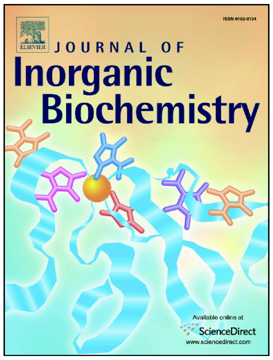

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The Fe^{II}(citrate) Fenton reaction under physiological conditions

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ABSTRACT

The Fenton reaction of Fe^{II}(citrate) in the presence and absence of bicarbonate (HCO_3^-) is studied. It is found that the rate constant of the Fenton reaction (k_{obs}) increases with increasing [citrate]. k_{obs} also increase with increasing [HCO_3^-]; this effect is most significant at biological citrate concentrations. Methane and ethane gases are formed from $(CH_3)_2SO$ when the Fenton reaction is carried out in the presence of large [citrate] due to the reaction of the citrate radical, $(^-{}_2OC)CH_2C(OH)(CO_2^-)CH(CO_2^-)^\bullet/(^-{}_2OC)CH_2C(O)(CO_2^-)CH_2(CO_2^-)^\bullet$ with $(CH_3)_2SO$. In the absence of citrate $(CH_3)_2SO_2$ is the main product of the Fenton reaction. However, in the presence of 0.10 mM citrate, no $(CH_3)_2SO_2$ is formed, some $(CH_3)SOOH$ is formed, along with a low yield of beta-ketoglutaric acid. Formation of $(CH_3)SOOH$ and beta-ketoglutaric acid are due to the citrate radical and Fe^{IV}(citrate). In the presence of bicarbonate formation of abundant beta-ketoglutaric acid confirms the formation of carbonate radical anion ($CO_3^{\bullet -}$). Thus, bicarbonate affects the mechanism and kinetics of the reaction dramatically. Hydroxyl radicals ($OH^\bullet$) are not formed in the presence of bicarbonate and probably also not in its absence. These results point out that hydroxyl radicals, formed by the Fenton reaction, do not initiate oxidative stress in biological systems.

Keywords: Fenton reaction; Fe^{II}(citrate); bicarbonate; biological oxidative stress; Fe^{IV}=O; carbonate anion radical

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1. Introduction

The Fenton reaction[1] is of major importance in causing oxidative stress[2–4] and in advanced oxidation processes[5–7]. Originally the Fenton reaction is the reaction between $Fe(H_2O)_6^{2+}$ and H_2O_2[1], but over the years, many other Fenton like reactions have been studied by changing the metal ion, water ligand, and peroxide. However, the mechanism of the Fenton reactions is still in debate. In 1932 two mechanisms were proposed: Haber and Weiss[8] proposed that the mechanism involves the formation of hydroxyl radicals ($OH^{\bullet}$) via:

(1) $$Fe(H_2O)_6^{2+} + H_2O_2 \rightarrow Fe(H_2O)_6^{3+} + OH^{\bullet} + OH^{-}$$

While Bray and Gorin[9] proposed that the mechanism of the Fenton reaction is:

(2) $$Fe(H_2O)_6^{2+} + H_2O_2 \rightarrow (H_2O)_5Fe{=}O^{2+} + 2H_2O$$

The debate about which of these mechanisms is correct lasted for decades. It was shown that these reactions could not proceed via the outer-sphere mechanism and the minimal mechanism has to be written as follows:[10,11]

(3) $$M^nL_m + H_2O_2 \leftrightarrows L_{m-1}M^n(O_2H) + H_3O^+ + L$$

(4) $$\begin{array}{lll} & \xrightarrow{H^+} L_{m-1}M^{n+2}{=}O + H_2O & (a) \\ L_{m-1}M^n(O_2H) & \xrightarrow{\quad} L_{m-1}M^{n+1}(OH) + OH^{\bullet} & (b) \\ & \xrightarrow{RH} L_{m-1}M^{n+1}(OH) + R^{\bullet} + H_2O & (c) \end{array}$$

It is now clear that for M = Fe both mechanisms (4a) and (4b) are correct, thus for $Fe(H_2O)_6^{2+}$ reaction (4b) is correct at pH < 4 whereas reaction (4a) is correct in neutral solutions[12]. For $[H_2O_2] >> [Fe(H_2O)_6^{2+}]$ it was shown that at least in acidic solutions reaction (5) contributes to the process:[13,14]

(5) $Fe^{II}(H_2O)_6^{2+} + Fe^{III}(H_2O)_5(HO_2^{-})^{2+} \rightarrow Fe^{III}(H_2O)_6^{3+} + (H_2O)_5Fe^{IV}{=}O^{2+}$

Furthermore, different mechanisms are reported for other central cations and other ligands. For example, central cations with a high redox potential (e.g., Al,[15] and Co[16]), the mechanism is as follows:

(6) $(H_2O)_{6\text{-}l\text{-}1}M^n(O_2H^{-})_l(H_2O_2)_{n\text{-}l} \rightarrow (H_2O)_{6\text{-}l\text{-}1}M^n(O_2H^{-})_{l\text{-}1}(O_2H^{\bullet})(OH^{-})_{n\text{-}l} + OH^{\bullet}$

Whereas for central cations with somewhat lower oxidation potential (Mo[17]) it is:

(7) $Mo^VO_4^{3-} + H_2O_2 \rightarrow O_3Mo^V(O_2^{2-}) + H_2O$
(8) $O_3Mo^V(O_2^{2-})^{3-} + H_2O_2 \rightarrow Mo^{VI}O_4^{2-} + O_2^{\bullet-} + H_2O$

Finally, it was shown, theoretically, that transient complexes of the types: $(H_2O)_{6\text{-}l\text{-}k}L_lM^n(O_2H^{-})$ or $(H_2O)_{6\text{-}l\text{-}1}L_lM^n(O_2H^{-})_k$ are expected to decompose via the oxidation of an L ligand if that is mechanistically and/or thermodynamically favorable[18]. This was proposed for carboxylate ligands and carbonate. Indeed, it was demonstrated experimentally that carbonate radical anion ($CO_3^{\bullet-}$) are formed in the Fenton like reactions of $Co(H_2O)_6^{2+}$ and $Fe(H_2O)_6^{2+}$ in the presence of bicarbonate.[14,19]

This short discussion points out that one cannot assume a priori that under given conditions, the product of the Fenton like reaction would be a strong oxidizing agent. Oxidative stress in biological systems is commonly induced by the reactions of iron in the mobile pool of non-transferrin bound iron[20,21]. Fe^{III}(citrate), and Fe^{II}(citrate) are the main iron-containing species in this pool[22,23]. The Fenton reaction in the presence of citrate was studied often. The following are some relevant systems in which this reaction was studied: a. In biological systems[21,24–27]. b. In advanced oxidation processes[28–30]. In all these studies, it is proposed that $OH^{\bullet}$ radicals are the product of the reaction of Fe^{II}(citrate) with peroxides. One study raise the question of whether the products are $OH^{\bullet}$ radicals or $Fe^{IV}{=}O^{2+}_{aq}$[27]. In none of these studies, the role of bicarbonate in the process was considered. It seemed, therefore, of interest to check the mechanism of the Fenton like reaction in solutions containing citrate and bicarbonate at concentrations present in the body.

2. Experimental

2.1. Materials, instruments, and methods

The chemicals used were all of the analytical grade, purchased from the following companies; Iron(II) perchlorate, Sigma-Aldrich; hydrogen peroxide (H_2O_2), J.T.Baker; sodium bicarbonate, J.T.Baker; trisodium citrate, Alfa Aesar; dimethyl sulfoxide, $(CH_3)_2SO$, TCI and D_2O, Tzamal D-Chem Laboratories Ltd.

UV-Vis kinetic measurements were performed using an Applied Photophysics SX20 stopped-flow instrument. The kinetics were followed at 270 nm due to the formation of Fe^{III} complex[31]. The ^1H NMR spectra were recorded on a 400 MHz Bruker AvanceIII spectrometer. GC determinations of methane and ethane were performed using an Agilent 7890B GC System with FID and TCD detectors and a GS Gaspro column. Pulse radiolysis of the aqueous solutions was done using 1.5 μs pulses from the linear electron accelerator of Ariel University. The pH was measured using a Schott Instrument Lab 850 pH meter.

All experiments were performed using the citrate as the buffer. Its capacity is enough to keep the pH at 7.2[32,33]. $HClO_4$ was used for adjusting the pH to 7.2 ± 0.1. A 7.8 mM H_2O_2 stock solution was prepared from 30 wt. % H_2O_2. Low concentrations of the reactants were used in order to minimize the ionic strength effect. All the solutions were purged in glass syringes with argon during the preparation and before mixing. Stock solutions of iron(II) perchlorate in buffered Milli-Q H_2O (Millipore) were prepared freshly before each set of experiments. The exact amounts of iron(II) perchlorate crystals were added to the saturated argon solutions while the argon purging was running to avoid any contact of the iron(II) with oxygen. $Iron^{II}$(citrate) complexes were prepared in one syringe by adding trisodium citrate to the Milli-Q water, adjusting the pH with $HClO_4$ solution, purging the solution with Ar, and then finishing by the addition of the deaerated iron(II) stock solution. In the case of individual ^1H NMR and GC measurements dimethyl-sulfoxide, $(CH_3)_2SO$, was added to the iron(II) solution at the end of the preparation. Concentrated sodium bicarbonate solutions were prepared and added to the diluted H_2O_2 solutions, to form solutions containing the desired compositions. Then the pH was set to the required pH 7.2 by adding $HClO_4$.

At this pH, CO_2 was also present in the solutions. Thus, while O_2 is removed by purging an inert gas, it decreases the HCO_3^- concentration considerably. To overcome this problem, Ar was passed through a gas-washing bottle containing a solution of HCO_3^- at the same concentration. This method and its effectiveness were previously reported.[14] In all the kinetic measurements, the Fe^{II} (with or without citrate) and the H_2O_2 (with or without bicarbonate) solutions in different concentrations were mixed in a 1:1 volumetric ratio. Thus, the pH in the kinetic runs was always pH of 7.2 ± 0.1. The concentrations stated in the manuscript are those in the final solutions. Kinetic experiments were performed at 25 ± 0.10 °C. The experiments were repeated at least three times in order to check the reproducibility.

Dimethyl sulfoxide, $(CH_3)_2SO$, was added to the solutions in order to analyze the products formed by the Fenton reaction, and different products were monitored by ^1H NMR and gas chromatography (GC). ^1H NMR spectra of the products of oxidation of $(CH_3)_2SO$ via the Fenton reaction were measured in the absence and presence of bicarbonate. It is known that $(CH_3)_2SO$ reacts with $Fe^{IV}{=}O_{aq}$ by oxygen atom transfer forming dimethyl sulfone, $(CH_3)_2SO_2$[12], while $OH^\bullet$ generates methyl-sulfinic acid, (CH_3SOOH) and ethane (*via* methyl radicals).[12,14,34] As the concentrations of the products of the Fenton reaction are too low to measure by NMR, the reactions were performed in bulk. The final solution (reaction mixture) was then acidified and concentrated using a rotary evaporator at room temperature.

Further, in order to investigate the reactions of $CO_3^{\bullet-}$ with $(CH_3)_2SO$ and citrate, the solutions were irradiated in a ^{60}Co γ source with a dose rate of ~4 Gy/min. The Milli-Q H_2O (Millipore) was used for all the experimental measurements, while 10% D_2O was added to record ^1H NMR spectra. Fenton reactions were performed for $[H_2O_2] > [Fe^{II}]$. The Fe^{II} and the H_2O_2 concentrations were 0.020 and 0.39 mM. The $(CH_3)_2SO$ concentration was 12.5 mM or 25 mM in all experiments. This means high excess over Fe^{II} and H_2O_2 in all cases.

3. Results and discussion

3.1. Rate constant (k_{obs}) dependence on the citrate concentration

The effect of [citrate] on the observed rate constant of the Fenton reaction is determined; the results are summed up in Fig. 1. The results demonstrate that the rate of the Fenton reaction depends on [citrate]. Even at the physiological concentration of 0.1 mM citrate[35], the observed rate constant is considerably higher than that in the absence of citrate. The effect of the citrate on the rate constant is due to the formation of the $Fe(Cit)^{-}$;

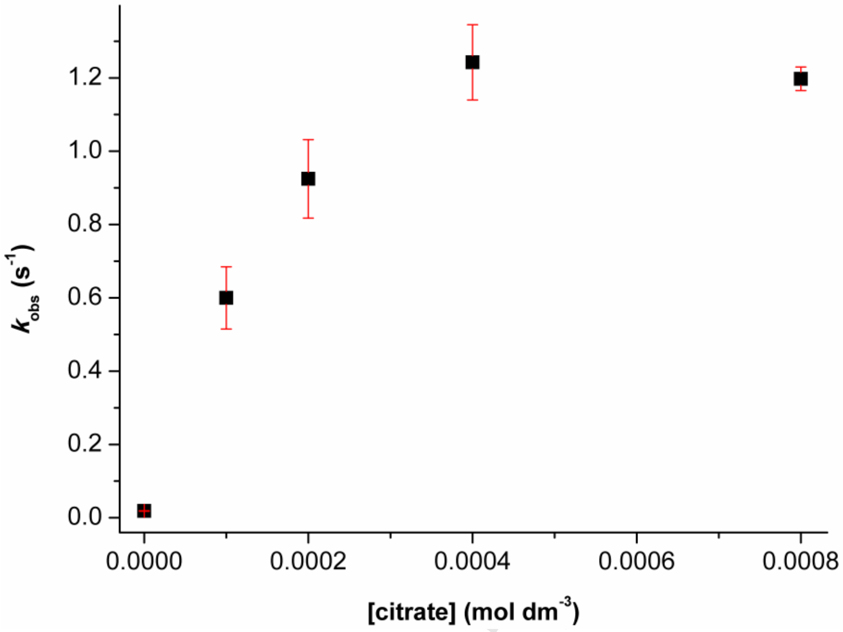

Fig. 1. Observed rate constant dependence on the citrate concentration during the Fenton-like reaction of Fe^{II}(citrate) complex with H_2O_2. The solutions contained: 0.020 mM Fe^{II}, 0.10-0.80 mM trisodium citrate, 0.39 mM H_2O_2. The pH was 7.2, adjusted with $HClO_4$.

$Fe(CitH)^0$ and $Fe(Cit)_2^{4-}$ complexes (where CitH, Cit are di and tri negative citrate ligand respectively) that lower the redox potentials of the corresponding $Fe^{III/II}$ and $Fe^{IV/II}$ couples. The stability constants of the citrate complexes are poorly known; see results reported in the following references.[36–42] Therefore it is impossible to determine the rate constants of the reactions of each component in the solution with H_2O_2. The measured rate constant ($(4.8\pm0.6)\times10^3$ $M^{-1}s^{-1}$) of the Fenton reaction of Fe^{II}(citrate) matches well with the previously reported ($(4.9\pm0.3)\times10^3$ $M^{-1}s^{-1}$) value[32].

3.2. Rate constant (k_{obs}) dependence on the HCO_3^{-} concentration

The effect of bicarbonate on the reaction rate of the Fenton reaction of the Fe^{II}(citrate) complex with H_2O_2 at pH 7.2 at [citrate] = 0.10; 0.50 and 2.0 mM was studied. These concentrations of citrate were chosen in order to determine the effect under conditions where the citrate affects the kinetics differently. The results are summed up in Fig. 2.

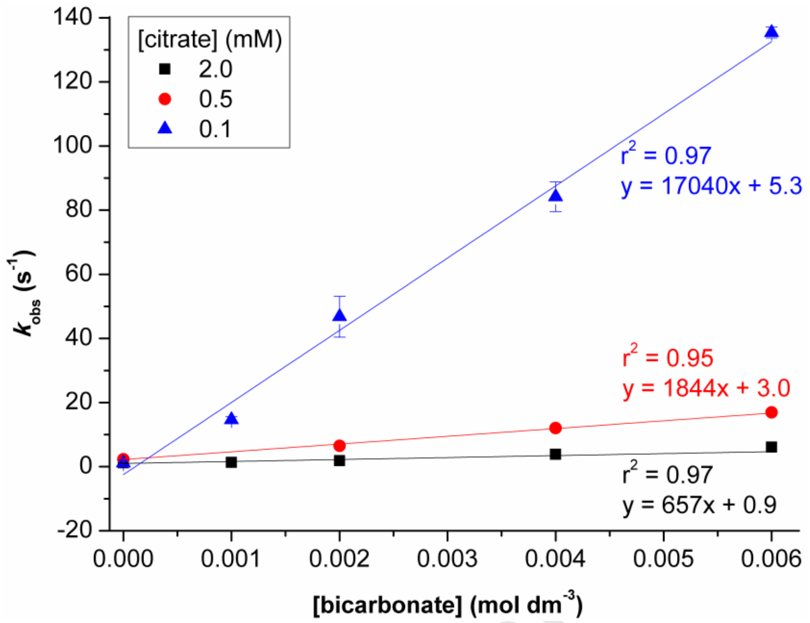

Fig. 2 Observed rate constant dependence on the bicarbonate concentration at different citrate concentrations during the Fenton-like reaction of the Fe^{II}(citrate) complexes with H_2O_2. The solutions contained: 0.020 mM Fe^{II}, 0.10-2.0 mM trisodium citrate, 0.39 mM H_2O_2, 0.0-8.0 mM $NaHCO_3$. The pH was 7.2, adjusted with $HClO_4$.

The presence of bicarbonate unambiguously increases the rate constant of the reaction considerably. The dependence of the observed rate constant on $[HCO_3^{-}]$ is affected by the citrate concentration. Under near-physiological conditions, in the presence of 0.10 mM citrate, where some $Fe(H_2O)_6^{2+}$ is present in the solution, the slope of the linear line is the largest, 17,040 mol^{-1}dm^{3}s^{-1}. In the absence of citrate, the slope of the linear line is considerably larger, 105,400 mol^{-1}dm^{3}s^{-1} [14]. Thus, the reaction rate is much faster in the presence of only bicarbonate. The effect of [citrate] on the results is attributed to its effect on the concentrations of the following complexes in the solutions: $Fe(CO_3)^0$, $Fe(HCO_3)^+$, $Fe(Cit)^-$, $Fe(Cit)(CO_3)^{3-}$ $Fe(Cit)(HCO_3)^{2-}$, $Fe(CitH)^0$, $Fe(CitH)(HCO_3)^-$, $Fe(CitH)(CO_3)^{2-}$, and $Fe(Cit)_2^{4-}$. In this context, it should be noted that Adam et. al.[25] have shown that with the addition of ascorbate to the Fe^{II}(citrate) complex the reaction rate increases.

Also, the stability constants of $Fe(CO_3)^0$ and $Fe(HCO_3)^+$ are poorly known; see discussion by Illés *et. al*.[14] The stability constants of the mixed complexes $Fe(Cit)(HCO_3)^{2-}$; $Fe(CitH)(CO_3)^{2-}$; $Fe(Cit)(CO_3)^{3-}$ and $Fe(CitH)(HCO_3)$ are unknown. Therefore, it is impossible to determine the rate constants of the reactions of each component in the solution with H_2O_2. The nature of the reactive oxidizing species in these reactions is unclear at present. In principle, the formation of the corresponding (Fe^{III} +$OH^{\bullet}$), Fe^{IV}, and/or $Fe^{II}(CO_4{}^{2-})$ [19] complexes can be envisaged. Clearly with the increase in [$HCO_3{}^{-}$], the concentrations of all the complexes containing bicarbonate/carbonate as a ligand increases: This lowers the redox potential of the central Fe^{II} ion and increases the chance to form per-carbonate complexes.

3.3. Gas-chromatographic measurements of methane end ethane after the Fenton reaction

The remaining question is what the products of these reactions are. For this purpose, it was decided to add $(CH_3)_2SO$ to the solutions as it is known that $OH^{\bullet}$ radicals react with $(CH_3)_2SO$ to form methyl radicals via:

(9) $(CH_3)_2SO + OH^{\bullet} \rightarrow (CH_3)_2S(OH){=}O^{\bullet}$ $k_9 = 7.0\text{x}10^9$ $mol^{-1}dm^3s^{-1}$ [43]

(10) $(CH_3)_2S(OH){=}O^{\bullet} \rightarrow CH_3SOOH + CH_3{}^{\bullet}$ $k_{10} = 1.5\text{x}10^7$ s^{-1} [43]

It should be pointed out that also other radicals react with $(CH_3)_2SO$ to form methyl radicals.[44,45] The methyl radicals thus formed react to form ethane and methane via:

(11) $CH_3{}^{\bullet} + (CH_3)_2SO \rightarrow CH_4 + CH_2(CH_3)SO^{\bullet}$ $k_{11} = 100$ $mol^{-1}dm^3s^{-1}$ [46]

(12) $CH_3{}^{\bullet} + RH \rightarrow CH_4 + R^{\bullet}$ k_{12} = depending on RH (Where RH is an aliphatic compound.)[47]

(13) $2CH_3{}^{\bullet} \rightarrow C_2H_6$ $k_{13} = 1.6\text{x}10^9$ $mol^{-1}dm^3s^{-1}$ [47]

On the other hand, $Fe^{IV}{}_{aq}$ reacts with $(CH_3)_2SO$ to form $(CH_3)_2SO_2$[12]. Therefore, the yields of methane, ethane, and $(CH_3)_2SO_2$ under different conditions were determined.

The Fenton reaction in the presence of 0.10 mM Fe^{II}(citrate), where over 90% of the reaction proceeds via the citrate complex, Fig. 1, no methane and ethane formation takes place, Fig. 3. However, when higher citrate concentrations are used, 1.0 mM, methane, and ethane formation are observed. It is assumed that the citrate radical, $({}^{-}{}_2OC)CH_2C(OH)(CO_2{}^{-})CH(CO_2{}^{-})^{\bullet}/({}^{-}{}_2OC)\text{-}CH_2C(O)(CO_2{}^{-})CH_2(CO_2{}^{-})^{\bullet}$[48] reacts with $(CH_3)_2SO$ to yield methyl radicals that form methane and some ethane.

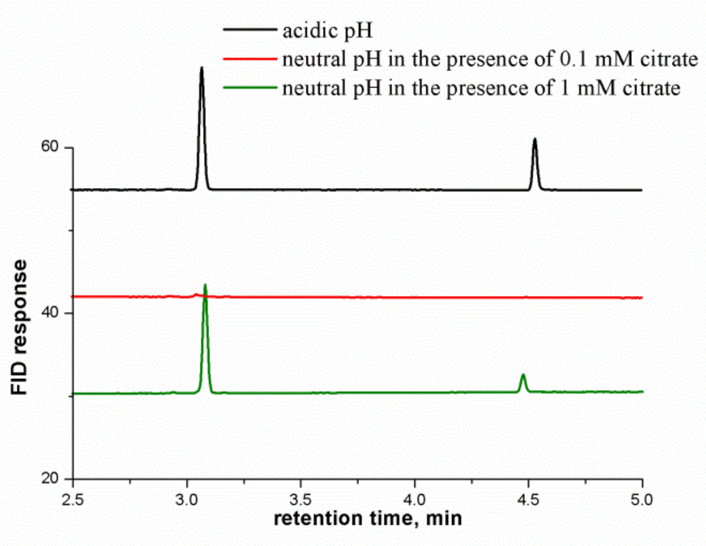

Fig. 3 Methane (t_r = 3.1) and ethane (t_r = 4.5) determination by GC in $(CH_3)_2SO$, 25 mM, containing solutions after Fenton reaction under different conditions. The initial concentrations were $[Fe^{II}]$ = 0.020 mM, $[H_2O_2]$ = 0.39 mM in all of the samples. The pH was 2.2 in the acidic solution, and 7.2 in the neutral solutions.

During the Fenton reaction of Fe^{II} with H_2O_2 in neutral pH (pH 7.2), no methane or ethane formation was observed, Fig. 4. The presence of bicarbonate does not enhance

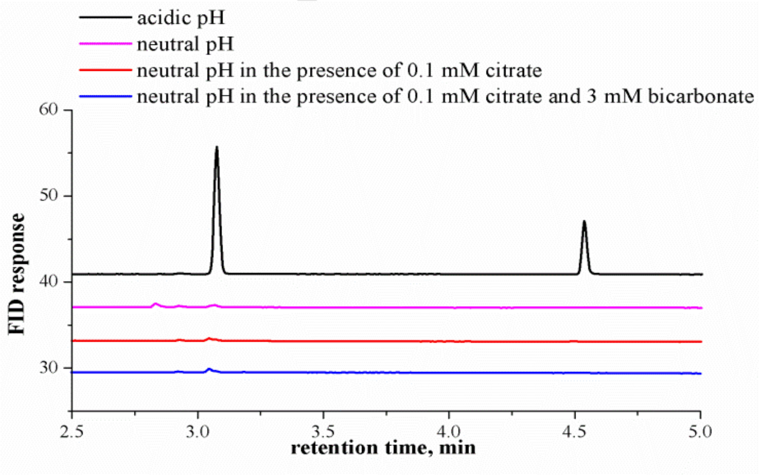

Fig. 4 Methane (t_r = 3.1) and ethane (t_r = 4.5) determination by GC in $(CH_3)_2SO$, 25 mM, containing solutions after Fenton reaction under different conditions. The initial concentrations were $[Fe^{II}]$ = 0.020 mM, $[H_2O_2]$ = 0.39 mM in all of the samples. The pH was 2.2 in the acidic solution, and 7.2 in the neutral solutions.

the methane and ethane formation in the Fenton-like reaction of the Fe^{II}(citrate) complexes, Fig. 4. These experiments point out that there is no $OH^{\bullet}$ radical formation during the Fenton reaction of Fe^{II}(citrate) complexes and in the presence of bicarbonate in these reactions. However, it must be checked whether the active intermediate in the presence of bicarbonate is the $CO_3^{\bullet -}$ anion radical.

3.4. Yields of methane and ethane in γ irradiated solutions

Two experiments to check the nature of the reactive intermediate were performed: 1. The yields of methane and ethane gas are measured after ^{60}Co γ irradiation of samples in the presence of $(CH_3)_2SO$ and citrate. 2. The final organic products after the Fenton reaction under different conditions were determined by ^{1}H NMR analysis.

N_2O saturated solutions containing either 1.0 M citrate or 0.0050 M $(CH_3)_2SO$ or 1.0 M Na_2CO_3 or mixtures of these compounds were irradiated in the ^{60}Co γ source to a dose of 1.0 kGy (This dose produces ca. $6x10^{-4}$ M of radicals in the sample). The yields of methane and ethane obtained in these experiments are summed up in Fig. 5.

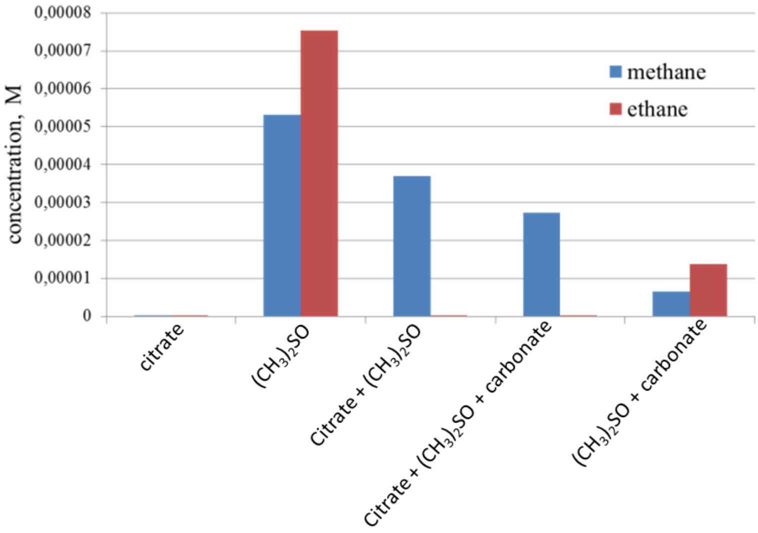

Fig. 5 Methane and ethane determination by GC of $(CH_3)_2SO$, 0.0050 M, citrate, 1.0 M and/or Na_2CO_3, 1.0 M containing N_2O saturated solutions after irradiation by a ^{60}Co γ source, total dose 1.0 kGy. The pH was not adjusted.

In order to analyze the results presented in Fig. 5 one has to note that when N_2O neutral solutions are irradiated the only reactive radical formed is the $OH^{\bullet}$ radical[43].

The $OH^{\bullet}$ radicals thus formed react with the solutes present via reactions (9), (10), (14), and (15):

(14) citrate + $OH^{\bullet} \rightarrow$

$$({}^{-}{}_2OC)CH_2C(OH)(CO_2{}^{-})CH(CO_2{}^{-})^{\bullet}/({}^{-}{}_2OC)CH_2C(O)(CO_2{}^{-})CH_2(CO_2{}^{-})^{\bullet} + H_2O$$

$k_{14} = 5.0\text{x}10^{7}\ \text{mol}^{-1}\text{dm}^{3}\text{s}^{-1}$ [49]

(15) $CO_3{}^{2-} + OH^{\bullet} \rightarrow CO_3{}^{\bullet-} + H_2O$ $\quad k_{15} = 3.0\text{x}10^{8}\ \text{mol}^{-1}\text{dm}^{3}\text{s}^{-1}$ [47]

Thus, at the concentrations used in these experiments $k_9[(CH_3)_2SO] = 3.5\text{x}10^{7}\ \text{s}^{-1}$, $k_{14}[\text{citrate}] = 5.0\text{x}10^{7}\ \text{s}^{-1}$ and $k_{15}[(CO_3{}^{2-}] = 3.0\text{x}10^{8}\ \text{s}^{-1}$. When citrate solutions are irradiated, no methane and ethane are formed as expected. The yields of methane and ethane in the $(CH_3)_2SO$ irradiated solution are as expected according to reactions (11) & (13). The total yield of methyl radicals ($[CH_4] + 2[C_2H_6]$) in the solutions containing both $(CH_3)_2SO$ and citrate is ~19 % of that observed in solutions containing only $(CH_3)_2SO$. This value is considerably smaller than the yield of methyl radicals calculated via $100k_9[(CH_3)_2SO]/(k_9[(CH_3)_2SO] + k_{14}[\text{citrate}]) = 41$ %. The decrease in the yield of methyl radicals *vs.* the calculated one is attributed to reactions (16) and (17).

(16) $CH_3{}^{\bullet} + ({}^{-}{}_2OC)CH_2C(OH)(CO_2{}^{-})CH(CO_2{}^{-})^{\bullet} \rightarrow$

$$({}^{-}{}_2OC)CH_2C(OH)(CO_2{}^{-})CH(CH_3)(CO_2{}^{-})^{\bullet}$$

(17) $CH_3{}^{\bullet} + ({}^{-}{}_2OC)CH_2C(O)(CO_2)CH_2(CO_2{}^{-})^{\bullet} \rightarrow ({}^{-}{}_2OC)CH_2C(OCH_3)(CO_2)CH_2(CO_2{}^{-})^{\bullet}$

The contribution of the latter reactions inhibits the verification of the suggestion that the radicals $({}^{-}{}_2OC)CH_2C(OH)(CO_2{}^{-})CH(CO_2{}^{-})^{\bullet}/({}^{-}{}_2OC)CH_2C(O)(CO_2{}^{-})CH_2(CO_2{}^{-})^{\bullet}$ react with $(CH_3)_2SO$ to form methyl radicals, as suggested above. The yield of methyl radicals, ($[CH_4] + 2[C_2H_6]$), in the solutions containing all three components is ~14.5 % of the value obtained in the $(CH_3)_2SO$ solutions. This value is considerably larger than that calculated via $100k_9[(CH_3)_2SO]/(k_9[(CH_3)_2SO] + k_{14}([\text{citrate}] + k_{15}[CO_3{}^{2-}]) = 9$ %. This is attributed to the reaction (18) that was reported earlier[44].

(18) $CO_3{}^{\bullet-} + (CH_3)_2SO \rightarrow CH_3{}^{\bullet} + CH_3SOO^{-} + CO_3{}^{2-}$ [44]

The same conclusion is supported by the yield of methyl radicals in solutions containing $(CH_3)_2SO$ and carbonate that is ~ 60 % larger than that calculates assuming that all the methyl radicals are formed via reaction (9).

3.5. Analysis of the products of oxidation of $(CH_3)_2SO$ via the Fenton reaction by ^1H NMR

In order to get more information on the oxidation products in these Fenton reactions, the oxidation products of $(CH_3)_2SO$ were determined, the results are summed up in Fig. 6. In the absence of citrate $(CH_3)_2SO_2$ is the main product, as expected[12].

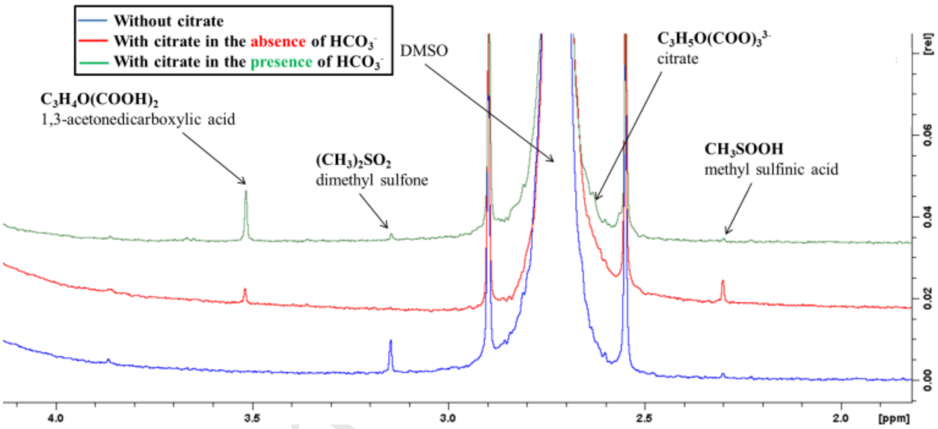

Fig. 6 ^1H NMR spectra after the Fenton reaction in the presence of $(CH_3)_2SO$, 25 mM). The experimental conditions were the following: [citrate] = 0.10 mM, $[HCO_3^-]$ = 0 or 4 mM, $[Fe^{II}]$ = 0.020 mM, $[H_2O_2]$ = 0.39 mM, the pH was 6.4 in the solution without citrate and 7.2 in the solutions with citrate.

When the Fenton reaction is performed in the presence of 0.10 mM citrate, some $(CH_3)SOOH$ and a low yield of beta-ketoglutaric acid (1,3-acetone-dicarboxylic acid) are obtained. But $(CH_3)_2SO_2$ is not formed. Since it is not known that Fe^{IV}(citrate) always oxidizes $(CH_3)_2SO$ to $(CH_3)_2SO_2$ we can not state that Fe^{IV}(citrate) is not formed in the reaction. Maybe Fe^{IV}(citrate) is formed but oxidizes the citrate ligand to beta-ketoglutaric acid with a low yield. However, as proposed above the citrate radicals, $(^-{}_2OC)CH_2C(OH)(CO_2^-)CH(CO_2^-)^{\bullet}/(^-{}_2OC)CH_2C(O)(CO_2^-)CH_2(CO_2^-)^{\bullet}$, probably react with $(CH_3)_2SO$ to form methyl radicals and $(CH_3)SOOH$. When 4.0

mM HCO_3^- is present, the products change again, and the only product observed is beta-ketoglutaric acid in considerably higher yield than in the absence of bicarbonate. As the addition of bicarbonate does not shift anodically the potential of the reactive intermediate formed, this result suggests that the bicarbonate changes the mechanism probably by forming a per-carbonate as the active intermediate.

In order to verify that the formation of the beta-ketoglutaric acid is due only to the presence of bicarbonate and that the $(CH_3)_2SO$ is not involved, the experiment is repeated in the absence of $(CH_3)_2SO$. The results summed up in Fig. 7 clearly points out that indeed the formation of the beta-ketoglutaric acid is due to the presence of bicarbonate.

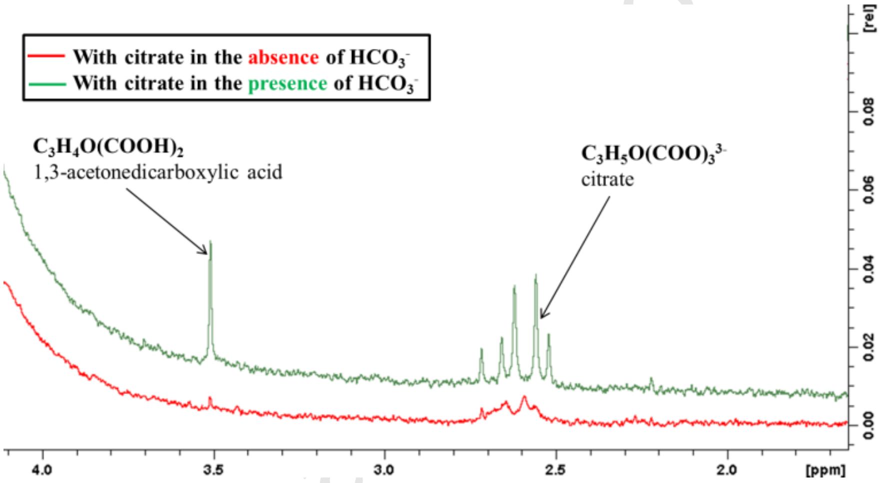

Fig. 7 ^1H NMR spectra after Fenton reaction. The experimental conditions were the following: [citrate] = 0.10 mM, $[HCO_3^-]$ = 0 or 4 mM, $[Fe^{II}]$ = 0.020 mM, $[H_2O_2]$ = 0.39 mM, the pH of the solutions was 7.2.

4. Conclusions

The results obtained in this study point out that the presence of low concentrations of HCO_3^-, *i.e.*, physiological conditions, affect the mechanism and kinetics of the Fenton reaction of Fe^{II}(citrate) dramatically. Under these conditions clearly, $OH^•$ radicals are not formed. $OH^•$, radicals are probably not formed also in this Fenton reaction in the absence of bicarbonate. These results reiterate our conclusion[14] that the source of oxidative stress in biological systems is not hydroxyl radicals.

The results obtained in this study do not enable a definite conclusion concerning the nature of the active oxidizing species formed in this system. As no hydroxyl radicals are formed in the absence of bicarbonate, it is reasonable to suggest that the Fenton reaction in the presence of citrate proceeds via:

(19) $Fe^{II}(citrate)^{-} + H_2O_2 \rightarrow Fe^{IV}(citrate)^{+}{}_{aq} + 2OH^{-}$

Whether one should write $Fe^{IV}(citrate)^{+}{}_{aq}$ or $(citrate)Fe^{IV}{=}O^{-}{}_{aq}$ is not clear at present. In the presence of bicarbonate, the reaction rates increase dramatically; this can, in principle, be due to the following:

1. Reaction 19 is not an outer sphere process [11] and should be written as:

(19) $Fe^{II}(citrate)^{-} + H_2O_2 \leftrightarrows (citrate)Fe^{II}(H_2O_2)^{-} \rightarrow Fe^{IV}(citrate)^{+}{}_{aq} + 2OH^{-}$

and in the presence of bicarbonate:

(20) $Fe^{II}(citrate)(HCO_3)^{2-} + H_2O_2 \leftrightarrows (citrate)Fe^{II}(H_2O_2)(HCO_3)^{2-}$

$\rightarrow Fe^{IV}(citrate)(HCO_3)_{aq} + 2OH^{-}$

and as the bicarbonate/carbonate ligands lower the redox potential of the central cation[50] it accelerates the reaction rate. The mechanism of decomposition of the $Fe^{IV}(citrate)(HCO_3)_{aq}$ thus formed is not clear at present: It can decompose via the formation of $CO_3^{\cdot -}$ or via oxidation of the citrate ligand.

2. Alternatively, the $(citrate)Fe^{II}(H_2O_2)(HCO_3)^{2-}$ formed as an intermediate in reaction (21) can isomerize via:

(21) $(citrate)Fe^{II}(H_2O_2)(HCO_3)^{2-} \rightarrow (citrate)Fe^{II}(CO_4)^{3-} + H_3O^{+}$

in analogy to many earlier reports, see, for example, reference [19]. The $(citrate)Fe^{II}(CO_4)^{3-}$ thus formed is expected to decompose via: [19]

(22) $(citrate)Fe^{II}(CO_4)^{3-} + H_2O \rightarrow Fe^{III}(citrate) + CO_3^{\bullet -} + 2OH^{-}$

The latter mechanism seems to fit better the experimental results, although the other mechanisms cannot be ruled out.

Conflicts of interest

There are no conflicts to declare.

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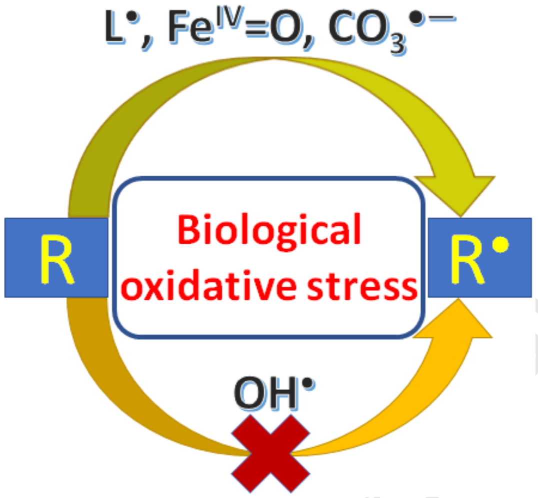
Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Graphical abstract



Synopsis

The kinetics and mechanism of the Fenton reaction of Fe^{II}(citrate) are significantly affected by bicarbonate. The products of oxidation of $(CH_3)_2SO$ by the Fenton reaction are analyzed. Biological oxidative stress is caused by $Fe^{IV}=O$, citrate radical and/or carbonate radical anion ($CO_3^{\bullet-}$) but not by the hydroxyl radical ($OH^{\bullet}$).

Highlights

- The Fenton reaction is crucial in causing oxidative stress under biological conditions.
- The Fenton reaction of Fe^{II}(citrate) in the presence of bicarbonate is studied.
- Bicarbonate affects the mechanism and kinetics of the Fenton reaction dramatically.
- Hydroxyl radicals ($OH^{\bullet}$) are not formed in the Fe^{II}(citrate) Fenton reaction.
- $OH^{\bullet}$ radicals do not initiate oxidative stress in biological systems.