A COMPARATIVE STUDY OF SEVERAL TRANSITION METALS IN FENTON-LIKE REACTION SYSTEMS AT CIRCUM-NEUTRAL pH

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Abstract

The *N,N'*-(5-nitro-1,3-phenylene)bisglutaramide hydroxylation assay for spectrophotometric determination of the rate of oxidising species generation in Fenton-like systems was used to obtain comparative data for Cd(II), Co(II), Cr(III), Cu(II), Fe(III), Mn(II), Ni(II), and Zn(II). The pH range of interest was 5.5-9.5 and was controlled by addition of an appropriate phosphate buffer. The temperature of the reaction mixture was controlled in the range 25-80 °C. The rates of production of oxidising species at pH 7 decrease in the following order: Cu(II) > Cr(III) > Co(II) > Fe(III) > Mn(II) > Ni(II), while Cd(II) and Zn(II) did not exhibit any catalytic activity and Ni(II) only led to a significant production of oxidising species at pH > 7.5. In mixtures of Cu(II) and Fe(III) the rate of oxidising species production may be considered as the sum of contributions of individual metals. This was not the case of a mixture containing additional small amounts of Zn(II), Co(II) and Mn(II). The later two had strong pro-oxidative effects, the addition of Zn(II) had an anti-oxidative effect. Apparent activation energies for oxidising species generation are in the range 75-110 kJ mol^{-1}, and decrease in the following order: Cu(II) > Ni(II) > Mn(II) > Fe(III) > Co(II).

Introduction

The sheer wealth of available literature demonstrates the importance of Fenton chemistry in a variety of reaction systems applicable in biology, ecology, food chemistry and material science. Several reviews are available on the subject.[1-3]

Fenton-like reagent system[4] denotes a mixture composed of a transition metal and hydrogen peroxide or other hydroperoxide or hypohalous acid leading to the production of oxidising species the identity of which is still a subject of scientific debate. In the classical Fenton system the three alternatives are either *free* hydroxyl radicals, *crypto* hydroxyl radicals or reactive complex iron(IV) intermediates, *e.g.* ferryl ion, FeO^{2+}.[5-11] Intermediates involving Cu(III), Co(IV) and Mn(IV) are also discussed.[12]

Beside the much-researched iron and copper,[13] the role of other transition metal ions may also be of interest. The oxidative chemistry involved in the toxicity of several transition metals of interest here were reviewed in the literature,[14] excluding cobalt and manganese. While cobalt is both toxic and carcinogenic, it is assumed that Co(I) and Co(II) may both enter the Fenton reaction, depending on chelation.[15] The generation of oxidising species by Co(II) is well known.[16,17] Cr(III) and hydrogen peroxide induce DNA strand breakage at pH 6-8.9, but not at pH 4, and it is assumed that Cr(III)[18,19] or Cr(II)[20] enter the Fenton reaction. The role of chromium is complex, as it may be oxidised to Cr(VI) via a number of intermediates[21] and Cr(V)-mediated generation of $HO^{\bullet}$ was also assumed.[22] Mn(II) is thought to be an ineffective catalyst[23] although the formal potentials are $E_{H2O2/HO\bullet} > E_{Mn(III)/Mn(II)} > E_{O2/O2\bullet-}$ if $pH > 7$.[24] Depending on the ligand and the substrate, however, Mn(III) may lead to generation of oxidising species under oxygenated conditions.[12] Manganese has been postulated to be capable of inducing brain injury through the generation of reactive oxygen species,[25] although antioxidative properties are also reported.[26,27] Similarly, cadmium is thought to be a non-Fenton metal, yet it may lead to production of hydroxyl radicals in the presence of metallothionein.[28] While cadmium induces lipid peroxidation in rat liver slices, it exhibits an inhibitory action *in vitro*.[29] Similar results were presented by other authors.[14] Considerable evidence in the literature suggests that oxidative mechanisms are involved in the toxicity of Ni(II).[14] With ESR, the presence of free radicals was indicated in the system Ni(II)/H_2O_2.[30] The authors also showed that the activity may be enhanced by chelation, the finding was also supported by other authors.[31] Zinc is a non-Fenton transition metal, yet it decreases the production of oxidising species in many systems, and it is assumed that displacement of iron or copper from binding sites leads to the protective effect.[14] However, even *in vitro*, zinc was shown to decrease the rate of generation of oxidising species.[32] Dietary Zn deficiency is known to increase susceptibility to oxidative damage.[33]

The rate of Fenton reaction itself is greatly affected by pH [34] and the choice of the buffer system is crucial as it may induce changes in kinetics,[35] and according to a study, phosphate buffer is the best choice unless it interferes with metal binding to proteins, in the order of decreasing interference: HEPES > MOPS > Tris >> phosphate.[36]

Aromatic hydroxylation may provide data on the kinetics of production of the oxidising species. In addition, it is non-demanding and rapid. It is assumed that the hydroxylation step proceeds at a diffusion controlled rate, and the yield of hydroxylated product is used to assess the kinetics of oxidising species production. Depending on the substrate (benzoate, salicylate, 4-nitrophenol, phenol) hydroxylation products may be numerous,[37] or even remove iron from the reaction mixture by formation of stable co-ordination compounds (*e.g.* with catechol species). Therefore, a substituted nitrobenzene substrate was synthesised,[38] *N*,*N'*-(5-nitro-1,3-phenylene)bisglutaramide (NPG), the two predominant products being *o*- and *p*-hydroxylated derivates (*i.e.* 4- or 6-hydroxy and 2-hydroxy, respectively). The hydroxylated products were later characterised regarding respective spectroscopic properties and yields at various pH values of reaction media and temperature.[39]

Considering that literature data on various transition metals are at times contradictory, aromatic hydroxylation assay may provide interesting comparative data on the behaviour of various metals in Fenton-like reaction systems. It is also of great interest to study how variations in the reaction parameters, such as pH and temperature influence the observed rates. We therefore undertook a study of the effects of pH (5.5 -9.5) and temperature (25 - 80 °C) on the rates of generation of oxidising species in Fenton-like systems containing either Cd, Co, Cr, Cu, Fe, Mn, Ni, or Zn.

Results and discussion

Iron and Copper

The role of both iron and copper was the subject of numerous studies. As in oxidative conditions, both metals are more stable in the oxidised state and therefore predominantly present as such in the environment, chlorides of Fe(III) and Cu(II) were used. Although the actual catalytically active species are Fe(II) or Cu(I), a steady state ratio $[Me^{n+}]/[Me^{(n+1)+}]$ is quickly achieved, and an initial curvature in the otherwise linear plot of absorbance *vs.* time is observed. An overview of the reaction scheme leading to metal reduction is out of scope of this contribution, as it has been treated exhaustively elsewhere.[1-3] The actual oxidising species may be hydroxyl radical produced according to the classical Fenton reaction:

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + HO^{\bullet} + OH^{-}, \qquad (1)$$

or some higher oxidation state transition metal species, *e.g.*

$$Fe^{2+} + H_2O_2 \longrightarrow FeO^{2+} + H_2O. \qquad (2)$$

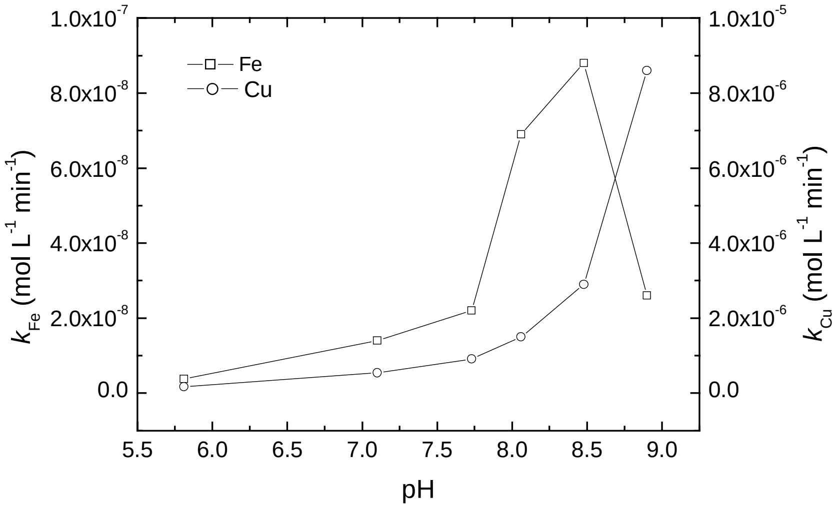

Figure 1. Rates of oxidising species production in Fenton-like reaction systems in the pH interval 5.5-9, at 25 °C for systems containing Fe(III) and Cu(II), initially.

A number of reactions are pH-dependent, the fact being mirrored in the pH dependence of oxidising species production demonstrated in Figure 1. Inactivation of Fe at pH > 8.5 is demonstrated. However, no visible precipitate is formed, although it should be stressed that all transition metals under study may also form bi- and polynuclear complexes. The consequence of chelation with phosphate at pH 7 was even described to promote oxygen consumption.[40] At increased pH, a decrease in activity is not observed for copper.

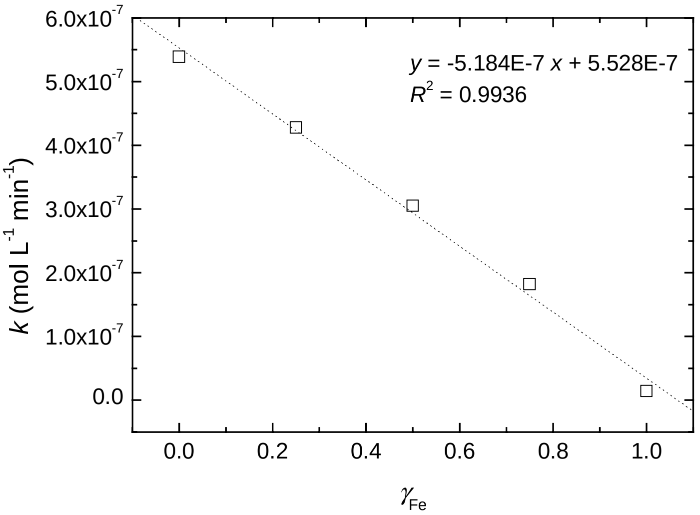

Figure 2. The effect of a mixture of Cu(II) and Fe(III) in Fenton-like reaction systems at pH 7, 25 °C on the rate of oxidising species production (γ_{Cu} + γ_{Fe} = 1, the summary concentration of transition metals 0.1 mmol L^{-1}).

In a salicylate hydroxylation study[41] it has been reported that small amounts of copper exhibit an inhibitory effect in Fenton-like systems containing both Fe and Cu. Such behaviour was not observed in our study of mixtures of both transition metals (Figure 2) as they appear to behave independently. Different pH of the reaction media used may be the reason for differences, but it should not be overlooked that the NPG substrate was designed in such a way that it does not interfere with the reaction system so that it is not able to remove the transition metal ion by complexation.[38] Salicylate (and also its oxidation products), on the other hand, chelates iron and thereby influences the oxidising species generation system.

Cobalt and Chromium

As outlined in the Introduction, the chemistry of cobalt and chromium in Fenton-like reaction systems is not well understood. Both are known to catalyse the production of hydroxyl radicals under certain conditions, yet it is not clear what is the actually active oxidation state. Only limited free radical formation in the Co(II)/H_2O_2 reaction system was shown in ESR studies.[30,42] Starting with lower oxidation states, *i.e.* Co(II), and Cr(III), a number of features may be outlined.

Cobalt(II) appears to be a better catalyst than iron, as the rates of generation of oxidising species are higher. The curves in Figure 3 may be modelled assuming a pseudo-zero order process, however, a rapid consumption of all NPG is clearly evident at pH 7.7-8.5. At pH < 7.1, the system behaves similarly to those containing iron or copper, as the curves may be approximated using linear models. At higher pH (*i.e.* 8.9), only a rapid initial process is observed leading to hydroxylation of NPG, and continuation of the experiment does not lead to any further significant increase of absorbance, *i.e.* formation of hydroxylated NPG products. However, if at this point Cu(II) is added, a rapid and linear increase of absorbance indicates that hydrogen peroxide is still present in the reaction system.

If Co(III) is formed, it is not able to oxidise hydrogen peroxide under the conditions used, as suggested for other systems.[43] It would seem therefore, that while the catalytical activity of Co(II) increases with pH, Co(III) cannot be recycled in the active form at too high pH values.

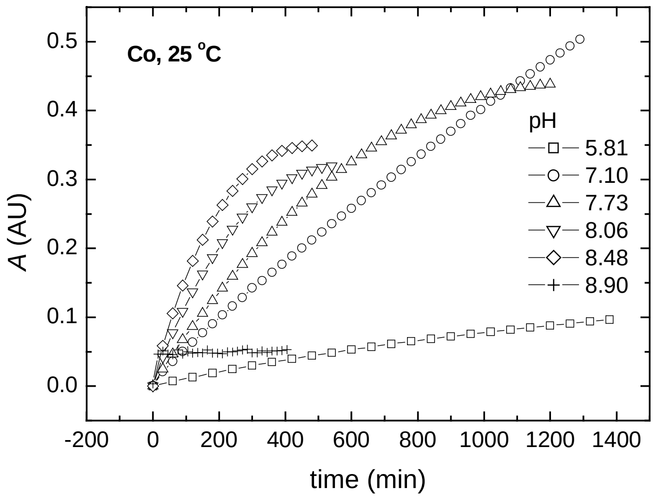

Figure 3. Formation of hydroxylated NPG derivatives followed by spectrophotometry at 431 nm, in Fenton-like reaction systems containing Co(II) at 25 °C, the solution pH as indicated.

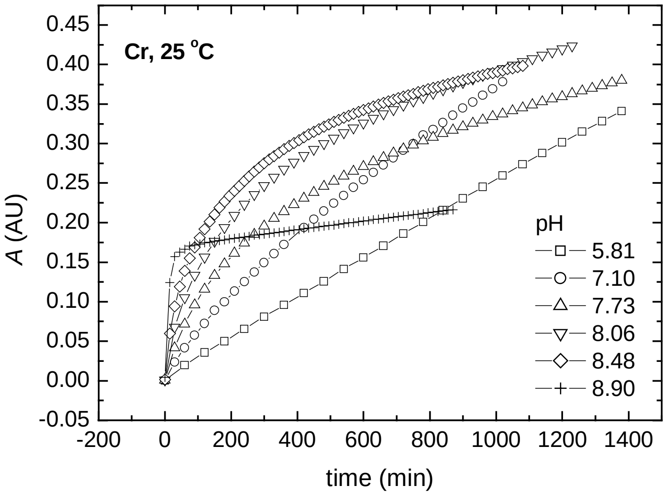

Figure 4. Formation of hydroxylated NPG derivatives followed by spectrophotometry at 431 nm, in Fenton-like reaction systems containing Cr(III) at 25 °C, the solution pH as indicated.

In the presence of Cr(III), the reaction system is even more complex. In alkaline solutions, a number of Cr(IV) and Cr(V) intermediates are supposed to be involved in the radical oxidation of Cr(III)[21] and in the Cr(III)/H_2O_2 reaction system the existence of both hydroxyl radical and superoxide was demonstrated using ESR at pH 7.5.[18] In our study, the linear model may still be used at pH 5.8 and 7.1 (Figure 4), indicating that low-oxidation state chromium species effectively participate in the generation of oxidising species, as there is no evidence of production of chromate(VI), which would otherwise be detected in the absorption spectra of the reaction solution. On the other hand, this is effectively the case at higher pH, *e.g.* at 8.9, where a rapid initial oxidation of Cr(III) is observed leading to a very high initial rate of NPG hydroxylation, yet after the concentration of chromate(VI) reaches a steady-state (as evidenced in the absorbance spectra), the production of oxidising species still proceeds at a measurable rate, which confirms that the redox pair Cr(V)/Cr(VI) is also catalytically active, and may lead to the generation of $HO^{\bullet}$ as discussed in the literature,[44] albeit at a slower rate than Cr(II) or Cr(III).[19,20] This is in accordance with ESR studies.[18]

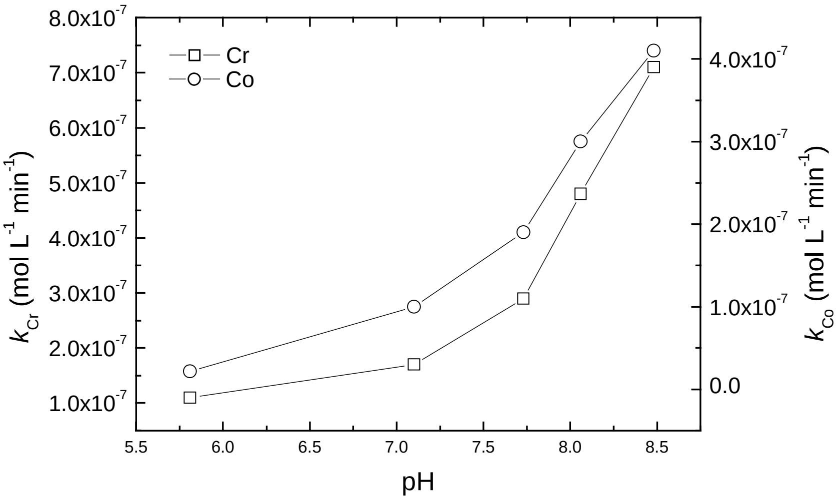

Figure 5. Rates of oxidising species production in Fenton-like reaction systems in the pH interval 5.5-9, at 25 °C for systems containing Co(II) and Cr(III), initially.

The initial rates of production of oxidising species leading to NPG hydroxylation may be approximated and the resulting dependence of the rates on the reaction system pH is demonstrated in Figure 5. The rates at pH 8.9 are too high to be determinable with the NPG hydroxylation assay. This is in line with the observation that the yield of hydroxyl radical is 15 times higher at pH 10 than that at pH 7.2.[19] A comparison of the data in Figures 1 and 5 shows that both chromium and cobalt are much more effective catalysts in Fenton-like reaction systems than iron.

Comparison of transition metals

Similar studies were performed with Zn(II), Cd(II), Mn(II), and Ni(II). A reaction system without an addition of transition metal was also studied, although a metal-free reaction system was not attempted. A comparison of the rates of oxidising species production in the pH interval 5.5-9.5 shows that Zn and Cd have no catalytic effect as the rates of hydroxylation are not statistically different from the system where no transition metal was added. This is in line with other studies – the reason for an increased cadmium concentration related generation of free radicals in tissues may well be an indirect one,[45] *e.g.* cadmium-induced displacement of iron or a decrease in glutathione content.[14] On the other hand, the rate of hydroxylation increases with pH in the case of Ni(II) – at pH 7.8 it is already statistically different from the blank. Most studies with Ni(II) were performed at physiological pH, where the rate of production of oxidising species is difficult to assess, and this fact may explain the sometimes controversial interpretations. *E.g.*, in an ESR study of the system Ni(II)/H_2O_2, no radical species were detected.[30]

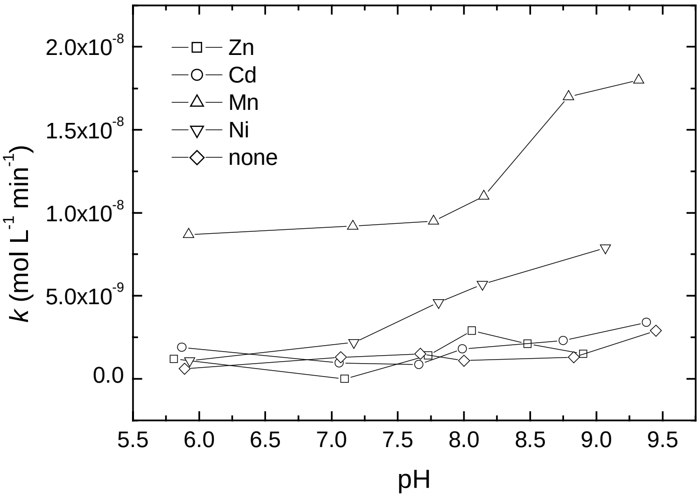

Figure 6. Rates of oxidising species production in Fenton-like reaction systems in the pH interval 5.5-9, at 25 °C with the addition of transition metals as indicated.

Mn(II) also leads to the production of oxidising species, although at pH > 7.5 not to a considerable extent in comparison with the catalytically more active metals such as cobalt, chromium, iron and copper. If Mn(III) is formed, it may be reduced not only by superoxide, but also by hydrogen peroxide.[46] In Figure 7, the rates at pH 7 are compared. At this pH, the reaction system containing Mn(II) exhibits a rate of production of oxidising species comparable to the Fe(III)-containing system. The catalytical activity of systems containing Co(II) and Cr(III) is superior, and still exceeded by Cu(II).

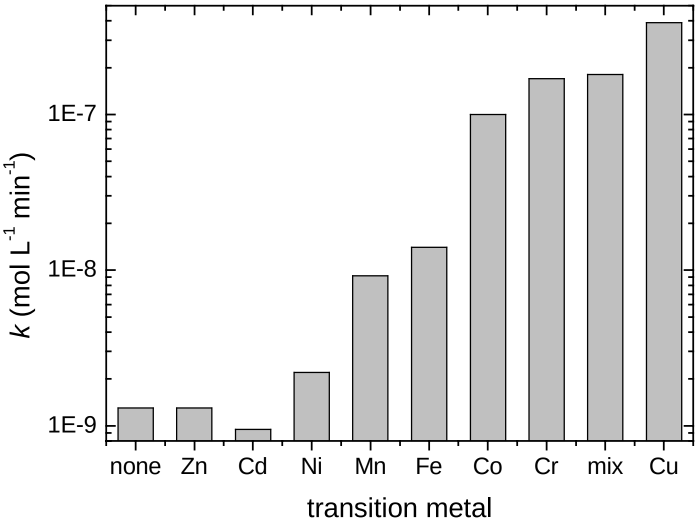

Figure 7. A comparison of rates of oxidising species production in Fenton-like reaction systems at pH 7 at 25 °C with transition metals as indicated.

Table 1. The rates of production of oxidising species in Fenton-like systems containing a mixture of transition metals [67.6% Fe(III), 16.4% Cu(II), 13.8% Zn, 1.3% Mn(II) and 1% Co(II)] at pH 7, 25 °C. All contents expressed in *n*/*n*.

Reaction system	*k* [nmol L^{-1} min^{-1}]	% relative to the observed rate
mixture (observed rate)	186	100
mixture (calculated rate)	99	53
mixture excluding Co	133	71
mixture excluding Mn	98	52
mixture excluding Zn	222	119

An arbitrary mixture composed predominantly of Cu(II) and Fe(III) was also used in this study, containing 67.6% Fe(III), 16.4% Cu(II), 13.8% Zn, 1.3% Mn(II) and 1% Co(II) (all contents expressed in *n*/*n*), the summary molar concentration remaining 0.1 mmol L^{-1}. As shown above, the catalytic effects of copper and iron are additive and it was of interest to examine whether such behaviour is exhibited by more complex systems also, especially if zinc or manganese are also present, which have been shown to exhibit an inhibitory effect in several studies. It is of interest to observe the surprisingly high rate of oxidising species production despite the fact that only 16.4% of copper is present in the mixture (Figure 7).

If no interactions are assumed, as was the case with Fe(III) and Cu(II), the rate of production of oxidising species may be calculated by summing up the respective contributions proportional to the content of an individual metal. However, the rate, calculated in this way, is only 53% of the observed one (Table 1), leading to the conclusion that the roles of metals in Fenton-like systems may be synergistic. By excluding one of the metals, this can be shown even more clearly: by excluding 1% of Co(II), the rate is reduced by 30%. Furthermore, by excluding 1.3% of Mn(II), the rate is reduced by even 48%, indicating that in the reaction mixture, manganese has a strongly pro-oxidative effect instead of the reported anti-oxidative.[26] On the other hand, by excluding 13.8% of Zn(II), the rate is increased by 20%. This is not surprising, as *e.g.* $ZnSO_4$ was shown to inhibit the production of oxygen radical species, *i.e.* both hydroxyl radicals and superoxide. By reducing the concentration of superoxide, the rate of reduction of Fe(III) and Cu(II) may take place thus reducing the overall rate of generation of oxidising species.

In our system, small additions of Mn(II) or Co(II) have a pronounced promoting effect, while the addition of Zn(II) has an inhibiting effect. Since in biological systems the metals are rarely isolated, such concerted effects should also be taken into account.

At pH 7, the rate of generation of oxidising species was studied at 25, 30, 40, 50, 60, 70, and 80 °C in order to obtain the apparent activation energies for all the reaction systems (Table 2). In several cases, the repeatability of experiments was lower, as demonstrated by higher standard deviations. The kinetic data for chromium could not be obtained due to the presence of a mixed mechanism not allowing the application of a *pseudo*-zero order model. As estimated by Barb *et al.*,[47] the reactions with Cu(II) are more endothermic than those with Fe(III). The apparent activation energies for iron and cobalt-containing systems are similar, which may indicate the same rate-controlling step. Due to the small differences in rates of generation of oxidising species at 25 °C and due to the different apparent activation energies, in manganese-containing system, the rate at 80 °C decreases to the level when no significant production of oxidising species is observed at pH > 7 in comparison with the system with no transition metal added.

Table 2. The apparent activation energies for production of oxidising species in Fenton-like systems at pH 7.

Reaction system	E_a (kJ mol K^{-1})	S.D. (kJ mol K^{-1})
Co	75	3
Fe	77	3
Mn	87	12
mixture	97	4
Ni	101	10
control (no metal)	107	7
Cu	108	2

A systematic study of the synergistic effects and the temperature dependences are of significant importance for studies of the role of transition metals in biological systems and further work is in progress.

Conclusions

A comparative aromatic hydroxylation study of rates of oxidising species generation in Fenton-like systems was performed with Cd, Co, Cr, Cu, Fe, Mn, Ni, and Zn in the pH range 5.5 - 9.5 and in the temperature range 25 - 80 °C and has lead to several conclusions:

- in the whole pH range, the presence of Cd(II) and Zn(II) does not lead to significant production of oxidising species;
- Ni(II) exhibits a very limited catalytic activity at pH > 7.5;
- there is a general trend of increasing rate of generation of oxidising species with pH for all the active transition metals, however, in the case of iron, a marked decrease in the activity is again evident at pH > 8.5;
- at pH 7, the rates of production of oxidising species decreases in the following order: Cu(II) > Cr(III) > Co(II) > Fe(III) > Mn(II) > Ni(II);
- no interactions between transition metals are evident in systems containing both iron and copper;
- in the Fenton-like reaction mixture containing 67.6% Fe(III), 16.4% Cu(II), 13.8% Zn, 1.3% Mn(II) and 1% Co(II) the small contents of Mn and Co had strong pro-oxidative effects, the addition of Zn had an anti-oxidative effect;

- apparent activation energies for oxidising species generation are in the range 75 - 110 kJ mol^{-1}, and decrease in the following order: Cu(II) > Ni(II) > Mn(II) > Fe(III) > Co(II).

Experimental

N,N'-(5-nitro-1,3-phenylene)bisglutaramide (NPG) was synthesised according to the literature.[38] The hydroxylation assay was performed in the following way. The reaction mixture (2.5 mL) contained 0.1 mmol L^{-1} transition metal, 1 mmol L^{-1} NPG, 20 mmol L^{-1} phosphate buffer, and 20 mmol L^{-1} H_2O_2 (non-stabilised, Fluka, Buchs). The transition metal solutions were prepared from the corresponding chlorides, all of p.a. quality: $FeCl_3{\cdot}6H_2O$ (Fluka, Buchs), $CuCl_2$ (Merck, Darmstadt), $MnCl_2{\cdot}4H_2O$ (Riedel-de Häen, Hannover), $CoCl_2{\cdot}6H_2O$ (Carlo Erba, Rodano), $CrCl_3{\cdot}6H_2O$ (Kemika, Zagreb), $NiCl_2{\cdot}6H_2O$ (Scharlau, Barcelona), or $CdCl_2{\cdot}H_2O$ (Zorka, Šabac). The buffers was prepared from corresponding mixtures of Na_3PO_4, Na_2HPO_4, and NaH_2PO_4, (all microselect quality, Fluka, Buchs), with nominal values of pH 6, 7, 8, 9 or 10. The actual pH values of the reaction mixtures were determined separately with a combined glass electrode and a pH-meter. All solutions were prepared with additionally purified deionised water (Millipore, Molsheim). In selected experiments, mixtures of transition metals were also used with the final molar concentration of metals always 0.1 mmol L^{-1}.

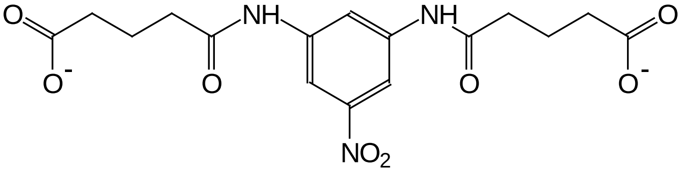

Figure 8. *N,N'*-(5-nitro-1,3-phenylene)bisglutaramide (NPG).

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. The rate of production of oxidising species (k [mol L^{-1} min^{-1}]) was determined from the plots of absorbance *vs.* time. After steady state is achieved, including the ratio of [Me^{n+}]/[Me$^{(n+1)+}$], temperature and concentration of oxygen in the solution, the curves may be modelled using *pseudo*-zero order kinetics for most reaction systems. In the case of Co and Cr, the simplified approach was not possible in several cases and is discussed separately. Repeated experiments were performed at pH 7, 25 °C, the typical RSD in the determination of k was 20% (n = 4). Since some reaction rates were extremely low, the cuvettes were well sealed including some air atmosphere allowing for oxygenated Fenton chemistry. From the slopes of plots ($\Delta A/\Delta t$), k was calculated using molar absorptivities and hydroxylated NPG product ratios at 432 nm and at respective pH observed for the particular reaction system.[39]

Acknowledgements

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Povzetek

V reakcijskih sistemih podobnih Fentonovemu, s Cd(II), Co(II), Cr(III), Cu(II), Fe(III), Mn(II), Ni(II) ali Zn(II), smo za določitev hitrosti nastajanja oksidirajoče zvrsti uporabili spektrofotometrično metodo hidroksilacije *N,N'*-(5-nitro-1,3-fenilen)bisglutaramida. Zanimalo nas je območje pH 5.5-9.5, kar smo uravnavali z dodatkom fosfatnega pufra, in območje temperature 25-80 °C. Hitrosti nastajanja oksidirajoče zvrsti pri pH 7 padajo v naslednjem zaporedju Cu(II) > Cr(III) > Co(II) > Fe(III) > Mn(II) > Ni(II), medtem ko Cd(II) in Zn(II) ne kažeta katalitske sposobnosti, Ni(II) pa le v območju pH > 7.5. V rakcijskih mešanicah z Cu(II) in Fe(III) lahko hitrost nastajanja oksidirajoče zvrsti obravnavamo kot vsoto prispevkov posameznih kovin. Drugačne lastnosti imajo mešanice, ki vsebujejo Zn(II), Co(II) ali Mn(II). Zadnja dva izkazujeta močno prooksidativno aktivnost, medtem ko ima Zn(II) antioksidativen učinek. Navidezne aktivacijske energije za nastajanje oksidirajoče zvrsti so v intervalu 75-110 kJ mol^{-1} in padajo v naslednjem zaporedju: Cu(II) > Ni(II) > Mn(II) > Fe(III) > Co(II).