Reactivity of catecholamine-driven Fenton reaction and its relationships with iron(III) speciation

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Introduction: Fenton reaction is the main source of free radicals in biological systems. The reactivity of this reaction can be modified by several factors, among these iron ligands are important. Catecholamine (dopamine, epinephrine, and norepinephrine) are able to form Fe(III) complexes whose extension in the coordination number depends upon the pH. Fe(III)-catecholamine complexes have been related with the development of several pathologies.

Methods: In this work, the ability of catecholamines to enhance the oxidative degradation of an organic substrate (veratryl alcohol, VA) through Fenton and Fenton-like reactions was studied. The initial VA degradation rate at different pH values and its relationship to the different iron species present in solution were determined. Furthermore, the oxidative degradation of VA after 24 hours of reaction and its main oxidation products were also determined.

Results: The catecholamine-driven Fenton and Fenton-like systems showed higher VA degradation compared to unmodified Fenton or Fenton-like systems, which also showed an increase in the oxidation state of the VA degradation product. All of this oxidative degradation takes place at pH values lower than 5.50, where the primarily responsible species would be the Fe(III) mono-complex.

Conclusion: The presence of Fe(III) mono-complex is essential in the ability of catecholamines to increase the oxidative capacity of Fenton systems.

Keywords: Fenton reaction, Catecholamines, Dopamine, Epinephrine, Norepinephrine, Catechol, Fenton-like reaction

Introduction

Fenton reactions are catalytic oxidation reactions starting with iron(II) precursors. A mixture of Fe(II) and H₂O₂ is known as Fenton's reagent that was described for the first time by H.J.H. Fenton in 1894.¹ The mechanism of this reaction was proposed by Haber and Weiss in 1934.² This mechanism includes the production of the hydroxyl radical (HO•) as the main oxidizing species (Equation (1))

As a product of the Fenton reaction, alternatives oxidizing species have also been suggested to the HO• radical, among which stand out the ferryl species⁴ [Fe=O]²⁺, and singlet oxygen.⁵ However, the conditions in which one sort or another specie that prevails is still unclear.

Fenton-like reactions are catalytic oxidation reactions starting with iron(III) precursors. Fe(III) with H₂O₂ is known as a Fenton-like reagent. The rate of this reaction is four orders magnitude lower than Fenton reactions and proceeds through the formation of hydroperoxyl radicals (HO₂•)⁶ (Equation (2)).

It should be noted that both Fenton and Fenton-like reactions proceed catalytically by using several sequential reactions.⁷ Among these, Equations (1) and (2) are involved in both types of reactions

The production of reactive species by a Fenton reaction can be enhanced by several iron ligands. Among these ligands are 2,4-dimethylaniline,⁸ ethylenediaminetetraacetic acid (EDTA), diethylene triamine pentaacetic acid (DTPA), desferal,⁹ humic acids,¹⁰ malonic acid, oxalic acid, ascorbic acid,¹¹ and dihydroxybenzenes (DHBs).¹² The DHB-driven Fenton

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Figure 1 Catecholamine-Fe(III) complexes at different pH values. R=H (catechol); CH₂-CH₂-NH₂ (dopamine); CHO-CH₂-NHCH₃ (epinephrine); and CHO-CH₂-NH₂ (norepinephrine).

reaction has been studied in different systems such as metabolic pathways^{13,14} and advanced oxidation processes for water or wastewater treatment.^{15,16} The main explanation for the increase in the production of reactive species is based on the ability of DHB to form complexes with Fe(III) and thereby reducing it to Fe(II) through inner-sphere mechanisms.¹⁷ This ability has been established only for 1,2- and 1,4-DHB and not for 1,3-DHB because a quinoid system has to be formed.¹⁸ In previous reports, the speciation of iron complexes has been associated with the reactivity of a 1,2-DHB-driven Fenton reaction against a recalcitrant substrate. Here, the highest oxidation of the substrate was predicted at a pH=3.4.¹⁹ At this pH, the concentrations of HO• in the 1,2-DHB-driven Fenton reactions were higher than in unmodified Fenton reactions.²⁰ The chemiluminescence of the 1,2-DHB-driven Fenton reactions was also higher and longer in time than for the unmodified Fenton reactions.²¹

The biological catecholamines (dopamine, epinephrine, and norepinephrine) are a type of 1,2-DHB. These compounds have been related with the etiology and development of Parkinson's disease,^{22–24} stress-induced arrhythmias, cardiopathies,²⁵ cardiac injuries by myocardial infarction,²⁶ and inflammatory damage caused by chronic stress.²⁷

The coordination number of iron complexes with catecholamines is pH-dependent²⁸ (Fig. 1). Mono-complexes are mainly formed at acidic pH values lower than 6.0 and have a maximum absorbance at approximately 700 nm. This complex is very unstable because Fe(III) quickly reduces to Fe(II) by the catecholamine through an inner-sphere mechanism.²⁹ At nearly neutral pH values, bis-complexes are formed (maximum absorbance at approximately 560 nm). These are more stable than the mono-complexes. At basic pH values, stable tris-complexes are formed (maximum absorbance at approximately 490 nm).²⁸

In vitro studies have reported that the Fe(III)-dopamine complex induces neuronal toxicity via oxidizing species such as HO•.²³ However, the capacities of catecholamines to induce and amplify Fenton or a Fenton-like reactions have not been directly studied.

In this paper, the effect of catecholamines on the reactivities of Fenton and Fenton-like reactions was established through analysis of 3,4-dimethoxybenzyl alcohol (veratryl alcohol (VA)) degradation in a pH ranging between 2.00 and 7.00. This substrate is hard to oxidize (1.36 V),³⁰ which makes it an appropriate compound to study the oxidizing ability of the modified and unmodified Fenton and Fenton-like systems.

Experimental methods

General procedures

All of the reagent solutions were prepared in the dark under argon atmosphere.

Reagents

All reagents were used without additional purification. The reagents were catechol (Sigma); dopamine hydrochloride (Sigma); (±)-epinephrine hydrochloride (Sigma); DL-norepinephrine hydrochloride; H₂O₂ 30% (w/w) (Merck); ferric nitrate (Merck); ferrous sulfate (Merck); 3,4-dimethoxybenzyl alcohol (VA, Sigma); formic acid (Fluka); and HPLC quality acetonitrile (Merck).

VA degradation

Oxidative VA degradation by Fenton and Fenton-like systems was performed between pH values of 2.00 and 7.00. The pH of each solution was adjusted just before each reaction using a 3 Start Thermo Orion pH meter. A 0.050 mol l⁻¹ bis-tris buffer was used for pH values from 6.00 to 7.00; a 0.050 mol l⁻¹ acetate buffer was used for pH values from 4.00 to 5.50; and HNO₃ was used to regulate pH values lower than 4.00.

The final concentrations in the systems were 5.00 μmol l⁻¹ FeSO₄ or Fe(NO₃)₃ (for Fenton or Fenton-like systems, respectively), 50.0 μmol l⁻¹ catechol or catecholamines, 500 μmol l⁻¹ H₂O₂ and 300 μmol l⁻¹ VA. The ionic strength of all the solutions was adjusted with KNO₃ to 0.100 mol l⁻¹. All of the experiments were performed at 20±0.1°C. The description of each system studied is shown in Table 1.

The VA concentration was quantified by high-performance liquid chromatography (HPLC) (Hitachi, Elite LaChrom). The mobile phase composition was water, acetonitrile, and formic acid in the ratio of

Table 1 Systems studied

Systems	Name
FeSO ₄ +H ₂ O ₂ +VA	Unmodified Fenton system
Fe(NO ₃) ₃ +H ₂ O ₂ +VA	Unmodified Fenton-like system
FeSO ₄ +H ₂ O ₂ +VA+catechol	Catechol-driven Fenton system
Fe(NO ₃) ₃ +H ₂ O ₂ +VA+catechol	Catechol-driven Fenton-like system
FeSO ₄ +H ₂ O ₂ +VA+catecholamine*	Catecholamine-driven Fenton system
Fe(NO ₃) ₃ +H ₂ O ₂ +VA+catecholamine*	Catecholamine-driven Fenton-like system

*Catecholamine: dopamine, epinephrine, or norepinephrine.

900:100:1 with a flow rate of 1.1 ml minute⁻¹. The stationary phase was a Merck C-18 column filled with 5 µm sized particles (Lichrospher 100 RP-18) with a stainless steel holder 12.5 cm long (Lichrocart 125-4). The photometric detector was set at 277 nm.

The VA concentration was monitored during 30 minutes after starting the reaction. Then 24 hours later the final VA concentration was determined. The initial VA degradation rate (I_R) was determined from the linear range in the first 30 minutes of the reaction. The maximum oxidative degradation of VA was determined after 24 hours of reaction time. These results are expressed as the percentage of VA degradation.

The uncertainty of each measurement was calculated from the calibration curve by the Eurachem method.³¹

Determination of VA degradation products

Five hundred milliliters of each system studied were reacted for 24 hours. Then, the samples were pre-concentrated by using solid phase extraction cartridges (60 mg of C18, OASIS, Waters). Afterward, the compounds in the column were eluted with 4.00 ml of dichloromethane. Finally, this extract was dried with N₂ gas to a 1.00 ml volume. The samples were methylated with 300 µmol l⁻¹ diazomethane and then injected into a Hewlett-Packard HP 5890 Series II GC equipped with an HP5972 MSD detector. The analytical column used was a HP 5 MS (30 m × 0.25 mm × 0.25 µm) with He as the carrier gas at a constant flow rate of 1 ml minute⁻¹. The oven temperature programed was as follows: 70°C × 1 minute; 60°C minute⁻¹, 200°C × 5 minutes; and 50°C minute⁻¹, 250°C × 8 minutes. The temperatures of the injector and interface were 250 and 280°C, respectively.

The identification of the VA oxidation products was performed by comparing each spectrum with a mass spectra library database (NIST/EPA/NIH7SK).

Speciation of Fe(III)

The speciation of Fe(III) at different pH values was calculated using 'CHEAQS proV.2004.1' software designed by Wilko Verweij (<http://home.tiscali.nl/cheaqs/>). The equilibrium constants used in this software were obtained from the NIST database 46, 2004.

Estimated initial VA degradation

The experimental results obtained for the I_R by Fenton-like systems at different pH values were fit with several functions (exponential, sigmoidal, and polynomial). The Gaussian function showed the best fit. The statistical validation was performed by an analysis of variance (ANOVA) test with 95% reliability. From this Gaussian function a curve was plotted. This curve will now on be referred to as an estimated I_R .

Relationship between iron speciation and the initial VA degradation rate

To obtain a quantitative parameter to establish the participation of various Fe(III) species in the oxidative degradation of VA, a linear polynomial was determined. This polynomial was obtained by using multiple linear regression (MLR) (Equation (3)) with algorithm of Equation (4). The obtained equation was validated by an ANOVA test with 95% reliability

$$Y = B \times X \quad (3)$$

$$B = (X^T X)^{-1} X^T Y \quad (4)$$

In MLR, the concentrations of different Fe(III) species at different pH values were used as independent variables (X matrix) and the estimated I_R was used as the dependent variable (Y matrix). The estimated I_R was used instead of the experimental I_R values. This is because the number of experimental values obtained for the I_R were insufficient to establish a relationship between this parameter of reactivity and the Fe(III) species present at different pH values.

From the MLR analysis, a vector of coefficients was obtained (matrix B). Each coefficient (B_i) was related to a specific independent variable (iron species). For each pH value, the contribution of each Fe(III) species (%) was determined. This value was calculated using Equation (5), where $[\text{FeL}]_i$ is the concentration of each Fe(III) species at each pH value, and $\sum B_i[\text{FeL}]_i$ is the summation of all of the products of each $[\text{FeL}]_i$ and its respective B_i

$$\text{Contribution (\%)} = \left(\frac{B_i[\text{FeL}]_i}{\sum B_i[\text{FeL}]_i} \right) 100 \quad (5)$$

Figure 2 (A) Percentage of oxidative degradation of VA after 24 hours of reaction in Fenton systems. (B) Initial VA degradation rate at different pH values in Fenton systems. Light gray bar: unmodified Fenton systems; red bar: catechol-driven Fenton systems; green bar: dopamine-driven Fenton systems; blue bar: epinephrine-driven Fenton systems; pink bar: norepinephrine-driven Fenton systems.

Results and discussion

VA degradation in unmodified Fenton and Fenton-like systems

In order to analyze the VA degradation by Fenton and Fenton-like systems, the initial VA degradation rate (I_R) and the percentage of VA degradation after 24 hours were determined.

Unmodified Fenton systems degrade less than 5% of the VA after 24 hours of reaction at a pH of 2.00 and pH values between 4.50 and 7.00 (Fig. 2A). The maximum oxidative degradations of VA at pH values of 3.00, 3.40, and 4.00 were $24.6 \pm 0.2\%$, $16.3 \pm 0.3\%$, and $7.5 \pm 0.3\%$, respectively. Among these, the highest I_R was at a pH of 3.40 ($0.59 \pm 0.05 \mu\text{mol l}^{-1} \text{ minute}^{-1}$; Fig. 2B).

In unmodified Fenton-like systems, the oxidative degradation of VA was not observed over a pH of 5.50 (Fig. 3A). At pH 3.00, highest degradation for

these systems was found to occur ($28.8 \pm 0.2\%$ of VA degradation).

The I_R for unmodified Fenton-like systems was determined at pH values from 2.00 to 4.50 (Fig. 3B). Since at pH value higher than 4.50 significant decreases in the VA concentration were not observed for the first 30 minutes of the reaction. For unmodified Fenton-like systems the highest I_R was observed at a pH of 3.00 ($0.71 \pm 0.04 \mu\text{mol l}^{-1} \text{ minute}^{-1}$).

In general, the I_R value for the unmodified Fenton system was lower than the I_R for the unmodified Fenton-like system. This could be explained by the main oxidizing species produced in the initial stages of the reaction in each system. In Fenton reactions, the main oxidizing species is $\text{HO}^\bullet$ which is highly reactive, and therefore it has a low diffusion capacity. The main oxidizing species in Fenton-like reactions is $\text{HO}_2^\bullet$ which is less reactive and therefore has a greater

Figure 3 (A) Percentage of degradation of VA after 24 hours of reaction in Fenton-like systems. (B) Initial VA degradation rate at different pH values in Fenton-like systems. Gray bar: unmodified Fenton-like systems; red bar: catechol-driven Fenton-like systems; green bar: dopamine-driven Fenton-like systems; blue bar: epinephrine-driven Fenton-like systems; pink bar: norepinephrine-driven Fenton-like systems.

diffusion capacity than $\text{HO}^\bullet$. Due to this difference in the oxidizing species, the VA oxidations performed by unmodified Fenton-like systems are more efficient than the oxidations performed by unmodified Fenton systems.

VA degradation in catecholamine-driven Fenton and Fenton-like reactions

Increases in the oxidative degradation of VA by Fenton and Fenton-like systems were observed when catecholamines drive these reactions (Figs. 2 and 3).

In unmodified Fenton and Fenton-like systems, the maximum oxidative degradation of VA does not exceed 30%, whereas in the presence of catecholamines, this degradation can reach up to 70%. In both catecholamine-driven Fenton and Fenton-like systems, the highest oxidative degradation of VA is reached at a pH of 3.40. This is consistent with the studies on catechol that was previously reported by Contreras et al.¹⁹

The oxidative degradation of VA by catecholamine-driven Fenton and Fenton-like systems was observed up to a pH of 5.50.

At the same pH value, no significant differences were observed in the maximum oxidative degradation of VA in Fenton and Fenton-like systems driven by the same 1,2-DHB (Figs. 2B and 3B, respectively). However, for the same systems at different pH values, significant differences in the I_R and maximum oxidative degradation of VA were observed.

In general, catechol-driven Fenton and Fenton-like systems have higher I_R values than catecholamine-driven Fenton and Fenton-like systems. Catecholamines have an electron withdrawing substituent.³² This substituent may have the following two parallel effects: one is to increase the formation constant of the Fe(III)-complex and the other to destabilize semiquinone intermediates formed by the reduction of Fe(III) to Fe(II). The combination of these two effects could be related to the decrease in reactivity of the catecholamine-driven systems.

It is noteworthy that catecholamine-driven Fenton-like systems have higher I_R values than catecholamine-driven Fenton systems. Similar results have been observed in the degradation of several substrates by 1,2-DHB-driven Fenton systems.^{33–35} This behavior could be related to the increase of the production of activated species by 1,2-driven Fenton-like systems.^{20,21}

Fe(III) speciation and its effect on the initial VA degradation rate

To compare the relative contributions of different Fe(III) species to the reactivity (I_R) values, a first-order polynomial was modeled. For this, the concentrations of Fe(III) species at different pH values were

Table 2 Coefficients obtained by MLR for each Fe(III) species present in the unmodified Fenton-like system

Fe(III) species	Coefficient (B_i) $\times 10^{-5}$
$[\text{Fe}]^{3+}$	19 ± 4
$[\text{Fe}(\text{OH})]^{2+}$	6.7 ± 0.7
$[\text{Fe}(\text{OH})_2]^+$	20 ± 4
$[\text{Fe}(\text{OOH})]^{2+}$	$(63 \pm 3) \times 10^3$

$\pm$ standard error of the mean.

considered as independent variable, and the estimated I_R values were considered as the dependent variable. Then, using MLR, a coefficient (B_i) associated with each Fe(III) species was obtained. These coefficients indicate the contribution of each Fe(III) species to I_R .

Just for catechol the formation constants for all complexes formed with Fe(III) are reported. Because of this, the MLR is performed only for the catechol-driven system. However, changes in the speciation of catechol and catecholamine should be small, considering their structural similarity and the similarity observed in the experimental degradation that results at pH function.

Table 2 shows the coefficients obtained for unmodified Fenton-like systems. Here, the coefficient for the peroxo-complex $[\text{Fe}(\text{OOH})]^{2+}$ can be highlighted. This value is three orders of magnitude higher than the other coefficients. Thus, the polynomial shows that $[\text{Fe}(\text{OOH})]^{2+}$ is the main species that contributes to the I_R . Therefore, the results obtained from the polynomial are consistent with previous reports by De Laat and Gallard, in which the $[\text{Fe}(\text{OOH})]^{2+}$ participation in the rate-limiting step of Fenton reactions was established from kinetic data.³

It should be noted that in unmodified Fenton-like systems, all coefficients are greater than zero (Table 2). This implies that all Fe(III) species present in the systems contribute to the estimated I_R .

Figure 4 Contribution percentages of Fe(III) species in VA degradation by a Fenton-like reaction. Black line: Fe(III) species, gray line: estimated initial VA degradation rate.

Table 3 Coefficients obtained by MLR for each Fe(III) species present in the catechol-driven Fenton-like system

Fe(III) species	Coefficient (B_i) $\times 10^{-5}$
$[\text{Fe}]^{3+}$	6.2 ± 0.1
$[\text{Fe}(\text{OH})]^{2+}$	1.3 ± 0.7
$[\text{Fe}(\text{OH})_2]^+$	5.5 ± 0.1
$[\text{Fe}(\text{OOH})]^{2+}$	$(23 \pm 3) \times 10^3$
$[\text{Fe}(\text{catechol})]^+$	8.9 ± 0.1
$[\text{Fe}(\text{catechol})_2]^+$	41 ± 1
$[\text{Fe}(\text{catechol})_3]^{3-}$	$-(53 \pm 3) \times 10^6$

$\pm$ standard error of the mean.

The contribution of each Fe(III) species in unmodified Fenton-like systems and the estimated I_R as a function of pH were plotted (Fig. 4). The estimated I_R maximum coincides with the maximum contribution of $[\text{Fe}(\text{OOH})]^{2+}$. This was an expected result because it was the largest coefficient (Table 2). At pH values lower than 2.70 and higher than 3.50, the species that contributes the most to the estimated I_R are $[\text{Fe}]^{3+}$ and $[\text{Fe}(\text{OH})_2]^+$, respectively. However, the I_R decreases in both pH ranges. This suggests that although $[\text{Fe}]^{3+}$ and $[\text{Fe}(\text{OH})_2]^+$ contributes to the I_R , their efficiency in the oxidative degradation of VA is, however, low.

In catechol-driven Fenton-like systems results similar to those of unmodified Fenton-like systems were obtained (Table 3). The coefficient for $[\text{Fe}(\text{OOH})]^{2+}$ is three orders of magnitude higher than the other coefficients. The coefficients of all the Fe(III) species are greater than zero with one exception, indicating that all the species contribute to the estimated I_R (Table 3). The *tris*-complex, $[\text{Fe}(\text{catechol})_3]^{3-}$, was the exception. This species showed a coefficient lower than zero and at least six orders of magnitude higher than the other coefficients. This is coherent with the antioxidant activity for catechol *tris*-complexes. In this complex, all the Fe(III) positions in the coordination sphere are occupied by a non-labile ligand, limiting the exchange of H_2O_2 by other ligands and thereby inhibiting the Fenton reaction, which is performed by an inner-sphere mechanism.^{36,37}

For catechol-driven Fenton-like systems, the contribution of each Fe(III) species and the estimated I_R as a function of pH is plotted in Fig. 5. In catechol-driven Fenton-like systems, the peroxo-complex remains the main species that contributes to the I_R when it reaches its maximum. The $[\text{Fe}]^{3+}$ provides the same contribution to the I_R as in the unmodified Fenton-like system. However, $[\text{Fe}(\text{OH})_2]^+$ shows a decrease in contribution to the I_R from pH >3.00 in comparison to the contribution observed in Fenton-like systems. At a pH of 4.50 in unmodified Fenton-like systems, $[\text{Fe}(\text{OH})_2]^+$ provides a contribution to the estimated I_R of 86.6%, and in catechol-driven Fenton-like systems, this contribution decreases to

Figure 5 Contribution percentages of Fe(III) species in VA degradation by a catechol-driven Fenton-like reaction. Black line: Fe(III) species, gray line: estimated initial VA degradation rate.

55.5%. The decrease in the contribution of $[\text{Fe}(\text{OH})_2]^+$ occurs due to the presence of Fe(III)-catechol species present in the catechol-driven Fenton-like systems.

The catechol presence in the studied system shifts the maximum I_R to a pH of 3.40. Together with this shift, at least a threefold increase in the I_R was observed over the pH range studied. Because $[\text{Fe}]^{3+}$ and $[\text{Fe}(\text{OH})_2]^+$ shows low efficiencies to promote the oxidative degradation of VA, it could be proposed that the Fe(III)-catechol species must be responsible for the increase in the I_R .

The Fe(III)-catechol species that contributes to the estimated I_R are the *bis*-complex ($[\text{Fe}(\text{catechol})_2]^+$) and the *mono*-complex ($[\text{Fe}(\text{catechol})]^+$). These species have coefficients of similar magnitude. However, Fig. 5 shows that $[\text{Fe}(\text{catechol})]^+$ is the Fe(III)-catechol species that contributes the most to estimated I_R at pH values lower than 5.50. In the pH range close to the maximum I_R , there is a linear correlation between the contribution of $[\text{Fe}(\text{catechol})]^+$ and the increase in the estimated I_R ($r = 0.996$). This indicates that $[\text{Fe}(\text{catechol})]^+$ would be the species primarily responsible for the changes observed in the I_R . $[\text{Fe}(\text{catechol})]^+$ would contribute to the oxidative degradation of VA through the reduction of Fe(III) into Fe(II).³⁸ This increases the rate of limiting step of the Fenton reaction, which is the reduction of Fe(III) to Fe(II) by the disproportionation of $[\text{Fe}(\text{OOH})]^{2+}$ to $\text{HO}_2^\bullet$ and Fe(II).³⁹

VA oxidation products

The VA oxidation products were determined from systems that showed high oxidative degradation of VA (24 hours of reaction at a pH of 3.40).

Oxidized compounds of VA were identified from methylated samples (Table 4). Differences were observed in the relative amount of VA oxidation products generated by the different systems studied

Table 4 VA oxidation products determined by GC-MS

N	Chemical structure	Compound (#CAS)	MW (g/mol)	Qual (%)	Main fragment ions, m/z (rel. Int.)
1		Benzaldehyde, 3,4-dimethoxy (120-12-9)	166	95	50(18),51(35),52(17),53(10),62(10),63(18),65(19), 67(12),77(38),79(33),90(12),95(52),151(14), 165(65),166(100),167(10)
2		Benzoic acid, 3,4-dimethoxy-methyl ester (2150-38-1)	196	99	50(11),51(23),59(11),63(10),77(18),79(30),94(10),107(10), 121(12),122(11),125(12),137(10),165(100),166(14), 196(81),197(10)
3		Benzaldehyde, 3,4,5-trimethoxy* (86-81-7)	196	97	51(13),53(11),65(16),66(10),77(14),79(10),93(18),95(23), 110(29),125(30),166(11),181(43),196(100)
4		Benzaldehyde, 2,4,5-trimethoxy* (4460-86-0)	196	98	50(14),51(15),53(22),59(11),65(11),66(10),69(25),77(14), 79(15),93(12),95(23),107(10),109(14),110(21), 123(11),125(29),136(10),137(10),139(10),150(27), 151(16),153(19),165(12),166(11),179(10),181(62), 195(20),196(100),197(12)

Table 5 Relative amounts of VA oxidation products from the different systems

	Relative amount (%)			
System	1	2	3	4
Unmodified Fenton system	96.7	3.3	0	0
Unmodified Fenton-like system	98.7	1.3	0	0
Catechol-driven Fenton system	86.4	11.0	1.2	1.4
Catechol-driven Fenton-like system	87.4	10.4	1.2	1
Dopamine-driven Fenton system	89.9	10.1	0	0
Dopamine-driven Fenton-like system	89.8	9.2	0	0
Epinephrine-driven Fenton system	90.9	9.1	0	0
Epinephrine-driven Fenton-like system	90.7	9.3	0	0
Norepinephrine-driven Fenton system	89.6	10.3	0.1	0
Norepinephrine-driven Fenton-like system	90.6	9.3	0.1	0

(Table 5). In unmodified Fenton and Fenton-like systems, approximately 95% of the VA oxidation compounds were veratryl aldehyde (Compound 1) and less than 5% were veratryl acid (Compound 2).

The oxidation state in the identified products was higher in catechol and catecholamine-driven systems than in unmodified Fenton and Fenton-like systems. It was determined that over 99% of the oxidation compounds were veratryl acid (Compound 2) in all of the catechol and catecholamine-driven systems. The differences in relative amounts of the compounds determined by gas chromatography–mass spectrometry (GC-MS) between the systems studied are not statistically significant. This is consistent with the results obtained in the percentage of VA degradation after 24 hours in which no significant difference was

observed in the VA degradation by catechol or catecholamines-driven Fenton and Fenton-like systems.

Hydroxylation products were detected at the C2 and C3 positions of the ring from the catechol and norepinephrine-driven systems (Compounds 3 and 4). It was found that the hydroxylated species also had alcohols oxidized into aldehyde. This is consistent with the higher reactivity of the alcohol group.

Ligands, such as 1,2-DHB, can increase the production of reactive species in Fenton and Fenton-like systems, and they can also promote the formation of other oxidizing species such as singlet oxygen, semiquinone,⁵ and ferryl species.⁴⁰ This can be an explanation for the increase in the oxidation state of VA degradation products obtained from catecholamine-driven Fenton and Fenton-like systems.

Conclusions

The oxidative degradation of VA by catechol and catecholamine-driven Fenton and Fenton-like reactions is pH-dependent. Catecholamines have the ability to significantly amplify Fenton and Fenton-like reactions at pH values ranging between 2.00 and 5.50. This implies that catecholamines would promote oxidative stress by Fenton or Fenton-like reactions at pH values lower than 5.50 because the mono-complex $[\text{Fe}(\text{catecholamine})]^+$ must be formed.

Disclaimer statements

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Conflicts of interest None.

Ethics approval None.

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