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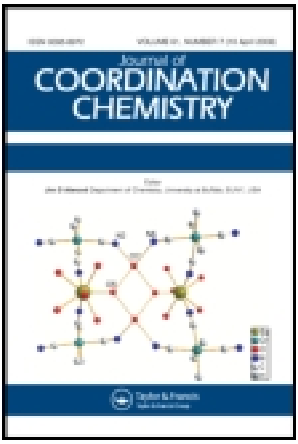

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S. Torres [a], G. Ferraudi [b], N.P. Chandía [a] & B. Matsuhiro [a]
[a] Departamento de Ciencias del Ambiente, Facultad de Química y Biología, Universidad de Santiago de Chile, Av. B. O'Higgins 3363, Santiago, Chile
[b] Department of Chemistry, Radiation Research Building, University of Notre Dame, Notre Dame, IN 46556-0579, USA

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Observations on the mechanisms of the thermal and photoinduced oxidation of D-mannitol and fucoidan by transition metal complexes and inorganic radicals

S. TORRES†, G. FERRAUDI*‡, N.P. CHANDÍA† and B. MATSUHIRO†

†Departamento de Ciencias del Ambiente, Facultad de Química y Biología,
Universidad de Santiago de Chile, Av. B. O'Higgins 3363, Santiago, Chile
‡Department of Chemistry, Radiation Research Building, University of Notre Dame,
Notre Dame, IN 46556-0579, USA

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The mechanism of the D-mannitol and fucoidan oxidation by $^{\bullet}OH$, peroxyl radicals, $PRX^{\bullet} = O_2^{\bullet -}$, $HOCH_2O_2^{\bullet}$, and $CH_2(O_2^{\bullet})CO_2^{-}$, inorganic radicals, $NO_3^{\bullet}$, $Cl_2^{\bullet -}$, and $[Ni^{III}(Me_6\text{-}[14]aneN_4)H_2O]^{3+}$ was investigated by pulse radiolysis. Relative to the diffusion-controlled reaction of the $^{\bullet}OH$ radical, the reactions of $PRX^{\bullet}$, $NO_3^{\bullet}$, and $Cl_2^{\bullet -}$ are slow, with a 10^5–$10^6\,(\mathrm{mol\,L^{-1}})^{-1}\,\mathrm{s^{-1}}$ order of magnitude. The formation of saccharide–Ni(III) complexes also accounts for oxidation of the D-mannitol and fucoidan by $[Ni^{III}(Me_6\text{-}[14]aneN_4)H_2O]^{3+}$. A rate constant $k = 2.2 \times 10^7\,(\mathrm{mol\,L^{-1}})^{-1}\,\mathrm{s^{-1}}$ was estimated for the complexation of $[Ni^{III}(Me_6\text{-}[14]aneN_4)H_2O]^{3+}$ by D-mannitol. In addition, both carbohydrates accelerate the decay of the Ni(III) complex which occurs with half life $t_{1/2} \sim 10^2$ ms. Consistent with the formation of fucoidan–Cu^{2+} complexes, the flash irradiations of these complexes at 351 nm produces transient spectra assigned to Cu_n^{+} ($n = 2, 3$) species. The non-linear dependence of the product concentration on the flash intensity shows that formation of the species is a biphotonic process.

Keywords: D-mannitol; Fucoidan; Copper complexes; Radicals; Photolysis

1. Introduction

Convincing evidence has been presented showing that some oxygen species, e.g., $^{\bullet}OH$, $O_2^{\bullet -}$, and $HO_2^{\bullet}$, play a central role in animal and plant pathogen defenses [1–3]. In animals, production of such species by pathogenic leukocytes is a well-characterized antimicrobial defense mechanism. On the other hand, the microbe's suppression of this defense mechanism involves secretion of enzymes, e.g., superoxide dismutase which traps the reactive oxygen species and converts them to less reactive products.

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*Corresponding author. Email: Ferraudi.1@ND.EDU

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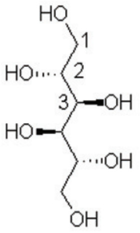
A non-enzymatic mechanism is also possible when substances such as D-mannitol, **1**, are present in the required quantities [4, 5]. Fucoidan, **2**, is another antioxidant which has commanded a great deal of attention as a nutritional and pharmaceutical "miracle drug". It has been communicated that fucoidan induces apoptosis in human lymphoma cell lines [6].



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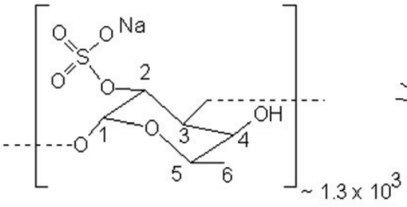

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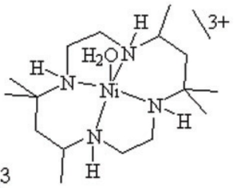

(3)

Some D-mannitol and fucoidan properties are linked to their antioxidant activity and chemical methods, based on H_2O_2 reactions, are used to test the antioxidant activities in a global manner [7]. Because of the biochemical and analytical interests in redox processes of D-mannitol and fucoidan, we have investigated the kinetics and mechanism of their reactions with transition metal complexes and radicals. Reactions much slower than the reaction of the $^{\bullet}OH$ radicals with the carbohydrates were observed when they reacted with oxygen-centered radicals ($PRX^{\bullet} = O_2^{\bullet -}$, $HOCH_2O_2^{\bullet}$, and $CH_2(O_2^{\bullet})CO_2^{-}$), and inorganic oxidants $NO_3^{\bullet}$, $Cl_2^{\bullet -}$, and $[Ni^{III}(Me_6\text{-}[14]aneN_4)H_2O]^{3+}$ (**3**). Moreover, a biphotonic process of fucoidan–Cu^{2+} complexes produced unstable Cu clusters, Cu_n^{+} ($x = 2, 3$) when it was irradiated at 351 nm.

2. Experimental

2.1. *Pulse radiolysis procedures*

The instrument for the pulse radiolysis experiments and the computerized data collection for time-resolved UV-Vis spectroscopy and reaction kinetics have been described [8]. The solutions were saturated with streams of O_2-free N_2O or with the mixtures of O_2 and N_2O required for the experiment. Partial pressures of O_2 and N_2O in the gas mixtures were used for the estimation of the dissolved O_2 concentration. The oxidants $O_2^{\bullet -}$ [9–12], $HOCH_2O_2^{\bullet}$ [13], $CH_2(O_2^{\bullet})CO_2^{-}$ [14], $Cl_2^{\bullet -}$ [15], $NO_3^{\bullet}$ [16], and $[Ni^{III}(Me_6\text{-}[14]aneN_4)OH]^{2+}$ [17, 18] were pulse radiolytically generated following specific procedures in the literature. Experimental conditions were optimized on the basis of the reaction kinetics communicated in the literature for the radicals generated in this study [9–17]. The concentrations of D-mannitol and fucoidan were chosen to prevent reactions between carbohydrates and the radiolytically generated radicals, $^{\bullet}OH$, $H^{\bullet}$, and e_{sol}^{-} used for preparation of the oxidants. The concentrations and other experimental conditions of the pulse radiolysis experiments are communicated together with the results.

2.2. *Photochemical procedures*

Absorbance changes, ΔA, occurring in a time scale longer than 15 ns were investigated at room temperature with a flash photolysis apparatus described elsewhere [19]. In these experiments, 15-ns flashes of 351-nm light were generated with a Lambda Physik SLL-200 excimer laser. The intensity of the laser flash was attenuated to values equal to or less than 35 mJ by absorbing some of the laser light in a filter solution of $Ni(ClO_4)_2$ having the desired optical transmittance, $T = I_t/I_0$ where I_0 and I_t are, respectively, the intensities of the light arriving to and transmitted from the filter solution. The transmittance, $T = 10^{-A}$, was routinely calculated using the spectrophotometrically measured absorbance, A, of the filter solution. A right angle configuration was used for the pump and the probe beams. Concentrations of the chromophores in solution were adjusted to provide homogeneous concentrations of photogenerated intermediates over the optical path, $l = 1$ cm, of the probe beam. To satisfy this optical condition, solutions were made with an absorbance equal to or less than 0.1 over the 0.2-cm optical path of the pump. These solutions were deaerated with streams of N_2 before and during the photochemical experiments. Other conditions for these measurements are given in section 3.

2.3. *Materials*

D-mannitol was obtained by ethanol extraction of the brown seaweed *Lessonia vadosa* [20]. Fucoidan was extracted from the brown seaweed *L. vadosa* with a 2% aqueous solution of $CaCl_2$. The structural characterization has been published elsewhere [21]. Other materials were of reagent grade and used without purification.

2.4. *Data treatment*

The reaction kinetics were investigated by following the absorbance change at given wavelengths of the spectrum and incorporating those changes in $\xi = (\Delta A_{inf} - \Delta A_t)/(\Delta A_{inf} - \Delta A_0)$ [22]. In the expression of the dimensionless parameter ξ, ΔA_0 is the absorbance change at the beginning of the reaction, ΔA_t is determined at an instant t of the reaction and ΔA_{inf} is determined at the end of the reaction. Values of ξ were fitted to the integrated rate law by a non-linear least squares method.

To correct for diminished concentration of fucoidan after oxidation by the $^\bullet OH$ radical, a term $(A(\lambda)_0/[\text{fucoidan}]_0)$ [fucoidan] was added to the absorbance change, ΔA, of the difference spectrum. In this term $[\text{fucoidan}]_0$ and $A(\lambda)_0$ are, respectively, the concentration of fucoidan and the absorbance of the solution at the monitoring wavelength λ before the irradiation. The concentration of fucoidan remaining after the reaction with $^\bullet OH$ radical was estimated as $[\text{fucoidan}] = [\text{fucoidan}]_0 - [^\bullet OH]_{pulse}$ and the concentration $[^\bullet OH]_{pulse}$ produced by the irradiation was obtained from the dose.

3. Results and discussion

Before studying the responses of D-mannitol and fucoidan to the thermal and photochemical oxidations by transition metal complexes, it was necessary to investigate their reactions with simpler oxidants, i.e., $^{\bullet}$OH, PRX$^{\bullet}$, $NO_3^{\bullet}$, and $Cl_2^{\bullet-}$ radicals, using pulse radiolysis. Once the unstable products of the reactions of the radicals with the carbohydrates were observed, the reactions of the carbohydrates with a Ni(III) complex, **3**, and Cu^{2+} ions could be understood. To emphasize that the oxidation reactions produce dehydrogenation of the alcohol groups in D-mannitol and fucoidan, the corresponding radicals will be represented with a subscript –H in the formulas, i.e., D-mannitol$_{-H}$• and fucoidan$_{-H}$•.

3.1. *Oxidation of D-mannitol and fucoidan by PRX$^{\bullet}$, $NO_3^{\bullet}$, and $Cl_2^{\bullet-}$ radicals*

The reaction of the pulse radiolysis generated $^{\bullet}$OH radicals in a N_2O-saturated solution of $10^{-2}\,mol\,L^{-1}$ D-mannitol generates a transient spectrum with $\lambda_{max} \leq 250\,nm$ (figure 1). Moreover, the rate constant for the generation of the transient spectrum agreed within 10% with the value $1.7 \times 10^9\,(mol\,L^{-1})^{-1}\,s^{-1}$ in the literature for $^{\bullet}$OH reaction with mannitol [23]. The featureless transient spectrum in figure 1 is similar to those of the $RC^{\bullet}HOH$ and $R_2C^{\bullet}OH$ radicals produced by oxidation of primary and secondary alcohols [24]. Because of this similarity of spectral features and that no other C-centered radicals have absorption bands in this wavelength region, the attack of the radiolytically generated radicals must therefore produce hydroxyalkyl radical centers, $>C^{\bullet}OH$ and $–C^{\bullet}HOH$, in D-mannitol and the saccharide units of fucoidan. Based on the values in the literature for the rate constants of $^{\bullet}$OH radicals with alcohols [25], one could expect that in D-mannitol, the $>C^{\bullet}OH$ and $–C^{\bullet}HOH$ will be produced in a 1 : 4 molar relationship.

The decay of the transient spectrum was followed at $\lambda_{ob} = 250\,nm$, where it was verified that the reaction takes place in a single step. Plots of the inverse of the

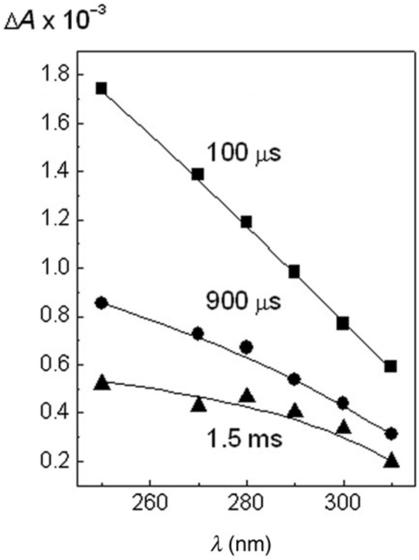

Figure 1. Decay of the transient spectra observed when radiolytically generated $^{\bullet}$OH radicals react with D-mannitol. The decay of the D-mannitol radicals is kinetically second order with $2k/\varepsilon = 2.5 \times 10^5\,cm\,s^{-1}$.

absorbance change, $1/\Delta A$, *versus* time were linear making the process appear as a reaction that is second order on concentration of D-mannitol radicals. From the oscillographic traces recorded with $\lambda_{ob} = 250$ nm, $t_{1/2} = 2.8 \times 10^{-4}$ s was calculated for the decay of the absorbance associated with D-mannitol$_{-H}$• radicals from the initial value $\Delta A_0 \sim 3.5 \times 10^3$. The process can be represented by equation (1), where the products are the result of reactions where the D-mannitol$_{-H}$• radicals disproportionate and/or dimerize.

$$\text{D-mannitol}_{-H}\bullet + \text{D-mannitol}_{-H}\bullet \rightarrow \text{products} \tag{1}$$

In contrast to the reaction of $^{\bullet}$OH radicals with D-mannitol, the reaction with fucoidan exhibited a more complex mechanism, consistent with the polyelectrolyte nature of fucoidan. To record the spectra of the radicals produced by $^{\bullet}$OH oxidation of fucoidan, a N_2O-saturated solution containing $1.5 \times 10^{-5}\,\text{mol}\,\text{L}^{-1}$ polysaccharide ($\sim 10^{-2}\,\text{mol}\,\text{L}^{-1}$ repeating units) was pulse radiolyzed. Changes in the solution's absorption spectrum were investigated in a 1.0 µs to 0.1 s time scale. The radicals produced by $^{\bullet}$OH reaction with fucoidan were formed in a time scale shorter than 1 µs, consistent with a diffusion-controlled rate of reaction (figure 2 and equation 2).

$$\begin{aligned} &^{\bullet}\text{OH} + \text{fucoidan} \rightarrow \text{fucoidan}_{-H}\bullet + H_2O \\ &k > 10^9\ (\text{molL}^{-1})^{-1}\,\text{s}^{-1} \end{aligned} \tag{2}$$

$$\text{fucoidan}_{-H}\bullet \rightarrow \text{fucoidan}_{-H}\bullet' \, k \sim 5 \times 10^3\,\text{s}^{-1} \tag{3}$$

In a longer time domain of 1 µs to 4 ms, changes in the shape of the difference spectrum are consistent with a biphasic decay of the spectrum. The biphasic decay of the spectrum can also be clearly seen in the oscillographic trace in the inset to figure 2 which shows the decay of the 245 nm absorbance where the progressive appearance of a

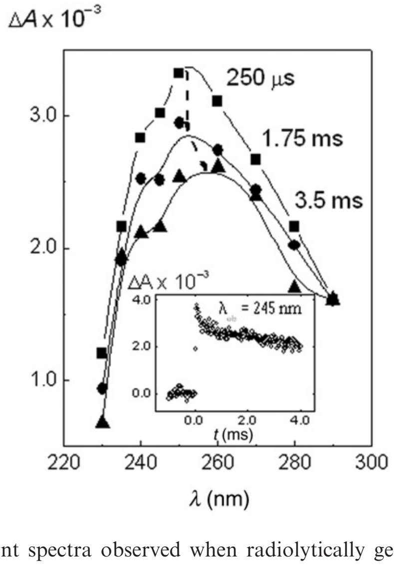

Figure 2. Decay of the transient spectra observed when radiolytically generated $^{\bullet}$OH radicals react with fucoidan. The trace in the inset shows the conversion between fucoidan radicals with $k = 5 \times 10^3\,\text{s}^{-1}$. The dotted arrows emphasize the time-dependent displacement of the maximum.

shoulder can be seen in the spectra. To rationalize these experimental observations, it is necessary to accept that the first radicals produced in the oxidation of fucoidan, denoted as fucoidan$_{-\mathrm{H}^\bullet}$ in equation (2), are converted to radicals denoted as fucoidan$_{-\mathrm{H}^{\bullet\prime}}$ in equation (3).

Based on the independence of the rate of equation (3) on the fucoidan$_{-\mathrm{H}^\bullet}$ concentration and on the good fit of oscillographic traces to a single exponential, it was concluded that equation (3) is first order in radical concentration with a rate constant $k = 5 \times 10^3\,\mathrm{s}^{-1}$. Decay of the fucoidan$_{-\mathrm{H}^{\bullet\prime}}$ radicals, equation (4), takes place in a time scale, i.e., $t > 1 \times 10^{-2}\,\mathrm{s}$, much longer than the formation of the fucoidan$_{-\mathrm{H}^{\bullet\prime}}$.

$$\begin{gathered}\text{fucoidan}_{-\mathrm{H}^{\bullet\prime}} \rightarrow \text{products} \\ t_{1/2} \gg 1 \times 10^{-2}\,\mathrm{s}\end{gathered} \tag{4}$$

A semiquantitative correction of the spectrum in figure 2 for the bleaching of the solution due to the disappearance of fucoidan *via* equation (1) resulted in spectra with maxima at $\lambda_{\max} \leq 250\,\mathrm{nm}$ that resemble the spectra of hydroxyalkyl radicals (figure 1) [8]. Therefore, it is possible to say that fucoidan$_{-\mathrm{H}^\bullet}$ and fucoidan$_{-\mathrm{H}^{\bullet\prime}}$ are radicals resulting from oxidations centered at carbons 1, 2 and 4 of **2**. On the basis of this assignment, two different mechanisms explain the observed ~10-nm bathochromic shift occurring between 1 μs and 1 ms in figure 2. In one mechanism, equation (3) can be the slow reorganization of the polysaccharide morphology induced by oxidation of reactive centers in the chain. To account for changes in the spectrum, this rearrangement changes the solvation of the $>\mathrm{C}^\bullet\mathrm{OH}$ and $-\mathrm{C}^\bullet\mathrm{HOH}$ radical centers. Indeed, the displacement of the absorbance maximum is consistent with solvent-induced changes of the electronic spectrum. A different mechanism assumes the formation of both fucoidan$_{-\mathrm{H}^\bullet}$ and fucoidan$_{-\mathrm{H}^{\bullet\prime}}$ radicals in the initial $^\bullet$OH attack of fucoidan. The initial concentration of fucoidan radicals will be determined by the rate of the $^\bullet$OH attack of the most accessible positions in the helical structure of fucoidan. Because these reactions are irreversible, the radical concentrations of the fucoidan radicals will be determined by the respective formation rate constants. They will not correspond to concentrations based on the thermodynamic equilibrium. These initial concentrations will be subsequently transformed to the concentrations dictated by the thermochemical equilibrium. In this mechanism, the observed ~10-nm shift of the absorption spectrum maximum must be attributed to structural differences between fucoidan$_{-\mathrm{H}^\bullet}$ and fucoidan$_{-\mathrm{H}^{\bullet\prime}}$ radicals that are reflected in the absorption spectrum. For example, the difference in the spectra of primary and secondary hydroxyalkyl radicals [24–27] which are radicals serving as models of the fucoidan$_{-\mathrm{H}^\bullet}$ and fucoidan$_{-\mathrm{H}^{\bullet\prime}}$ radicals.

In contrast to the reactions of D-mannitol and fucoidan with $^\bullet$OH radicals, their reactions with PRX$^\bullet$, $\mathrm{NO}_3^\bullet$, and $\mathrm{Cl}_2^{\bullet-}$ radicals (table 1) are far from diffusion-controlled processes. Solutions containing a high concentration of O_2 were used for the pulse radiolysis generation of the PRX$^\bullet$ radicals in concentrations between 10^{-5} and $10^{-6}\,\mathrm{mol\,L^{-1}}$. Concentrations of D-mannitol and the repeating units of fucoidan were varied between 10^{-1} and $10^{-2}\,\mathrm{mol\,L^{-1}}$ for these studies. The calculated rate constants of the reactions of PRX$^\bullet$ with D-mannitol, equation (5), are in the range $k \sim 10^5$–10^6 $(\mathrm{mol\,L^{-1}})^{-1}\,\mathrm{s}^{-1}$ and are very close to those of fucoidan.

$$\text{D-mannitol} + \mathrm{PRX}^\bullet \rightarrow \text{D-mannitol}_{-\mathrm{H}^\bullet} + \text{PRX-H} \tag{5}$$

Table 1. A comparison of the rate constants of the reactions of OCR$^\bullet$ and inorganic radicals with D-mannitol, fucoidan, and ascorbate.

	$k/10^5$ ((mol L^{-1})$^{-1}$ s^{-1})				
	$O_2^{\bullet -}$	$HOCH_2O_2^\bullet$	$CH_2(O_2^\bullet)CO_2^-$	$NO_3^\bullet$	$Cl_2^{\bullet -}$
D-mannitol	3.0	3.0	1.7	67	3.1
Fucoidan	8.6	–	–	1.4	~5
Ascorbate [10]	2.7 (pH = 7.4)	47 (pH = 7)	22 (pH = 7)	–	600 (pH = 2)
(or ascorbic acid)	0.16 (pH = 1)				

Moreover, the values of the rate constants in table 1 show that with the exception of the reaction of D-mannitol and fucoidan with $O_2^{\bullet -}$, their reactions with PRX$^\bullet$, $NO_3^\bullet$, and $Cl_2^{\bullet -}$ radicals are slower than the reactions of these radicals with ascorbic acid.

Because the pulse radiolysis generation of the perhydroxyl radicals took place in solutions containing a high concentration of oxygen, the D-mannitol$_{-H}$• radicals produced in these reactions, equation (5), reacted with O_2, equation (6).

$$\begin{aligned}&\text{D-mannitol}_{-H}\bullet \xrightarrow{O_2} \text{D-mannitol-}O_2^\bullet \rightarrow \text{products}\\ &k = 3.0\times 10^7 (\text{mol L}^{-1})^{-1}\,\text{s}^{-1}\end{aligned} \tag{6}$$

In this reaction with O_2, the concentration was several orders of magnitude larger than the concentration of D-mannitol$_{-H}$• radicals and equation (6) was kinetically pseudo first-order on D-mannitol$_{-H}$• radical concentration. A second-order rate constant, $k = 3.0\times 10^7$ (mol L^{-1})$^{-1}$ s^{-1}, was calculated dividing the pseudo first-order rate constant by the estimated concentration of O_2 (2.6×10^{-4} mol L^{-1} at 760 Torr and 298 K).

3.2. *Thermal redox reactions of D-mannitol and fucoidan with $[Ni^{III}(Me_6\text{-}[14]aneN_4)OH]^{2+}$*

When transition metal complexes oxidize the carbohydrates, an inner sphere reaction mechanism may exist where the carbohydrate coordinates to the oxidant. Carbohydrates are known to form complexes with diverse substrates, which include transition metal complexes. To investigate the formation of these intermediate complexes, D-mannitol and fucoidan were, respectively, oxidized with a Ni(III) complex, **3**. Pulse radiolytically generated $^\bullet$OH radicals were used for oxidation of $[Ni^{II}(Me_6\text{-}[14]aneN_4)]^{2+}$. No association between D-mannitol and $[Ni^{II}(Me_6\text{-}[14]aneN_4)]^{2+}$ could be discerned from the UV-Vis spectrum of solutions containing millimolar concentrations of both species. Transient spectra were recorded when $^\bullet$OH radicals reacted with the Ni(II) complex in two N_2O-saturated solutions (figure 3). One solution, (a), used as a blank, which contained 10^{-2} mol L^{-1} $[Ni^{II}(Me_6\text{-}[14]aneN_4)]^{2+}$ and the other, (b), contained 10^{-2} mol L^{-1} $[Ni^{II}(Me_6\text{-}[14]aneN_4)]^{2+}$ together with 10^{-4} mol L^{-1} D-mannitol. The first product is $[Ni^{III}(Me_6\text{-}[14]ane\ N_4)OH]^{2+}$, rapidly produced in both solutions by $^\bullet$OH oxidation of the Ni(II) complex, equation (7).

$$[Ni^{II}(Me_6\text{-}[14]aneN_4)]^{2+} + {}^\bullet OH \rightarrow [Ni^{III}(Me_6\text{-}[14]aneN_4)OH]^{2+} \tag{7}$$

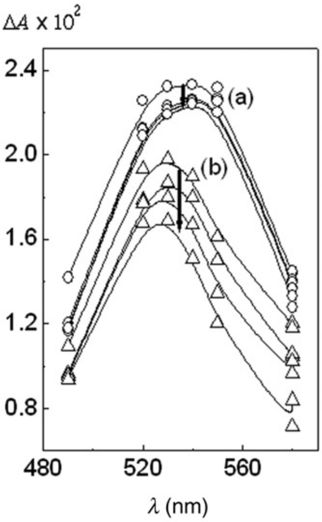

Figure 3. Transient spectra of the Ni(III) complex generated when $^{\bullet}$OH radicals react with $[Ni^{II}(Me_6\text{-}[14]aneN_4)]^{2+}$ (a) and with $[Ni^{II}(Me_6\text{-}[14]aneN_4)]^{2+}$ in the presence of D-mannitol (b). Delays with respect to the radiolysis pulse in (a) are: 10 µs, 70 µs, 155 µs, and 10 ms from top to bottom curve and in (b): 15 µs, 125 µs, 200 µs, and 2 ms from top to bottom curve. The arrows indicate the sense of the spectroscopic change with the delay.

Concentrations of $[Ni^{II}(Me_6\text{-}[14]aneN_4)]^{2+}$ and D-mannitol used in the experiment were calculated on the basis of the rate constants of the respective reactions with $^{\bullet}$OH radicals. These concentrations prevented $^{\bullet}$OH radicals reacting with D-mannitol in the presence of the Ni(II) complex. The recorded spectrum of $[Ni^{III}(Me_6\text{-}[14]aneN_4)OH]^{2+}$ was in good agreement with those reported in the literature [17, 18]. A 10-nm ipsochromic shift and a smaller absorbance at λ_{max} are seen in the maximum of the spectrum of the Ni(III) recorded in the presence of D-mannitol *versus* the spectrum recorded in the absence of D-mannitol (figure 3). Initial changes in the absorption spectrum are consistent with transformation of $[Ni^{III}(Me_6\text{-}[14]aneN_4)OH]^{2+}$ to $[Ni^{III}(Me_6\text{-}[14]aneN_4)H_2O]^{3+}$. Moreover, oscillographic traces collected at different monitoring wavelengths, $\lambda_{ob} = 490$, 560, and 580 nm, were fitted to a biexponential, i.e., $\Delta A = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2)$, where $\tau_1 = 45\,\mu s$ and $\tau_2 = 480\,\mu s$. The first step of the Ni(III) transformation was observed in both solutions. It has been ascribed in previous reports to the protonation of the OH^- ligand, equation (8), with a diffusion-controlled rate [17].

$$[Ni^{III}(Me_6\text{-}[14]aneN_4)OH]^{2+} \xrightarrow{H^+,\,\tau=45\,\mu s} [Ni^{III}(Me_6 - [14]aneN_4)H_2O]^{3+} \quad (8)$$

The second step of the Ni(III) transformation, namely the shift in the spectrum of the Ni(III) complex, was only observed when D-mannitol was present. This is associated with substitution of the H_2O by D-mannitol, equation (9), prior to the decay of the Ni(III) moiety.

$$\text{D-mannitol} + [Ni^{III}(Me_6\text{-}[14]aneN_4)H_2O]^{3+} \xrightarrow{\tau=480\,\mu s} [Ni^{III}(Me_6\text{-}[14]aneN_4)(\text{D-mannitol})]^{3+} \quad (9)$$

Similar complexation reactions of metal ions by carbohydrates are known and have been used as an analytical tool [27, 28]. On the basis of the D-mannitol concentration, a rate constant, $k = 2.2 \times 10^7 (\mathrm{mol\,L^{-1}})^{-1}\,\mathrm{s^{-1}}$, was estimated for the complexation reaction. In addition, oxidation of D-mannitol by the Ni(III) complex accelerates the decay of the latter, i.e., half-life periods $t_{1/2} \sim 90\,\mathrm{ms}$ and $t_{1/2} \geq 120\,\mathrm{ms}$ of the Ni(III) species were estimated in the presence and the absence of D-mannitol. A reaction, equation (10), faster than the bimolecular decay of $[\mathrm{Ni^{III}(Me_6\text{-}[14]aneN_4)H_2O}]^{3+}$ [17, 18] explains the accelerated decay observed in the presence of D-mannitol.

$$[\mathrm{Ni^{III}(Me_6\text{-}[14]aneN_4)(D\text{-}mannitol)}]^{3+} \xrightarrow{t_{1/2}=90\,\mathrm{ms}} [\mathrm{Ni^{II}(Me_6\text{-}[14]aneN_4)}]^{2+} + \cdots \qquad (10)$$

Because oxidation of the alcohol groups to produce alkoxy radicals, $>\mathrm{CHO^{\bullet}}$ and $-\mathrm{CH_2O^{\bullet}}$ by Ni(III) is improbable, equation (10) must have a complex mechanism.

Decay of the radiolytically generated Ni(III) in the presence of fucoidan exhibited minor differences with the decay observed in the presence of D-mannitol. The reactions of the Ni(III) complex with fucoidan were investigated in a N_2O-saturated solution containing $10^{-2}\,\mathrm{mol\,L^{-1}}$ $[\mathrm{Ni^{II}(Me_6\text{-}[14]aneN_4)}]^{2+}$ and $8 \times 10^{-7}\,\mathrm{mol\,L^{-1}}$ ($\sim 1.3 \times 10^{-3}\,\mathrm{mol\,L^{-1}}$ repeating units) fucoidan. Compared with the reactions of D-mannitol, the formation of the complex with fucoidan is slightly slower, $\tau \sim 290\,\mu\mathrm{s}$, corresponding to a second-order rate constant $k = 3.4 \times 10^6\,\mathrm{mol\,L^{-1-1}\,s^{-1}}$ when the pseudo first-order rate constant is normalized to the concentration of repeating units. The decay of the Ni(III) complex in the presence of fucoidan takes place with $t_{1/2} \sim 56\,\mathrm{ms}$.

3.3. *Photoinduced redox reactions of a fucoidan–Cu^{2+} complex*

The previous observations show that oxidations of carbohydrates with powerful oxidants are not necessarily fast reactions. In contrast, the photochemical oxidation of the carbohydrates can be achieved with weaker and/or sluggish oxidants. If the carbohydrate coordinates to the metal ion, ligand-to-metal charge transfer excited states can be used to photoinduce such oxidation. The formation of a fucoidan–Cu^{2+} complex was confirmed by the UV-Vis absorption spectrum of a solution containing $8 \times 10^{-7}\,\mathrm{mol\,L^{-1}}$ ($\sim 1.0 \times 10^{-3}\,\mathrm{mol\,L^{-1}}$ repeating units) fucoidan and $10^{-3}\,\mathrm{mol\,L^{-1}}$ $Cu(ClO_4)_2$. By contrast to the formation of the fucoidan–Cu^{2+} complex, the UV-Vis spectrum of solutions containing millimolar concentrations of D-mannitol and Cu^{2+} did not show formation of complexes in agreement with the poor coordinating ability of D-mannitol toward Cu^{2+} [27]. Coordination of Cu(II) to the alcohol groups of fucoidan must be assisted therefore by the sulfate groups of fucoidan. The electrostatic attraction between the polysaccharide highly charged anionic chains and Cu(II) must be one stabilizing factor in the formation of the complexes.

Photoredox reactions of the fucoidan–Cu^{2+} complex show also the polyelectrolyte nature of the complex. Flash irradiation at 351 nm of deaerated solutions containing $8 \times 10^{-7}\,\mathrm{mol\,L^{-1}}$ ($\sim 1.0 \times 10^{-3}\,\mathrm{mol\,L^{-1}}$ repeating units) fucoidan and $10^{-3}\,\mathrm{mol\,L^{-1}}$ $Cu(ClO_4)_2$, $Cu(F_4B)_2$, or $CuSO_4$ generate a transient spectrum with an intense absorption band in the 600–700 nm region (figure 4), which cannot be attributed to Cu(I) complexes, e.g., Cu_2^{2+} ions, or complexes with Cu–C bonds. Moreover, a quadratic dependence of the photogenerated concentration of the transient on the light

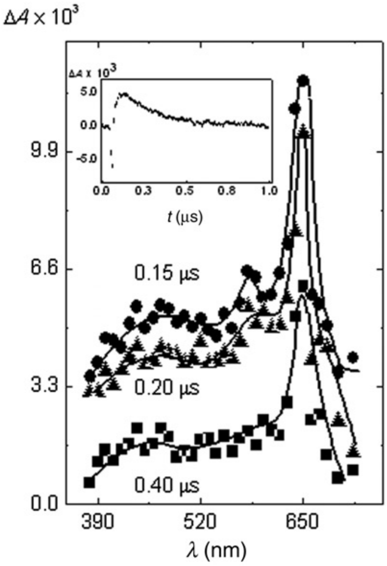

Figure 4. Transient spectra recorded after the 351-nm flash irradiation of a de-aerated solution containing 8×10^{-7} mol L^{-1} (~1.3×10^{-3} mol L^{-1} repeating units) fucoidan and 10^{-3} mol L^{-1} Cu(ClO$_4$)$_2$ with an integrated pulse intensity ~25 mJ. The delays for the 15-ns flash are indicated in the figure. The inset is an average of 20 oscillographic traces recorded at $\lambda_{ob} = 650$ nm with an integrated pulse intensity ~20 mJ.

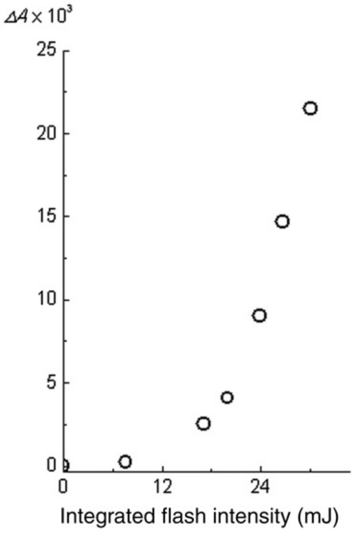

Figure 5. Dependence of the 650 nm absorbance change on the integrated flash intensity. The absorbance change was recorded with a 50-ns delay from the laser flash.

intensity (figure 5) reveals that the species photogeneration occurs following the sequential absorption of two photons. Transient Cu_n^+ ($n \geq 2$) species with absorption bands in the vis–NIR (NIR, near-infrared) region are known to be formed when Cu_2^{2+} ions are reduced, for example, by solvated electron [29, 30]. We propose that related species, i.e., Cu_n^+ $n \leq 3$, are formed as a consequence of the biphotonic process. The formation of Cu_n^+ can be explained on the basis that not all the photogenerated Cu(I), equation (11), is consumed *via* equation (12). Curly brackets are used in the equations to indicate which species remain around the polyelectrolyte chain.

$$\{\text{fucoidan}, Cu^{2+}\} \xrightarrow{h\nu} \{\text{fucoidan}^{\bullet+}, Cu^{+}\} \tag{11}$$

$$\{\text{fucoidan}^{\bullet+}, Cu^{+}\} \xrightarrow{h\nu} \{\text{fucoidan}^{2+}, Cu^{0}\} \tag{12}$$

$$\{\text{fucoidan}^{2+}, Cu^{0}\} \underset{\leftarrow}{\xrightarrow{Cu^{+}}} \{\text{fucoidan}^{2+}, Cu_2^{+}\} \tag{13}$$

In another step of the mechanism, the Cu(I) not consumed in equation (12) remains in and/or nearby the oxidized fucoidan chain reacting later with Cu(0), equation (13).

It was estimated on the basis of the oscillographic traces that the formation of Cu_n^+ species *via* equation (12) take place with a lifetime, $\tau < 10$ ns. Formation of the Cu_n^+ species is therefore fast, i.e., in a period equal to or less than 30 ns. The decay of the Cu_2^+ spectrum takes place with a lifetime $\tau = 250$ ns and without the formation of metallic copper. Also, no metallic copper was produced when a similar solution was irradiated with ~300 laser flashes having each ~25 mJ, i.e., the power required for driving the biphotonic process. These experimental observations point to the conclusion that the Cu_2^+ species formed in equations (11)–(13) reduce the solvent or are transformed to stable complexes of Cu(I) within the oxidized fucoidan chain. It is possible, for example, that Cu^0, equations (12) and (13), can be transformed *via* equation (14) into Cu(I) species inside the oxidized fucoidan chain.

$$\{\text{fucoidan}^{2+}, Cu^{0}\} \xrightarrow{Cu^{2+}} \{\text{fucoidan}^{2+}, Cu_2^{2+}\} \tag{14}$$

Reactions such as equation (14) explain that no metallic copper is detected in the flash irradiation of these solutions. Otherwise, metallic copper will be the expected product of Cu(I) disproportionation if the copper products of equations (11)–(13) escape from the oxidized fucoidan chains to the bulk of the solution.

4. Concluding remarks

Both D-mannitol and fucoidan are, respectively, a polyhydric alcohol and a sulfated polysaccharide whose various reactive centers are engaged in reactions with radiolytically generated radicals and transition metal oxidants. Although the reactions of these carbohydrates with radicals are relatively slow, coordination to a toxin such as the Ni(III) complex, **3** [31], kinetically enhances the antioxidant response. Except for the reactions of D-mannitol and fucoidan with $O_2^{\bullet-}$, the rate constants for the scavenging of various radicals in table 1 show that D-mannitol and fucoidan are not as good scavengers of radicals as ascorbic acid. The rate constants of the three compounds for the scavenging of $O_2^{\bullet-}$ have similar values and they trap $^{\bullet}OH$ radicals with a diffusion-controlled rate. On the basis of these rate constants, they appear to be equally good as scavengers of the most common biochemically deleterious radicals, i.e., $O_2^{\bullet-}$ and $^{\bullet}OH$.

Based on the known reactivity of the $^\bullet$OH radical [25], the mechanism of the $^\bullet$OH radicals' oxidation of D-mannitol and fucoidan must be hydrogen atom abstractions occurring at various reaction centers of the carbohydrates. In D-mannitol, for example, the centers will be the 1, 2, and 3 carbons of **1**. Electron transfers are most likely the mechanisms of some of the $PRX^\bullet$, $NO_3^\bullet$, and $Cl_2^{\bullet-}$ reactions with the carbohydrates. These electron transfer processes will occur in the same reaction centers, and, in the D-mannitol example, will form D-mannitol$_{-\mathrm{H}^\bullet}$ $= C^\bullet HOH(CHOH)_4CH_2OH$, $CH_2OHC^\bullet OH(CHOH)_3CH_2OH$, and $CH_2OH\ CHOHC^\bullet OH\ (CHOH)_2CH_2OH$ radicals. From a reaction kinetics stand point, the decay of the D-mannitol$_{-\mathrm{H}^\bullet}$ and fucoidan$_{-\mathrm{H}^{\bullet\prime}}$ radicals are markedly different. The disappearance of the D-mannitol$_{-\mathrm{H}^\bullet}$ radicals is a fast process with characteristics of a reaction that is kinetically second order, i.e., the characteristic expected in a radical–radical annihilation, i.e., dimerization or disproportionation. It must be assumed that the annihilation reactions of the D-mannitol$_{-\mathrm{H}^\bullet}$ occur between radicals with similar and dissimilar $>C^\bullet OH$ and $-C^\bullet HOH$ centers. On the other hand, it is expected that polymeric fucoidan$_{-\mathrm{H}^{\bullet\prime}}$ radicals will be unable to make the fast diffusive displacements of the D-mannitol$_{-\mathrm{H}^\bullet}$ radicals. The slow diffusive motions of the fucoidan$_{-\mathrm{H}^{\bullet\prime}}$ radical explains their long lifetimes and that the decay reaction resembles a reaction with first-order kinetics. The conversion of fucoidan$_{-\mathrm{H}^\bullet}$ to fucoidan$_{-\mathrm{H}^{\bullet\prime}}$, equation (3), has a rate constant, $k \sim 5 \times 10^3\,\mathrm{s}^{-1}$, which is close to the rate constants for reactions of C-centered radicals with protons of primary and secondary alcohols [25]. This observation suggests that equation (3) involves an intrachain conversion of radicals rather than a chain conformation reorganization.

The photogeneration of Cu_n^+, $n \leq 3$, species is consistent with the light intensity of the laser flash with an estimated limit $n \leq 3$ based on the amount of Cu(0) that can be photoproduced. Departure from a linear dependence of the Cu_n^+ concentration on the number of absorbed photons resembles other examples of polymer biphotonic photolysis, e.g., polymers containing photoactive Re(I) pendent groups [32]. Prompt appearance of the Cu_n^+ species with $\tau < 10\,\mathrm{ns}$, equation (14), contrasts with the slow formation of products *via* disproportionation reactions in the photolysis of complexes of Cu(II) with poly(acrylate) [33].

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References

[1] D.B. Jennings, M. Ehrenshaft, D.M. Pharr, J.D. Williamson. *Proc. Natl. Acad. Sci.*, **95**, 15129 (1998).
[2] D. Rotrosen, J.I. Gallin. *Annu. Rev. Immunol.*, **5**, 127 (1987).
[3] I. Apostol, D.F. Heinstein, P.S. Low. *Plant Physiol.*, **90**, 109 (1989).
[4] A.I. Tauber, B.M. Babior. *J. Clin. Invest.*, **60**, 374 (1977).

[5] V. Chaturvedi, B. Wong, S.L. Newman. *J. Immunol.*, **179**, 157 (1996).
[6] Y. Aisa, Y. Miyakawa, T. Nakazato, H. Shibata, K. Saito, Y. Ikeda, M. Kizaki. *Am. J. Hematol.*, **78**, 7 (2005).
[7] K. Shimada, K. Fujikawa, K. Yahara, T. Nakamura. *J. Agric. Food. Chem.*, **40**, 945 (1992).
[8] G.L. Hug, Y. Wang, C. Schöneich, P.-Y. Jiang, R.W. Fessenden. *Radiat. Phys. Chem.*, **54**, 559 (1999).
[9] J. Rabani, S.O. Nielsen. *J. Phys. Chem.*, **73**, 3736 (1969).
[10] G. Czapski. *Annu. Rev. Phys. Chem.*, **22**, 171 (1971).
[11] D. Behar, G. Czapski, L.M. Dorfman, J. Rabani, H.A. Schwarz. *J. Phys. Chem.*, **74**, 3209 (1970).
[12] A. Fojtik, G. Czapski, A. Henglein. *J. Phys. Chem.*, **74**, 3204 (1970).
[13] J. Rabani, D. Klug-Roth, A. Henglein. *J. Phys. Chem.*, **78**, 2089 (1974).
[14] S. Abramovitch, J. Rabani. *J. Phys. Chem.*, **80**, 1562 (1976).
[15] B.K. Broszkiowicz. *Bull. Pol. Acad. Sci., Ser. Sci. Chim.*, 24, 128 (1976).
[16] Z.B. Alfassi, R.E. Huie, M. Kumar, P. Neta. *J. Phys. Chem.*, **96**, 767 (1992).
[17] P. Maruthamuthu, L.K. Patterson, G. Ferraudi. *Inorg. Chem.*, **17**, 3157 (1978).
[18] G. Ferraudi, S. Muralidharan. *Inorg. Chem.*, **20**, 4262 (1981).
[19] J. Guerrero, G.E. Piro, E. Wolcan, M.R. Feliz, G. Ferraudi, S.A. Moya. *Organometallics*, **20**, 2842 (2001).
[20] N.P. Chandía, B. Matsuhiro, J.S. Ortiz, A. Mansilla. *J. Chil. Chem. Soc.*, **50**, 501 (2005).
[21] N.P. Chandía, B. Matsuhiro. *Int. J. Biol. Macromol.*, **42**, 235 (2008).
[22] A.A. Frost, R.G. Pearson. *Kinetics and Mechanism*, John Wiley & Sons, New York (1953).
[23] S. Goldstein, G. Czapski. *Int. J. Radiat. Biol. Relat. Phys. Chem. Chem. Med.*, **46**, 725 (1984).
[24] M. Simic, P. Neta, E. Hayon. *J. Phys. Chem.*, **73**, 3794 (1969).
[25] G.V. Buxton, C.L. Greenstock, W.P. Hellman, A.B. Ross, W. Tsang. *J. Phys. Chem. Ref. Data*, **17**, 513 (1988) and references therein.
[26] S.B. Aoun, G.S. Bang, T. Koga, Y. Nonaka, T. Sotomura, I. Taniguchi. *Electrochem. Commun.*, **5**, 317 (2003).
[27] J. Dolegal, K.S. Klausen, F.J. Langmywr. *Anal. Chim. Acta*, **63**, 71 (1973).
[28] S. Striegler. *Macromolecules*, **36**, 1310 (2003).
[29] G.R. Dey. *Radiat. Phys. Chem.*, **74**, 172 (2005).
[30] M. Kumar. *Radiat. Phys. Chem.*, **64**, 99 (2002).
[31] S.K. Dutta, G. Ferraudi. *J. Phys. Chem. A*, **105**, 4241 (2001), and references therein.
[32] E. Wolcan, M.R. Feliz, J.L. Alessandrini, G. Ferraudi. *Inorg. Chem.*, **45**, 6666 (2006), and references therein.
[33] E. Baumgartner, S. Ronco, G. Ferraudi. *Inorg. Chem.*, **29**, 4747 (1990).