

# The influence of halide and pseudo-halide antioxidants in Fenton-like reaction systems containing copper(II) ions

Jasna Malešič<sup>a,\*</sup>, Matija Strlič<sup>b</sup>, Jana Kolar<sup>a</sup>, Slovenko Polanc<sup>b</sup>

<sup>a</sup> National and University Library, Leskoškova 12, SI-1000 Ljubljana, Slovenia

<sup>b</sup> University of Ljubljana, Faculty of Chemistry and Chemical Technology, Aškerčeva 5, SI-1000 Ljubljana, Slovenia

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## Abstract

Application of the *N,N'*-(5-nitro-1,3-phenylene)bisglutaramide (NPG) hydroxylation assay for spectrophotometric determination of the rate of generation of oxidizing species in systems containing copper(II) ions/phosphate buffer/H<sub>2</sub>O<sub>2</sub> in the presence of halide and pseudo-halide antioxidants was evaluated. A satisfactory correlation was demonstrated between the kinetics of NPG hydroxylation, determined both spectrophotometrically and chromatographically.

Employing the assay, efficiency of various halide and pseudo-halide antioxidants was studied in solutions of pH 7 or 9. Among the halides tested, iodide proved to have the highest antioxidant efficiency. The efficiency of thiocyanate is pH-dependent, exhibiting even pro-oxidant properties at pH 7 at lower concentrations. Various bromides exhibited comparative antioxidant behavior at pH 7 and 9, although the reaction rate constants were much lower at pH 7. A pronounced effect of the cation is evident, with the rates of oxidizing species production affected by the length of alkyl chain on the quaternary ammonium bromide. The use of tetrabutylammonium chloride proved that in the case of tetrabutylammonium cation the influence of anion can be neglected.

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## 1. Introduction

Fenton chemistry [1–6] is of immense importance in biological systems [7–10], ecology [11,12], food chemistry [13,14] and also material ageing [15–17]. The production of oxidizing species, often assumed to be hydroxyl radicals, in Fenton-like systems may lead to extensive unwanted oxidative damage in a variety of systems and antioxidants are often employed to minimize it. Their effectiveness may be studied using aromatic hydroxylation assays. Hydroxyl radicals, produced in Fenton-like reaction systems composed of transition metal ions and hydrogen peroxide, react with aromatic radical scavengers and the yield of hydroxylated products is used to assess the kinetics of oxidizing species production. Therefore, a substituted nitrobenzene substrate

*N,N'*-(5-nitro-1,3-phenylene)bisglutaramide (NPG) was used [18], the two predominant products of its hydroxylation being *o*- and *p*-hydroxylated derivatives (i.e. 4- or 6-hydroxy and 2-hydroxy, respectively, designated as *o*- and *p*-HNPG). The assay was used to evaluate antioxidant efficiency of various halides and a pseudo-halide in an aqueous reaction system at 70 °C containing Fe(III) ions and hydrogen peroxide in phosphate buffer pH 9 [19]. The NPG hydroxylation assay for determination of the rate of oxidizing species generation can also be used in pseudo-Fenton reaction mixtures containing other transition metals, such as copper ions [20]. These are more catalytically active than iron ions [21,22], therefore, a higher rate of NPG hydroxylation is obtained at 25 °C and pH between 5.5 and 9, if Cu(II) ions are used compared to systems containing Fe(III) ions [20,23].

Production of oxidizing species in Fenton-like reaction systems containing copper(II) ions is pH-dependent, it was

\* Corresponding author. Tel.: +386 15861300; fax: +386 15861311.  
E-mail address: [jasna.malesic@nuk.uni-lj.si](mailto:jasna.malesic@nuk.uni-lj.si) (J. Malešič).

shown recently that the rates are increasing with the pH of the reaction mixture [20].

The choice of buffer system is crucial, as it may induce changes in kinetics [24] and according to the literature, phosphate buffer is the best choice unless it interferes with metal binding to proteins [25].

In the present paper, the aromatic hydroxylation assay is used to evaluate antioxidant efficiency of various halides and a pseudo-halide in an aqueous reaction system containing Cu(II) ions and hydrogen peroxide in phosphate buffers of pH 7 or 9.

## 2. Experimental

The reaction mixture was composed of 0.1 mmol L<sup>-1</sup> CuCl<sub>2</sub> (Merck, Darmstadt), 1 mmol L<sup>-1</sup> *N,N'*-(5-nitro-1,3-phenylene)bisglutaramide, 20 mmol L<sup>-1</sup> phosphate buffer (composed of Na<sub>3</sub>PO<sub>4</sub> and Na<sub>2</sub>HPO<sub>4</sub>, microselect quality, Fluka, Buchs), 20 mmol L<sup>-1</sup> H<sub>2</sub>O<sub>2</sub> (Fluka, Buchs, non-stabilized). Various halides and a pseudo-halide were added in four different concentrations (0.1, 0.5, 2.5 or 12.5 mmol L<sup>-1</sup>). Due to high reaction rates and in order for these to be comparable with previous studies [19], the temperature of the reaction mixture was set to 40 °C. The rates of NPG hydroxylation in Fenton-like systems catalyzed with copper(II) ions are several orders of magnitude higher than those catalyzed with iron(III) ions [20].

The substrate, *N,N'*-(5-nitro-1,3-phenylene)bisglutaramide was synthesized according to the literature [18]. The solution of NPG with pH 7 and 9 was prepared in phosphate buffer.

The halides and pseudo-halides studied in this work were potassium iodide (p.a. quality, Carlo Erba, Milano), potassium bromide (p.a. quality, Riedel de Haen, Seelze), potassium thiocyanate (p.a. quality, Merck, Darmstadt), tetrabutylammonium bromide (purum, Fluka, Buchs), tetrabutylammonium chloride (purum, Fluka, Buchs). Benzyltributylammonium bromide, dodecyltrimethylammonium bromide, tetramethylammonium bromide, tetrapropylammonium bromide, tetraethylammonium bromide and methyltriphenylphosphonium bromide were all from Dishman, Ahmedabad, India. The pH of quaternary ammonium and phosphonium bromide solutions was adjusted with NaOH to the desired pH of reaction mixtures.

The actual pH of the reaction mixtures (8.75 ± 0.05 if using phosphate buffer pH 9 and 7.17 ± 0.06 if using phosphate buffer pH 7) was determined with a combined glass electrode and a pH-meter. All solutions were prepared with additionally purified deionized water (MilliQ water purification system, Millipore, Molsheim).

The photometric experiments were conducted in a 3-mL Peltier-thermostated cuvette equipped with a magnetic stirrer and a Pt 1000 resistance thermometer in a Cary 50 Probe spectrophotometer.

On hydroxylation, the substrate yields colored derivatives, the mixture exhibiting an absorption maximum at 431 nm. The description of separation, isolation and UV–vis absorption properties of the hydroxylated derivatives are described in the literature [23] and the extinction coefficients were used for calculation of rate constants from slopes of absorbance versus reaction time curves. The concentration ratios of *o*- and *p*-hydroxylated derivatives was determined by HPLC for each reaction system.

For liquid chromatographic analyses, reaction mixtures (5 mL) were prepared as described above and maintained at 40 °C. The reaction was terminated by an addition of 0.5 mL of catalase solution to 0.5 mL of reaction mixture. The catalase solution was prepared by dissolving 4 μL catalase suspension (1,300,000 u mg<sup>-1</sup>; Merck, Darmstadt) in 100 mL phosphate buffer of pH 7.

High-pressure liquid chromatography was performed using a Hewlett-Packard 1100 Series chromatographic system equipped with a diode-array detector. A Hypersil ODS column was used (250 mm × 4 mm, 5 μm particle size) and 100 μL of sample was injected. Gradient elution of 1.5 mL min<sup>-1</sup> was used and consisted of 3–8% acetonitrile and 97–92% of phosphate buffer (corresponding mixture of KH<sub>2</sub>PO<sub>4</sub>/Na<sub>2</sub>HPO<sub>4</sub>, pH 7, 20 mmol L<sup>-1</sup>; Kemika, Zagreb) in 0–5 min, after which the non-reacted NPG was flushed out of the column with a 30% acetonitrile/70% phosphate buffer eluent.

For ion-chromatographic analyses, the Fenton-like reaction mixture was prepared as described above. Potassium iodide or potassium bromide was added to the reaction mixture in various concentrations (0.02, 0.05, 0.1 and 0.2 mmol L<sup>-1</sup>). In some experiments, the mixtures were prepared without NPG. The reaction mixtures were maintained at 40 °C. The reaction was terminated by an addition of 5 μL catalase solution to 495 μL of the mixture. The catalase solution was prepared by dissolving 10 μL of catalase suspension (1,300,000 u mg<sup>-1</sup>; Merck, Darmstadt) in 5 mL of phosphate buffer pH 7. Ion-chromatography was performed using a Merck Hitachi chromatographic system (D-6000 Interface, AS-2000A Autosampler, L-6200A pump) equipped with a UV–vis detector (L-4250). An Ion Pac AS4A-SC analytical column with an IonPac AG4A-SC guard column were used (Dionex) and 100 μL of sample was injected. Isocratic elution at 2 mL min<sup>-1</sup> of carbonate buffer (1.8 mmol L<sup>-1</sup> Na<sub>2</sub>CO<sub>3</sub> and 1.7 mmol L<sup>-1</sup> Na(HCO<sub>3</sub>)<sub>2</sub> in deionized water was used. Iodide and bromide were detected at 200 nm.

## 3. Results and discussion

It may be assumed that NPG reacts with hydroxyl radicals at a virtually diffusion-controlled rate, so that in a pseudo-Fenton reaction system with an excess of NPG, hydroxyl radical production rate can be approximated by the rate of NPG hydroxylation. When metal-chelating preventive

antioxidants are introduced to the reaction system, the so obtained rates may provide comparative data on the kinetics of production of oxidizing species. However, if hydroxyl radicals formed are scavenged by antioxidants, the resulting rate of NPG hydroxylation cannot be equaled with the rate of hydroxyl radical production due to the competitive role of NPG and the antioxidant.

The yield of hydroxylated product may be determined chromatographically or spectrophotometrically, taking into account the product concentration ratio ( $p$ -HNPG/ $o$ -HNPG) and the ratio of molar absorptivities of both derivatives [23].

The rate of formation of hydroxylated derivatives is initially constant. After most of the hydrogen peroxide is consumed in the reaction mixture, the otherwise linear plot of absorbance versus time begins to level off. Curvature of the plot was observed after ca. 10 min reaction time at 40 °C and pH 9 and after ca. 100 min at pH 7. The initially linear dependence of absorbance versus time was used to calculate the rate constants of hydroxylation and, conversely, oxidizing species production, although the reactions at pH 9 were followed during a longer period of time, which was 60 min. However, in order to enable rapid and non-demanding spectrophotometric determination of relative antioxidant efficiency, changes in the following parameters, which may influence the quality of the obtained results, have to be evaluated:

- concentration of the halide during an experiment;
- $p$ -HNPG/ $o$ -HNPG concentration ratio.

#### 4. Evaluation of the NPG hydroxylation assay for pseudo-Fenton reaction mixtures containing various halides

##### 4.1. Antioxidant concentration

During the course of reaction, the concentration of potential halide antioxidants may change, adversely affecting interpretation of results. In order to exclude erroneous interpretation arising from possible removal of halide from the reaction mixture, experiments were performed with and without NPG and the concentrations of bromide and iodide in phosphate buffer pH 9 were followed using ion-chromatography.

The concentration of iodide and bromide ions could decrease due to reactions with various components of the reaction mixture. Copper(II) ions may precipitate with halide ions forming sparingly soluble copper(I) iodide while iodine is liberated [26]. However, during the experiments no visual precipitation was observed in reaction mixtures. In addition, reactions could take place between iodide and hydrogen peroxide leading to formation of various products, such as iodine, iodate(I), triiodide, etc. [27]. Nevertheless, our results indicate that the concentration of iodide in reaction systems without an addition of NPG was not affected in the time interval of our study. Although a gradual decrease in iodide concentration is observed during 60 min of reaction (from

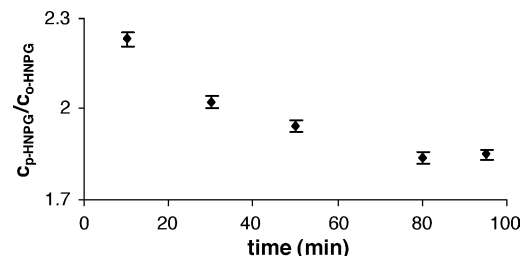


Fig. 1. The ratio of  $p$ -HNPG/ $o$ -HNPG products at 40 °C in a reaction system containing copper(II) ions, NPG and phosphate buffer at pH 7. The error bars present standard deviation of chromatographic measurements ( $n=3$ ).

0.199 to 0.187 mmol L<sup>-1</sup>, typical R.S.D. of iodide concentration determination was 3%,  $n=5$ ), the concentration of iodide did not change significantly in the initial period, during which the hydroxylation rate was determined (10 min). Contrary to iodide, concentration of bromide did not change during 60 min at all.

##### 4.2. Ratio of derivatives

As demonstrated in the literature, extinction coefficients of  $o$ -HNPG and  $p$ -HNPG products are different ( $o$ -HNPG:  $\epsilon_{222} = 8710 \text{ L cm}^{-1} \text{ mol}^{-1}$ ,  $\epsilon_{431} = 5952 \text{ L cm}^{-1} \text{ mol}^{-1}$ ;  $p$ -HNPG:  $\epsilon_{222} = 9950 \text{ L cm}^{-1} \text{ mol}^{-1}$ ,  $\epsilon_{431} = 2296 \text{ L cm}^{-1} \text{ mol}^{-1}$ ) [23]. In order to be able to determine the reaction rates from spectrophotometric data, it is preferable if the molar ratio of  $p$ -HNPG to  $o$ -HNPG derivatives remains constant during reaction. Also, the ratio should not change significantly if antioxidants are introduced.

To evaluate the possible error arising from differences in ratios of hydroxylated products, liquid chromatography was used. While the  $p$ -HNPG/ $o$ -HNPG ratio remains unchanged during 60 min of reaction time at pH 9, a minor decrease was observed at pH 7 (Fig. 1). If these changes were significant and the ratio of molar absorption coefficients were not taken into account, the resulting rates would be burdened with a systematic error.

During the time interval of the reaction at pH 7, where the rate constant is determined, the decrease of  $p$ -HNPG/ $o$ -HNPG product ratio from 2.23 to 1.85 accounts for a systematic error of +1.00% to the rate constant determined spectrophotometrically (typically, 1.86% R.S.D.,  $n=4$ ). This systematic error can still be considered acceptable. To account for it, during the initial 100 min of an experiment at pH 7, five determinations of  $p$ -HNPG/ $o$ -HNPG product ratio were performed and average values were used for calculations of the rate constants from spectrophotometric data.

As evident from the data in Table 1, variations in concentrations of KBr in the reaction mixture do not affect the average ratio of NPG derivatives. On the other hand, it is affected by the type of antioxidant used.

The average values of product ratio in phosphate buffer at pH 9 are between 1.55 and 1.71. However, this is reflected in a systematic error in determination of rate constant of

Table 1

The chromatographically determined average *p*-HNPG/*o*-HNPG ratios in reaction systems containing different halides in concentration 0.5 mmol L<sup>-1</sup>, compared to the system without halides at pH 7 ( $n=5$ , determined during initial 100 min of reaction) and pH 9 ( $n=3$ , determined during initial 60 min of reaction)

Antioxidant	pH 7		pH 9	
	$c_{p\text{-HNPG}}/c_{o\text{-HNPG}}$	S.D.	$c_{p\text{-HNPG}}/c_{o\text{-HNPG}}$	S.D.
–	2.0	0.2	1.70	0.02
KI	1.7	0.5	1.58	0.04
KSCN	1.8	0.1	1.55	0.02
KBr 0.1	2.0	0.1	1.71	0.02
KBr 0.5	2.0	0.2	1.69	0.02
KBr 2.5	2.0	0.2	1.67	0.02
KBr 12.5	1.9	0.4	1.71	0.07
Bu <sub>4</sub> NCl	2.0	0.3	1.65	0.01
Bu <sub>4</sub> NBr	2.0	0.3	1.63	0.02
Me <sub>4</sub> NBr	2.0	0.1	1.64	0.04
Et <sub>4</sub> NBr	2.0	0.2	1.70	0.01
Pr <sub>4</sub> NBr	2.0	0.2	1.66	0.00
C <sub>12</sub> H <sub>25</sub> Me <sub>3</sub> NBr	2.0	0.5	1.60	0.07
BnBu <sub>3</sub> NBr	1.9	0.3	1.66	0.03
MePh <sub>3</sub> PBr	2.0	0.2	1.72	0.01

Only KBr was added to reaction mixtures in four different concentrations as indicated (in mmol L<sup>-1</sup>).

only +0.3%. This is considerably smaller if compared to the experimentally determined R.S.D. for spectrophotometric determination of the rate constant for NPG hydroxylation, which is typically 5.2% ( $n=7$ ).

#### 4.3. Spectrophotometrically and chromatographically determined rate constants

The presumption that the error arising from variations in ratios of the two derivatives may be neglected was confirmed by a good agreement of the changes in absorbance of reaction mixtures obtained at 432 nm and the sum of concentrations for *o*-HNPG and *p*-HNPG derivatives as determined by HPLC (Table 2).

Basing on the data in Table 2, it is evident that the *N,N'*-(5-nitro-1,3-phenylene)bisglutaramide hydroxylation assay may be used for rapid comparative assessment of rates of oxidizing species generation in Fenton-like systems containing various halide and pseudo-halide antioxidants.

Table 2

Calibration data (absorbance (AU) vs. concentration (mmol L<sup>-1</sup>)) during initial 60 min of reaction time at pH 9 and 100 min of reaction at pH 7 in reaction systems containing Cu(II) or additionally 0.5 mmol L<sup>-1</sup> of KBr, KSCN or KI

Antioxidant	pH 7			pH 9		
	Calibration curve equation	$R^2$	$n$	Calibration curve equation	$R^2$	$n$
–	$y=0.1201x+0.0006$	0.9987	6	$y=0.107x-0.002$	0.9994	5
KI	$y=0.081x+0.002$	0.9976	6	$y=0.048x-0.0002$	0.9967	5
KBr	$y=0.114x+0.004$	0.9967	6	$y=0.107x+0.003$	0.9946	5
KSCN	$y=0.126x-0.001$	0.9898	6	$y=0.108x-0.002$	0.9973	5

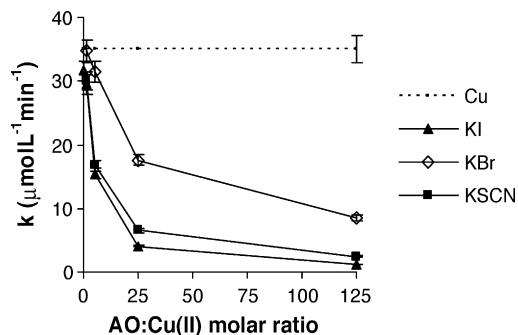


Fig. 2. The effect of concentration of antioxidants in reaction mixtures in phosphate buffer pH 9 on the rate of NPG hydroxylation. The denotation “Cu” represents reaction system without addition of antioxidants, KI, KBr and KSCN addition of potassium iodide, bromide and thiocyanate as indicated. Error bars represent standard deviation of rate constants determination.

## 5. Comparative study of the antioxidative effect of various halides and a pseudo-halide

The effect of additions of KI, KBr and KSCN to reaction mixtures at pH 9 is presented in Fig. 2. It is evident that their efficiency at this pH decreases in the order  $I^- \geq SCN^- > Br^-$ . Also, the effect of antioxidant concentration is demonstrated, as higher concentrations result in lower rate constants of NPG hydroxylation.

The results obtained are consistent with those obtained by Elliot and Simsons [28], who determined the rate constants for reactions of hydroxyl radicals ( $k_{OH}$ ) with thiocyanate and iodide ions also as a function of temperature. The  $k_{OH}$  for both thiocyanate and iodide ions are nearly diffusion-controlled; at 39 °C the values for  $k_{OH}$  are higher for potassium iodide ( $k_{OH} = 18.9 \times 10^9 \text{ L mol}^{-1} \text{ s}^{-1}$ ) in comparison to potassium thiocyanate ( $k_{OH} = 14.7 \times 10^9 \text{ L mol}^{-1} \text{ s}^{-1}$ ).

The relative rate constants for reaction of hydroxyl radicals with iodide are more than 10 times higher than with bromide [28–31]. For example, the relative rate constant for reaction of  $HO^\bullet$  at pH 9 and room temperature with iodide is  $1.14 \times 10^{12} \text{ L mol}^{-1} \text{ s}^{-1}$  and with bromide  $8.9 \times 10^{10} \text{ L mol}^{-1} \text{ s}^{-1}$  [29]. Chloride anion, which is also the subject of our study, has a relative rate constant for reaction with hydroxyl radicals lower than  $0.01 \times 10^{10} \text{ L mol}^{-1} \text{ s}^{-1}$ .

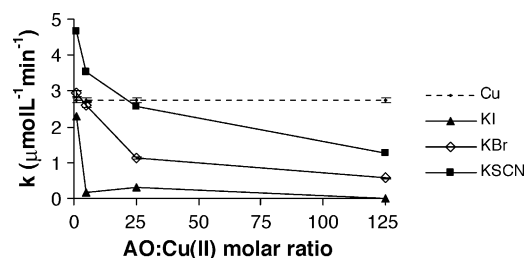


Fig. 3. The effects of concentration of halides and KSCN in reaction mixture at pH 7 on the rate of NPG hydroxylation. The denotation “Cu” represents reaction system without addition of antioxidant, KI, KBr and KSCN addition of potassium iodide, bromide and thiocyanate as indicated. Error bars present standard deviation of rate constants determination.

On the other hand, it was found that NaBr has a strong inhibitory effect during the autoxidation of 6-hydroxydopamine with oxygen in the presence of copper ions [32]. The authors explained the effect on the basis of reduction potential. The binding of bromide to copper cations increases the reduction potential of Cu(II) from  $E_0 = 0.153$  to  $0.640$  V, rendering the Cu(I) complex less prone to reaction with oxygen [32].

Further surprising results were obtained on comparison of the effect of the different anions at pH 7. As evident from Fig. 3, the addition of KSCN even promotes hydroxylation of NPG if the concentration ratio to copper ions is lower than 25:1. On the other hand, KBr has better antioxidative properties, while KI slows down the production of hydroxylated NPG in the most efficient manner. The rate of reaction of hydroxyl radicals with iodide in neutral solution as determined by Thomas [30] is  $1.02 \times 10^{10} \text{ L mol}^{-1} \text{ s}^{-1}$ .

The described data indicate that cations exhibit a pronounced effect on the rate of hydroxyl radical production (Fig. 4) as already demonstrated in the case of iron-catalyzed reactions [19]. The results indicate that the addition of tetrabutylammonium bromide to reaction mixtures decreases the rate of hydroxyl radical production more effectively than KBr at pH 9 (Fig. 4). Tetrabutylammonium chloride has a similar

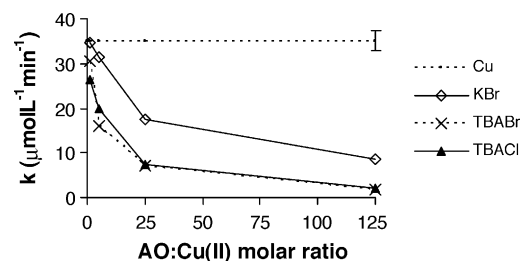


Fig. 4. The effect of halide concentration (potassium bromide, KBr; tetrabutylammonium bromide, TBABr; tetrabutylammonium chloride, TBACl) on the rate of NPG hydroxylation in reaction mixtures at pH 9. The denotation “Cu” represents reaction system without addition of an antioxidant. Error bars present standard deviation of rate constants determination.

effect to tetrabutylammonium bromide, indicating a prevailing influence of the cation on the rate of hydroxylation.

The results for reaction mixtures at pH 7 or 9 are comparable. At the molar ratio of bromide to copper ions 1:1, the differences in rates of NPG hydroxylation are not very informative (Table 3). However, all bromides at pH 9 decrease the rate of hydroxyl radical production in comparison to the control experiment (Fig. 5). At pH 7 and bromide to copper(II) concentration ratio of 5:1, tetramethylammonium bromide and tetraethylammonium bromide do not affect hydroxyl radical production (Fig. 6). Other tested bromides slow down the production of hydroxylated derivatives. The most effective in both cases are the ones containing longer aliphatic chains on the quaternary ammonium bromide. The results are consistent with studies in pseudo-Fenton reaction systems containing iron ions [19].

The differences in rates of hydroxylated derivatives production due to effect of cations connected to the quaternary ammonium salts is difficult to explain because no studies of the reaction mechanism were performed.

A similar effect was observed when corrosion inhibition in acidic solutions of copper and iron with various surfactants was studied. On the positively charged copper or iron surface, quaternary ammonium ions can be electrostatically

Table 3

Rates of oxidizing species production,  $k$  ( $\mu\text{mol L}^{-1} \text{ min}^{-1}$ ), in reaction systems containing phosphate buffer pH 9 or 7, copper ions, various halides or a pseudo-halide in different concentrations ( $c_{\text{hal}}$ ), as indicated

Antioxidant	$c_{\text{hal}}$ (mmol L <sup>-1</sup> ) at pH 9					$c_{\text{hal}}$ (mmol L <sup>-1</sup> ) at pH 7			
	0.02	0.1	0.5	2.5	12.5	0.1	0.5	2.5	12.5
KI	32	30	15	4.1	1.1	2.30	0.17	0.32	0.00
KBr	–	35	31	18	8.6	2.94	2.62	1.14	0.57
KSCN	–	30	17	6.6	2.4	4.67	3.51	2.56	1.25
Bu <sub>4</sub> NBr	–	30	16	7.1	1.6	2.19	1.15	0.35	0.10
Bu <sub>4</sub> NCl	–	26	20	7.4	2.0	2.35	1.39	0.37	0.17
Me <sub>4</sub> NBr	–	29	25	19	7.6	2.93	2.85	1.54	0.71
Et <sub>4</sub> NBr	–	32	28	21	7.9	2.74	3.05	1.35	0.66
Pr <sub>4</sub> NBr	–	31	26	12	3.8	2.89	2.34	0.79	0.14
(C <sub>12</sub> H <sub>25</sub> )Me <sub>3</sub> NBr	–	26	14	4.5	1.6	1.82	0.73	0.40	0.14
BnBu <sub>3</sub> NBr	–	28	19	6.3	1.7	2.84	0.40	0.40	0.12
MePh <sub>3</sub> PBr	–	21	20	8.8	2.4	2.73	1.39	0.84	0.29

Rate constants for systems without addition of antioxidants are  $35 \text{ mmol L}^{-1}$  at pH 9 and  $2.74$  at pH 7. Typical R.S.D. for determination of rate constants was 5.2% at pH 9 and 1.86% at pH 7.

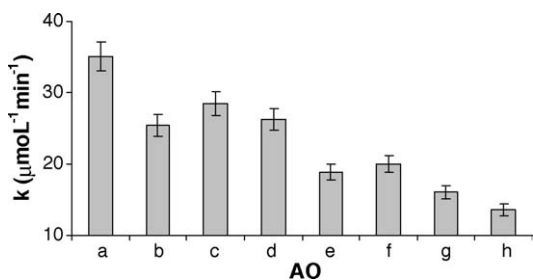


Fig. 5. Effect of various bromides ( $n_{\text{bromide}}:n_{\text{Cu(II)}}=5:1$ ) added to the pseudo-Fenton reaction mixture containing phosphate buffer pH 9 on NPG hydroxylation assay. *Samples*: (a) reaction mixture without an addition of bromide; (b) tetramethylammonium bromide; (c) tetraethylammonium bromide; (d) tetrapropylammonium bromide; (e) benzyltributylammonium bromide; (f) methyltriphenylphosphonium bromide; (g) tetrabutylammonium bromide; (h) dodecyltrimethylammonium bromide. Error bars represent standard deviation for determination of rate constants.

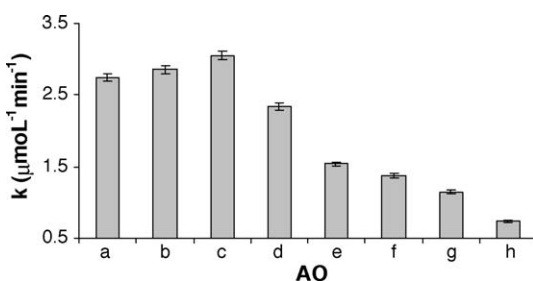


Fig. 6. Effect of various bromides ( $n_{\text{bromide}}:n_{\text{Cu(II)}}=5:1$ ) added to the pseudo-Fenton reaction mixture containing phosphate buffer pH 7 on NPG hydroxylation assay. *Samples*: (a) reaction mixture without addition of bromide; (b) tetramethylammonium bromide; (c) tetraethylammonium bromide; (d) tetrapropylammonium bromide; (e) benzyltributylammonium bromide; (f) methyltriphenylphosphonium bromide; (g) tetrabutylammonium bromide; (h) dodecyltrimethylammonium bromide. Error bars represent standard deviation for determination of rate constants.

adsorbed on a primarily adsorbed layer of bromide or iodide ions [33–35]. A higher efficiency of corrosion inhibition was observed when the length of alkyl chain increased and the concentration of halides was increased.

However, in the studied reaction systems, it is likely that hydroxyl radicals react with alkyl chains, thus, forming water and alkyl radicals, which may react further or form stable products via termination reactions.

## 6. Conclusions

The aromatic substrate *N,N'*-(5-nitro-1,3-phenylene) bisglutaramide was used to obtain comparative rate constants of  $\text{HO}^\bullet$  production in buffered aqueous solutions of  $\text{H}_2\text{O}_2$  and copper(II) ions in the presence of iodide, thiocyanate and bromide antioxidants. The results demonstrate that if the same cation is used, the antioxidant efficiency increases in the order  $\text{Br}^- < \text{SCN}^- < \text{I}^-$  in an alkaline environment. In neutral conditions,  $\text{SCN}^-$  promotes  $\text{HO}^\bullet$  production if the concentration ratio to copper(II) ions is lower than 25:1. A strong

effect of cation is demonstrated by some water-soluble bromides, with the most pronounced antioxidant effect exhibited by bromides with longer aliphatic chains on the quaternary ammonium ion.

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## References

- [1] C. Walling, *Acc. Chem. Res.* 31 (1998) 155.
- [2] S. Goldstein, D. Meyerstein, *Acc. Chem. Res.* 32 (1999) 547.
- [3] P.A. MacFaul, D.D.M. Wayner, K.U. Ingold, *Acc. Chem. Res.* 31 (1998) 159.
- [4] C. Walling, *Acc. Chem. Res.* 8 (1975) 125.
- [5] C.E. Thomas, S.D. Aust, in: J. Miquel, A.T. Quintanilha, H. Weber (Eds.), *Handbook of Free Radicals and Antioxidants in Biomedicine: Role of Metals in Oxygen Radical Reactions and Oxidative Stress*, CRC Press, Boca Raton, FL, USA, 1989, p. 37.
- [6] P. Wardman, L.P. Candeias, *Radiat. Res.* 145 (1996) 523.
- [7] B. Halliwell, J.M.C. Gutteridge, *Arch. Biochem. Biophys.* 246 (1986) 501.
- [8] A. Singh, in: J. Miquel, A.T. Quintanilha, H. Weber (Eds.), *Handbook of Free Radicals and Antioxidants in Biomedicine, Chemical and Biochemical Aspects of Activated Oxygen: Singlet Oxygen, Superoxide Anion, and Related Species*, CRC Press, Boca Raton, FL, USA, 1989, p. 17.
- [9] E. Hideg, C. Spetea, I. Vass, *Biochim. Biophys. Acta* 1186 (1994) 143.
- [10] G. Lubec, *J. Invest. Med.* 44 (1996) 324.
- [11] R.G. Zepp, B.C. Faust, J. Hoigne, *Environ. Sci. Technol.* 26 (1992) 313.
- [12] B.C. Faust, R.G. Zepp, *Environ. Sci. Technol.* 27 (1993) 2517.
- [13] R. Kahl, A.G. Hildebrandt, *Food Chem. Toxicol.* 24 (1986) 1007.
- [14] J.K. Donnelly, D.S. Robinson, *Free Radic. Res.* 22 (1994) 147.
- [15] Z. Osawa, *Polym. Degrad. Stabil.* 20 (1988) 203.
- [16] M. Strlič, J. Kolar, B. Pihlar, *Polym. Degrad. Stabil.* 72 (2001) 535.
- [17] J. Kolar, M. Strlič, G. Novak, B. Pihlar, *J. Pulp Pap. Sci.* 24 (1998) 89.
- [18] S. Singh, R. Hider, in: C. Rice-Evans, B. Halliwell (Eds.), *Free Radicals, Methodology and Concepts, Aromatic Hydroxylation: A Sensitive Method for Detection of Hydroxyl Radical Production by Various Iron Complexes*, Richelieu Press, London, UK, 1988.
- [19] J. Malešič, J. Kolar, M. Strlič, S. Polanc, *J. Mol. Catal. A Chem.* in press.
- [20] M. Strlič, J. Kolar, V.S. Šelih, D. Kočar, B. Pihlar, *Acta Chim. Slov.* 50 (2003) 619.
- [21] S. Goldstein, D. Meyerstein, G. Czapski, *Free Radic. Biol. Med.* 15 (1993) 435.
- [22] W.H. Koppenol, in: B.R.H. Rice-Evans (Ed.), *Free Radical Damage and Its Control, Chemistry of Iron and Copper in Radical Reactions*, Elsevier, Amsterdam, 1994.
- [23] M. Strlič, J. Kolar, B. Pihlar, *Acta Chim. Slov.* 46 (1999) 555.
- [24] H. Iwahashi, T. Ishii, R. Sugata, R. Kido, *Arch. Biochem. Biophys.* 276 (1990) 242.

- [25] B.R.V. Dyke, D.A. Clopton, P. Saltman, *Inorg. Chim. Acta* 242 (1996) 57.
- [26] D.A. Skoog, D.M. West, F.J. Holler, *Fundamentals of Analytical Chemistry*, Saunders College Publishing, USA, 1992.
- [27] K.J. Morgan, *Quat. Rev. (Lond.)* 8 (1954) 123.
- [28] A.J. Elliot, A.S. Simons, *Radiat. Phys. Chem.* 24 (1984) 229.
- [29] I. Kraljić, C.N. Trumbore, *J. Am. Chem. Soc.* 87 (1965) 2547.
- [30] J.K. Thomas, *Trans. Faraday Soc.* 61 (1965) 702.
- [31] R.W. Matthews, D.F. Sangster, *J. Phys. Chem.* 69 (1965) 1938.
- [32] B. Bandy, P.B. Walter, J. Moon, A.J. Davison, *Arch. Biochem. Biophys.* 389 (2001) 22.
- [33] D.P. Schweinsberg, V. Ashworth, *Corros. Sci.* 28 (1988) 539.
- [34] H. Ma, S. Chen, B. Yin, S. Zhao, X. Liu, *Corros. Sci.* 45 (2003) 867.
- [35] V. Branzoi, F. Branzoi, M. Baibarac, *Mater. Chem. Phys.* 65 (2000) 288.