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Reduction of hypervalent chromium in acidic media by alginic acid

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ABSTRACT

Selective oxidation of carboxylate groups present in alginic acid by Cr^{VI} affords CO_2, oxidized alginic acid, and Cr^{III} as final products. The redox reaction afforded first-order kinetics in [alginic acid], [Cr^{VI}], and [H^+], at fixed ionic strength and temperature. Kinetic studies showed that the redox reaction proceeds through a mechanism which combines $Cr^{VI} \rightarrow Cr^{IV} \rightarrow Cr^{II}$ and $Cr^{VI} \rightarrow Cr^{IV} \rightarrow Cr^{III}$ pathways. The mechanism was supported by the observation of free radicals, CrO_2^{2+} and Cr^V as reaction intermediates. The reduction of Cr^{IV} and Cr^V by alginic acid was independently studied and it was found to occur more than 10^3 times faster than alginic acid/Cr^{VI} reaction, in acid media. At pH 1–3, oxo-chromate(V)–alginic acid species remain in solution during several hours at 15 °C. The results showed that this abundant structural polysaccharide present on brown seaweeds is able to reduce $Cr^{VI/V/IV}$ or stabilize high-valent chromium depending on pH value.

1. Introduction

The major structural polysaccharide of brown seaweeds (*Phaeophyta*) is alginic acid, a linear copolymer of (1 → 4)-linked β-D-mannopyranuronic acid (M) and (1 → 4)-linked α-L-gulopyranuronic acid (G) residues, arranged in heteropolymeric and homopolymeric blocks (Scheme 1) (Larsen, Salem, Sallam, Misheikey, & Beltagy, 2003; Leal, Matsuhiro, Rossi, & Caruso, 2008). The content of uronic acids varies with species and tissues types, and partial acid hydrolysis of alginic acids allows the preparation of fractions enriched in hetero- and homopolymeric blocks (Craigie, Morris, Rees, & Thom, 1984). The presence of carboxylic acid groups in both monomeric units makes possible it interaction with different metal ions, and in this sense its complexation ability has been studied with heavy metal ions present in wastewaters for water purification. Several authors have proposed interaction models between alginic acid and specific metal ions (De Stefano, Gianguzza, Piazzese, & Sammartano, 2005; Emmerichs, Wingender, Flemming, & Mayer, 2004; Maureira & Rivas, 2009).

Cr^{VI} is a very important pollutant, and its derivative compounds represent a potential environmental hazard because of their mammalian toxicity and carcinogenicity (Cood, Irwin, & Lay, 2003; Levina, Zhang, & Lay, 2010). It is well established that reduction of Cr^{VI} to Cr^{III} with a variety of organic and inorganic reductants can occur by a multiplicity of mechanisms which depend on the nature of the reducing agent (Gheju & Iovi, 2006; Mangiameli, González, Bellú, Bertoni, & Sala, 2014). The existence of different species of chromium in acid media, Cr^V and Cr^{IV}, and the tendency of Cr^{III} to form a variety of complexes, all combine to give systems of considerable complexity (Levina & Lay, 2005).

Polyoxygenated compounds, such as polyalcohols and hydroxycarboxylic acids, are effective as non-enzymatic reductants (at low pH) and can stabilize the labile oxidation states of chromium (Codd, Dillon, Levina, & Lay, 2001; Ciéslak-Golonka & Daszkiewicz, 2005). Due to the potential biological and ecological relevance of these kinds of biopolymers, the reduction and stabilization of hypervalent chromium by naturally occurring polysaccharides can provide useful information on the role that these polyoxygenated compounds play in the uptake and transport of chromium (Bellú, González, García, Signorella, & Sala, 2008). Although the reduction of hypervalent chromium by low molecular weight saccharides has been extensively studied (González et al., 2004; Mangiameli et al., 2010, 2011), little is known on the reaction of polysaccharides with Cr^{VI}. Kinetic and mechanistic studies of chromic acid oxidation onto kappa-karrageen; carboxymethyl cellulose and chondroitin-4-sulfate polysaccharides as natural polymers has been reported by Zaafarany, Khairou, and Hassan (2009), Hassan et al. (2010, 2013).

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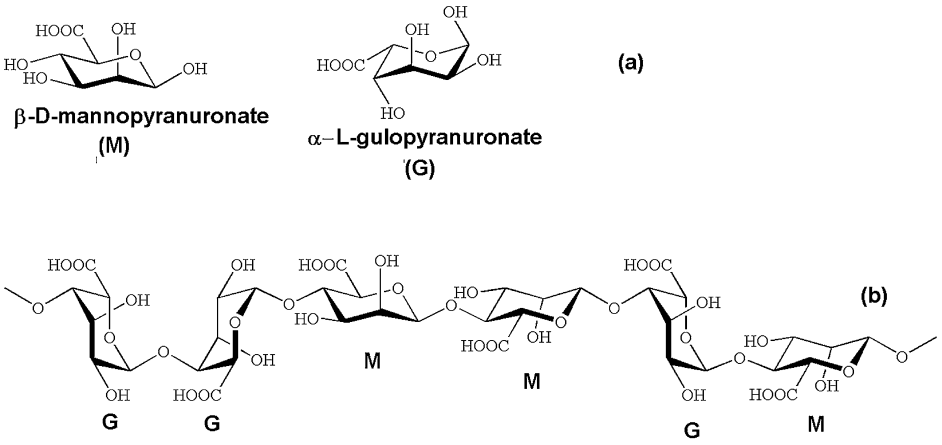


Scheme 1. Structure of alginic acid (a) monomers (b) polymeric chain.

Cellullose, hemicellullose and chitin reactions with Cr^{VI} were also studied at acid media (Lin & Wang, 2012) but no redox mechanistic studies were performed in this case. In a previous work we report the mechanism of oxidation of apple pectin by Cr^{VI} in aqueous acid medium (Bellú et al., 2008). The determination of the ability of alginic acid to reduce or stabilize hypervalent chromium will contribute to understand the potential role of this polysaccharide in the biochemistry of this metal. In this work, we report the study of the redox reaction of alginic acid with Cr^{VI} providing information related to the relative reactivity of alginic acid toward Cr^{VI}, Cr^{V}, and Cr^{IV}, the influence of pH on the redox reactions, and the formation of long-lived oxo-Cr^{V}–alginic acid complexes, being characterized by paramagnetic electronic resonance.

2. Experimental

2.1. Materials

Alginic acid sodium salt (Sigma, p.a.), GSH = L-glutathione reduced (Sigma, 98.0%), potassium dichromate (Mallinckrodt p.a.), sodium perchlorate monohydrate (Fluka 98.0%), oxygen (99.99%), nitrogen (99%), perchloric acid (A. C. S. Baker), Ammonium iron(II) sulfate hexahydrate (Sigma, 99%), acrylamide (Merck, 99.0%), sodium hydroxide (Cicarelli, p.a.), diphenylpicryllhydrazyl (dpph) (Sigma, 99.9%), formic acid (Sigma, 80%), acetone (Anedra 99.5%), ethanol absolute (Cicarelli, p.a.), ehba = 2-ethyl-hydroxybutanoic acid (Aldrich 99.0%), H_2SO_4 (Sigma, HPLC), were used without further purification.

Aqueous solutions were prepared in milliQ deionized water. Solutions of alginic acid sodium salt were prepared by stepwise addition of the reagent powder to milliQ deionized water while rapidly stirring the solution to avoid the formation of lumps, which dissolves with difficulty.

$Na[Cr^{V}O(ehba)_2]\cdot H_2O$, $[Cr^{IV}O(ehbaH)_2]$ and $K_3[Cr^{V}(O)(GSH)_2]$ were synthesized according to the method described in the literature (Krumpolc, Roček, Haight, & Merrill, 1980; Ghosh & Gould, 1991; Levina, Zhang, & Lay, 2003). For experiments performed in the 1–6 pH range, the pH of the solutions was adjusted by addition of $HClO_4$. In experiments performed at constant ionic strength (μ = 0.50 M) and different hydrogen ion concentrations; sodium perchlorate and perchloric acid solution mixtures were used. The concentration of stock solutions of perchloric acid was determined by titration employing standard analytical methods.

Caution: Cr^{VI}, $Na[Cr^{V}O(ehba)_2]\cdot H_2O$, $[Cr^{IV}O(ehbaH)_2]$ and dpph are human carcinogens. Contact with skin and inhalation must be avoided.

2.2. Alginic acid stability

Stability of polysaccharide in acidic conditions was studied by HPLC. The chromatograms were obtained on a Varian Polaris 200 chromatograph provided with a cc Star 9000 HPLC pump. The separation was carried out on an Aminex HPX-87X (300 × 7.8 mm^2, Bio-Rad Lab) HPLC column, using H_2SO_4 as eluent (pH 1.5) and a flow rate of 0.6 mL/min, at 30 °C. The samples were filtered through a 0.2 mm membrane prior to the injection into the chromatographic system. The effluent was monitored with a UV–vis detector (Prostar 325 UV–vis detector, λ = 220 nm). Chromatograms, recorded after incubation of the standard sample in 1.0 M $HClO_4$ (higher than the highest $[H^+]$ used in the kinetic measurements) at 60 °C during 3.0 h, showed only one peak (*tr*: 5.65 min) assigned to alginic acid. No others peaks were observed suggesting the stability of the polysaccharide under the present experimental conditions.

2.3. Measurement of free carboxylic groups of alginic acid

Measurements of free carboxylic groups of alginic acid were performed by acid base titration employing NaOH as titrant and phenolphthalein as visual indicator (Kolthoff, Sandell, Meehan, & Bruckenstein, 1969). Titration of 10.00 mL solution containing 20.00 g/L alginic acid with 0.08704 M NaOH until change color from colorless to pink afforded 10.75 mL of NaOH consumed which corresponds to 87.6 mM of free carboxylic groups. The employed polysaccharide contains an average of 4.38 mmol free carboxylic groups per gram of polymer.

2.4. Product analysis

Carbon dioxide was measured from a mixture of alginic acid (33.1 mg/mL), Cr^{VI} (5.0 mM) and $HClO_4$ (0.50 M). The temperature was kept constant at 60 °C and the reaction mixture was continuously stirred and flushed with pure nitrogen. The gaseous products were passed through three flasks containing NaOH (0.02 M). After reaction, in order to determine the yield of carbon dioxide, the NaOH solutions were titrated with standard HCl (0.0232 M). Aliquots of the reaction mixture of alginic acid/Cr^{VI} were analyzed by HPLC, using the same experimental conditions and column mentioned at Section 2.2. No peak corresponding to HCOOH (*tr*: 13.2 min) was observed.

2.5. Polymerization test

Detection of free organic radical generation, during the oxidation of alginic acid by hypervalent chromium, was tested employing

acrylamide. In a typical experiment, a solution of Cr^{VI} (1.0 mL, 0.010 M) was added to 10.0 mL of a solution 2.70 g/L of alginic acid, 0.50 M $HClO_4$ and 0.70 M of acrylamide. When [Cr^{VI}] became negligible, the precipitation of a white polymer of polyacrylamide was observed. Control experiments showed that no polymerization of acrylamide took place under the same experimental conditions with either $K_2Cr_2O_7$ or alginic acid alone. Possible reactions of Cr^V and Cr^{IV} with acrylamide were tested with Na[$Cr^VO(ehba)_2$] and [$Cr^{IV}O(ehbaH)_2$]. No precipitation occurred on mixing Cr^V or Cr^{IV} complexes with acrylamide under the conditions used in the Cr^{VI}/alginic acid reaction.

2.6. Kinetic measurements

Kinetic measurements were performed by monitoring absorbance changes on a Jasco V-550 spectrophotometer with fully thermostated cell compartments (±0.2 °C). The reactions were followed under pseudo-first-order conditions, at 60 °C; using excess of alginic acid over Cr^{VI}/Cr^V. Reactant solutions were thermally equilibrated at 60 °C prior to the experiment and $NaClO_4$ was used to maintain a constant ionic strength (μ).

2.6.1. Alginic acid/Cr^{VI} reactions

Disappearance of Cr^{VI} was followed by DPC method (Clesceri, Greenberg, & Eaton, 1998); monitoring the absorbance at 540 nm until at least 80% conversion. In the kinetic measurements, the concentration of Cr^{VI} and μ were kept constant at 1.0 mM and 0.50 M, respectively, while carboxylic group/Cr^{VI} ratio was varied from 20:1 to 60:1, at various [$HClO_4$]. The experimental pseudo-first-order rate constants (k_{6exp}), obtained from nonlinear least-square fits of kinetic data, were averages of at least three determinations and were within ±5% of each other. The first-order dependence of the rate upon [Cr^{VI}] was verified in a set of experiments where the $[Cr^{VI}]_0$ was varied between 0.30 and 2.0 mM, keeping temperature, [alginic acid], [H^+], and μ constant. Ionic strength effect was studied in a set of experiments where $[Cr^{VI}]_0$, [alginic acid], [H^+] and T were kept constant and μ was varied from 0.20 M to 1.0 M.

2.6.2. Alginic acid/Cr^V reactions

Kinetic measurements for the oxidation of alginic acid by Cr^V was performed by ligand exchange reaction, employing $K_3[Cr^V(O)(GSH)_2]$. In a typical experiment, 100 μL of the oxo-Cr^V complex solution (24 mM) were mixed with 2.3 mL of a solution containing alginic acid (4.57 g/L), [H^+] (0.30 M) and μ = 0.50 M ($NaClO_4$) at 15 °C. The reaction was followed at 350 nm, until at least 80% conversion.

2.6.3. Alginic acid/Cr^{IV} reactions

The oxidation of alginic acid by Cr^{IV} (CrO^{2+}) was spectrophotometrically monitored following the appearance of CrO_2^{2+} as a final redox product. Alginic acid/CrO^{2+} mixtures showed an increase of two intense absorption bands at 290 and 247 nm, with relative intensity Abs_{247}/Abs_{290} = 2.2 characteristic of CrO_2^{2+} (Scott, Bakac, & Espenson, 1991). The kinetic data were collected spectrophotometrically by following the formation of CrO_2^{2+} at 290 nm (ε = 3100 $(M\,cm)^{-1}$) at 25 °C (Scott, Bakac, & Espenson, 1992). At this wavelength, neither alginic acid nor the oxidized products absorb. In the kinetics measurements, [Cr^{IV}], μ, [O_2], and temperature were kept constant at 0.207 mM, 0.50 M, 1.26 mM and 25 °C, respectively. The concentration range of alginic acid used was chosen in order to avoid CrO^{2+} disproportionation. The carboxylic group/Cr^{IV} ratio was varied from 50:1 to 400:1. The experimental pseudo first order rate constants (k_{4exp}), obtained from nonlinear least square fits of absorbance data at 290 nm were averages of at least five determinations and were within ±10% of each other. Data used to calculate the kinetic constant, k_{4exp}, correspond to 80% of the total experimental values. The first order dependence of the rate upon [Cr^{IV}] was verified in a set of experiments where the $[Cr^{IV}]_0$ was varied between 0.10 and 0.50 mM, but T, [alginic acid], and μ were kept constant.

2.7. Detection of superoxo-Cr^{III} during the reaction of alginic acid with Cr^{VI}

The possible formation of Cr^{II} in alginic acid/Cr^{VI} mixtures was examined by UV–vis periodic scanning (220–600 nm) of solutions of 0.78 g/L alginic acid, 0.0797 mM Cr^{VI} and 3.0 M $HClO_4$ saturated with oxygen ([O_2] = 1.26 mM), at 25 °C. Spectra were collected every 2 min. If Cr^{II} forms, it is converted rapidly by dioxygen to CrO_2^{2+}, which has characteristic absorption bands (Scott et al., 1991, 1992) and can be detected at low [Cr^{VI}]. Periodic scanning of the reaction mixture showed that the Cr^{VI} band at 350 nm decreased in intensity, while two new peaks at 290 nm and 247 nm – characteristic of CrO_2^{2+} – grew in. When [Cr^{VI}] was negligible, 0.091 mM Fe^{2+} was added.

2.8. Chromate esters

Chromate esters were investigated by UV–vis spectrophotometry in the 320–450 nm region in which these compounds show characteristic absorption bands. Reactions were performed at pH 6.0, where the redox reaction is slow enough to enable the observation of the ester formation. The instrument was zeroed to an arrangement of the reference and sample beams passing through matched cell, both containing 0.5 mM Cr^{VI} at pH 6.0. The solution in the sample cell was replaced with the reaction solution containing 0.5 mM Cr^{VI} and 0.228–0.457 g/L alginic acid at pH = 6.0, μ = 0.50 M and T = 25 °C. Spectra obtained at 30 min after mixing showed a characteristic absorption at 375 nm.

2.9. EPR measurements

The EPR spectra were obtained with a Bruker ESP 300 E computer-controlled spectrometer operating at X-band frequencies (~9.4–9.8 GHz). Microwave generation was by means of a klystron (ER041MR) and frequencies were measured with a built-in frequency-counter. Spectra were recorded as first derivatives of the microwave absorption in 1024 points at 288 K, using 10 mW microwave power and 100 kHz modulation frequency. Power values used in the EPR experiments did not overcome 10 mW in order to avoid signal saturation. In EPR measurements, scanning speed and number were fixed in order to reduce the time used in each measurement. This was done to avoid fluctuations in the EPR signal during the sample scanning. The g-values were determined by reference to diphenylpicrylhydrazyl (dpph) (g_{iso} = 2.0036) as an external standard. Long-lived oxo-Cr^V–alginic acid complexes were generated in two different ways: a) by reaction of $K_2Cr_2O_7$ with an aqueous solution containing alginic acid; b) by mixing reduced glutathione (GSH) and $K_2Cr_2O_7$ where [Cr^{VI}] = [GSH] and an excess of alginic acid.

3. Results and discussion

3.1. Oxidation of alginic acid by Cr^{VI}

3.1.1. Rate studies

The reaction kinetic of alginic acid with Cr^{VI} was examined by the diphenylcarbazide (DPC) (Clesceri et al., 1998) method, in excess of alginic acid over Cr^{VI}, in the 0.10–0.50 M $HClO_4$ range. DPC can react with Cr^V and probably with Cr^{IV} (Eckert, Judd, Lay, &

Table 1
Experimental pseudo-first-order rate constants for different [$HClO_4$] and [alginic acid][a].

[H^+](M)	0.1	0.2	0.3	0.4	0.5
[alginic acid] (g/L)			$10^2 \times k_{6exp}$[b]		
4.57	0.817 ± 0.006	1.00 ± 0.04	1.57 ± 0.18	1.80 ± 0.03	2.28 ± 0.04
6.85	1.26 ± 0.01	1.53 ± 0.04	2.05 ± 0.07	2.49 ± 0.14	3.12 ± 0.19
9.13	1.62 ± 0.02	2.01 ± 0.02	2.63 ± 0.06	3.53 ± 0.09	4.16 ± 0.26
11.4	2.10 ± 0.04	2.35 ± 0.08	3.55 ± 0.17	4.43 ± 0.02	5.63 ± 0.13
13.7	2.64 ± 0.03	3.02 ± 0.05	4.22 ± 0.09	5.30 ± 0.06	6.60 ± 0.14

[a] $T = 60\,^{\circ}C$; [Cr^{VI}] = 1.0 mM; μ = 0.50 M. The values of k_{6exp} are expressed in min^{-1}.
[b] Mean values from multiple determinations (estimated errors are lower than 10%). Rate constants were obtained using Sigma Plot 11.0 Program.

Symons, 1991), therefore it is very important to probe that no accumulation of these species occurs in the alginic acid/Cr^{VI} reaction mixtures. Under experimental conditions employed in this work, both species Cr^{IV} and Cr^{V}, decay rates are much higher than Cr^{VI} (as shown below) and do not accumulate in the reaction alginic acid/Cr^{VI}. This result allows the use of DPC method for measuring Cr^{VI} without any interference by other chromium species. A monophasic decrease of [Cr^{VI}] with time was observed, and the kinetic profiles could be adequately described by a single exponential decay from which Cr^{VI} pseudo-first-order rate constants (k_{6exp}) were calculated. Table 1 summarizes k_{6exp} values for various concentrations of alginic acid and $HClO_4$.

The value of k_{6exp} did not vary with different [Cr^{VI}]$_0$ at fixed T, μ, [alginic acid] and [$HClO_4$], confirming the first-order rate dependence on [Cr^{VI}]. When μ is varied from 0.20 M to 1.0 M but [Cr^{VI}]$_0$, T, [alginic acid] and [$HClO_4$] are fixed, the value of k_{6exp} increased with the increase of μ. The effect of ionic strength on the rate constants of reactions involving ions is well understood applying the Bronsted–Debye–Hückel equation (Espenson, 2002):

$$\log_{10} k = \log_{10} k_0 + 2A Z_A \; Z_B \, F_\mu \qquad (1)$$

where A is a constant equals to 0.73 for water at 333 K ($A = 1.87 \times 10^6/(\varepsilon T)^{3/2}$, z_A, z_B are the charges of reactive A and B, respectively, and F_μ is the ionic strength function $\mu^{1/2}/(1+\mu^{1/2})$. The quantity k_0 is the limiting rate constant at zero ionic strength. The value of $z_A z_B$ obtained from the slope of the plot $\log_{10} k$ vs F_μ was 1.01 ± 0.01 (see Supplementary material Fig S1), which is in agreement with two reactive ionic species with a charge value of $z_A = z_B = -1$.

It is well known that the trivalent metal cation Cr^{III} have a high tendency to make complexation with alginic acid or its derivatives to give the corresponding coordination biopolymeric cross-linked trivalent metal–alginate complexes (Ibáñez & Umetsu, 2004). Since Cr^{III} is one of the oxidation products, its influence on the oxidation rates was examined. Table 2 shows the values of k_{6exp} obtained with and without Cr^{III} perchlorate salts added to the reaction mixtures.

As is shown in Table 2, no significant variation of k_{6exp} values was obtained at fixed [H^+], [alginic acid], T and μ. These results demonstrate that Cr^{III}–alginates, if it is formed, they don't modify the oxidation rates of alginic acid by Cr^{VI}.

Table 2
Effect of [Cr^{III}]$_0$ over k_{6exp}[a].

[Cr^{III}]$_0$ (M)	0.000	0.010	0.050
[alginic acid] (g/L)		$10^2 \times k_{6exp}$[b]	
4.57	1.00 ± 0.04	1.06 ± 0.05	1.07 ± 0.08
13.7	3.02 ± 0.05	3.08 ± 0.08	3.03 ± 0.06

[a] $T = 60\,^{\circ}C$; [Cr^{VI}] = 1.0 mM; [H^+] = 0.20 M; μ = 0.50 M. The values of k_{6exp} are expressed in min^{-1}.
[b] Mean values from multiple determinations (estimated errors are lower than 10%). Rate constants were obtained using Sigma Plot 11.0 Program.

Plots of k_{6exp} versus [alginic acid] afforded good straight lines (Fig. 1) from which values of k_{6S} were determined. A linear dependence of k_{6S} values on [$HClO_4$] was observed, (**inset**, Fig. 1).

The rate constant k_6 and k_{6H} calculated from plot of k_{6S} versus [H^+] were found to be $(0.21 \pm 0.03)\,min^{-1}\,M^{-1}$ and $(1.72 \pm 0.08)\,min^{-1}\,M^{-2}$, respectively. Consequently, the complete rate law for the Cr^{VI} consumption can be expressed as:

$$-\frac{d[Cr^{VI}]}{dt} = (k_6 + k_{6H}[H^+])[\text{alginic acid}][Cr^{VI}]_T \qquad (2)$$

Under conditions used in the kinetic studies, CO_2 was detected as product of oxidation. Quantitative analysis of CO_2 showed that the reaction yielded 0.60 mol of CO_2 per mole of Cr^{VI}. In alginic acid structure, the functional group being oxidized is the carboxylic group and can be rationalized taking into account the relative reactivity of functional groups in saccharides toward Cr^{VI}:

$-(H)C(OR)OH_{hemiacetal}$ (Signorella et al., 1999) > $-CO_2H$ (Bellú et al., 2008) > $-H_2COH_{primary}$ (Roldán et al., 2000) ≫ $(H)COH_{secondary}$ (Santoro et al., 2007); $(H)COR_{glycoside}$ (Signorella et al., 2000); RCO_2R_{ester} (Sala, Palopoli, Alba, & Signorella, 1993).

Stoichiometry of the oxidation of alginic acid by Cr^{VI} is shown in Scheme 2.

3.1.2. Detection of Cr^{VI} esters

Chromate esters were investigated by differential UV–vis spectra of alginic acid/Cr^{VI} mixtures. The mixtures exhibited an absorption band with λ_{max} = 375 nm consistent with that described for oxo-Cr^{VI}-ester (Mitewa & Bontchev, 1985; Mangiameli et al., 2010, 2014). At pH 6.0, the redox reaction of Cr^{VI} with alginic acid proceeds very slowly, with negligible reduction of Cr^{VI}. Thus, at this pH value, the ester formation step can be clearly distinguished from the electron transfer reaction. Spectra obtained within 30.0 min after mixing revealed a distinctive absorption band at 375 nm, Fig. 2.

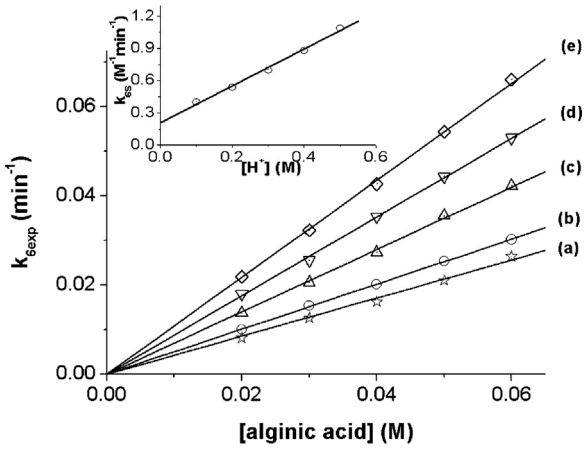
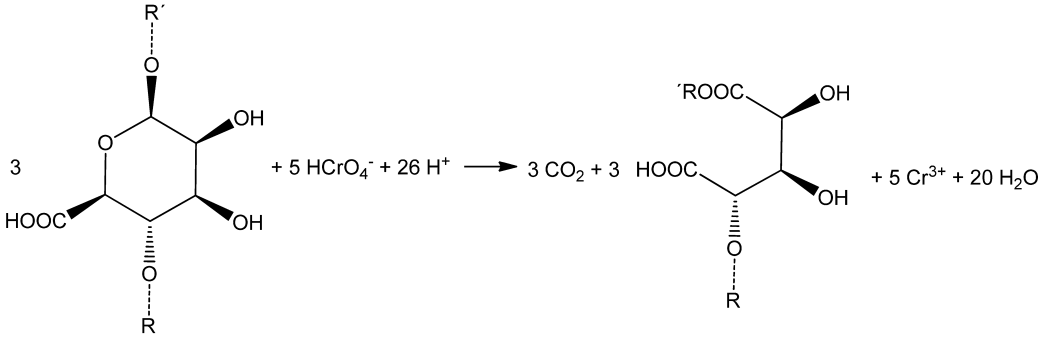


Fig. 1. Effect of [alginic acid] on k_{6exp}. $T = 60\,^{\circ}C$, μ = 0.50 M and [H^+]: (a) 0.10, (b) 0.20, (c) 0.30, (d) 0.40, (e) 0.50 M. Inset: dependence of k_{6S} on [H^+].



Scheme 2. Stoichiometry of the redox reaction between alginic acid and Cr^{VI} in acid media.

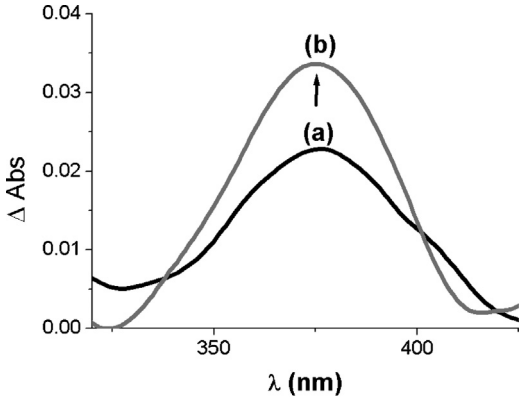


Fig. 2. UV–vis differential spectra of alginic acid/Cr^{VI} mixtures at pH 6.0, showing the increasing band at 375 nm with increasing [alginic acid]. (a) [alginic acid] = 0.228 g/L, (b) [alginic acid] = 0.457 g/L. [Cr^{VI}] = 0.5 mM, μ = 0.50 M, T = 25 °C.

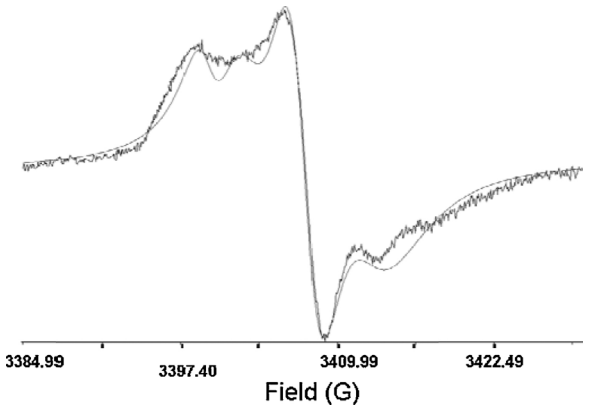


Fig. 3. Experimental and simulated X-band EPR spectra of oxo-Cr^{V}–alginic acid species. T = 15 °C; [alginic acid] = 4.57 g/L; [Cr^{VI}] = 1.0 mM; μ = 0.50 M; pH = 2; center field = 3410 G; modulation amplitude = 2.0 G; resolution = 1024 points; frequency = 9.4312 GHz. Spectra were collected after 1 h of mixing reactants.

Alginic acid/Cr^{VI} mixtures with [alginic acid] 0.228–0.457 g/L, at pH 6.0, showed that the absorbance at 375 nm increased with increasing [alginic acid], probably as a result of a shift toward the ester in the esterification equilibrium.

3.1.3. *Intermediacy of Cr^{V}*

EPR spectroscopy is the most specific and sensitive technique to detect micro molar concentrations of paramagnetic ions such as Cr^{V} species. Therefore, the presence of oxo-Cr^{V} complexes can be identified with great sensitivity by EPR spectroscopy at room temperature in X-band spectra. The EPR spectral parameter, g_{iso}, values of the EPR signal of Cr^{V} complexes depend on the Cr^{V} coordination number and the nature of the donor groups bound to oxo-Cr^{V} (Barr-David et al., 1995). EPR spectra of alginic acid/Cr^{VI} reaction mixtures showed complex and less resolved signals than in the other systems (Sala, González, García, Frascaroli, & Van Doorslaer, 2011). In general, the signals were very broad, which indicates the contribution of several Cr^{V} isomers; Fig. 3. The signal belongs to species where the oxo-Cr^{V} was coordinated to alcoholate and carboxylate groups which may belong to the same or different polymeric chain, thus affording a complex EPR signal.

After 30 min of reaction multiplicity of EPR signals of alginic/Cr^{VI} reaction mixture at pH 1–3 could not be resolved, even with low modulation amplitude, because the superhyperfine coupling constants were lower than the line width. Spectra were simulated with four singlets in order to obtain information from the g_{iso} values (Table 2) of oxo-Cr^{V} species in the reaction mixture. The simulation of the EPR spectra showed that the complex signal of the alginic acid/Cr^{VI} reaction mixture came from the contribution of, at least, four species of oxo-Cr^{V} species. Additionally, it must be noted that the average g_{iso} value were in the range of that expected for five-coordinated oxochromate(V) complexes (see Supplementary material Fig. S2 and Table 3). The signal with average g_{iso} 1.9780 (species A) is consistent with those for five-co-ordinated oxo-Cr^{V} complexes having two carboxylate and two alcoholate donor groups, (Barr-David et al., 1995) [Cr^{V}O(R-COO^{-},R-OH)$_2$]. Meanwhile the signal with average g_{iso} 1.9790 belongs to five-co-ordinated oxo-Cr^{V} mixed species [Cr^{V}O(R-COO^{-},OH)(R-OH)$_2$] (species B) where the oxo-Cr^{V} group is being coordinated by three alcoholate and one carboxylate groups.

Unlike the results observed in the acid sacharides/Cr^{V} complexes, (González et al., 2009; González et al., 2010), at pH 2.0, there

Table 3
g_{iso} values for proposed Cr^{V}-alginic acid complexes.

Species	Coordination	g_{iso}	g_{iso} (calculated)	Line width (G)	%
A	[Cr^{V}O(R-COO^{-},R-OH)$_2$]	1.9780	1.9783	2.90	45.0
B	[Cr^{V}O(R-COO^{-},OH)(R-OH)$_2$]	1.9790	1.9791	3.90	35.4
C	[Cr^{V}O(R-OH)$_2$ (R-OH)$_2$]	1.9803	1.9800	2.40	14.7
D	[Cr^{V}O(R-COO^{-})$_2$ (H_2O)$_2$]	1.9750	1.9749	5.00	4.6

T = 15 °C; [alginic acid] = 4.57 g/L; [Cr^{VI}] = 1.0 mM; μ = 0.50 M; pH = 2.0; center field = 3410 G; modulation amplitude = 2.0 G; resolution = 1024 points; frequency = 9.4312 GHz.

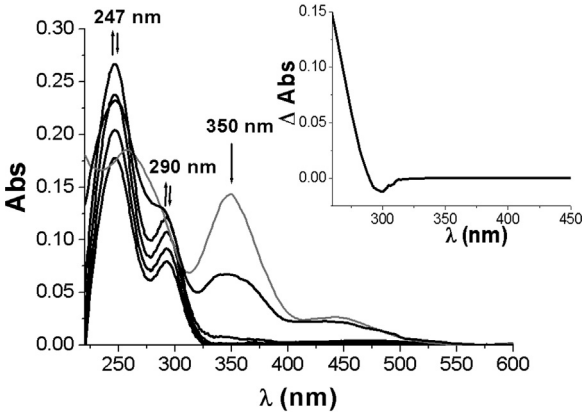


Fig. 4. CrO_2^{2+} formation from the reaction between alginic acid and Cr^{VI}. [alginic acid] = 0.78 g/L, $[HClO_4]$ = 3.0 M, $[O_2]$ = 1.26 mM, $[Cr^{VI}]$ = 0.0797 mM, μ = 3.0 M, T = 25 °C. Spectra were recorded every 3.0 min. Inset: differential spectrum after addition of Fe^{II}.

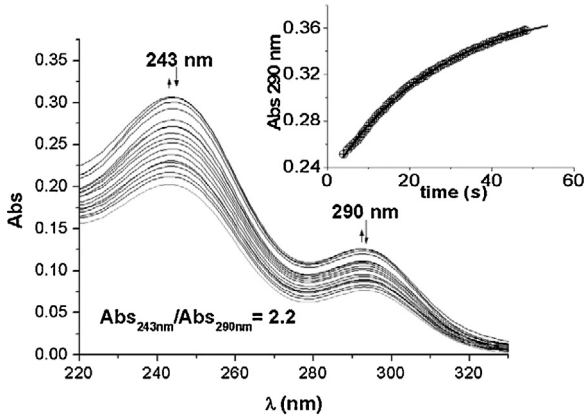


Fig. 5. Formation of CrO_2^{2+} from the reaction 2.28 g/L alginic acid, $[H^+]$ = 0.30 M, $[O_2]$ = 1.26 mM, μ = 0.50 M, T = 25 °C, $[Cr^{IV}]$ = 0.207 mM. Inset: absorbance at 290 vs. time. Fitted lines were calculated using Eq. (4) and SigmaPlot 11.0 program.

was approximately a 15% of species, where the oxo-Cr^V group is being coordinated with four alcoholate groups (species C). Finally, a fourth species (D) with a g_{iso} 1.9750 could be related with oxo-Cr^V coordinated by two carboxylates and molecules of waters.

Anyway, the predominant species with g_{iso} values 1.9780 and 1.9791, during reaction time, indicated that the coordination mode of oxo-Cr^VO by carboxylate and diolates (belonging to β-D-mannopyranuronic and α-L-gulopyranuronic acid residues,) was the most suitable from the thermodynamic point of view at acidic pH.

3.1.4. Intermediacy of Cr^{II}

The fact that Cr^{II} is involved in the oxidation mechanism of several alcohols and tiols by Cr^{IV} and Cr^{VI} in $HClO_4$ was demonstrated by conversion to CrO_2^{2+} upon reaction with dioxygen (Scott et al., 1991, 1992; Al-Ajlouni, Bakac, & Espenson, 1994; Pérez Benito, Arias, & Lamrhari, 1994; Pérez Benito & Arias, 1993). In appropriate experimental conditions, such as high $[O_2]$ = 1.26 mM and low $[Cr^{VI}]$ = 0.08 mM, the reaction of Cr^{II} (if any) with O_2 to give CrO_2^{2+} can compete successfully with: a) the reaction of Cr^{II} with Cr^{VI} and b) the autocatalytic consumption of CrO_2^{2+} by Cr^{II}. If Cr^{II} is an intermediate species in the alginic acid/Cr^{VI} redox reaction, CrO_2^{2+} should be detected (Scott et al., 1992).

We examined the presence of intermediate Cr^{II} in the reaction of alginic acid with Cr^{VI}, by monitoring the formation of CrO_2^{2+}, using $[Cr^{VI}]_0$ low enough to avoid the $Cr^{VI} + Cr^{II}$ competitive reaction, and 3.0 M $HClO_4$ to accelerate the alginic acid/Cr^{VI} reaction at a temperature lower than used in the kinetics experiments.

A periodic scanning of the O_2-saturated solution (1.26 mM O_2) of a alginic acid/Cr^{VI} reaction mixture in 3.0 M $HClO_4$ over a period of 60 min, showed that the band at 350 nm, characteristic of Cr^{VI}, decreased in intensity meanwhile two absorption bands at λ_{max} 247 and 290 nm appeared (Fig. 4).

These two bands are characteristic of CrO_2^{2+} formed as a long-lived intermediate (Scott et al., 1991), that then slowly transforms into the final Cr^{III} (as shown below). Since CrO_2^{2+} can be exclusively formed by reaction of Cr^{II} with O_2, and, in turn, Cr^{II} had previously been demonstrated to form exclusively through two-electron reduction of Cr^{IV} (Scott et al., 1992; Al-Ajlouni et al., 1994), our spectroscopic results provide evidence that Cr^{II} and Cr^{IV} are involved in the redox mechanism of the reaction between Cr^{VI} and alginic acid. When the absorbance at 350 nm was negligible, 0.091 mM of Fe^{II} was added to bring about the following reaction:

$$CrO_2^{2+} + 3Fe^{2+} + 4H^+ \rightarrow Cr^{3+} + 3Fe^{3+} + 2H_2O \quad (3)$$

The spectrum of the reaction mixture was subtracted from the corresponding one prior to Fe^{II} addition. As shown in Fig. 4 inset, there is a negative absorbance difference around 290 nm, consistent with the presence of CrO_2^{2+}.

3.2. Oxidation of alginic acid by Cr^V

The reaction of alginic acid with Cr^V was examined by ligand exchange employing the complex $K_3[Cr^V(O)(GSH)_2]$, and following the decrease in absorption at 350 nm. The ligand exchange reaction was verified by EPR measurements and it was completed in a few seconds. Comparing the alginic acid/Cr^{VI} and alginic acid/Cr^V reactions, at the same conditions (μ, [alginic acid] and $[Cr^{VI/V}]_0$) except T (15 °C for Cr^V reaction and 60 °C for Cr^{VI} reaction) it was observed that alginic acid/Cr^V reaction is very fast (see Supplementary material Fig S3).

As it is showed in Fig. S3, Cr^V decays with time very fast and is expected that no accumulation of this species occurs in the alginic acid/Cr^{VI} systems. After 2.0 min, only 6% of Cr^{VI} consumption was achieved while 85% of Cr^V consumption occurs. Fig. S3 showed a monophasic decrease of $[Cr^V]$ with time. The oxo-Cr^V–alginic acid species kinetic profile could be adequately fitted to a single exponential decay from which Cr^V pseudo-first-order rate constant (k_{5exp}) was calculated. The value of k_{5exp} estimated in this way resulted to be $(2.14 \pm 0.06) \times 10^{-2}$ s^{-1}. EPR and electronic spectroscopy show that Cr^{III} is the ultimate fate of chromium in these reactions.

3.3. Reaction of CrO^{2+} with alginic acid

The addition of variable quantities of alginic acid to O_2-saturated solutions containing 0.30 mM of CrO^{2+} resulted in the increase of the CrO_2^{2+} spectrum intensity. The formation of CrO_2^{2+} was followed at 290 nm and the monotonic increase of absorbance was found to follow first-order kinetics. The experimental rate constants k_{4exp} were calculated by nonlinear least-square fit of absorbance-time data to the equation:

$$Abs_t = Abs_\infty + (Abs_0 - Abs_\infty)\, e^{(-k4exp\, t)} \quad (4)$$

where Abs_∞ and Abs_0 were absorbance at infinite time and initial absorbance, respectively.

Fig. 5 shows spectral scanning of alginic acid/Cr^{IV} mixtures and the right fitting of the absorbance data vs. time at 290 nm employing Eq. (4), (inset Fig. 5).

The value of k_{4exp} did not vary with different $[Cr^{IV}]_0$, but fixed T, μ, [alginic acid], and $[HClO_4]$, confirming the first-order

Table 4
Experimental pseudo-first-order rate constants (k_{4exp}) for different concentrations of $HClO_4$ and alginic acid[a].

$[H^+]$(M)	0.2	0.3	0.4	0.5
[alginic acid] (g/L)	$10^2 \times k_{4exp}$[b]			
2.28	1.80 ± 0.11	1.90 ± 0.18	1.89 ± 0.12	1.85 ± 0.15
4.57	3.20 ± 0.16	3.00 ± 0.17	2.80 ± 0.14	3.05 ± 0.08
6.85	4.04 ± 0.04	4.10 ± 0.06	3.89 ± 0.13	4.06 ± 0.05
9.13	4.45 ± 0.18	4.60 ± 0.15	4.50 ± 0.12	4.52 ± 0.13
11.4	4.90 ± 0.12	5.20 ± 0.11	5.00 ± 0.09	5.05 ± 0.10

[a] $T = 25\,^{\circ}C$; $[Cr^{IV}] = 0.20$ mM; $\mu = 0.50$ M. The values of k_{4exp} are expressed in s^{-1}.
[b] Mean values from multiple determinations (estimated errors are lower than 10%). Rate constants were obtained using Sigma Plot 11.0 Program.

dependence of rate on $[Cr^{IV}]$. Table 4 summarizes values of k_{4exp} for various concentrations of alginic acid and $HClO_4$.

Experimental conditions were chosen so that the alginic acid/Cr^{IV} reaction was much faster than Cr^{IV} disproportionation into Cr^{VI} and Cr^{III}. In the absence of alginic acid, or when the alginic acid concentration was too low, disproportionation of Cr^{IV} was evidenced by the grow-up of absorbance at 350 nm due to formation of Cr^{VI}. Using reactant concentrations of Table 4, Cr^{VI} was not detected. Values of k_{4exp} are independent of $[H^+]$ in the range 0.20–0.50 M, followed by saturation kinetic with [alginic acid] (see Supplementary material Fig. S4).

Non-linear curve fit of kinetic data allowed to calculate values of k_1 and k_2 applying the equation:

$$k_{4exp} = \frac{k_1[\text{alginic acid}]}{1 + k_2[\text{alginic acid}]} \quad (5)$$

Consequently, the complete rate law for the Cr^{IV} consumption can be expressed as in the equation:

$$\frac{d[CrO_2^{2+}]}{dt} = -\frac{d[Cr^{IV}]}{dt} = \left(\frac{k_1[\text{alginic acid}]}{1 + k_2[\text{alginic acid}]}\right)[Cr^{IV}]_T \quad (6)$$

with $k_1 = 66 \pm 1\,(M\,s)^{-1}$ and $k_2 = 1120 \pm 30\,M^{-1}$.

Reaction mixtures containing excess of alginic acid over CrO^{2+} in 0.20 M $HClO_4$ were analyzed for CO_2 as reaction product. Quantitative analysis of CO_2 showed that the reaction yielded 1.0 mol of CO_2 per mole of Cr^{IV}.

Scheme 3 shows a proposed reaction mechanism for the oxidation of alginic acid by CrO^{2+}.

We proposed that a reactive alginic acid–Cr^{IV} ester is formed in a first step of the reaction pathway. This very reactive intermediate is in stationary state so its formation and decomposition rates are equals. Taking into account the proposed mechanism, the rate law takes the form:

$$-\frac{d[Cr^{IV}]}{dt} = k_4\left[Cr^{IV} - \text{ester}\right] \quad (10)$$

Applying stationary state concept:

$$[Cr^{IV} - \text{ester}] = \frac{k_a[\text{alginic acid}][Cr^{IV}]}{k_{-a} + k_4} \quad (11)$$

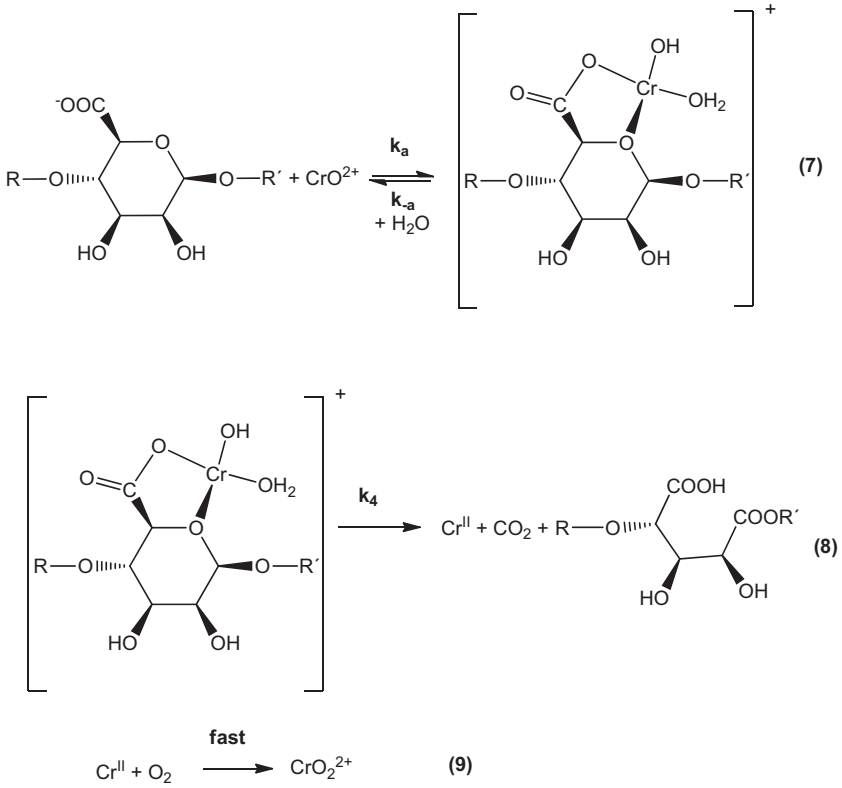
Considering total analytical concentration of Cr^{IV}:

$$\left[Cr^{IV}\right]_T = \left[Cr^{IV}\right] + \left[Cr^{IV} - \text{ester}\right] \quad (12)$$

Replacing and making the corresponding mathematical arrangements, Eq. (10) transforms into Eq. (13).

$$-\frac{d\left[Cr^{IV}\right]}{dt} = \frac{k_a k_4\,[\text{alginic acid}]\left[Cr^{IV}\right]_T}{k_{-a} + k_4 + k_4[\text{alginic acid}]} \quad (13)$$

The rate law for the disappearance of Cr^{IV} takes the form of Eq. (13) which is in total agreement with the experimental rate law, Eq. (6) where $k_1 = k_a k_4 / k_{-a} + k_4$ and $k_2 = k_4 / k_{-a} + k_4$.



Scheme 3. Proposed mechanism for the oxidation of alginic acid by CrO2+.

3.4. Reaction mechanism for the oxidation of alginic acid by Cr^{VI}

In the range of substrate and acid concentration used in the kinetic measurements, the oxidation of alginic acid by Cr^{VI} is a complex reaction that yields Cr^{III}, CO_2, and oxidized alginic acid as final redox products. CrO_2^{2+} was detected in the reaction of alginic acid with Cr^{VI} under aerobic conditions. This fact indicates that Cr^{IV} and Cr^{II} were involved in the mechanism of the redox reaction Cr^{VI}/alginic acid. Cr^{II} was originated exclusively by two-electron reduction of Cr^{IV} species affording indirect evidence of participation of Cr^{IV} in the redox mechanism. The observation of oxo-Cr^{V} species at pH higher than used in the kinetic measurements, indicate that oxo-Cr^{V}–alginic acid intermediate species were formed in the reaction of Cr^{VI} with alginic acid. However, under conditions used in the kinetic measurements, Cr^{IV} and Cr^{V} react with alginic acid much faster than Cr^{VI} and do not accumulate in the reaction mixture. Reduction of Cr^{IV} by alginic acid at 0.30 M $HClO_4$ and 25 °C is 5.5×10^3 times faster than reduction of Cr^{VI} at 60 °C. Cr^{V} also oxidizes alginic acid faster than Cr^{VI} does. In 0.30 M $HClO_4$, and T = 15 °C, Cr^{V} oxidizes alginic acid 160 times faster than Cr^{VI} at 60 °C.

Thus, the concentration–time profiles of the Cr^{VI}–alginic acid mixtures reflect the [Cr^{VI}] monotonic decay without interference of [Cr^{IV}] and/or [Cr^{V}]. Oxidation of alginic acid by $HClO_4$ was excluded because this strong acid acts as oxidant only at high concentration (higher than 50%) and at high temperature (near to the boiling point) (Diehl & Smith, 1959).

In Scheme 4, we propose a mechanism that combines $Cr^{VI} \rightarrow Cr^{IV} \rightarrow Cr^{II}$ and $Cr^{VI} \rightarrow Cr^{IV} \rightarrow Cr^{III}$ pathways, and takes into account: (a) kinetic results, (b) the polymerization of acrylamide added to the reaction mixture, (c) detection of intermediate of oxo-Cr^{V}–alginic acid species, (d) spectroscopy detection of CrO_2^{2+}, and (e) formation of CO_2, Cr^{III}, and oxidized alginic acid as final redox products.

The reduction of Cr^{VI} by alginic acid follows two pathways, one independent of [H^+] and another dependent of [H^+], because the acid base behavior of the polymer. The reactive species are alginate and chromate acid, both anionic species with charge = −1 as suggested by the dependence of k_{6exp} with the ionic strength.

In the [H^+] range under study, Cr^{VI} exists predominantly as $HCrO_4^-$ and in acidic medium Cr^{VI} presents dimerization equilibrium (Brasch, Buckingham, Evans, & Clark, 1996):

$$2HCrO_4^- \rightleftharpoons Cr_2O_7^{2-} + H_2O \quad K_D = 70\,(T = 333\,K) \tag{26}$$

The value of the second acidic constant of chromic acid is very low:

$$HCrO_4^- \rightleftharpoons CrO_4^{2-} + H^+ \quad Ka_2 = 10^{-7} \tag{27}$$

Combining Eqs. (26) and (27), it is possible to calculate [$HCrO_4^-$] and [$Cr_2O_7^{2-}$]. This method was proposed by Sen Gupta and co workers (Sen Gupta, Samanta, & Samanendra, 1985) in order to identify the reactive chromium specie.

Table 5 shows the dependence of k_{6exp} with [$HCrO_4^-$] and [$Cr_2O_7^{2-}$].

The values in Table 5 shows a first- order dependence of k_{6exp} with [$HCrO_4^-$] and not with [$Cr_2O_7^{2-}$]. This results together with the ionic strength effect supports that $HCrO_4^-$ is the chromium reactive specie.

It is known that oxidation of polysaccharides by Cr^{VI} is preceded by the formation of a chromate ester (Bellú et al., 2008; Zaafarany et al., 2009; Hassan et al., 2010; Lin & Wang, 2012; Hassan et al., 2013). The observation of the absorbance bands characteristic of chromate oxy-esters around 375 nm, few minutes after mixing alginic acid and Cr^{VI}, under conditions where the redox reaction is slow, indicates that an intermediate Cr^{VI} complex is formed rapidly prior to the redox steps. Thus, the second and third step of the mechanism proposed in Scheme 4 involved the formation of alginic acid–Cr^{VI} monochelate with alginic acid and alginate acting as bidentate ligands, which is also consistent with the first order dependence of the reaction on [Cr^{VI}].

Oxidation rate of alginic acid by Cr^{V} and Cr^{IV} are faster than Cr^{VI} at the same experimental conditions. Therefore, Cr^{IV} and Cr^{V}, although formed in the Cr^{VI}/alginic acid reaction, should be involved in fast steps of the reaction pathway.

The slow redox step proposed in Scheme 4 involves C—C bond cleavage through a two-electron redox process to yield Cr^{IV}, CO_2, and oxidized alginic acid (Eq. (17)). The initial two-electron reduction of Cr^{VI} by alginic acid is in agreement with previous reports on various acid saccharides that were selectively oxidized by Cr^{VI} to the lower homologous (Bellú et al., 2008; González et al., 2009; Mangiameli et al., 2014).

The rate law for the Cr^{VI} consumption derived from Eq. (14)–(17) in Scheme 4 is given by Eq. (28), where [Cr^{VI}]$_T$ refers to the total [Cr^{VI}] in the reaction mixture.

$$-\frac{d\left[Cr^{VI}\right]}{dt} = \frac{\left(k_6K_2 + k_6K_3K_1\left[H^+\right]\right)[\text{alginate}]\left[Cr^{VI}\right]_T}{1 + K_2\,[\text{alginate}] + K_3[\text{alginic acid}]} \tag{28}$$

If K_2 [alginate] $<<$ 1, and K_3 [alginic acid] $<<$ 1 then Eq. (28) agrees perfectly with the experimental rate law, Eq. (2).

In the mechanism, we have included two competitive one- and two-electron reductions of Cr^{IV} by alginic acid. Thus, Cr^{IV} is proposed to react with excess alginic acid to yield Cr^{II}, CO_2, and oxidized alginic acid or Cr^{III} and alginic acid radical (R•), through two alternate fast steps, Eqs. (19) and (20). The first supposition was supported by the observation of CrO_2^{2+} (the product of the reaction of Cr^{II} with O_2), while the second one, by the observed polymerization of acrylamide when it is added to the Cr^{VI}–alginic acid reaction mixtures. Cr^{V} was generated by fast reaction of Cr^{II} with Cr^{VI}, Eq. (21), and, alternatively, by rapid reaction of the alginic acid radical with Cr^{VI}, Eq. (22). Cr^{V} can further oxidize alginic acid to yield Cr^{III}, CO_2, and oxidized alginic acid as final redox products, Eq. (23).

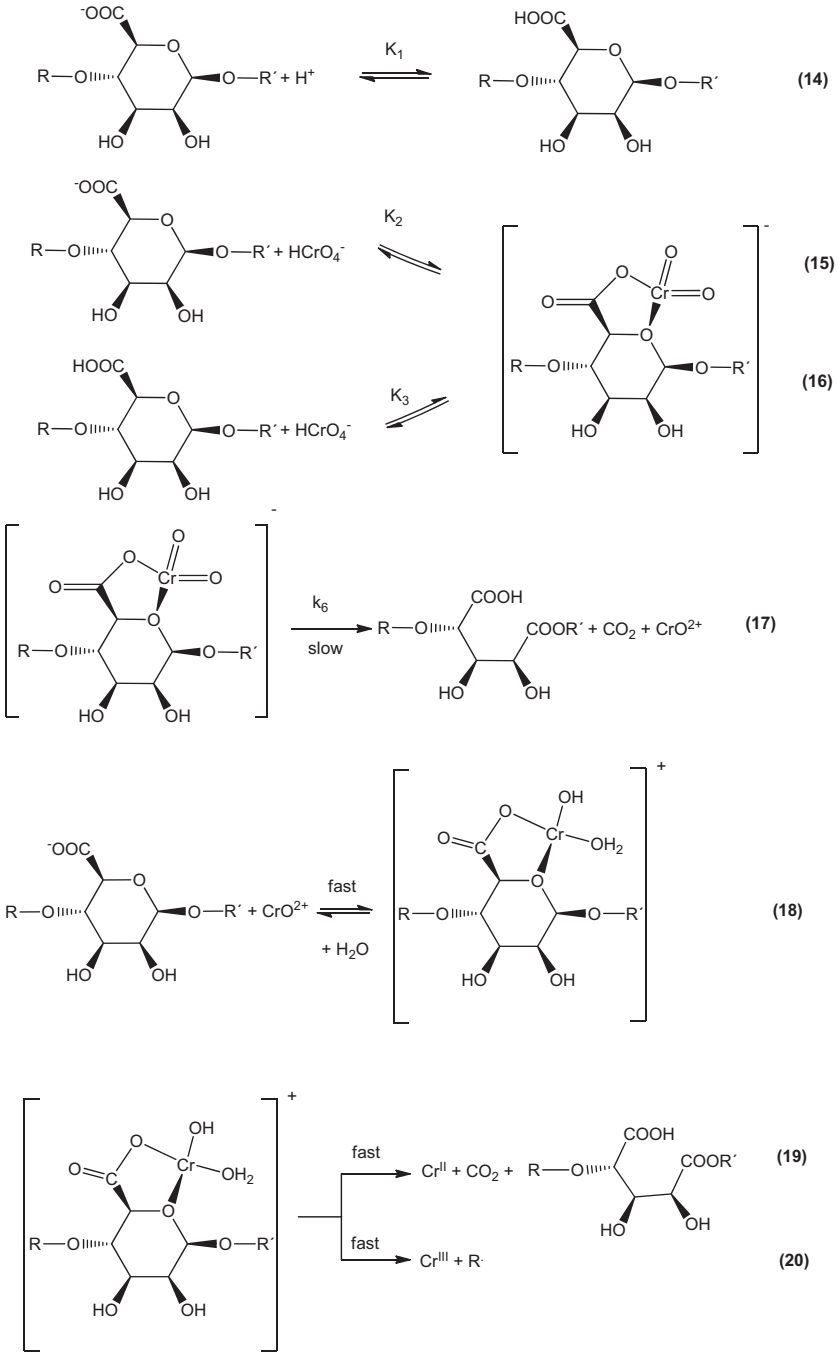
In O_2-saturated solutions (1.26 mM) and [Cr^{VI}]$_0 \leq$ 0.06 mM, reactions (21) and (22) can be neglected because R• and Cr^{II} intermediates formed in reactions (19) and (20) should be rapidly trapped by O_2, Eqs. (24) and (25) (Bakac, Scott, Espenson, & Rodgers, 1995). The proposed mechanism is in accordance with the observation that O_2 has no kinetic effect on this reaction, because when [Cr^{VI}]$_0 \geq$ 0.06 mM (as employed in the kinetic measurements), both Cr^{II} and R• react with Cr^{VI} faster than they do with O_2, and therefore Eqs. (24) and (25) could be neglected (Scott et al., 1992; Lay & Levina, 1998).

Detection of free radicals and CrO_2^{2+} in the Cr^{VI}/alginic acid reaction, provided information supporting that the reduction of Cr^{IV} by alginic acid took place through Eqs. (19) and (20).

The reaction of Cr^{V} with alginic acid was also much faster than Cr^{VI}/alginic acid. Only a few seconds were required to reduce Cr^{V} in acid solution, at 15 °C. The redox reaction involves two-electrons. At higher pH, the redox reaction was slower, and oxo-Cr^{V}–alginic acid species remain in solution for hours. At pH 1.0–3.0, alginic acid is a good scavenger of oxo-Cr^{V}, and the EPR parameter, g_{iso} values observed, suggests that the donor sites of alginic acid involved in coordination to the metal are carboxylate and hydroxyl groups.

The overall reaction stoichiometry deduced from Eqs. (14) to (23) of Scheme 4 agrees with the experimental 0.60:1 CO_2/Cr^{VI} ratio found in mixtures of Cr^{VI} and alginic acid.

The present results suggest that Cr^{VI} (as well as Cr^{IV} and Cr^{V}) selectively oxidizes the free carboxylic groups of alginic acid through C—C bond break. Zaafarany et al. (2009), Hassan et al. (2009, 2013) had reported the oxidation of similar polysaccharides by this oxidant and they found that the oxidation processes led to the oxidation of the hydroxyl groups on C_2—C_3 bonds to its corresponding



$Cr^{II} + Cr^{VI} \rightarrow Cr^{III} + Cr^{V}$ (fast) **(21)**

$Cr^{VI} + R^{\cdot} \rightarrow Cr^{V} + CO_2$ + oxidized alginic acid (fast) **(22)**

Cr^{V} + alginic acid $\rightarrow Cr^{III} + CO_2$ + oxidized alginic acid (fast) **(23)**

$Cr^{II} + O_2 \rightarrow CrO_2^{2+} \rightarrow\rightarrow Cr^{III}$ (fast) **(24)**

$R^{\cdot} + O_2 \rightarrow CO_2$ + oxidized alginic acid (fast) **(25)**

Scheme 4. Proposed mechanism for the oxidation of alginic acid by Cr^{VI}.

keto forms without the occurrence of any bond cleavage. The difference between our work and that of Hassan and Co-workers is due principally by differences in the experimental conditions. We work at higher temperature (60 °C) and lower [H^+] (lower than 0.5 M), whereas Hassan and Co-workers use lower temperatures (around 40 °C) and higher [H^+] (higher than 2.0 M). Experimental conditions employed in our work increases the oxidant power of $HCrO_4^-$ allowing the cleavage of C—C bond with the release of CO_2.

Table 5
Identification of the chromium reactive specie[a].

$[Cr^{VI}]_0$ (μM)	$[Cr_2O_7^{2-}]$ (μM)	$[HCrO_4^-]$ (μM)	k_{6exp}[b] × 10^2	$k_{6exp} \times A$	$k_{6exp}B \times 100$
300	16	268	2.32 ± 0.04	1.16 ± 0.01	2.60 ± 0.10
500	20	459	2.33 ± 0.06	0.76 ± 0.01	2.54 ± 0.10
1000	55	889	2.27 ± 0.04	0.41 ± 0.01	2.55 ± 0.10
2000	125	1750	2.29 ± 0.08	0.24 ± 0.01	2.62 ± 0.10

[a] [alginic acid] = 9.13 g/L; $[H^+]$ = 0.30 M; μ = 0.50 M; T = 60 °C; $[Cr^{VI}]_0$ = 0.30–2.0 mM.
[b] k_{6exp} Values are expressed in min^{-1}. $A = [Cr^{VI}]_0/[Cr_2O_7^{2-}]$; $B = [Cr^{VI}]_0/[HCrO_4^-]$.

This pattern of selectivity distinguishes chromic oxidation of polyuronic acids from that observed by others oxidizer agents. Thus, $NaVO_3$ oxidizes the terminal unit of the polymeric chain (Gessa, De Cherchi, Dessi, Deiana, & Micera, 1983), $KMnO_4$ yields ketoderivatives upon oxidation of vicinal hydroxyl groups (Abdel-Hamid, Khairou, & Hassan, 2003), and $NaIO_4$ oxidizes vicinal hydroxyl groups to yields aldehyde groups (Gomez, Rinaudo, & Villar, 2007).

4. Conclusions

The reaction of alginic acid with Cr^{VI} strongly depends on pH. In acid media, redox reaction occurs and reactive Cr^V, Cr^{II}, and Cr^{IV} intermediate species are generated in the redox process, together with CO_2, free radicals, and oxidized forms of alginic acid. At pH 1–3, alginic acid slowly reduces Cr^{VI}, and stabilizes Cr^V yielding long-lived oxo-Cr^V–alginic acid species, that, if the medium becomes acid, rapidly affords redox processes. The Cr-binding sites of alginic acid are mainly the free carboxylic groups that can undergo redox processes with $Cr^{VI–IV}$ in acid media or stabilize Cr^V together with hydroxo donor groups of the polysaccharide. The present results evidence that alginic acid can act as reducing agent of chromate acid (particularly at low pH), with formation of highly toxic free radicals, CrO^{2+} and Cr^V intermediate species, or can be involved in the transport of higher oxidation state of chromium at pH > 2.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.07.065.

References

Abdel-Hamid, M. I., Khairou, K. S., & Hassan, R. M. (2003). Kinetics and mechanism of permanganate oxidation of pectin polysaccharide in acid perchlorate media. *European Polymer Journal*, *39*, 381–387.
Al-Ajlouni, A., Bakac, A., & Espenson, J. (1994). Hydride abstraction from 1,2-diols by the pentaaqua(oxo)chromium(IV) ion. *Inorganic Chemistry*, *33*, 1011–1014.
Bakac, A., Scott, S. L., Espenson, J. H., & Rodgers, K. R. (1995). Interaction of chromium(II) complexes with molecular oxygen. Spectroscopic and kinetic evidence for eta.1-superoxo complex formation. *Journal of the American Chemical Society*, *117*, 6483–6488.
Barr-David, G., Charara, M., Codd, R., Farell, R. P., Irwin, J. A., Lay, P. A., Ramley, R., Brumby, S., Ji, J. Y., & Hanson, G. R. (1995). EPR characterisation of the Cr^V intermediates in the $Cr^{VI/V}$ oxidations of organic substrates and of relevance to Cr-induced cancers. *Journal of Chemistry Society Faraday Transaction*, *91*, 1207–12016.
Bellú, S. E., González, J. C., García, S. I., Signorella, S. R., & Sala, L. F. (2008). Kinetics and mechanism of oxidation of apple pectin by CrVI in aqueous acid medium. *Journal of Physical Organic Chemistry*, *21*, 1–10.
Brasch, N. E., Buckingham, D. A., Evans, A. B., & Clark, C. R. (1996). ^{17}O NMR study of chromium(VI) ions in water. *Journal of American Chemical Society*, *118*, 7969–7980.
Ciéslak-Golonka, M., & Daszkiewicz, M. (2005). Coordination geometry of Cr(VI) species: Structural and spectroscopic characteristics. *Coordination Chemistry Reviews*, *249*, 2391–2407.
Clesceri, L., Greenberg, A., & Eaton, A. (1998). *Standard methods for the examination of water and wastewater* (20th ed., pp. 366). Washington, D.C.: American Public Health Association, American Water Association, Water Environment Federation.
Codd, R., Dillon, C. T., Levina, A., & Lay, P. A. (2001). Studies on the genotoxicity of chromium: from the test tube to the cell. *Coordination Chemistry Reviews*, *216–217*, 537–582.
Cood, R., Irwin, J. A., & Lay, P. A. (2003). Sialoglycoprotein and carbohydrate complexes in chromium toxicity. *Current Opinion in Chemical Biology*, *7*, 213–219.
Craigie, J. S., Morris, E. R., Rees, D. A., & Thom, D. (1984). Alginate block structure in phaeophyceae from Nova Scotia: Variation with species, environment and tissue-type. *Carbohydrate Polymers*, *4*, 237–252.
De Stefano, C., Gianguzza, A., Piazzese, D., & Sammartano, S. (2005). Modelling of proton and metal exchange in the alginate biopolymer. *Analytical Bioanalysis Chemistry*, *383*, 587–596.
Diehl, H., & Smith, G. F. (1959). Wet oxidation of organic matter employing mixed perchloric and sulphuric acids at controlled temperatures and graded high potentials. *Talanta*, *2*, 209–219.
Eckert, J. M., Judd, R. J., Lay, P. A., & Symons, A. D. (1991). Response of chromium(V) to the diphenylcarbazide spectrophotometric method for the determination of chromium(VI). *Analytica Chimica Acta*, *255*, 31–33.
Emmerichs, N., Wingender, J., Flemming, H-C., & Mayer, C. (2004). Interaction between alginates and manganese cations: identification of preferred cation binding sites. *International Journal of Biological Macromolecules*, *34*, 73–79.
Espenson, J. H. (2002). *Chemical kinetics and reactions mechanism* (2nd ed., pp. 206–214). New York, NY: McGraw Hill.
Gessa, C., De Cherchi, M. L., Dessi, A., Deiana, S., & Micera, G. (1983). The reduction of Fe(III) to Fe(II) and V(V) to V(IV) by polygalacturonic acid: A reduction and complexation mechanism of biochemical significance. *Inorganica Chimica Acta*, *80*, L53–L55.
Gheju, M., & Iovi, A. (2006). Kinetics of hexavalent chromium reduction by scrap iron. *Journal of Hazardous Materials*, *B135*, 66–73.
Ghosh, M. C., & Gould, E. S. (1991). Electron transfer. 106. Stabilized aqueous chromium(IV), as prepared from the chromium(VI)–arsenic(III) reaction. *Inorganic Chemistry*, *30*, 491–494.
Gomez, C. G., Rinaudo, M., & Villar, M. A. (2007). Oxidation of sodium alginate and characterization of the oxidized derivatives. *Carbohydrate Polymers*, *67*, 296–304.
González, J. C., Daier, V., García, S., Goodman, B. A., Sala, L. F., & Signorella, S. (2004). Redox and complexation chemistry of the Cr^{VI}/Cr^V-D-galacturonic acid system. *Dalton Transaction*, 2288–2296.
González, J. C., García, S. I., Bellú, S., Atria, A. M., Salas- Peregrín, J. M., Rockenbauer, A., Korecz, L., Signorella, S., & Sala, L. (2009). Oligo and polyuronic acids interactions with hypervalent chromium. *Polyhedron*, *28*, 2719–2729.
González, J. C., García, S., Bellú, S., Salas Peregrín, J. M., Atria, A. M., Sala, L. F., & Signorella, S. (2010). Redox and complexation chemistry of the $Cr^{VI}/Cr^V/Cr^{IV}$-D-glucuronic acid system. *Dalton Transaction*, *39*, 2204–2217.
Hassan, R. M., Ahmed, S. A., Fawzy, A., Abdel-Kader, D. A., Ikeda, Y., & Takagi, H. D. (2010). Acid-catalyzed oxidation of carboxymethyl cellulose polysaccharide by chromic acid in aqueous perchlorate solutions. A kinetics study. *Catalysis Communications*, *11*, 611–615.
Hassan, R., Ibrahim, S., Dahy, A. R., Zaafarany, I., Tirkistani, F., & Takagi, H. (2013). Kinetics and mechanism of oxidation of chondroitin-4-sulfate polysaccharide by chromic acid in aqueous perchlorate solutions. *Carbohydrate Polymers*, *92*, 2321–2326.
Ibáñez, J. P., & Umetsu, Y. (2004). Uptake of trivalent chromium from aqueous solutions using protonated dry alginate beads. *Hydrometallurgy*, *72*, 327–334.
Kolthoff, I. M., Sandell, E. B., Meehan, E. J., & Bruckenstein, S. (1969). *Quantitative Chemical Analysis* (4th ed., pp. 777–794). London: The Macmillan Company Collier-Macmillan Limited.
Krumpolc, M., Roček, J., Haight, G. P., Jr., & Merrill, P. (1980). Sodium bis[2-ethyl-2-hydroxybutyrato(2-)]-oxochromate(V). *Inorganic Synthesis*, *20*, 63–65.

Larsen, B., Salem, D. M. S. A., Sallam, M. A. E., Misheikey, M. M., & Beltagy, A. I. (2003). Characterization of the alginates from algae harvested at the Egyptian Red Sea coast. *Carbohydrate Research*, *338*, 2325–2336.

Lay, P. A., & Levina, A. (1998). Activation of molecular oxygen during the reactions of chromium(VI/V/IV) with biological reductants: Implications for chromium-induced genotoxicities. *Journal of the American Chemical Society*, *120*, 6704–6714.

Leal, D., Matsuhiro, B., Rossi, M., & Caruso, F. (2008). FT-IR spectra of alginic acid block fractions in three species of brown seaweeds. *Carbohydrate Research*, *343*, 308–316.

Levina, A., Zhang, L., & Lay, P. A. (2003). Structure and reactivity of a chromium(V) glutathione complex. *Inorganic Chemistry*, *42*, 767–784.

Levina, A., & Lay, P. A. (2005). Mechanistic studies of relevance to the biological activities of chromium. *Coordination Chemistry Reviews*, *249*, 281–298.

Levina, A., Zhang, L., & Lay, P. A. (2010). Formation and reactivity of chromium(V)–thiolato complexes: A model for the intracellular reactions of carcinogenic chromium(VI) with biological thiols. *Journal of American Chemical Society*, *132*, 8720–8731.

Lin, Y., & Wang, S. (2012). Chromium(VI) reactions of polysaccharide biopolymers. *Chemical Engineering Journal*, *181–182*, 479–485.

Mangiameli, M. F., González, J. C., Bellú, S., Bertoni, F., & Sala, L. F. (2014). Redox and complexation chemistry of the Cr^{VI}/Cr^{V}-D-glucaric acid system. *Dalton Transactions*, *43*, 9242–9254.

Mangiameli, M. F., González, J. C., García, S. I., Bellú, S., Santoro, M., Caffaratti, E., Frascaroli, M. I., Salas- Peregrín, J. M., Atria, A. M., & Sala, L. F. (2010). Redox, kinetics, and complexation chemistry of the $Cr^{VI}/Cr^{V}/Cr^{IV}$-D-glycero-D-gulo-heptono-1,4-lactone system. *Journal of Physical Organic Chemistry*, *23*, 960–971.

Mangiameli, M. F., González, J. C., García, S. I., Frascaroli, M. I., Van Doorslaer, S., Salas Peregrín, J. M., & Sala, L. F. (2011). New insights on the mechanism of oxidation of D-galacturonic acid by hypervalent chromium. *Dalton Transaction*, *40*, 7033–7045.

Maureira, A., & Rivas, B. L. (2009). Metal ions recovery with alginic acid coupled to ultrafiltration membrane. *European Polymer Journal*, *45*, 573–581.

Mitewa, M., & Bontchev, P. (1985). Chromium(V) coordination chemistry. *Coordination Chemistry Reviews*, *61*, 241–272.

Pérez Benito, J., & Arias, C. (1993). A kinetic-study on the reactivity of chromium(IV). *Canadian Journal of Chemistry*, *71*, 649–655.

Pérez Benito, J., Arias, C., & Lamrhari, D. (1994). Chromium(II) is an intermediate in the reduction of carcinogenic chromate with biological thiols. *New Journal of Chemistry*, *18*, 663–666.

Roldán, V., Daier, V., Goodman, B., Santoro, M., González, J. C., Calisto, N., Signorella, S., & Sala, L. (2000). Kinetics and mechanism of the reduction of chromium(VI) and chromium(V) by D-glucitol and D-mannitol. *Helvetica Chimica Acta*, *83*, 3211–3228.

Sala, L. F., González, J. C., García, S. I., Frascaroli, M. I., & Van Doorslaer, S. (2011). Detection and structural characterization of oxo-chromium(V)-sugar complexes by electron paramagnetic resonance. *Advances Carbohydrate Chemistry Biochemistry*, *66*, 69–120.

Sala, L., Palopoli, C., Alba, V., & Signorella, S. (1993). A secondary reaction in the oxidation of L-methionine by chromium(VI) in acidic medium. *Polyhedron*, *12*, 2227–2234.

Santoro, M., Caffaratti, E., Salas-Peregrin, J. M., Korecz, L., Rockenbauer, A., Sala, L. F., & Signorella, S. (2007). Kinetics and mechanism of the chromic oxidation of *myo*-inositol. *Polyhedron*, *26*, 169–177.

Scott, S., Bakac, A., & Espenson, J. (1991). Preparation and reactivity of the aquachromium(IV) ion. *Journal of American Chemical Society*, *113*, 7787–7788.

Scott, S., Bakac, A., & Espenson, J. (1992). Oxidation of alcohols, aldehydes, and carboxylates by the aquachromium(IV) ion. *Journal of American Chemical Society*, *114*, 4205–4213.

Sen Gupta, K. K., Samanta, T., & Samanendra, N. B. (1985). Kinetics of oxidation of methanol and mono-deutero-methanol by chromium(VI) in perchloric acid medium. *Tetrahedron*, *41*, 205–208.

Signorella, S., Daier, V., García, S., Cargnello, R., González, J. C., Rizzotto, M., & Sala, L. F. (1999). The relative ability of aldoses and deoxyaldoses to reduce Cr^{VI} and Cr^{V}. A comparative kinetic and mechanistic study. *Carbohydrate Research*, *316*, 14–25.

Signorella, S., Frascaroli, M. I., García, S., Santoro, M., González, J. C., Palopoli, C., Casado, N., & Sala, L. F. (2000). Kinetics and mechanism of the chromium(VI) oxidation of methyl-alpha-D-glucopyranoside and methyl-alfa-D-mannopyranoside. *Journal of Chemical Society Dalton Transaction*, 1617–1623.

Zaafarany, I. A., Khairou, K. S., & Hassan, R. M. (2009). Acid-catalysis of chromic acid oxidation of kappa-carrageenan polysaccharide in aqueous perchlorate solutions. *Journal of Molecular Catalysis*, *302*, 112–118.