Uranium reactions with hydrogen peroxide studied by EPR–spin trapping with DMPO †

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Uranyl nitrate [$UO_2(NO_3)_2$] (1.0×10^{-3} M) is reacted with hydrogen peroxide (H_2O_2, 5×10^{-3} M) in the presence of the spin trap 5,5-dimethyl-4,5-dihydro-3*H*-pyrrole *N*-oxide (DMPO, 5×10^{-2} M) in acidic solutions. The reaction generates a 1 : 2 : 2 : 1 quartet with hyperfine coupling constants, $a_N = a_H^\beta = 1.50$ mT. These values are consistent with reported values for the DMPO–OH spin adduct. It is possible that the uranous ion (UO^{2+}), which corresponds to U^{IV}, generates hydroxyl radicals ($^{\bullet}OH$) to give the observed DMPO–OH. This is suggested by two observations: (1) the intensity of the EPR spectrum is dependent on pH, reaching a maximum at pH = 0.6, which is consistent with the formation of UO^{2+} from UO_2^{2+} at lower pH values; and (2) the uranyl ion (UO_2^{2+}) corresponds to U^{VI} and cannot be further oxidized. To determine whether $^{\bullet}OH$ radicals generate DMPO–OH, the reaction is carried out in the presence of varying concentrations of ethanol (EtOH). The results indicate that at a low ratio of [EtOH]/[DMPO] the EPR signal corresponds to DMPO–OH, while at a [EtOH]/[DMPO] ratio *ca.* 1 the signal is mixed and equally intense for DMPO–OH and the hydroxyethyl adduct to DMPO (DMPO–EtOH). When the [EtOH]/[DMPO] ratio is *ca.* 10 the EPR signal is mainly that of the DMPO–EtOH adduct. This confirms that uranium reacts with H_2O_2 generating hydroxyl radicals.

Introduction

The isotopic composition of naturally occurring uranium is ^{238}U (99.28%), ^{235}U (0.718%) and ^{234}U (0.0056%). Depleted uranium is obtained as a byproduct in the enrichment process during the production of nuclear fuel. It is also obtained in the recycling of spent fuel. The word depleted refers to depletion of the ^{235}U isotope, thus raising the purity of ^{238}U from 99.28 to 99.8%. Although containing low radioactivity (0.4 μCi g^{-1}), depleted uranium is continuously emitting α-particles, β-particles and γ-rays. In the decay process from ^{238}U to ^{234}U depleted uranium emits two α-particles (≈4.2 MeV). The β (0.076–2.8 MeV) and γ (0.0633–1.001 MeV) components are less significant.[1] Therefore, the toxicity of uranium may originate from two sources, one having a chemical component and the other a radiological component. The toxicity of uranium has been studied since the discovery of nuclear fission.[2–6] These studies investigated the effects of uranium either by inhalation, ingestion or parenteral administrations of uranium compounds ranging from insoluble oxides to soluble salts. There is little or no information of the toxicity of embedded depleted uranium in its metallic form.[7] It is generally assumed that chemical toxicity originates from dissolved uranium. However, radiological toxicity can originate from metallic and dissolved uranium. There are several questions that need to be addressed when uranium is ingested or finds its way into the body in a metallic form: (1) Will this uranium dissolve? (2) Does the oxidation state of uranium play a role in the uranium toxicity? (3) What are the mechanisms of transport to the target tissues? (4) Will the toxicological effects be chemical or radiological in nature?

Uranium's most stable and soluble state is in its sixth and highest oxidation state, U^{VI}. This form of uranium is usually associated with oxygen as the uranyl cation, UO_2^{2+}. However, in solution UO_2^{2+} is usually in equilibrium with small quantities of U^{IV} in the form of the uranous cation, UO^{2+}. The equilibrium can readily be shifted toward UO^{2+} in the presence of acid or a mild reducing agent (sodium hydrosulfite).[8] Due to the reducing environment inside cells, it is possible that U^{IV} as the UO^{2+} cation is the predominant form of ingested or embedded dissolved uranium. Furthermore, the cycling between U^{VI} and U^{IV} may lead to various reactions involving free radical intermediates which may be detrimental to biological systems.

One such reaction is the reaction with hydrogen peroxide (H_2O_2) to yield hydroxyl radicals ($^{\bullet}OH$). Several other metal ions (*e.g.* Fe^{2+} and VO^{2+}) react in this way with H_2O_2 generating $^{\bullet}OH$ in what is known as a Fenton-type reaction.[9] It was of interest to investigate whether uranium reacts in a similar fashion with H_2O_2. The reactions were studied by EPR–spin trapping. Spin trapping is a technique which uses a spin trap, usually a nitrone or nitroso compound, to trap short lived free radicals generating a longer lived aminoxyl ‡ spin adduct which can be measured and identified by EPR. In this work the reaction between uranium and H_2O_2 was studied in the presence of the nitrone 5,5-dimethyl-4,5-dihydro-3*H*-pyrrole *N*-oxide (DMPO).

Materials and methods

Uranyl nitrate [$UO(NO_3)_2$], vanadyl sulfate [$VOSO_4$], ferrous ammonium sulfate [$FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$] and potassium permanganate ($KMnO_4$) were obtained from Fisher Scientific Co. (Fair Lawn, NJ). Sodium oxalate ($Na_2C_2O_4$) was obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ). The concentration of $UO(NO_3)_2$ was determined by phosphorescence using a Kinetic Phosphorescence Analyzer (KPA) (Chemchek, WA).[10] The concentration of VO^{2+} in the $VOSO_4$ solution was determined spectrophotometrically ($\lambda = 750$ nm, $\varepsilon = 18$ M^{-1} cm^{-1}).[11] $KMnO_4$ was standardized against measured quantities of dried $Na_2C_2O_4$. $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$ and hydrogen peroxide (H_2O_2) were titrated with the standard $KMnO_4$ solution to determine their concentration.[12] H_2O_2, sodium hydrosulfite ($Na_2S_2O_4$) and Sephadex DEAE were obtained from Sigma (St. Louis, MO). The spin trap DMPO and nitric acid (HNO_3) were obtained from Aldrich Chemical Co. (Milwaukee, WI). The concentration of DMPO was determined spectro-

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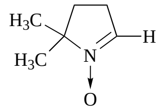
‡ Formerly known as nitroxide.

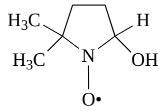
photometrically ($\lambda = 227$ nm, $\varepsilon = 8 \times 10^{-3}$ M^{-1} cm^{-1}).[13] The DMPO was determined free of radical impurities by EPR spectroscopy. Solutions of $UO(NO_3)_2$ (U^{IV}) were prepared by reducing $UO_2(NO_3)_2$ with $Na_2S_2O_4$. The excess $S_2O_4^{2-}$ was removed by addition of the anion exchange resin Sephadex DEAE.

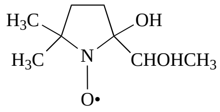
Experiments were carried out under air-saturated conditions, mixing metal ion solutions (UO_2^{2+}/UO^{2+}, VO^{2+} or Fe^{2+}), DMPO and ethanol (when required) immediately prior to addition of the appropriate amount of hydrogen peroxide. Controls containing uranium were also carried out under air-free nitrogen saturated solutions following the procedure described by Russell *et al.*[14] and Evans.[15] The pH of the metal ion solutions were adjusted with HNO_3 prior to addition of other reactants to the mixture. Solutions of $UO(NO_3)_2$ are usually pH 3.6. After addition of the H_2O_2, the samples were rapidly mixed and transferred to an EPR quartz flat cell (60 × 10 × 0.25 nm) and their EPR spectra recorded.

The EPR quartz flat cells were soaked in a concentrated mixture of HNO_3–H_2SO_4 (1:1), to eliminate all metal ion contamination. These were rinsed with deionized water from a Sybron Barnstead NANO pure system. This water was also used to make the various solutions required for the experiments. All other labware used was plastic and required no further treatment.

All EPR spectra were recorded on a Varian E-9 X-band spectrometer at 100 kHz magnetic field modulation. The magnetic field was set at 338.50 mT; microwave power, 10 mW; modulation amplitude, 0.1 mT; microwave frequency, 9.510 GHz; time constant, 0.5 s; scan time, 4 min. The hyperfine coupling constants of the spin adducts (DMPO–OH and DMPO–EtOH)


DMPO


DMPO–OH


DMPO–EtOH

were obtained by computer simulation, generating a theoretical EPR spectrum that matched the experimental spectrum. The chemical structures of DMPO, DMPO–OH and DMPO–EtOH are shown.

Results and discussion

When H_2O_2 is mixed with a $UO_2(NO_3)_2$ solution containing spin trap DMPO, an EPR spectrum consisting of 1:2:2:1 quartet is obtained (Fig. 1). Figs. 1(*a*)–1(*c*) are the control experiments yielding EPR spectra of DMPO in 250 mM HNO_3 [Fig. 1(*a*)], $UO_2(NO_3)_2$ mixed with 250 mM HNO_3 [Fig. 1(*b*)] and H_2O_2 mixed with 250 mM HNO_3 [Fig. 1(*c*)]. The pH of these solutions was 0.6. Fig. 1(*d*) shows the results obtained at pH 0.6 after mixing H_2O_2 (5 mM) with a $UO_2(NO_3)_2$ (1 mM) solution containing DMPO (50 mM). The 1:2:2:1 quartet with hyperfine coupling constants, $a_N = a_H^{\beta} = 1.50$ mT, corresponds to the DMPO–OH spin adduct.[16] The intensity of the EPR spectra from the solutions [Figs. 1(*a*)–1(*c*)] resulted in less than 10% of the intensity observed in Fig. 1(*d*).

The formation of ${}^{\bullet}OH$ in the reaction of uranium ions and hydrogen peroxide can be by three reaction mechanisms: (1) the radiation emitted by uranium (α-particles, γ-rays) reacting with water to yield ${}^{\bullet}OH$ [reaction (1)]; (2) the reaction of uranium

$$UO_2^{2+} \rightsquigarrow \alpha,\gamma \rightsquigarrow H_2O \longrightarrow {}^{\bullet}OH, {}^{\bullet}H, e^-_{aq} \qquad (1)$$

ions with H_2O_2 to yield ${}^{\bullet}OH$ in a Fenton-type reaction [reactions (2a) and (2b)]; and (3) the reaction of U^{VI} in $UO_2(NO_3)_2$

$$UO_2^{2+} + 2H^+ + 2e^- \longrightarrow UO^{2+} + H_2O \qquad (2a)$$

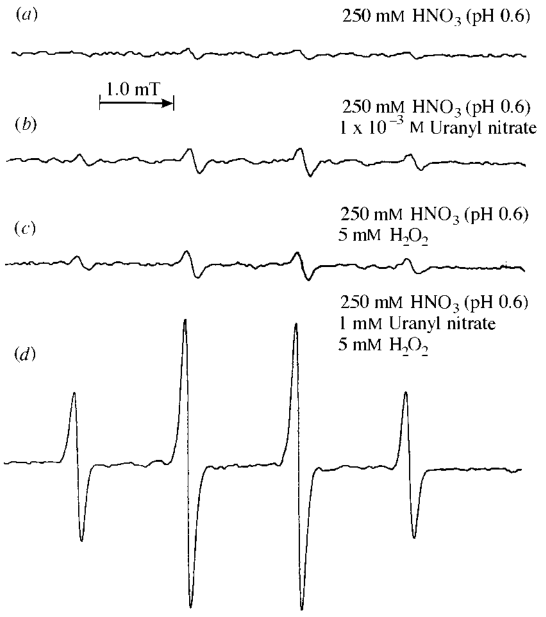


Fig. 1 (*a*)–(*c*) Controls. (*d*) Addition of H_2O_2 to a solution of $UO_2(NO_3)_2$ in the presence of DMPO (50 mM). Receiver gain 1.25×10^4.

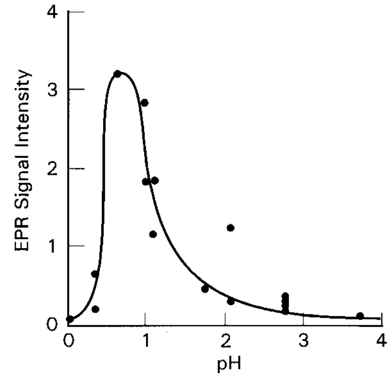


Fig. 2 DMPO–OH EPR signal intensity plotted as a function of pH

$$UO^{2+} + H_2O_2 \longrightarrow UO_2^{2+} + {}^{\bullet}OH + H^+ \qquad (2b)$$

with dissolved oxygen to yield superoxide anion radicals ($O_2^{\bullet -}$) [reaction (3)]. Superoxide reacts directly with DMPO to form an unstable adduct (DMPO–O_2^-) which decomposes forming DMPO–OH.

$$UO_2^{2+} + 4H^+ \longrightarrow U^{5+} + 2H_2O \qquad (3a)$$

$$U^{5+} + O_2 + 2H_2O \longrightarrow UO_2^{2+} + O_2^{\bullet -} + 4H^+ \qquad (3b)$$

Several experiments were conducted to obtain insight on the actual reaction mechanism. Since it is known that the formation of U^{IV} is facilitated at lower pH, the DMPO–OH EPR signal intensity as a function of pH was plotted (Fig. 2). Fig. 2 shows the peak-to-peak heights of the low field EPR line plotted as a function of pH. Provided that the EPR line shape does not change from sample to sample, the peak-to-peak height is proportional to the actual EPR signal intensity. The EPR signal intensity is obtained by double integration of the complete spectrum and is directly proportional to the concentration of the species being measured. Therefore, the peak-to-peak height of an EPR spectrum is also related to the species concentration, providing that the EPR line shapes remain constant from spectrum to spectrum. In addition, it must be noted that there are two competing reactions occurring over the pH range plotted, the formation of DMPO–OH and the decay of the DMPO–OH due to its instability at lower pH values. When the rate of form-

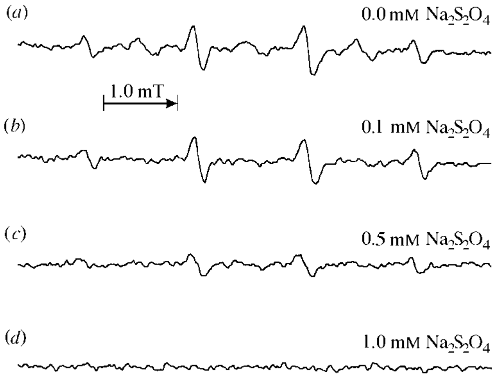


Fig. 3 DMPO–OH EPR at pH 0.6 as a function of varying concentrations of $Na_2S_2O_4$. $UO_2(NO_3)_2$ concentration was 1.0 mM, DMPO concentration was 50 mM, H_2O_2 concentration was 5 mM. Receiver gain 2.5×10^4.

ation of DMPO–OH is faster than the rate of decay, the DMPO–OH EPR signal intensity will increase, however, when the rate of decay is faster than the rate of formation the EPR signal intensity will decrease. For these reasons, the profile (Fig. 2) increases reaching a maximum at pH 0.6, then rapidly decreases due to the short lifetime (instability) of the DMPO–OH adduct in extreme acidic solutions. As the equilibrium between U^{VI} and U^{IV} is shifted toward the formation of U^{IV} in acidic environments, the results suggest that the observed DMPO–OH originates from the reaction of UO^{2+} (U^{IV}) with H_2O_2 to form $^{\bullet}OH$ [reaction (2)]. Furthermore, the oxygen exchange between oxyions and metal ions is faster in acid because of the enhanced reactivity of protonated oxyions.[17] Thus, the rate of formation of hydroxyl radicals should increase with increasing acidity of the solution. This is consistent with the observed increase in the DMPO–OH signal as the pH is lowered (Fig. 2).

The pH profile shown in Fig. 2 virtually eliminates the radiological mechanism shown in reaction (1), which is independent of pH. It would be expected that if the formation of DMPO–OH originates from the $^{\bullet}OH$ generated in the radiation of water, the EPR of DMPO–OH would have equal intensity under any conditions. The emission of α-particles, γ-rays and β-particles is continuously occurring, as part of the natural decay of uranium, and therefore the production of DMPO–OH would remain equal and would not increase as the pH is lowered (Fig. 2). A similar radiological mechanism was also discarded for plutonium. The oxidative damage caused by plutonium was found not to be due to α-particle emission by this element.[18]

The mechanism in reaction (3) is also unlikely for two reasons: (1) the uranyl ion corresponds to the highest oxidation state or uranium (U^{VI}) and cannot transfer an electron directly to oxygen unless the uranium is in a lower oxidation state [reactions (3a) and (3b)]; and (2) the formation of the DMPO–OH adduct occurs significantly only in the presence of H_2O_2. Furthermore, in the control experiments shown [Fig. 1(*b*)] the intensity of the DMPO–OH EPR signal is identical in the presence and absence of oxygen (nitrogen saturated).

This leaves the mechanism in reactions (2a) and (2b) as the likely explanation for the observed formation of the DMPO–OH adduct. Since $UO_2(NO_3)_2$ corresponds to U^{VI}, the highest valence state of uranium, it is not possible for UO_2^{2+} to react directly with H_2O_2 and form $^{\bullet}OH$. For this reason in addition to the pH profile [Fig. 2], the mechanism in reaction (2) was further investigated by reducing the U^{VI} to U^{IV} using $Na_2S_2O_4$ and then reacting the resulting U^{IV} with H_2O_2. $Na_2S_2O_4$ is known to specifically reduce U^{VI} to U^{IV}. The solution first takes on a pink color due to U^{V}, then turns green due to the formation of U^{IV}, at which point there is no further reduction to the lower oxidation states.[8] The purpose of the experiment was to reduce all uranyl ions UO_2^{2+} (U^{VI}) to uranous ions UO^{2+} (U^{IV}) prior to reacting it with hydrogen peroxide in the presence of DMPO. It would be expected that if the formation of DMPO–OH originated from $^{\bullet}OH$ produced in the reaction of UO^{2+} (U^{IV}) with H_2O_2 [reaction (2)], the DMPO–OH EPR signal intensity would significantly increase after addition of $Na_2S_2O_4$. Fig. 3 shows the results after adding $Na_2S_2O_4$ (various concentrations) to solutions containing 1 mM $UO_2(NO_3)_2$ and 50 mM DMPO and reacting these mixtures at pH 0.6 with 5 mM H_2O_2. These results suggest that the $Na_2S_2O_4$ also reduced the aminoxyl to the hydroxylamine, therefore eliminating the EPR signal altogether at higher concentrations of $Na_2S_2O_4$. To verify this, the experiment was repeated in the presence of an anion exchange resin (Sephadex-DEAE) in order to chelate all excess of $S_2O_4^{2-}$. The results in Fig. 4 show that after Sephadex-DEAE treatment of a solution at pH 0.6 containing 1 mM $UO_2(NO_3)_2$, 1 mM $Na_2S_2O_4$ and 50 mM DMPO, and subsequently reacting the supernatant mixture with 5 mM H_2O_2, a significant increase (approximately two-fold) in the DMPO–OH EPR signal intensity was observed [Fig. 4(*b*)]. Fig. 4(*a*) is the control experiment carried out in the absence of $Na_2S_2O_4$. The results in Fig. 4 strongly suggest that the direct reaction of UO^{2+} (U^{IV}) with H_2O_2 is occurring, as shown in reaction (2).

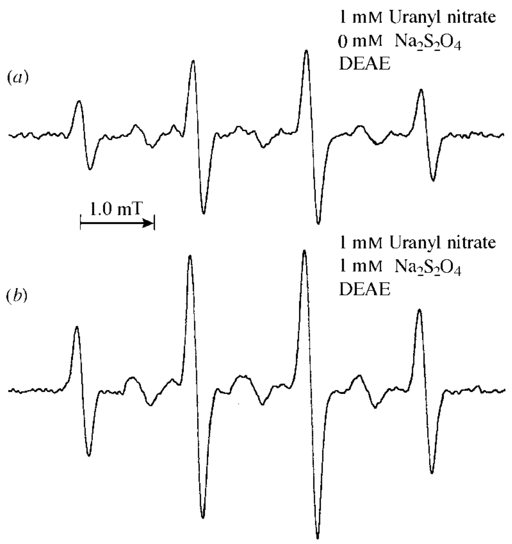


Fig. 4 Sephadex-DEAE treatment of the $UO_2(NO_3)_2$ solutions (1.0 mM, pH 0.6) (*a*) control, without $Na_2S_2O_4$ and (*b*) after addition of $Na_2S_2O_4$ (1.0 mM) and subsequently adding DMPO (50 mM) and H_2O_2 (5 mM) to the supernatant mixture. Receiver gain 2.5×10^4.

A free radical scavenging experiment using ethanol (EtOH) was carried out to verify that the DMPO–OH spin adduct originates from the direct formation of $^{\bullet}OH$ and its reaction with DMPO. EtOH and DMPO react with $^{\bullet}OH$ at approximately equal rates ($k > 10^9$ M^{-1} s^{-1}).[19] The reaction of EtOH with $^{\bullet}OH$ yields the hydroxyethyl radical ($EtOH^{\bullet}$). DMPO competes for the $^{\bullet}OH$ and the $EtOH^{\bullet}$ formed when the reaction between UO^{2+} and H_2O_2 is carried out in the presence of EtOH. Furthermore, the relative EPR intensities of the DMPO–OH and DMPO–hydroxyethyl (DMPO–EtOH) adducts vary depending on the [DMPO]/[EtOH] ratio in the solution. Fig. 5 shows the results obtained after adding 5 mM H_2O_2 to a solution containing 1 mM $UO_2(NO_3)_2$, 50 mM DMPO and varying concentrations of EtOH. The EPR spectra from these solutions change from the DMPO–OH spectrum [Fig. 5(*a*)] to a mixed DMPO–OH/DMPO–EtOH [Fig. 5(*b*)] to predominantly the DMPO–EtOH spectrum [Fig. 5(*c*)]. In Fig. 5(*a*), [EtOH]/[DMPO] = 0. The ratio [EtOH]/[DMPO] ≈ 1 [Fig. 5(*b*)] and [EtOH]/[DMPO] ≈ 10 [Fig. 5(*c*)], respectively. The EPR spectra, consisting of a triplet of doublets [Fig. 5(*b*) and 5(*c*)], correspond to the DMPO–EtOH adduct. This was verified by com-

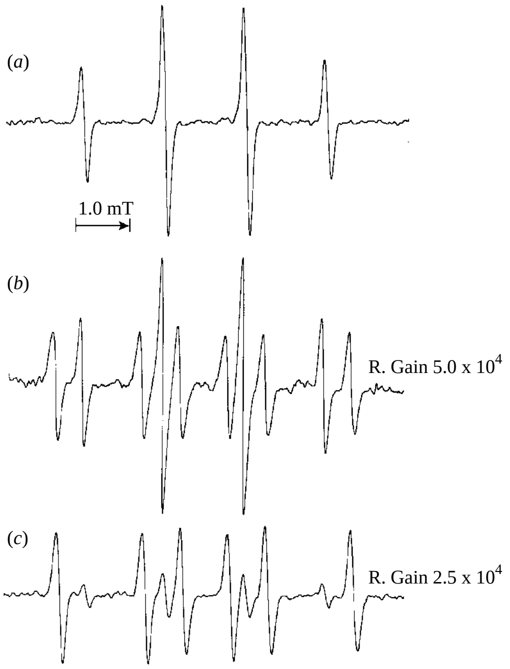


Fig. 5 •OH scavenging experiments at pH 0.6 using varying concentrations of ethanol (EtOH). [DMPO] = 50 mM; (*a*) [EtOH]/[DMPO] = 0; (*b*) [EtOH]/[DMPO] = 1; (*c*) [EtOH]/[DMPO] = 10. $UO_2(NO_3)_2$ and H_2O_2 concentrations were 1.0 mM and 5 mM respectively.

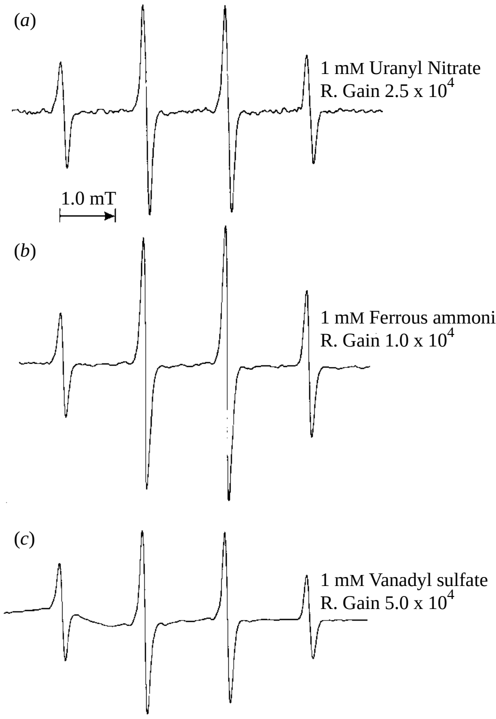


Fig. 6 Capacity of various metal ions to generate •OH at pH 0.6. (*a*) UO_2^{2+}/UO^{2+}; (*b*) Fe^{2+}; (*c*) VO^{2+}. The concentration of metal ions in each solution was 1.0 mM. The final concentration of H_2O_2 added to each solution was 5 mM.

puter simulation using hyperfine coupling constants $a_N = 1.58$ and $a_H^\beta = 2.28$ mT, which correspond to the reported values for the DMPO–EtOH adduct.[16] The results obtained in Fig. 5 confirm that •OH is formed in the reaction between uranium ions and H_2O_2 and this reaction occurs *via* the mechanism described in reactions (2a) and (2b).

It is of interest to determine how the uranium ions compare with other known metal ions that participate in Fenton-type reactions. For this reason uranium was compared with Fe^{2+} and VO^{2+}. The results are shown in Fig. 6. The reactions were carried out at pH 0.6 under the same conditions with regard to metal ion (1 mM) concentration, DMPO (50 mM) and H_2O_2 (5 mM) concentrations. The results show that the relative order of the capacity to generate •OH for the three metal ions is $VO^{2+} > Fe^{2+} > UO^{2+}$. In addition, from the EPR intensities of the DMPO–OH spectra (Fig. 6) VO^{2+} is approximately twice as efficient as Fe^{2+}, which in turn is approximately twice as efficient as UO^{2+} in their capacity to generate •OH in Fenton-type reactions. In a previous report comparing the capacity of VO^{2+} and Fe^{2+} to generate •OH in Fenton-type reactions at pH 5.5, the order was reversed and $Fe^{2+} > VO^{2+}$ by a factor of two.[9]

For biological and toxicological purposes, the knowledge that uranium participates in a Fenton-type reaction is important. Furthermore, the equilibrium between UO_2^{2+} (U^{VI}) and UO^{2+} (U^{IV}) is interesting because it suggests that cycling between U^{VI} and U^{IV} is possible and requires only a reactant such as H_2O_2 to drive the reaction and cycle. Although the valence state of uranium in biological systems remains to be determined, uranium(IV) is the likely species in the cells reducing environment. It must be kept in mind that the uranium reactions described in this work were carried out using solutions of plain inorganic salts in nitric acid. It remains to be determined if this property is true when these metal ions are chelated by complex biological systems and at a higher pH.

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