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Oxidation and chemiluminescence of catechol by hydrogen peroxide in the presence of Co(II) ions and CTAB micelles

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ABSTRACT: The oxidation of catechol in neutral and slightly alkaline aqueous solutions (pH 7–9.6) by excess hydrogen peroxide (0.002–0.09 mol/L) in the presence of Co(II) (2.10^{-7}–2.10^{-5} mol/L) is accompanied by abrupt formation of red purple colouration, which is subsequently decolourized within 1 h. The electron spectra of the reaction mixture are characterized by a broad band covering the whole visible range (400–700 nm), with maximum at 485 nm. The reaction is initiated by catechol oxidation to its semiquinone radical and further to 1,2-benzoquinone. By nucleophilic addition of hydrogen peroxide into the *p*-position of benzoquinone C=O groups, hydroperoxide intermediates are formed, which decompose to hydroxylated 1,4-benzoquinones. It was confirmed by MS spectroscopy that monohydroxy-, dihydroxy- and tetrahydroxy-1,4-benzoquinone are formed as intermediate products. As final products of catechol decomposition, muconic acid, its hydroxy- and dihydroxy- derivatives and crotonic acid were identified. In the micellar environment of hexadecyltrimethylammonium bromide the decomposition rate of catechol is three times faster, due to micellar catalysis, and is accompanied by chemiluminescence (CL) emission, with maxima at 500 and 640 nm and a quantum yield of 1×10^{-4}. The CL of catechol can be further sensitized by a factor of 8 (maximum) with the aid of intramicellar energy transfer to fluorescein. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS*:* catechol; chemiluminescence; micelles

INTRODUCTION

Catechols are biologically important substances known for their antioxidant activity (1, 2). Along with this beneficial action, toxic effects of catechols were also demonstrated (3, 4). Catechol causes skin erosion, induces prehaemolytic and haemolytic changes in human erythrocytes (3) and promotes the formation of stomach and kidney carcinoma in rodents (4). The interaction of catechols with DNA increases the amount of 8-oxo-7,8-dihydro-2-deoxyguanosine (8-oxodG) (4), which is known to correlate with the incidence of cancer. Therefore, catechol is classified as a potential human Group 2B carcinogen according to the International Agency for Research on Cancer (IARC). There is much evidence that the toxicity of catechols is increased during their oxidation (5, 6). Semiquinone radicals were detected in cigarette tar and their presence is related to the incidence of lung cancer in smokers (7). The process of activation of catechol and hydroquinone (two major constituents in cigarette smoke) toxicity during autooxidation includes the generation of hydrogen peroxide and the formation of 8-oxodG in DNA (8). Similar toxic products are formed in the course of the water purification process by the catalytical oxidation of phenols and catechol using hydrogen peroxide or oxygen (9). It is known that catechol toxicity is not associated with the brown-coloured products of its oxidation (1,2-benzoquinone) but rather to 1,4-benzoquinone, whose toxicity is much higher (9). Recently, the chemiluminescence (CL) technique for catechol and hydroquinone determination, based on the quenching of $KMnO_4$-initiated luminol CL by catechol or hydroquinone, has been suggested. The strong quenching effect was assured by using cyclodextrin as a microheterogeneous reaction medium (10).

In this paper we report that catechol oxidative decomposition is accompanied by the formation of excited states. Their formation can be visualized as CL emission, which can only be observed if the reaction is carried out in the micellar environment of CTAB. The intermediate and final products of catechol oxidation by hydrogen peroxide catalysed by Co(II) were identified using mass spectrometry.

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MATERIALS AND METHODS

Reagents

Catechol (CAT) was purchased from Aldrich; fluorescein sodium salt, hydrogen peroxide and hexadecyltrimethylammonium bromide (CTAB) were obtained from Fluka. All solutions were prepared using deionized water. Cobalt chloride was a Lachema Brno (Czech Republic) compound. All reagents were for analysis or better purity.

Instruments

A BioOrbit 1250 luminometer (Finland) was used for CL measurements. UV-vis spectra were recorded on a Beckman DU 7500 spectrometer (USA). EPR measurements were performed on a Magnettech MS200 (Germany) spectrometer, using capillaries. MS spectra were measured on an ESI–MS instrument (Finnigan MATLCQ, Finnigan, CA, USA).

RESULTS AND DISCUSSION

Electron spectra and CL emission accompanying catechol oxidation by the Co^{2+}–H_2O_2 system

Both alkaline and alkaline micellar solutions of CAT undergo spontaneous auto-oxidation in the presence of Co(II), which manifests itself as a formation of green catechol semiquinone. The semiquinone is probably stabilized by coordination to Co(II) or Co(III), present in the reaction mixture. A similar stabilization by Al^{3+} cation was described previously (11). In ESR spectra a single-line signal without fine structure is observed near the *g*-value of the free electron (not shown). When H_2O_2 is added into aqueous or micellar solutions containing CAT, an abrupt increase in the intensity of the CAT semiquinone spectral band occurs, followed by colour transition from green to red-purple. A new, broad band (400–700 nm) with the maximum at 485 nm and $\varepsilon \approx 10^3$ mol/L/cm arises in the spectrum. This colour change can be discerned if H_2O_2 concentration exceeds 0.002 mol/L and lasts for ca. 30–60 min. After this period of time complete decolouration occurs (Fig. 1a, c). In micellar solutions of CTAB, the rates are increased ca. three times for both formation and decay of the 485 nm peak (Fig. 1b, c). A similar absorption band centring at 490 nm was observed by de Lucia *et al.* (12) during oxidation of 2-(3,4-dihydroxyphenyl)ethanol by horseradish peroxidase (HRP)–H_2O_2 (hydroxyquinone species were found in the reaction mixture, including hydroxy-(1,4)-quinone derivative). Muňoz *et al.* (13) observed the dependence of decomposition mechanism on H_2O_2 concentration. At H_2O_2 concentrations >0.1 mmol/L, 2,5-dihydroxy-1,4-benzoquinone is formed, while at lower (0.01–0.1 mmol/L) peroxide concentrations only 1,2-benzoquinone, with a characteristic electron spectrum containing first long-wavelength bands at 410 nm (ε = 1623 mol/L/cm) and 430 nm (ε = 560 mol/L/cm), has been found. Similar results have also been obtained by others (14). A coincidence between the absorption spectra of the products of catechol and hydroquinone oxidation in an excess of H_2O_2 allows us to conclude that hydroxylated 1,4-benzoquinones are also formed as intermediate products of catechol oxidative decomposition.

Catechol is not regarded as a chemiluminescent compound and, as a typical antioxidant, it is a quencher of luminol and phthalic hydrazide CL. Nevertheless, in the micellar environment of CTAB we have observed relatively intense CL emission, which could be further sensitized up to eight-fold by fluorescein. Similar to the electron spectrum, the CL is observed only after limiting hydrogen peroxide concentration is exceeded. The overall CL intensity increases with CAT concentration and passes through maximum at an approximately equimolar CAT:micelles concentration ratio. The maximum CL quantum yield is 1×10^{-4}. The CL time profiles for 1×10^{-4} mol/L (trace A) and 1.5×10^{-5} mol/L (trace B) CAT solutions are shown in Fig. 2.

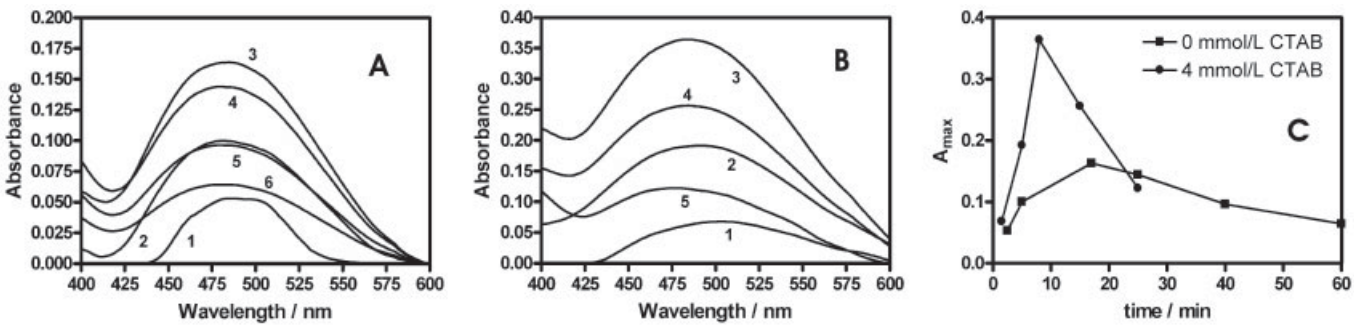

Figure 1. UV-vis spectra measured during the course of catechol oxidative decomposition by H_2O_2 (0.09 mol/L) in the presence of Co^{2+} (2×10^{-5} mol/L) without (A) and with (B) 4 mmol/L CTAB in the reaction mixture. The reaction was carried out in TRIS–HCl buffer, pH 8. (C) Time dependences of absorbance at peak wavelength (485 nm).

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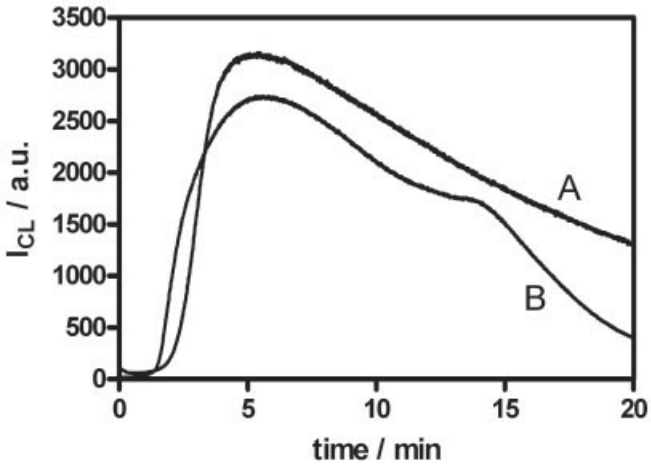

Figure 2. The CL time profiles measured for 1×10^{-4} mol/L (trace A) and 1.5×10^{-5} mol/L (trace B) catechol. Concentrations of reactants: H_2O_2, 0.09 mol/L; Co^{2+}, 2×10^{-5} mol/L. The reaction was carried out in TRIS–HCl buffer, pH 8.

A 2–4 min induction period can be observed on the CL time profiles, followed by the formation and decay portions of the CL curves. Further increase in CAT concentration leads to a decrease in the induction period and quenching of CL emission. Presumably this is caused by the combined effect of quenching due to higher occupancy of micelles by CAT and an inner filter effect in more intensely coloured solution.

The CL can be sensitized by the addition of fluorescein into the reaction mixture. The optimum fluorescein concentration is 1.2×10^{-4} mol/L for 4×10^{-3} mol/L CTAB solution, which is the same value as found in our previous study (15).

MS spectra

By means of mass spectroscopy, the products, which have higher molecular weights than CAT itself, were identified in the initial stages of the process of CAT oxidation. In these intermediates, the number of carbons is preserved and the increase in mass is caused by attachment of hydroxyl and hydroperoxyl groups. The identified products are summarized in Table 1, in which the products (whose mass increases by one oxygen atom; $\Delta m/z = 16$) are listed. Each row contains a pair of signals, characterized by mass difference ($\Delta m/z = 34$), which corresponds to the attachment of H_2O_2. The identified products listed in the table confirm that hydroxylation of the original 1,2-benzoquinone (CAT) takes place, yielding hydroxy-1,4-benzoquinone, which immediately continues to give di- and tetrahydroxybenzoquinone (TQH). The presence of trihydroxy-1,4-benzoquinone was questionable – only a weak signal of ca. S:N = 2 was detected. A further reaction that occurs in the process of catechol oxidation is H_2O_2 addition to quinones, accompanied by extradiol ring cleavage. Apparently, muconic acid ($m/z = 141$) and its hydroxy and dihydroxy derivatives ($m/z = 157$ and 171) are formed. Moreover, the signal with $m/z = 85$, which can be attributed to the TQH decomposition product (crotonic acid), was also found in the MS spectrum. From the time-dependences of the MS signals' intensities, it can be concluded that hydroxylation is stepwise, and within 1 h only colourless final products (muconic acid and its mono- and dihydroxylated derivatives) are present in the reaction mixture. The signals of rhodizonic acid ($m/z = 169$) and purpurogallin were not detected and there were no signs of dimerization reactions. MS spectra and a closer look at the time evolution of their most important features are documented in Figs 3 and 4.

Proposed mechanism of CL accompanying quinone hydroxylation

The oxidative degradation of catechol in the presence of Co(II) begins by one-electron oxidation, yielding green catechol semiquinone radical. Immediately after the addition of H_2O_2, more catechol semiquinone radical is formed, which further reacts to form a mixture of red hydroxylated *p*-quinone derivatives. The semiquinone

Table 1. Main intermediate and final products of CAT decomposition[a]

m/z	Quinones	Signal stability	*m/z*	Muconic acids	Signal stability	Remark
(108)	*o*-Quinone ($C_6H_4O_2$)	Disappears after the initiation of the reaction	141	Muconic acid ($C_6H_6O_4$)	Appears after 5 min, stable for ca. 10 min	$\Delta m = 34$
123	Hydroxy-quinone ($C_6H_4O_3$)	Disappears within 5 min	157	Hydroxy-muconic acid ($C_6H_6O_5$)	Weak signal disappears after ca. 10 min	$\Delta m = 34$
139	Dihydroxy-quinone ($C_6H_4O_4$)	One-third of the original intensity after 10 min	173	Dihydroxy-muconic acid ($C_6H_6O_6$)	Appears after 5 min, goes through maximum at 10 min	$\Delta m = 34$
171	Tetrahydroxy-quinone ($C_6H_4O_6$)	Detectable after 5 min, maximum intensity at 10 min, decays completely after 20 min				

[a]Conditions: CAT, 1×10^{-4} mol/L; H_2O_2, 0.09 mol/L; CO(II), 2×10^{-5} mol/L; TRIS–HCl buffer, pH 8.

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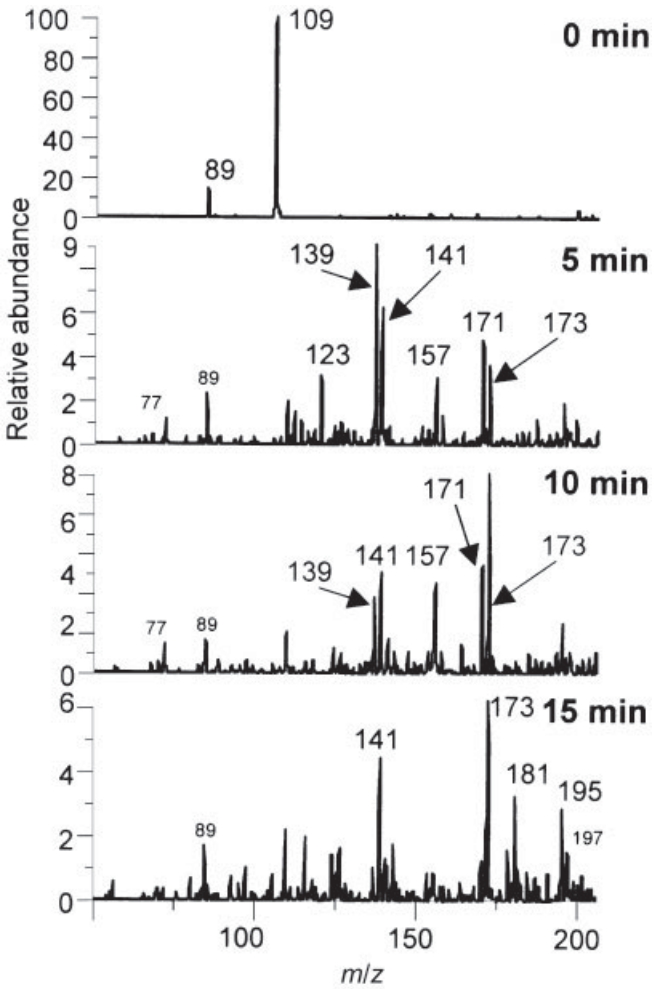

Figure 3. MS spectra recorded during the course of catechol oxidation. The reaction was carried out in TRIS–HCl buffer, pH 8. Concentrations of reactants: CAT, 1×10^{-4} mol/L; H_2O_2, 0.09 mol/L; Co^{2+}, 2×10^{-5} mol/L; CTAB, 4 mmol/L. Aliquots of the reaction mixture were injected into the injection port of the MS spectrometer at the time indicated in the figure.

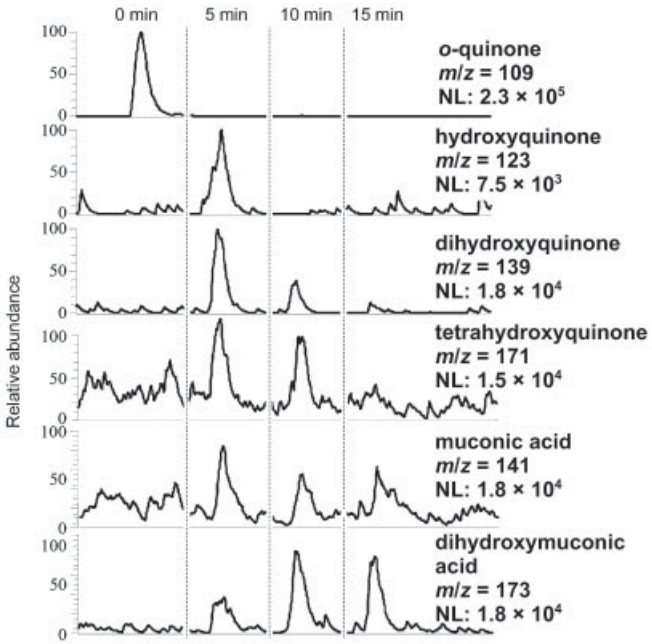

Figure 4. Time evolution of selected MS peaks. Conditions as described in Fig. 3.

radical is transformed into 1,2-benzoquinone by dismutation reaction. This reaction stage is accelerated by micellar catalysis, which significantly affects the interactions of short-lived species (e.g. radicals and excited states). Hydrogen peroxide attacks 1,2-benzoquinone at the *p*-position with respect to the C=O group. The hydroperoxide intermediate formed in this way decomposes into water and 2-hydroxy-1,4-benzoquinone. Analogous reactions proceed consecutively through the stage of 2,5-dihydroxy-1,4-benzoquinone to the final tetrahydroxy-1,4-benzoquinone. Only traces of trihydroxy-1,4-benzoquinone can be detected in the reaction mixture, presumably due to the rapid follow-up reaction yielding tetrahydroxy-1,4-benzoquinone. The proposed reaction scheme is shown in Fig. 5.

The CL time profile has a form of formation and decay, with cooperative onset of CL emission and maximum CL intensity in 2–3 min. This indicates that the CL emission is a result of reactions that follow primary oxidation (equation 1).

$$\mathrm{CAT} \xrightarrow{H_2O_2/Co^{2+}} Q_0 \xrightarrow{H_2O_2} Q_1^* \xrightarrow{H_2O_2} Q_2^* \rightarrow\rightarrow (Q_3) \rightarrow\rightarrow Q_4^* \quad (1)$$

where Q_0 is 1,2-benzoquinone and Q_i^* are actual concentrations of excited forms of individual hydroxy-1,4-benzoquinones. The CL emission starts when hydroxy-1,4-benzoquinone is formed in the reaction mixture, but it still proceeds even when hydroxy-1,4-benzoquinone is consumed by follow-up reactions. Therefore, the CL is composed of contributions from individual hydroxy-1,4-benzoquinones (equation 2):

$$I(\mathrm{CL}) = \sum \Phi_{\mathrm{CL}i} k_i [\mathrm{d}Q^*(t)]_i / \mathrm{d}t \quad (2)$$

The stepwise character of CL emission associated with catechol decomposition is further supported by observation of the resolved CL peaks at reduced (1.5×10^{-5} mol/L) CAT concentration (curve B in Fig. 2).

The magnitudes of CTAB micellar effects on reaction kinetics and CL emission differ significantly. In the micellar phase, the rates of both the development of the red colour and its decay are increased ca. three-fold compared to aqueous solutions free of micelles. Such enhancement factors are typical for the micellar catalysis of thermally activated reactions. On the other hand, the enhancement effect of micelles on the CL intensity has more than three orders of magnitude. It must be pointed out that it is difficult to detect the catechol CL

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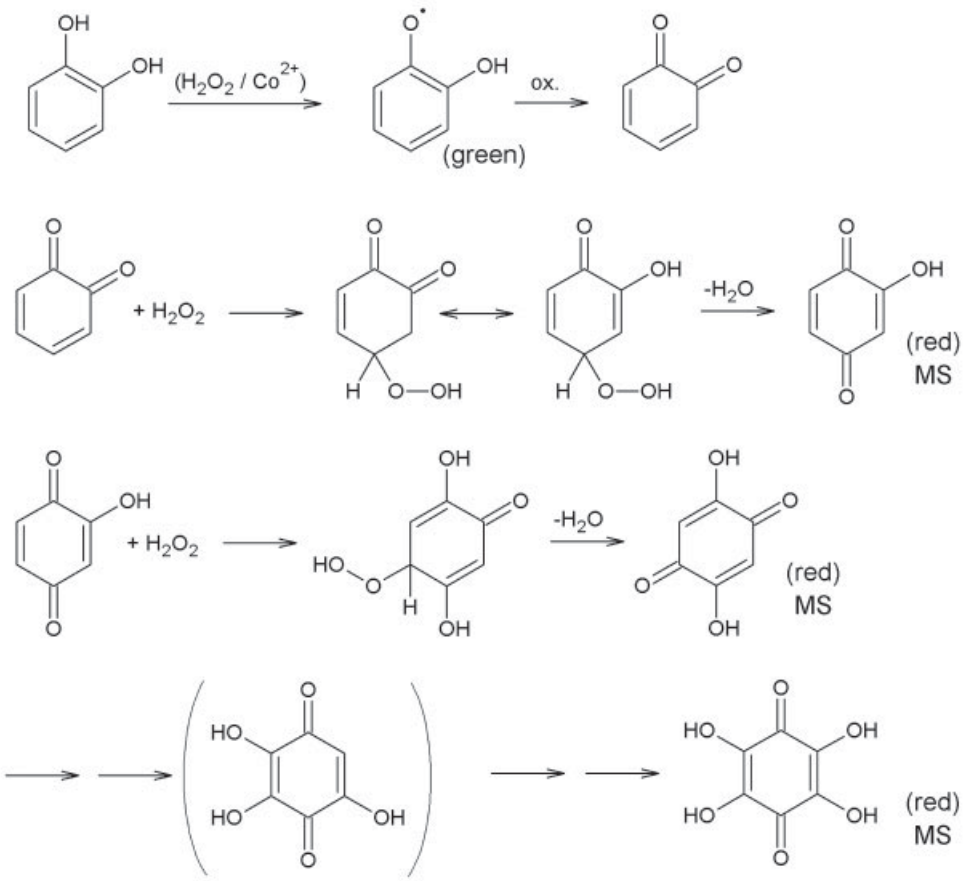

Figure 5. Proposed reaction scheme of CAT oxidative decomposition based on MS data.

on a common luminometer. The catechol CL emission is a bimolecular reaction with low activation energy. In such a case, the reaction rate, and consequently the CL emission, are governed by the interaction (collision rate) of short-lived intermediates – typically radical and/or excited states. From a simple calculation, it follows that within 10^{-8} s a molecule diffuses across the area equivalent to the surface of a micelle (a micelle of 3 nm diameter has a surface area of 28.3 nm^2; a molecule with $D = 10^{-9}$ m^2/s bound to the micellar surface will traverse this area in 1.4×10^{-8} s).

An ultraweak CL ($\Phi_{CL} \approx 10^{-8}$, $\lambda = 485$–530 nm) was observed by Brunmark and Cadenas (16, 17) for *p*-benzoquinone oxidized by H_2O_2 in slightly alkaline aqueous solution, which was described as the reaction between hydroxy-1,4-benzosemiquinone and OH• radical. The OH• radical in our system may originate from a Fenton-like reaction between H_2O_2 and Co(II) present in the reaction mixture, or from an 'organic Fenton reaction' (18, 19), which is the reaction between H_2O_2 and 1,2-benzosemiquinone or other semiquinone intermediates. The short lifetime of the OH• radical is in accord with the high enhancing effect of CTAB micelles. The above conclusions may be supported by the emission spectrum of the CL that accompanies oxidation of pyrocatechol violet (a catechol-like species, which exhibits stronger CL emission than catechol itself). The CL emission spectrum of this compound is characterized by a broad band at 500–640 nm, centring at 535 nm (not shown). Such an emission, occurring from $^3C{=}O^*$ and singlet oxygen, is typical for hydroperoxide decompositions, including the reaction described in (16). Decolourization of the reaction mixtures is caused by quinone reactions with hydrogen peroxide. Muconic acid is a frequently registered product of 1,2-benzoquinone oxidation by hydrogen peroxide. Hydroxylated muconic acids are formed by 'back' tautomerization of hydroxy-1,4-benzoquinones into an intermediate with 1,2-benzoquinoid structure followed by another addition of H_2O_2; 20 min after the initiation of the catechol oxidation, crotonic acid ($CH_3CHCHCOOH$; $m/z = 85$), which is the decomposition product of tetrahydroxy-1,4-quinone (THQ), is formed in the reaction mixture.

CONCLUSION

The oxidation of catechol by the Fenton-like system Co(II)–H_2O_2 is accompanied by the formation and decay of a red-purple colouration caused by intermediary

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formation of hydroxylated 1,4-benzoquinones. In the UV-vis spectrum, a band at 400–600 nm, with a maximum at 490 nm, is observed. Using mass spectroscopy, the formation of hydroxy-*p*-quinone (*m*/*z* 123), 2,5-dihydroxy-*p*-quinone (*m*/*z* 139) and tetrahydroxy-*p*-quinone (*m*/*z* 171) intermediates were confirmed. The MS signals of quinones disappear within several tens of minutes, simultaneously with decolouration of the reaction mixture. After the completion of this process stable signals of muconic acid ($m/z = 141$) and its monohydroxylated ($m/z = 157$) and dihydroxylated ($m/z = 173$) derivatives are found. In micellar solutions of CTAB, the above-mentioned reactions are accelerated ca. threefold compared with aqueous solutions free of micelles. Micelle-enhanced catechol oxidation is accompanied by CL ($\Phi_{CL} = 1 \times 10^{-4}$). The mechanism of catechol oxidation, based on hydrogen peroxide addition to quinones, yielding hydroperoxide intermediates that decompose to hydroxylated quinones, is outlined. The source of CL is probably the excited triplets and singlet oxygen of hydroxylated quinones. In a micellar system, the CL can be sensitized up to eight-fold by fluorescein.

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