$OH scavengers are salicilate and 4-hydroxybenzoate [7]. In all the aforementioned studies the use of a scavenger is a constant and the employment of a sophisticated analytical technique becomes compulsory in order to identify the reaction products of the scavenger. These methods, although highly accurate, also offer some inherent disadvantages like complexity and cost. This encouraged this work to be dedicated to develop a rather simple and less costly method based on UV-Vis Spectrophotometry to establish the production of hydroxyl radicals in an AOP. In this case the employed AOP was electrochemical and

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a Pt electrode was used since it has been reported that generates $^{\bullet}$OH without reaching mineralization [8].

2. Experimental

2.1. Reagents

Analytical grade Salicylic Acid (SA), 2,3-dihidroxybenzoic Acid (2,3-DHBA) and 2,5-dihydroxibenzoic Acid (2,5-DHBA) were purchased from Sigma Aldrich. Reagent grade Sulfuric acid was purchased from ACS and deionized water was obtained from a Millipore Direct Q3 UV with resistivity >18 MΩ at room temperature. Standard solutions of SA (1×10^{-3} *M*), 2,3-DHBA (1×10^{-3} *M*), 2,5-DHBA (8×10^{-4} *M*) and H_2SO_4 (0.5 *M*) were prepared with deionized water.

2.2. Production and scavenging of $^{\bullet}$OH

The production and scavenging of $^{\bullet}$OH radicals was conducted in a 5 ml typical electrochemical cell (not shown). For this purpose, 3 mL of a 1×10^{-3} *M* SA solution were mixed with 1 mL of 0.5 M H_2SO_4 solution. After mixing the SA concentration was 8×10^{-4} *M*. The experiments were carried out at 0.2 *A* and 4 *V*, 0.4 *A* and 5 *V* with a platinum electrode as anode and a graphite electrode as cathode. Both experiments were performed at room temperature, pH of 1.4 and a reaction time of 105 *min*. The power source was GWINSTEK GPR-1820HD. pH was monitored with a HI 9811 HANNA instruments potenciometer. Chemical oxygen demand was established with a HACH DR/4000U and an Orion COD 165 thermoreactor. Samples were taken every 15 minutes and analyzed in a UV-Vis Perking Elmer Precisely Lambda 25 spectrophotemeter.

2.3. Chemical Oxygen Demand (COD)

Chemical Oxygen Demand measurements were carried out according to the Standard Methods for the Examination of Water and Wastewater [9]. The closed reflux with calorimetric measurements was the followed standard method. This analysis was employed to verify the effectiveness of the conducted electrolysis. In this case, however, the aim was not to decrease the COD but to establish the operating conditions to achieve only the partial oxidation of SA towards 2,3-DHBA and 2,5-DHBA.

3. Results and discussion

3.1. Analysis of salicylic acid and hydroxylated derivatives standards

Standard solutions of SA, 2,3-DHBA and 2,5-DHBA with 1×10^{-3}, 1×10^{-3} y 8×10^{-4} *M* concentrations, respectively, were prepared and analyzed in order to establish the wavelength at which the maximum absorption is observed for every analyte. Fig. 1 depicts the resulting absorption spectra. The aforementioned compounds are carboxylic acids and therefore present $n\pi^*$ transitions. Since these compounds can be classified as chromophores, their maximum absorbance is expected to occur at a wavelength higher than 230 *nm*. Indeed, it can be observed in Fig. 1 that the maximum absorbance of SA, 2,3-DHBA and 2,5-DHBA is at 303 *nm*, 310 *nm* and 329 *nm*, respectively. This hypsochromical displacement of the $n\pi^*$ bands is expected and is due to the mobility of an electron from a n to a π^* orbital. When a carbonyl group, like the one in the SA, becomes in contact with $^{\bullet}$OH radicals, the free electrons pair of the carbonyl oxygen can bond with $^{\bullet}$OH groups by hydrogen bridges. The molecules configuration will be the one that favors such interaction. The basic energy of the system is then stabilized

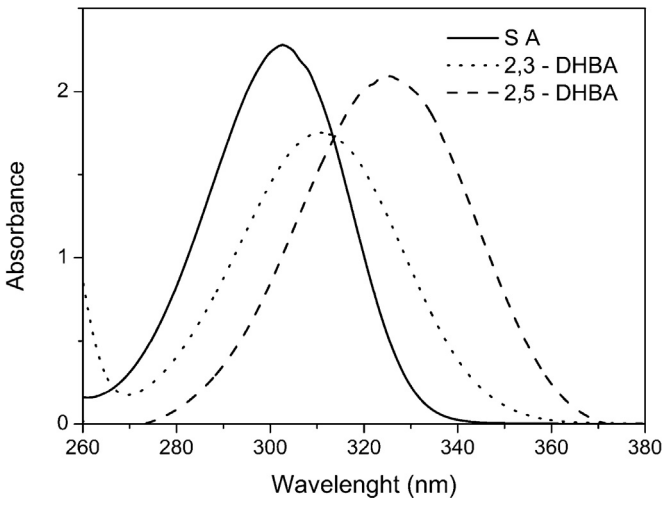

Fig. 1. Absorption spectra of SA, 2,3-DHBA and 2,5-DHBA.

and its energy decreases proportionally to the strength of the formed hydrogen bridge bond. The electronic excitation occurs in such a short time that the system is not able to adapt to that new electronic distribution. Thus the mobility of an electron from the n to the π^* orbital eliminates the possibility of stabilization of the excited state and the global result is the aforementioned hypsochromical displacement.

3.2. Calibration curve

Different calibration curves were built for the three analytes in the concentration range given in Table 1. These calibration curves were fitted to linear models (column 3, Table 1). The determination coefficient of such models and their concentration range of linearity are also presented in Table 1.

Once the calibration curves for the three analytes were established, the quantification of $^{\bullet}$OH was carried out.

3.3. Identification of species

In previous studies, when employing SA as free $^{\bullet}$OH scavenger, the following reaction products were identified, 2,3-DHBA, 2,5-DHBA and catechol [5,7,10–12]. However, 2,5-DHBA and catechol have been reported to occur in very small quantities [10] or to do not appear at all [11]. It has been reported that 2,3-DHBA is produced in higher quantity than 2,5-DHBA when $^{\bullet}$OH radicals are chemically produced while 2,5-DHBA is favored over 2,3-DHBA when $^{\bullet}$OH radicals are electrochemically produced [13]. The produced hydroxylated compounds at 0.2 *A*, 4 *V* and 0.4 *A*, 5 *V*, were analyzed in a UV-Vis Spectrophotometer and the obtained spectra are presented in Figs. 2 and 3. In these Figures, it can be observed that the maximum absorbance gradually diminishes and presents a hypsochromic displacement due to the reaction between Salicylic Acid and $^{\bullet}$OH radicals. The maximum absorbances were found at 303 *nm* for SA, 310 *nm* for 2,3-DHBA and 329 *nm* for 2,5-DHBA (see Fig. 1). As can be observed in Figs. 2 and 3, the maximum absorbance hypsochromic displacement tends to the maximum absorbance of the corresponding hydroxilated products. It is worth noticing that these products are due to the reaction between SA and $^{\bullet}$OH radicals. These radicals are produced during the water oxidation that is expected to occur in the platinum anode according to Ec. (1) [12].

$$H_2O \rightarrow {}^{\bullet}OH + H^+ + e^- \quad (1)$$

Table 1
Regression equation and statistics.

Analyte	Linear Range (*M*)	Regression Equation	R^2
SA	6×10^{-5}–1×10^{-3}	A^{303nm}= 3047[SA] ± 90 + 0.06 ± 0.05	0.995
2,3-DHBA	6×10^{-5} - 1×10^{-3}	A^{310nm} = 2720[2,3-DHBA] ± 32.36 + 0.08 ± 0.018	0.999
2,5-DHBA	6×10^{-5} - 8×10^{-4}	A^{329nm} = 3335[2,5-DHBA] ± 36.8 + 0.06 ± 0.016	0.999

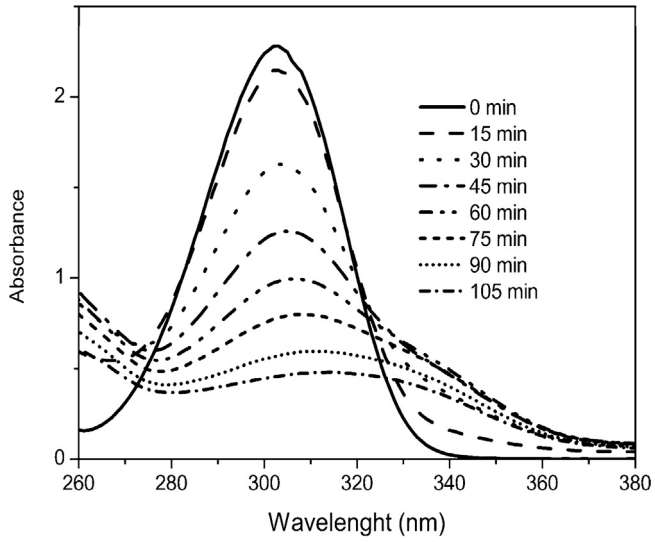

Fig. 2. Effect of reaction time on mixture UV-Vis spectrum. Reaction conditions: 0.2 A and 4 V, pH = 1.4, SA initial concentration (t = 0 min) = 8×10^{-4} M.

3.4. Hydroxyl radicals quantification

A method to quantify hydroxyl radicals has been previously reported [14] and is based on employing SA as $^{\bullet}$OH scavenger to produce 2,3-DHBA and 2,5-DHBA. According to this method and to the reaction stoichiometry, the sum of the hydroxilated compounds concentration is equal to the hydroxyl radicals concentration. Thus, the problem of quantifying $^{\bullet}$OH radicals is solved when the hydroxilated compounds concentration is established. To do so by UV-Vis, however, it should be taken into account that the mixture of the three analytes absorbs energy of only one wavelength (see Figs. 2 and 3). According to quantitative analytical chemistry basic literature [15], in order to establish the concentration of every analyte from this type of spectra a special method should be followed. In such a method, the total absorbance of a mixture is equal to the sum of the absorbances of each individual compound (Eq. 2),

$$A_T = Ax + Ay + Az \tag{2}$$

By employing the Beer-Lambert Law, Eq. (2) can be written in terms of molar absorptivity and concentration of every analyte. Thus, if the unknown variable is the concentration of every analyte, this can be known by establishing an equations system with many equations as unknown analytes concentrations. To do so, a number of pure standards equal to the number of analytes is prepared and the wavelength at which they present the maximum absorbance of energy is recorded. In this case there are three analytes so that three wavelengths for maximum absorbance were determined. At these wavelengths, when analyzing a mixture, three absorbances can also be determined. Thus a system with three equations was established and was solved in a matricial way at every reaction time. At this point it is worth noticing that this method is limited to the feasibility of establishing linearly independent equations. This is plausible in cases where experimentation is conducted under careful control. In addition, special attention should be given to the significance of the selected absorbance values so that they are not strongly influenced by signal noise or measurement errors.

Formally, let us define $\varepsilon^{*}i = X, Y$ or Z and the indice $i = 1,2,3$ corresponds to wavelengths λ_1, λ_2 and λ_3, respectively. Therefore we have,

$$\begin{pmatrix} A_1(t) \\ A_2(t) \\ A_3(t) \end{pmatrix} = b \begin{pmatrix} \varepsilon X_1 & \varepsilon Y_1 & \varepsilon Z_1 \\ \varepsilon X_2 & \varepsilon Y_2 & \varepsilon Z_2 \\ \varepsilon X_3 & \varepsilon Y_3 & \varepsilon Z_3 \end{pmatrix} \begin{pmatrix} [X](t) \\ [Y](t) \\ [Z](t) \end{pmatrix} \tag{3}$$

which can be solved as,

$$\begin{pmatrix} [X](t) \\ [Y](t) \\ [Z](t) \end{pmatrix} = b^{-1} \begin{pmatrix} \varepsilon X_1 & \varepsilon Y_1 & \varepsilon Z_1 \\ \varepsilon X_2 & \varepsilon Y_2 & \varepsilon Z_2 \\ \varepsilon X_3 & \varepsilon Y_3 & \varepsilon Z_3 \end{pmatrix}^{-1} \begin{pmatrix} A_1(t) \\ A_2(t) \\ A_3(t) \end{pmatrix} \tag{4}$$

where, A = Absorbance of mixture

t = reaction time
ε = Molar Absorptivity, $M^{-1}cm^{-1}$
b= Cell optical length = 1 *cm*
[X]= Molar salicilic acid concentration in the mixture
[Y]= Molar 2,3-DHBA concentration in the mixture
[Z]= Molar 2,5-DHBA concentration in the mixture

The absortivities for every compound were determined by a commonly employed procedure [15] at the wavelengths of 290, 310 and 330 *nm* (where the pure standards show a maximum of absorbance). Table 2 shows the value of the calculated absortivities.

The absorbances were measured at the same wavelengths but at various reaction times. At these reaction times the concentrations of the analytes were also determined by solving (4) and Figs. 4 and 5 were generated. The evolution of the reagent and products concentration when applying 0.2 *A* and *4* V can be observed in Fig. 4. It is observed that the production of $^{\bullet}$OH radicals is instantaneous since the SA concentration decays as soon as an electrical current

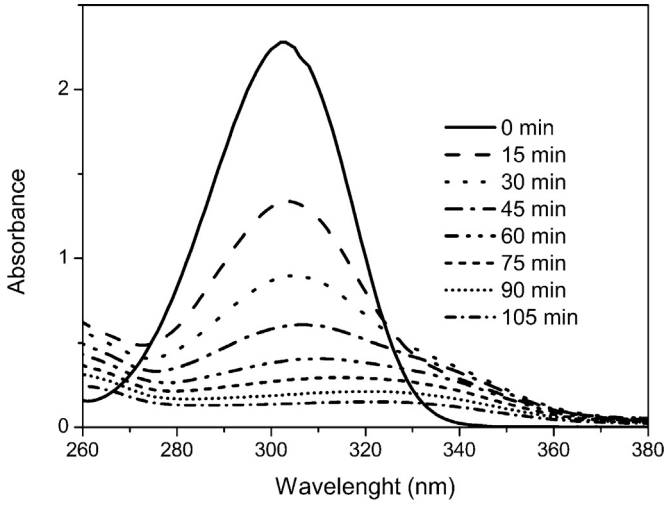

Fig. 3. Effect of reaction time on mixture UV-Vis spectrum. Reaction conditions: 0.4 A and 5 V, pH = 1.4, SA initial concentration (t = 0 min) = 8×10^{-4} M.

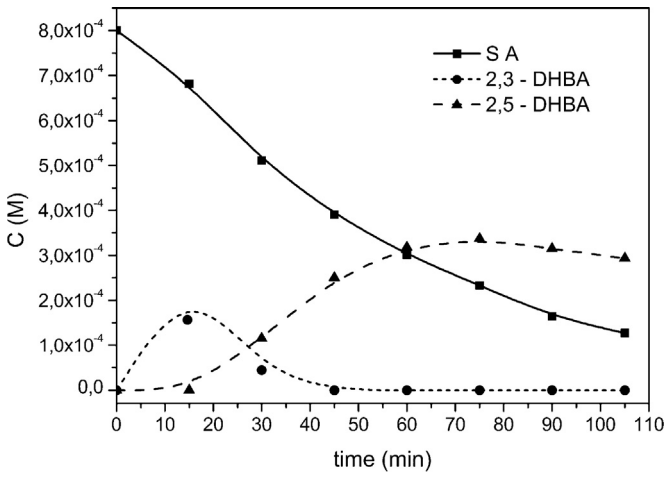

Fig. 4. Concentration profiles of Salicylic Acid, 2,3-DHBA and 2,5-DHBA. Operating conditions: 0.2 A and 4 V.

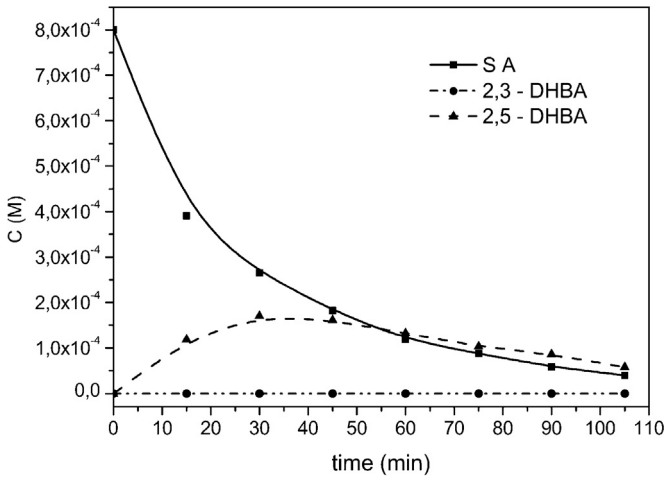

Fig. 5. Concentration profiles of Salicylic Acid, 2,3-DHBA and 2,5-DHBA. Operating conditions: 0.4 A and 5 V.

is applied. In the first 15 minutes of reaction, this decay can be ascribed to the oxidation of SA by ●OH radicals and the evidence of this reaction is the appearance of its main products 2,3-DHBA and 2,5-DHBA. 2,3-DHBA concentration reaches a maximum after 15 minutes of reaction. At this point this concentration starts to decay and practically extinguishes after 45 minutes. It could be

Table 2
Molar absortivities for every analyte.

Analyte	λ (nm)	Absortivity, ε (M^{-1} cm^{-1})
S A	290	2434.70
2,3–DHBA	290	1316.13
2,5–DHBA	290	635.14
S A	310	2591.04
2,3–DHBA	310	2706.30
2,5–DHBA	310	2402.49
S A	330	216.97
2,3–DHBA	330	1694.40
2,5–DHBA	330	3238.56

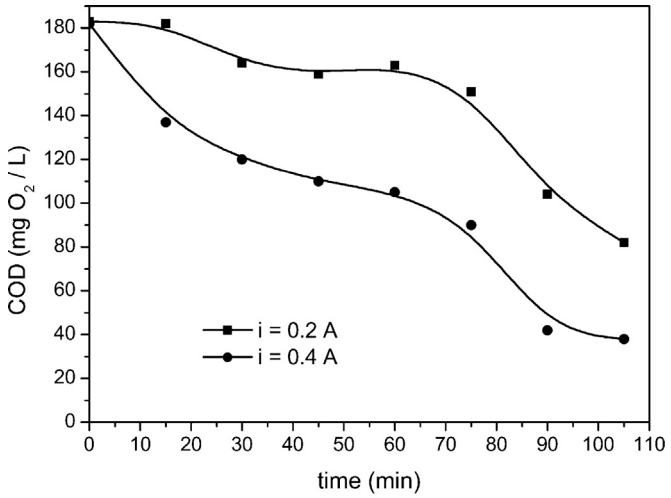

Fig. 7. Effect of time on chemical oxygen demand (COD).

thought that this observed 2,3-DHBA concentration diminishment is due to the appearance of 2,5-DHBA. This would suggest that the occurrence of these products is consecutive rather than in parallel. However, by analyzing Fig. 7, it can be concluded that the diminishment of 2,3-DHBA concentration is due to mineralization (COD decreases) and therefore the production of both acids occurs in parallel rather than in series and this is in concordance with previous reports, [4,13,15], the mechanism is illustrated in Fig. 6. In concordance to the stoichiometry of this reaction scheme, in the first stage of the reaction when only 2,3-DHBA is formed (before 15 minutes), ●OH radical concentration is equal to 2,3-DHBA concentration. According to previous reports [14], in the second stage of the reaction, when 2,5-DHBA appears, ●OH radical concentration

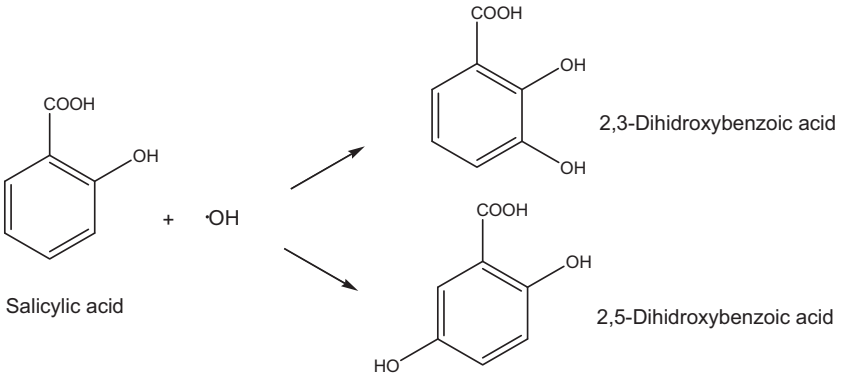

Fig. 6. Reaction of Salicylic Acid and hydroxyl radical.

would be equal to the sum of 2,3-DHBA and 2,5-DHBA concentrations. In this case, however, COD results (Fig. 7) shows that •OH radicals are not only consumed to produce the hydroxilated products but also are being consumed in their mineralization. It can also be inferred from Fig. 4, that reaction 1 is more rapid than reaction 2. When 0.4 A and 5 V are applied to the system, both reactions may be accelerated and this may explain why only 2,5-DHBA was detected (see Fig. 5). The concentration of this compound is observed to reach a maximum around 30 minutes and after this time a slight diminishment is observed.

It is observed in both experiments that the hydroxyl radicals production increases with reaction time. Unlike other reports[12,14], however, we believe that the quantification of the acid products is not enough to establish the hydroxyl radicals concentration. Fig. 7 shows that there is a certain degree of mineralization since the very beginning when employing i = 0.4 *A* and after 15 minutes at i =0.2 *A*. This suggests that after this point the amount of produced •OH should not be only related to the acids concentration but to the degree of mineralization also. Thus, to apply this method of quantification is necessary to establish besides the acids concentration the chemical oxygen demand at every point. Albeit this restriction, this method is still useful to compare advanced oxidation processes with initial reaction data only. At initial conditions (<15 minutes), the production rate of •OH radicals is 2×10^{-5} $mol{\cdot}L^{-1}{\cdot}min^{-1}$.

4. Conclusions

UV-Vis spectrophotometry can be used to indirectly quantify •OH radicals production when using Salicylic Acid as scavenger. This method can also be applied in other advanced oxidation processes. At any case, mineralization degree quantification is also required for this method to be valid. In addition, for this method to be applied, special attention should be paid to establish linearly independent equations of absorbance as function of analytes concentrations. As long as there is not mineralization at all of the two main products of salicylic acid hydroxylation (2,3-dihidroxybenzoic Acid and 2,5-dihydroxibenzoic Acid) then the •OH radicals concentration is the sum of the acid products. In this study, this could only be applied at the initial stage (<15 min) of the electrolysis conducted at the lowest voltage and current (0.2 *A* and 4 *V*). At these conditions, the initial production rate of •OH radicals is 2 × 10-5 $mol{\cdot}L^{-1}{\cdot}min^{-1}$.

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