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Author: Fernando A. Bertoni Sebastian E. Bellú Juan C. González Luis F. Sala



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# 1 Reduction of hypervalent chromium in acidic media by alginic acid

2 Fernando A. Bertoni<sup>a</sup>, Sebastian E. Bellú<sup>a\*</sup>, Juan C. González<sup>a</sup>, Luis F. Sala<sup>a</sup>

3 <sup>a</sup>Área Química General, Departamento de Químico-Física, Facultad de Ciencias Bioquímicas y  
4 Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, S2002LRK, Rosario, Santa  
5 Fe, Argentina. Instituto de Química de Rosario-CONICET. Suipacha 570, S2002LRK, Rosario,  
6 Santa Fe, Argentina. e-mail:bellu@iquir-conicet.gov.ar; Fax: +54 341 4350214; Tel: +54 341  
7 4350214

8 \*Corresponding author. Tel./fax: +54 341 4350214, extension 121.

9 e-mail address: bellu@iquir-conicet.gov.ar (S.E. Bellú).

## 10 Abstract

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

## 23 Keywords:

24 alginic acid  
25 oxidation  
26 chromium  
27 kinetics

## 29 1. Introduction

30 The major structural polysaccharide of brown seaweeds (*Phaeophyta*) is alginic  
31 acid, a linear copolymer of (1→4)-linked β-D-mannopyranuronic acid (M) and  
32 (1→4)-linked α-L-gulopyranuronic acid (G) residues, arranged in  
33 heteropolymeric and homopolymeric blocks (**scheme 1**) (Larsen et al., 2003;  
34 Leal et al., 2008). The content of uronic acids varies with species and tissues

1 types, and partial acid hydrolysis of alginic acids allows the preparation of  
2 fractions enriched in hetero- and homopolymeric blocks (Craigie et al., 1984).  
3 The presence of carboxylic acid groups in both monomeric units makes  
4 possible its interaction with different metal ions, and in this sense its  
5 complexation ability has been studied with heavy metal ions present in  
6 wastewaters for water purification. Several authors have proposed interaction  
7 models between alginic acid and specific metal ions (De Stefano et al., 2005;  
8 Emmerichs et al., 2004; Maureira & Rivas, 2009).

9

10 **Insert scheme 1 here**

11

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

21 Polyoxygenated compounds, such as polyalcohols and hydroxycarboxylic acids,  
22 are effective as non-enzymatic reductants (at low pH) and can stabilize the  
23 labile oxidation states of chromium (Codd et al., 2001; Ciésłak-Golonka &  
24 Daszkiewicz, 2005). Due to the potential biological and ecological relevance of  
25 these kinds of biopolymers, the reduction and stabilization of hypervalent  
26 chromium by naturally occurring polysaccharides can provide useful information  
27 on the role that these polyoxygenated compounds play in the uptake and  
28 transport of chromium (Bellú, et al., 2008). Although the reduction of  
29 hypervalent chromium by low molecular weight saccharides has been  
30 extensively studied (González et al., 2004; Mangiameli et al., 2010 ; Mangiameli  
31 et al., 2011), little is known on the reaction of polysaccharides with  $\text{Cr}^{\text{VI}}$ . Kinetic

1 and mechanistic studies of chromic acid oxidation onto kappa-karrageen;  
2 carboxymethyl cellulose and chondroitin-4-sulfate polysaccharides as natural  
3 polymers has been reported by Hassan and coworkers (Zaafarany, Khairou, &  
4 Hassan, 2009; Hassan et al., 2010; Hassan et al., 2013). Cellulose,  
5 hemicellulose and chitin reactions with  $\text{Cr}^{\text{VI}}$  were also studied at acid media (Lin  
6 & Wang, 2012) but no redox mechanistic studies were performed in this case.  
7 In a previous work we report the mechanism of oxidation of apple pectin by  $\text{Cr}^{\text{VI}}$   
8 in aqueous acid medium (Bellu et al., 2008). The determination of the ability of  
9 alginic acid to reduce or stabilize hypervalent chromium will contribute to  
10 understand the potential role of this polysaccharide in the biochemistry of this  
11 metal. In this work, we report the study of the redox reaction of alginic acid with  
12  $\text{Cr}^{\text{VI}}$  providing information related to the relative reactivity of alginic acid toward  
13  $\text{Cr}^{\text{VI}}$ ,  $\text{Cr}^{\text{V}}$ , and  $\text{Cr}^{\text{IV}}$ , the influence of pH on the redox reactions, and the formation  
14 of long-lived oxo- $\text{Cr}^{\text{V}}$ -alginic acid complexes, being characterized by  
15 paramagnetic electronic resonance.

## 16 **2. Experimental**

### 17 **2.1. Materials**

18 Alginic acid sodium salt (Sigma, p.a.), GSH = L-glutathione reduced (Sigma,  
19 98.0%), potassium dichromate (Mallinckrodt p.a.), sodium perchlorate  
20 monohydrate (Fluka 98.0%), oxygen (99.99%), nitrogen (99 %), perchloric acid  
21 (A. C. S. Baker), Ammonium iron(II) sulfate hexahydrate (Sigma, 99%),  
22 acrylamide (Merck, 99.0%), sodium hydroxide (Cicarelli, p.a.),  
23 diphenylpicrylhydrazyl (dpph) (Sigma, 99.9%), formic acid (Sigma, 80%),  
24 acetone (Anedra 99.5 %), ethanol absolute (Cicarelli, p.a.), ehba = 2-ethyl-  
25 hydroxybutanoic acid (Aldrich 99.0%),  $\text{H}_2\text{SO}_4$  (Sigma, HPLC), were used  
26 without further purification.

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

1 Na[Cr<sup>V</sup>O(ehba)<sub>2</sub>].H<sub>2</sub>O, [Cr<sup>IV</sup>O(ehbaH)<sub>2</sub>] and K<sub>3</sub>[Cr<sup>V</sup>(O)(GSH)<sub>2</sub>] were synthesized  
2 according to the method described in the literature (Krumpolc et al, 1980;  
3 Ghosh & Gould,1991; Levina et al., 2003). For experiments performed in the 1-  
4 6 pH range, the pH of the solutions was adjusted by addition of HClO<sub>4</sub>. In  
5 experiments performed at constant ionic strength ( $\mu = 0.50$  M) and different  
6 hydrogen ion concentrations; sodium perchlorate and perchloric acid solution  
7 mixtures were used. The concentration of stock solutions of perchloric acid was  
8 determined by titration employing standard analytical methods.

9  
10 ***Caution: Cr<sup>VI</sup>, Na[Cr<sup>V</sup>O(ehba)<sub>2</sub>].H<sub>2</sub>O, [Cr<sup>IV</sup>O(ehbaH)<sub>2</sub>] and dpph are human***  
11 ***carcinogens. Contact with skin and inhalation must be avoided.***

## 12 13 **2.2. Alginate acid stability**

14 Stability of polysaccharide in acidic conditions was studied by HPLC. The  
15 chromatograms were obtained on a Varian Polaris 200 chromatograph provided  
16 with a cc Star 9000 HPLC pump. The separation was carried out on an Aminex  
17 HPX-87X (300 x 7.8mm<sup>2</sup>, Bio-Rad Lab) HPLC column, using H<sub>2</sub>SO<sub>4</sub> as eluent  
18 (pH 1.5) and a flow rate of 0.6 mL/min, at 30°C. The samples were filtered  
19 through a 0.2 mm membrane prior to the injection into the chromatographic  
20 system. The effluent was monitored with a UV-vis detector (Prostar 325 UV-vis  
21 detector,  $\lambda = 220$  nm). Chromatograms, recorded after incubation of the standard  
22 sample in 1.0 M HClO<sub>4</sub> (higher than the highest [H<sup>+</sup>] used in the kinetic  
23 measurements) at 60 °C during 3.0 h, showed only one peak (tr: 5.65 min)  
24 assigned to alginate acid. No others peaks were observed suggesting the stability  
25 of the polysaccharide under the present experimental conditions.

## 26 27 **2.3. Measurement of free carboxylic groups of alginate acid**

28  
29 Measurements of free carboxylic groups of alginate acid were performed by acid  
30 base titration employing NaOH as titrant and phenolphthalein as visual indicator  
31 (Kolthoff et al., 1969). Titration of 10.00 mL solution containing 20.00 g/L alginate

1 acid with 0.08704 M NaOH until change colour from colourless to pink afforded  
2 10.75 mL of NaOH consumed which corresponds to 87.6 mM of free carboxylic  
3 groups. The employed polysaccharide contains an average of 4.38 mmol free  
4 carboxylic groups per gram of polymer.

5

#### 6 **2.4. Product analysis**

7 Carbon dioxide was measured from a mixture of alginic acid (33.1 mg/mL), Cr<sup>VI</sup>  
8 (5.0 mM) and HClO<sub>4</sub> (0.50 M). The temperature was kept constant at 60°C and  
9 the reaction mixture was continuously stirred and flushed with pure nitrogen.  
10 The gaseous products were passed through three flasks containing NaOH (0.02  
11 M). After reaction, in order to determine the yield of carbon dioxide, the NaOH  
12 solutions were titrated with standard HCl (0.0232 M). Aliquots of the reaction  
13 mixture of alginic acid/Cr<sup>VI</sup> were analyzed by HPLC, using the same  
14 experimental conditions and column mentioned at Section 2.2. No peak  
15 corresponding to HCOOH (tr: 13.2 min) was observed.

16

#### 17 **2.5. Polymerization test**

18 Detection of free organic radical generation, during the oxidation of alginic acid  
19 by hypervalent chromium, was tested employing acrylamide. In a typical  
20 experiment, a solution of Cr<sup>VI</sup> (1.0 mL, 0.010 M) was added to 10.0 mL of a  
21 solution 2.70 g/L of alginic acid, 0.50 M HClO<sub>4</sub> and 0.70 M of acrylamide. When  
22 [Cr<sup>VI</sup>] became negligible, the precipitation of a white polymer of polyacrylamide  
23 was observed. Control experiments showed that no polymerization of  
24 acrylamide took place under the same experimental conditions with either  
25 K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> or alginic acid alone. Possible reactions of Cr<sup>V</sup> and Cr<sup>IV</sup> with acrylamide  
26 were tested with Na[Cr<sup>V</sup>O(ehba)<sub>2</sub>] and [Cr<sup>IV</sup>O(ehbaH)<sub>2</sub>]. No precipitation  
27 occurred on mixing Cr<sup>V</sup> or Cr<sup>IV</sup> complexes with acrylamide under the conditions  
28 used in the Cr<sup>VI</sup>/alginic acid reaction.

29

#### 30 **2.6. Kinetic measurements**

1 Kinetic measurements were performed by monitoring absorbance changes on a  
2 Jasco V-550 spectrophotometer with fully thermostated cell compartments ( $\pm 0.2$   
3  $^{\circ}\text{C}$ ). The reactions were followed under pseudo-first-order conditions, at  $60^{\circ}\text{C}$ ;  
4 using excess of alginic acid over  $\text{Cr}^{\text{VI}}/\text{Cr}^{\text{V}}$ . Reactant solutions were thermally  
5 equilibrated at  $60^{\circ}\text{C}$  prior to the experiment and  $\text{NaClO}_4$  was used to maintain a  
6 constant ionic strength ( $\mu$ ).

### 7 ***Alginic acid/ $\text{Cr}^{\text{VI}}$ reactions.***

8 Disappearance of  $\text{Cr}^{\text{VI}}$  was followed by DPC method (Clesceri, Greenberg, &  
9 Eaton, 1998); monitoring the absorbance at 540 nm until at least 80%  
10 conversion. In the kinetic measurements, the concentration of  $\text{Cr}^{\text{VI}}$  and  $\mu$  were  
11 kept constant at 1.0 mM and 0.50 M, respectively, while carboxylic group/ $\text{Cr}^{\text{VI}}$   
12 ratio was varied from 20:1 to 60:1, at various  $[\text{HClO}_4]$ . The experimental  
13 pseudo-first-order rate constants ( $k_{\text{6exp}}$ ), obtained from nonlinear least-square  
14 fits of kinetic data, were averages of at least three determinations and were  
15 within  $\pm 5\%$  of each other. The first-order dependence of the rate upon  $[\text{Cr}^{\text{VI}}]$  was  
16 verified in a set of experiments where the  $[\text{Cr}^{\text{VI}}]_0$  was varied between 0.30 and  
17 2.0 mM, keeping temperature, [alginic acid],  $[\text{H}^+]$ , and  $\mu$  constant. Ionic strength  
18 effect was studied in a set of experiments where  $[\text{Cr}^{\text{VI}}]_0$ , [alginic acid],  $[\text{H}^+]$  and T  
19 were kept constant and  $\mu$  was varied from 0.20 M to 1.0 M.

### 20 ***Alginic acid/ $\text{Cr}^{\text{V}}$ reactions.***

21 Kinetic measurements for the oxidation of alginic acid by  $\text{Cr}^{\text{V}}$  was performed by  
22 ligand exchange reaction, employing  $\text{K}_3[\text{Cr}^{\text{V}}(\text{O})(\text{GSH})_2]$ . In a typical experiment,  
23 100  $\mu\text{L}$  of the oxo- $\text{Cr}^{\text{V}}$  complex solution (24 mM) were mixed with 2.3 mL of a  
24 solution containing alginic acid (4.57 g/L),  $[\text{H}^+]$  (0.30 M) and  $\mu = 0.50$  M  
25 ( $\text{NaClO}_4$ ) at  $15^{\circ}\text{C}$ . The reaction was followed at 350 nm, until at least 80 %  
26 conversion.

### 27 ***Alginic acid/ $\text{Cr}^{\text{IV}}$ reactions.***

28 The oxidation of alginic acid by  $\text{Cr}^{\text{IV}}$  ( $\text{CrO}_2^{2+}$ ) was spectrophotometrically  
29 monitored following the appearance of  $\text{CrO}_2^{2+}$  as a final redox product. Alginic  
30 acid/ $\text{CrO}_2^{2+}$  mixtures showed an increase of two intense absorption bands at

1 290 and 247 nm, with relative intensity  $Abs_{247}/Abs_{290} = 2.2$  characteristic of  
2  $CrO_2^{2+}$  (Scott, Bakac, & Espenson, 1991). The kinetic data were collected  
3 spectrophotometrically by following the formation of  $CrO_2^{2+}$  at 290 nm ( $\epsilon = 3100$   
4  $M^{-1}cm^{-1}$ ) at 25 °C (Scott, Bakac, & Espenson, 1992). At this wavelength, neither  
5 alginic acid nor the oxidized products absorb. In the kinetics measurements,  
6  $[Cr^{IV}]$ ,  $\mu$ ,  $[O_2]$ , and temperature were kept constant at 0.207 mM, 0.50 M, 1.26  
7 mM and 25 °C respectively. The concentration range of alginic acid used was  
8 chosen in order to avoid  $CrO^{2+}$  disproportionation. The carboxylic group/ $Cr^{IV}$   
9 ratio was varied from 50:1 to 400:1. The experimental pseudo first order rate  
10 constants ( $k_{4exp}$ ), obtained from nonlinear least square fits of absorbance data  
11 at 290 nm were averages of at least five determinations and were within  $\pm 10\%$   
12 of each other. Data used to calculate the kinetic constant,  $k_{4exp}$ , correspond to  
13 80% of the total experimental values. The first order dependence of the rate  
14 upon  $[Cr^{IV}]$  was verified in a set of experiments where the  $[Cr^{IV}]_0$  was varied  
15 between 0.10- 0.50 mM, but T, [alginic acid], and  $\mu$  were kept constant.

16

### 17 **2.7. Detection of superoxo- $Cr^{III}$ during the reaction of alginic acid with $Cr^{VI}$**

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

28

### 29 **2.8. Chromate esters**



1 Chromate esters were investigated by UV-vis spectrophotometry in the 320-  
2 450 nm region in which these compounds show characteristic absorption  
3 bands. Reactions were performed at pH 6.0, where the redox reaction is slow  
4 enough to enable the observation of the ester formation. The instrument was  
5 zeroed to an arrangement of the reference and sample beams passing through  
6 matched cell, both containing 0.5 mM Cr<sup>VI</sup> at pH 6.0. The solution in the sample  
7 cell was replaced with the reaction solution containing 0.5 mM Cr<sup>VI</sup> and 0.228-  
8 0.457 g/L alginic acid at pH = 6.0,  $\mu$  = 0.50 M and T = 25 °C. Spectra obtained  
9 at 30 minutes after mixing showed a characteristic absorption at 375 nm.

10

## 11 2.9. EPR measurements

12 The EPR spectra were obtained with a Bruker ESP 300 E computer-controlled  
13 spectrometer operating at X-band frequencies (~ 9.4-9.8 GHz). Microwave  
14 generation was by means of a klystron (ER041MR) and frequencies were  
15 measured with a built-in frequency-counter. Spectra were recorded as first  
16 derivatives of the microwave absorption in 1024 points at 288 K, using 10 mW  
17 microwave power and 100 kHz modulation frequency. Power values used in the  
18 EPR experiments did not overcome 10 mW in order to avoid signal saturation.  
19 In EPR measurements, scanning speed and number were fixed in order to  
20 reduce the time used in each measurement. This was done to avoid fluctuations  
21 in the EPR signal during the sample scanning. *g*-values were determined by  
22 reference to diphenylpicrylhydrazyl (dpph) ( $g_{iso} = 2.0036$ ) as an external  
23 standard. Long-lived oxo-Cr<sup>V</sup>-alginic acid complexes were generated in two  
24 different ways: a) by reaction of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> with an aqueous solution containing  
25 alginic acid; b) by mixing reduced glutathione (GSH) and K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> where [Cr<sup>VI</sup>] =  
26 [GSH] and an excess of alginic acid.

27

## 28 3. Results and discussion

### 29 3.1. Oxidation of alginic acid by Cr<sup>VI</sup>

#### 30 3.1.1. Rate studies

1 The reaction kinetic of alginic acid with  $\text{Cr}^{\text{VI}}$  was examined by the  
 2 diphenylcarbazide (DPC) (Clesceri, Greenberg, & Eaton, 1998) method, in  
 3 excess of alginic acid over  $\text{Cr}^{\text{VI}}$ , in the 0.10- 0.50 M  $\text{HClO}_4$  range. DPC can  
 4 react with  $\text{Cr}^{\text{V}}$  and probably with  $\text{Cr}^{\text{IV}}$  (Eckert et al., 1991), therefore it is very  
 5 important to probe that no accumulation of these species occurs in the alginic  
 6 acid/ $\text{Cr}^{\text{VI}}$  reaction mixtures. Under experimental conditions employed in this  
 7 work, both species  $\text{Cr}^{\text{IV}}$  and  $\text{Cr}^{\text{V}}$ , decay rates are much higher than  $\text{Cr}^{\text{VI}}$  (as  
 8 shown below) and do not accumulate in the reaction alginic acid/ $\text{Cr}^{\text{VI}}$ . This result  
 9 allows the use of DPC method for measuring  $\text{Cr}^{\text{VI}}$  without any interference by  
 10 other chromium species. A monophasic decrease of  $[\text{Cr}^{\text{VI}}]$  with time was  
 11 observed, and the kinetic profiles could be adequately described by a single  
 12 exponential decay from which  $\text{Cr}^{\text{VI}}$  pseudo-first-order rate constants ( $k_{6\text{exp}}$ ) were  
 13 calculated. **Table 1** summarizes  $k_{6\text{exp}}$  values for various concentrations of  
 14 alginic acid and  $\text{HClO}_4$ .

15 **Table 1: Experimental pseudo-first-order rate constants for different**  
 16  **$[\text{HClO}_4]$  and  $[\text{alginic acid}]$ .**<sup>[a]</sup>

$[\text{H}^+](\text{M})$	0.1	0.2	0.3	0.4	0.5
$[\text{alginic acid}]$ (g/L)	$10^2 \times k_{6\text{exp}}^{[b]}$				
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

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

20

1 The value of  $k_{6\text{exp}}$  did not vary with different  $[\text{Cr}^{\text{VI}}]_0$  at fixed  $T$ ,  $\mu$ , [alginic acid]  
 2 and  $[\text{HClO}_4]$ , confirming the first-order rate dependence on  $[\text{Cr}^{\text{VI}}]$ . When  $\mu$  is  
 3 varied from 0.20 M to 1.0 M but  $[\text{Cr}^{\text{VI}}]_0$ ,  $T$ , [alginic acid] and  $[\text{HClO}_4]$  are fixed,  
 4 the value of  $k_{6\text{exp}}$  increased with the increase of  $\mu$ . The effect of ionic strength  
 5 on the rate constants of reactions involving ions is well understood applying the  
 6 Bronsted- Debye- Hückel equation (Espenson, 2002):

$$7 \log_{10} k = \log_{10} k_0 + 2 A Z_A Z_B F_\mu \quad (1)$$

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

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

21 **Table 2: Effect of  $[\text{Cr}^{\text{III}}]_0$  over  $k_{6\text{exp}}$ .**<sup>[a]</sup>

$[\text{Cr}^{\text{III}}]_0$ (M)	0.000	0.010	0.050
[alginic acid] (g/L)	$10^2 \times k_{6\text{exp}}$ <sup>[b]</sup>		
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

22 <sup>[a]</sup> $T = 60 \text{ }^\circ\text{C}$ ;  $[\text{Cr}^{\text{VI}}] = 1.0 \text{ mM}$ ;  $[\text{H}^+] = 0.20 \text{ M}$  ;  $\mu = 0.50 \text{ M}$ . The values of  $k_{6\text{exp}}$   
 23 are expressed in  $\text{min}^{-1}$ . <sup>[b]</sup>Mean values from multiple determinations

1 (estimated errors are lower than 10%). Rate constants were obtained  
2 using Sigma Plot 11.0 Program.

3

4 As it showed in **Table 2** no significant variation of  $k_{6\text{exp}}$  values was obtained at  
5 fixed  $[\text{H}^+]$ , [alginic acid], T and  $\mu$ . These results demonstrate that  $\text{Cr}^{\text{III}}$ -  
6 alginates, if it is formed, they don't modify the oxidation rates of alginic  
7 acid by  $\text{Cr}^{\text{VI}}$ .

8 Plots of  $k_{6\text{exp}}$  versus [alginic acid] afforded good straight lines (**Fig. 1**) from  
9 which values of  $k_{6\text{S}}$  were determined. A linear dependence of  $k_{6\text{S}}$  values on  
10  $[\text{HClO}_4]$  was observed, (**inset, Fig. 1**).

11 **Insert Fig. 1 here**

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

$$16 \quad -\frac{d[\text{Cr}^{\text{VI}}]}{dt} = (k_6 + k_{6\text{H}}[\text{H}^+])[\text{alginic acid}][\text{Cr}^{\text{VI}}]_T \quad (2)$$

17 Under conditions used in the kinetic studies,  $\text{CO}_2$  was detected as product of  
18 oxidation. Quantitative analysis of  $\text{CO}_2$  showed that the reaction yielded 0.60  
19 mol of  $\text{CO}_2$  per mole of  $\text{Cr}^{\text{VI}}$ . In alginic acid structure, the functional group being  
20 oxidized is the carboxylic group and can be rationalized taking into account the  
21 relative reactivity of functional groups in saccharides toward  $\text{Cr}^{\text{VI}}$ :

22  $-(\text{H})\text{C}(\text{OR})\text{OH}_{\text{hemiacetal}}$  (Signorella et al., 1999) >  $-\text{CO}_2\text{H}$  (Bellú et al., 2008) >  
23  $-\text{H}_2\text{COH}_{\text{primary}}$  (Roldán et al., 2000)  $\gg$   $(\text{H})\text{COH}_{\text{secondary}}$  (Santoro et al., 2007);  
24  $(\text{H})\text{COR}_{\text{glycoside}}$  (Signorella et al., 2000);  $\text{RCO}_2\text{R}_{\text{ester}}$  (Sala et al., 1993).

25 Stoichiometry of the oxidation of alginic acid by  $\text{Cr}^{\text{VI}}$  is shown in **Scheme 2**.

26 **Insert scheme 2 here**

27 **3.1.2. Detection of  $\text{Cr}^{\text{VI}}$  esters**

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

9 **Insert Fig. 2 here**

10 Alginic acid/Cr<sup>VI</sup> mixtures with [alginic acid] 0.228- 0.457 g/L, at pH 6.0, showed  
11 that the absorbance at 375 nm increased with increasing [alginic acid], probably  
12 as a result of a shift towards the ester in the esterification equilibrium.

13

### 14 **3.1.3. Intermediacy of Cr<sup>V</sup>**

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

28

29 **Insert Fig. 3 here**

30

1 After 30 min of reaction multiplicity of EPR signals of alginic/Cr<sup>VI</sup> reaction  
 2 mixture at pH 1- 3 could not be resolved, even with low modulation amplitude,  
 3 because the superhyperfine coupling constants were lower than the line width.  
 4 Spectra were simulated with four singlets in order to obtain information from the  
 5  $g_{iso}$  values (**Table 2**) of oxo-Cr<sup>V</sup> species in the reaction mixture. The simulation  
 6 of the EPR spectra showed that the complex signal of the alginic acid/Cr<sup>VI</sup>  
 7 reaction mixture came from the contribution of, at least, four species of oxo-Cr<sup>V</sup>  
 8 species. Additionally, it must be noted that the average  $g_{iso}$  value were in the  
 9 range of that expected for five-coordinated oxochromate(V) complexes (see  
 10 Supplementary material **Fig. S2** and **Table 2**). The signal with average  $g_{iso}$   
 11 1.9780 (species A) is consistent with those for five-co-ordinated oxo-Cr<sup>V</sup>  
 12 complexes having two carboxylate and two alcoholate donor groups, (Barr-  
 13 David, et al., 1995)  $[Cr^VO(R-COO^-,R-OH)_2]$ . Meanwhile the signal with average  
 14  $g_{iso}$  1.9790 belongs to five-co-ordinated oxo-Cr<sup>V</sup> mixed species  $[Cr^VO(R-COO^-$   
 15  $,OH)(R-OH)_2]$  (species B) where the oxo-Cr<sup>V</sup> group is being coordinated by  
 16 three alcoholate and one carboxylate groups.

17 **Table 2**

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

18 T = 15 °C; [alginic acid] = 4.57 g/L;  $[Cr^{VI}]$  = 1.0 mM;  $\mu$  = 0.50 M; pH = 2.0;  
 19 Center Field = 3410 G ; Modulation amplitude = 2.0 G ; resolution = 1024  
 20 points ; Frequency = 9.4312 GHz

21

22 Unlike the results observed in the acid sacharides/Cr<sup>V</sup> complexes, (Gonzalez et  
 23 al., 2009; Gonzalez et al., 2010), at pH 2.0, there was approximately a 15% of  
 24 species, where the oxo-Cr<sup>V</sup> group is being coordinated with four alcoholate  
 25 groups (species C). Finally, a fourth species (D) with a  $g_{iso}$  1.9750 could be  
 26 related with oxo-Cr<sup>V</sup> coordinated by two carboxylates and molecules of waters.

1 Anyway, the predominant species with  $g_{\text{iso}}$  values 1.9780 and 1.9791, during  
2 reaction time, indicated that the coordination mode of oxo-Cr<sup>VO</sup> by carboxylate  
3 and diolates (belonging to  $\beta$ -D-mannopyranuronic and  $\alpha$ -L-gulopyranuronic acid  
4 residues,) was the most suitable from the thermodynamic point of view at acidic  
5 pH.

6

#### 7 **3.1.4. Intermediacy of Cr<sup>II</sup>**

8 The fact that Cr<sup>II</sup> is involved in the oxidation mechanism of several alcohols and  
9 tiols by Cr<sup>IV</sup> and Cr<sup>VI</sup> in HClO<sub>4</sub> was demonstrated by conversion to CrO<sub>2</sub><sup>2+</sup> upon  
10 reaction with dioxygen (Scott et al., 1991; Scott et al., 1992; Al-Ajlouni et al.,  
11 1994; Pérez Benito et al., 1994; Pérez Benito & Arias, 1993). In appropriate  
12 experimental conditions, such as high [O<sub>2</sub>] = 1.26 mM and low [Cr<sup>VI</sup>] = 0.08 mM,  
13 the reaction of Cr<sup>II</sup> (if any) with O<sub>2</sub> to give CrO<sub>2</sub><sup>2+</sup> can compete successfully with:  
14 a) the reaction of Cr<sup>II</sup> with Cr<sup>VI</sup> and b) the autocatalytic consumption of CrO<sub>2</sub><sup>2+</sup>  
15 by Cr<sup>II</sup>. If Cr<sup>II</sup> is an intermediate species in the alginic acid/Cr<sup>VI</sup> redox reaction,  
16 CrO<sub>2</sub><sup>2+</sup> should be detected (Scott et al., 1992).

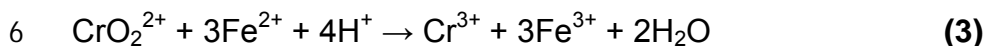
17 We examined the presence of intermediate Cr<sup>II</sup> in the reaction of alginic acid  
18 with Cr<sup>VI</sup>, by monitoring the formation of CrO<sub>2</sub><sup>2+</sup>, using [Cr<sup>VI</sup>]<sub>0</sub> low enough to  
19 avoid the Cr<sup>VI</sup> + Cr<sup>II</sup> competitive reaction, and 3.0 M HClO<sub>4</sub> to accelerate the  
20 alginic acid/Cr<sup>VI</sup> reaction at a temperature lower than used in the kinetics  
21 experiments.

22 A periodic scanning of the O<sub>2</sub>-saturated solution (1.26 mM O<sub>2</sub>) of a alginic  
23 acid/Cr<sup>VI</sup> reaction mixture in 3.0 M HClO<sub>4</sub> over a period of 60 min, showed that  
24 the band at 350 nm, characteristic of Cr<sup>VI</sup>, decreased in intensity meanwhile two  
25 absorption bands at  $\lambda_{\text{max}}$  247 and 290nm appeared (**Fig. 4**).

26 **Insert Fig. 4 here**

27 These two bands are characteristic of CrO<sub>2</sub><sup>2+</sup> formed as a long-lived  
28 intermediate (Scott et al., 1991), that then slowly transforms into the final Cr<sup>III</sup>  
29 (as shown below). Since CrO<sub>2</sub><sup>2+</sup> can be exclusively formed by reaction of Cr<sup>II</sup>  
30 with O<sub>2</sub>, and, in turn, Cr<sup>II</sup> had previously been demonstrated to form exclusively

1 through two-electron reduction of  $\text{Cr}^{\text{IV}}$  (Scott et al., 1992; Al-Ajlouni et al., 1994),  
 2 our spectroscopic results provide evidence that  $\text{Cr}^{\text{II}}$  and  $\text{Cr}^{\text{IV}}$  are involved in the  
 3 redox mechanism of the reaction between  $\text{Cr}^{\text{VI}}$  and alginic acid. When the  
 4 absorbance at 350 nm was negligible, 0.091 mM of  $\text{Fe}^{\text{II}}$  was added to bring  
 5 about the following reaction:



7 The spectrum of the reaction mixture was subtracted from the corresponding  
 8 one prior to  $\text{Fe}^{\text{II}}$  addition. As shown in **Fig. 4 inset**, there is a negative  
 9 absorbance difference around 290 nm, consistent with the presence of  $\text{CrO}_2^{2+}$ .

10

### 11 **3.2. Oxidation of alginic acid by $\text{Cr}^{\text{V}}$**

12 The reaction of alginic acid with  $\text{Cr}^{\text{V}}$  was examined by ligand exchange  
 13 employing the complex  $\text{K}_3[\text{Cr}^{\text{V}}(\text{O})(\text{GSH})_2]$ , and following the decrease in  
 14 absorption at 350 nm. The ligand exchange reaction was verified by EPR  
 15 measurements and it was completed in a few seconds. Comparing the alginic  
 16 acid/ $\text{Cr}^{\text{VI}}$  and alginic acid/ $\text{Cr}^{\text{V}}$  reactions, at the same conditions ( $\mu$ , [alginic acid]  
 17 and  $[\text{Cr}^{\text{VI}}]_0$ ) except T (15 °C for  $\text{Cr}^{\text{V}}$  reaction and 60 °C for  $\text{Cr}^{\text{VI}}$  reaction) it was  
 18 observed that alginic acid/ $\text{Cr}^{\text{V}}$  reaction is very fast (see Supplementary material  
 19 **Fig S3**).

20 As it is showed in **Fig. S3**,  $\text{Cr}^{\text{V}}$  decays with time very fast and is expected that  
 21 no accumulation of this species occurs in the alginic acid/ $\text{Cr}^{\text{VI}}$  systems. After 2.0  
 22 min, only 6 % of  $\text{Cr}^{\text{VI}}$  consumption was achieved while 85% of  $\text{Cr}^{\text{V}}$  consumption  
 23 occurs. **Fig. S3** showed a monophasic decrease of  $[\text{Cr}^{\text{V}}]$  with time. The oxo- $\text{Cr}^{\text{V}}$ -  
 24 alginic acid species kinetic profile could be adequately fitted to a single  
 25 exponential decay from which  $\text{Cr}^{\text{V}}$  pseudo-first-order rate constant ( $k_{5\text{exp}}$ ) was  
 26 calculated. The value of  $k_{5\text{exp}}$  estimated in this way resulted to be  $(2.14 \pm 0.06) \times$   
 27  $10^{-2} \text{ s}^{-1}$ . EPR and electronic spectroscopy show that  $\text{Cr}^{\text{III}}$  is the ultimate fate of  
 28 chromium in these reactions.

### 29 **3.3. Reaction of $\text{CrO}_2^{2+}$ with alginic acid**



1 The addition of variable quantities of alginic acid to O<sub>2</sub>-saturated solutions  
 2 containing 0.30 mM of CrO<sub>2</sub><sup>2+</sup> resulted in the increase of the CrO<sub>2</sub><sup>2+</sup> spectrum  
 3 intensity. The formation of CrO<sub>2</sub><sup>2+</sup> was followed at 290nm and the monotonic  
 4 increase of absorbance was found to follow first-order kinetics. The  
 5 experimental rate constants  $k_{4\text{exp}}$  were calculated by nonlinear least-square fit of  
 6 absorbance-time data to the equation:

$$7 \quad Abs_t = Abs_{\infty} + (Abs_0 - Abs_{\infty})e^{(-k_{4\text{exp}} t)} \quad (4)$$

8 where  $Abs_{\infty}$  and  $Abs_0$  were absorbance at infinite time and initial absorbance,  
 9 respectively.

10 **Fig. 5** shows spectral scanning of alginic acid/Cr<sup>IV</sup> mixtures and the right fitting  
 11 of the absorbance data vs. time at 290 nm employing **eqn 4**, (**inset Fig. 5**).

12 **Insert Figure 5 here**

13 The value of  $k_{4\text{exp}}$  did not vary with different [Cr<sup>IV</sup>]<sub>0</sub>, but fixed T,  $\mu$ , [alginic acid],  
 14 and [HClO<sub>4</sub>], confirming the first-order dependence of rate on [Cr<sup>IV</sup>]. **Table 3**  
 15 summarizes values of  $k_{4\text{exp}}$  for various concentrations of alginic acid and HClO<sub>4</sub>.

16 **Table 3: Experimental pseudo-first-order rate constants ( $k_{4\text{exp}}$ ) for different**  
 17 **concentrations of HClO<sub>4</sub> and alginic acid.**<sup>[a]</sup>

[H <sup>+</sup> ](M)	0.2	0.3	0.4	0.5
[alginic acid] (g/L)	$10^2 \times k_{4\text{exp}}^{[b]}$			
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

1 <sup>[a]</sup>T = 25 °C; [Cr<sup>IV</sup>] = 0.20 mM; μ = 0.50 M. The values of k<sub>4exp</sub> are expressed in  
 2 s<sup>-1</sup>. <sup>[b]</sup>Mean values from multiple determinations (estimated errors are lower than  
 3 10%). Rate constants were obtained using Sigma Plot 11.0 Program.

4 Experimental conditions were chosen so that the alginic acid/Cr<sup>IV</sup> reaction was  
 5 much faster than Cr<sup>IV</sup> disproportionation into Cr<sup>VI</sup> and Cr<sup>III</sup>. In the absence of  
 6 alginic acid, or when the alginic acid concentration was too low,  
 7 disproportionation of Cr<sup>IV</sup> was evidenced by the grow-up of absorbance at 350  
 8 nm due to formation of Cr<sup>VI</sup>. Using reactant concentrations of **Table 3**, Cr<sup>VI</sup> was  
 9 not detected. Values of k<sub>4exp</sub> are independent of [H<sup>+</sup>] in the range 0.20- 0.50 M,  
 10 followed by saturation kinetic with [alginic acid] (see Supplementary material **Fig.**  
 11 **S4**).

12 Non-linear curve fit of kinetic data allowed to calculate values of k<sub>1</sub> and k<sub>2</sub>  
 13 applying the equation:

$$14 \quad k_{4exp} = \frac{k_1[\text{alginic acid}]}{1+k_2[\text{alginic acid}]} \quad (5)$$

15 Consequently, the complete rate law for the Cr<sup>IV</sup> consumption can be expressed  
 16 as in the equation:

$$17 \quad \frac{d[\text{CrO}_2^{2+}]}{dt} = -\frac{d[\text{Cr}^{IV}]}{dt} = \left( \frac{k_1[\text{alginic acid}]}{1+k_2[\text{alginic acid}]} \right) [\text{Cr}^{IV}]_T \quad (6)$$

18 With k<sub>1</sub> = 66 ± 1 M<sup>-1</sup>s<sup>-1</sup> and k<sub>2</sub> = 1120 ± 30 M<sup>-1</sup>

19 Reaction mixtures containing excess of alginic acid over CrO<sup>2+</sup> in 0.20 M HClO<sub>4</sub>  
 20 were analyzed for CO<sub>2</sub> as reaction product. Quantitative analysis of CO<sub>2</sub>  
 21 showed that the reaction yielded 1.0 mol of CO<sub>2</sub> per mole of Cr<sup>IV</sup>.

22 **Scheme 3** shows a proposed reaction mechanism for the oxidation of alginic  
 23 acid by CrO<sup>2+</sup>.

24 **Insert scheme 3 here**

25 We proposed that a reactive alginic acid-Cr<sup>IV</sup> ester is formed in a first step of the  
 26 reaction pathway. This very reactive intermediate is in stationary state so its  
 27 formation and decomposition rates are equals. Taking into account the  
 28 proposed mechanism, the rate law takes the form:

$$1 \quad -\frac{d[\text{Cr}^{\text{IV}}]}{dt} = k_4[\text{Cr}^{\text{IV}} - \text{ester}] \quad (10)$$

2 Applying stationary state concept:

$$3 \quad [\text{Cr}^{\text{IV}} - \text{ester}] = \frac{k_a[\text{alginic acid}][\text{Cr}^{\text{IV}}]}{k_{-a}+k_4} \quad (11)$$

4 Considering total analytical concentration of  $\text{Cr}^{\text{IV}}$ :

$$5 \quad [\text{Cr}^{\text{IV}}]_T = [\text{Cr}^{\text{IV}}] + [\text{Cr}^{\text{IV}} - \text{ester}] \quad (12)$$

6 Replacing and making the corresponding mathematical arrangements, **eqn 10**  
7 transforms into **eqn 13**.

$$8 \quad -\frac{d[\text{Cr}^{\text{IV}}]}{dt} = \frac{k_a k_4 [\text{alginic acid}][\text{Cr}^{\text{IV}}]_T}{k_{-a} + k_4 + k_4 [\text{alginic acid}]} \quad (13)$$

9 The rate law for the disappearance of  $\text{Cr}^{\text{IV}}$  takes the form of **eqn 13** which is in  
10 total agreement with the experimental rate law, **eqn 6** where  $k_1 = \frac{k_a k_4}{k_{-a} + k_4}$  and

$$11 \quad k_2 = \frac{k_4}{k_{-a} + k_4}.$$

### 13 3.4. Reaction mechanism for the oxidation of alginic acid by $\text{Cr}^{\text{VI}}$

14 In the range of substrate and acid concentration used in the kinetic  
15 measurements, the oxidation of alginic acid by  $\text{Cr}^{\text{VI}}$  is a complex reaction that  
16 yields  $\text{Cr}^{\text{III}}$ ,  $\text{CO}_2$ , and oxidized alginic acid as final redox products.  $\text{CrO}_2^{2+}$  was  
17 detected in the reaction of alginic acid with  $\text{Cr}^{\text{VI}}$  under aerobic conditions. This  
18 fact indicates that  $\text{Cr}^{\text{IV}}$  and  $\text{Cr}^{\text{II}}$  were involved in the mechanism of the redox  
19 reaction  $\text{Cr}^{\text{VI}}$ /alginic acid.  $\text{Cr}^{\text{II}}$  was originated exclusively by two-electron  
20 reduction of  $\text{Cr}^{\text{IV}}$  species affording indirect evidence of participation of  $\text{Cr}^{\text{IV}}$  in the  
21 redox mechanism. The observation of oxo- $\text{Cr}^{\text{V}}$  species at pH higher than used  
22 in the kinetic measurements, indicate that oxo- $\text{Cr}^{\text{V}}$ -alginic acid intermediate  
23 species were formed in the reaction of  $\text{Cr}^{\text{VI}}$  with alginic acid. However, under  
24 conditions used in the kinetic measurements,  $\text{Cr}^{\text{IV}}$  and  $\text{Cr}^{\text{V}}$  react with alginic acid  
25 much faster than  $\text{Cr}^{\text{VI}}$  and do not accumulate in the reaction mixture. Reduction  
26 of  $\text{Cr}^{\text{IV}}$  by alginic acid at 0.30 M  $\text{HClO}_4$  and 25 °C is  $5.5 \times 10^3$  times faster than

1 reduction of Cr<sup>VI</sup> at 60 °C. Cr<sup>V</sup> also oxidizes alginate faster than Cr<sup>VI</sup> does. In  
 2 0.30 M HClO<sub>4</sub>, and T = 15 °C, Cr<sup>V</sup> oxidizes alginate 160 times faster than  
 3 Cr<sup>VI</sup> at 60 °C.

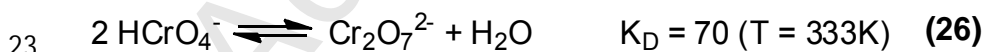
4 Thus, the concentration-time profiles of the Cr<sup>VI</sup>-alginate mixtures reflect the  
 5 [Cr<sup>VI</sup>] monotonic decay without interference of [Cr<sup>IV</sup>] and/or [Cr<sup>V</sup>]. Oxidation of  
 6 alginate by HClO<sub>4</sub> was excluded because this strong acid acts as oxidant  
 7 only at high concentration (higher than 50 %) and at high temperature (near to  
 8 the boiling point) (Diehl and Smith, 1959).

9 In **Scheme 4**, we propose a mechanism that combines Cr<sup>VI</sup>→Cr<sup>IV</sup>→Cr<sup>II</sup> and  
 10 Cr<sup>VI</sup>→Cr<sup>IV</sup>→Cr<sup>III</sup> pathways, and takes into account: (a) kinetic results, (b) the  
 11 polymerization of acrylamide added to the reaction mixture, (c) detection of  
 12 intermediate of oxo-Cr<sup>V</sup>-alginate species, (d) spectroscopy detection of  
 13 CrO<sub>2</sub><sup>2+</sup>, and (e) formation of CO<sub>2</sub>, Cr<sup>III</sup>, and oxidized alginate as final redox  
 14 products.

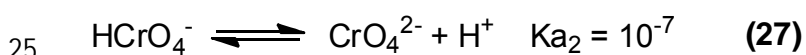
15 **Insert scheme 4 here**

16 The reduction of Cr<sup>VI</sup> by alginate follows two pathways, one independent of  
 17 [H<sup>+</sup>] and another dependent of [H<sup>+</sup>], because the acid base behavior of the  
 18 polymer. The reactive species are alginate and chromate acid, both anionic  
 19 species with charge = -1 as suggested by the dependence of k<sub>6exp</sub> with the ionic  
 20 strength.

21 In the [H<sup>+</sup>] range under study, Cr<sup>VI</sup> exists predominantly as HCrO<sub>4</sub><sup>-</sup> and in acidic  
 22 medium Cr<sup>VI</sup> presents dimerization equilibrium (Brasch, et al., 1996):



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



26 Combining **eqn 26** and **27**, it is possible to calculate [HCrO<sub>4</sub><sup>-</sup>] and [Cr<sub>2</sub>O<sub>7</sub><sup>2-</sup>]. This  
 27 method was proposed by Sen Gupta and co workers (Sen Gupta et al., 1985) in  
 28 order to identify the reactive chromium specie.

1 **Table 4** shows the dependence of  $k_{6\text{exp}}$  with  $[\text{HCrO}_4^-]$  and  $[\text{Cr}_2\text{O}_7^{2-}]$ .

2 **Table 4:** Identification of the chromium reactive specie<sup>a</sup>

$[\text{Cr}^{\text{VI}}]_0$ ( $\mu\text{M}$ )	$[\text{Cr}_2\text{O}_7^{2-}]$ ( $\mu\text{M}$ )	$[\text{HCrO}_4^-]$ ( $\mu\text{M}$ )	$k_{6\text{exp}}^b$ $\times 10^2$	$k_{6\text{exp}}$ $\times A$	$k_{6\text{exp}}$ $B \times 100$
300	16	268	$2.32 \pm 0.04$	$1.16 \pm 0.01$	$2.60 \pm 0.10$
500	20	459	$2.33 \pm 0.06$	$0.76 \pm 0.01$	$2.54 \pm 0.10$
1000	55	889	$2.27 \pm 0.04$	$0.41 \pm 0.01$	$2.55 \pm 0.10$
2000	125	1750	$2.29 \pm 0.08$	$0.24 \pm 0.01$	$2.62 \pm 0.10$

3 <sup>a</sup>[alginate] = 9.13 g/L;  $[\text{H}^+] = 0.30 \text{ M}$ ;  $\mu = 0.50 \text{ M}$ ;  $T = 60 \text{ }^\circ\text{C}$ ;  $[\text{Cr}^{\text{VI}}]_0 = 0.30$ -  
 4 2.0 mM. <sup>b</sup> $k_{6\text{exp}}$  values are expressed in  $\text{min}^{-1}$ .  $A = [\text{Cr}^{\text{VI}}]_0 / [\text{Cr}_2\text{O}_7^{2-}]$ ;  
 5  $B = [\text{Cr}^{\text{VI}}]_0 / [\text{HCrO}_4^-]$

6 The values in **Table 4** shows a first- order dependence of  $k_{6\text{exp}}$  with  $[\text{HCrO}_4^-]$   
 7 and not with  $[\text{Cr}_2\text{O}_7^{2-}]$ . This results together with the ionic strength effect  
 8 supports that  $\text{HCrO}_4^-$  is the chromium reactive specie.

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

19 Oxidation rate of alginate by  $\text{Cr}^{\text{V}}$  and  $\text{Cr}^{\text{IV}}$  are faster than  $\text{Cr}^{\text{VI}}$  at the same  
 20 experimental conditions. Therefore,  $\text{Cr}^{\text{IV}}$  and  $\text{Cr}^{\text{V}}$ , although formed in the  $\text{Cr}^{\text{VI}}$   
 21 /alginate reaction, should be involved in fast steps of the reaction pathway.

1 The slow redox step proposed in **Scheme 4** involves C—C bond cleavage  
 2 through a two-electron redox process to yield Cr<sup>IV</sup>, CO<sub>2</sub>, and oxidized alginic  
 3 acid (**eqn 17**). The initial two-electron reduction of Cr<sup>VI</sup> by alginic acid is in  
 4 agreement with previous reports on various acid saccharides that were  
 5 selectively oxidized by Cr<sup>VI</sup> to the lower homologous (Bellú, et al., 2008;  
 6 González, et al., 2009; Mangiameli, et al., 2014).

7 The rate law for the Cr<sup>VI</sup> consumption derived from **eqn 14-17** in **Scheme 4** is  
 8 given by **eqn 26**, where [Cr<sup>VI</sup>]<sub>T</sub> refers to the total [Cr<sup>VI</sup>] in the reaction mixture.

$$9 \quad -\frac{d[\text{Cr}^{\text{VI}}]}{dt} = \frac{(k_6 K_2 + k_6 K_3 K_1 [\text{H}^+])[\text{alginate}][\text{Cr}^{\text{VI}}]_{\text{T}}}{1 + K_2 [\text{alginate}] + K_3 [\text{alginic acid}]} \quad (26)$$

10 If  $K_2[\text{alginate}] \ll 1$ , and  $K_3[\text{alginic acid}] \ll 1$  then **eqn 26** agrees perfectly  
 11 with the experimental rate law, **eqn 2**.

12 In the mechanism, we have included two competitive one- and two-electron  
 13 reductions of Cr<sup>IV</sup> by alginic acid. Thus, Cr<sup>IV</sup> is proposed to react with excess  
 14 alginic acid to yield Cr<sup>II</sup>, CO<sub>2</sub>, and oxidized alginic acid or Cr<sup>III</sup> and alginic acid  
 15 radical (R·), through two alternate fast steps, **eqns 19** and **20**. The first  
 16 supposition was supported by the observation of CrO<sub>2</sub><sup>2+</sup> (the product of the  
 17 reaction of Cr<sup>II</sup> with O<sub>2</sub>), while the second one, by the observed polymerization  
 18 of acrylamide when it is added to the Cr<sup>VI</sup>- alginic acid reaction mixtures. Cr<sup>V</sup>  
 19 was generated by fast reaction of Cr<sup>II</sup> with Cr<sup>VI</sup>, **eqn 21**, and, alternatively, by  
 20 rapid reaction of the alginic acid radical with Cr<sup>VI</sup>, **eqn 22**. Cr<sup>V</sup> can further  
 21 oxidize alginic acid to yield Cr<sup>III</sup>, CO<sub>2</sub>, and oxidized alginic acid as final redox  
 22 products, **eqn 23**.

23 In O<sub>2</sub>-saturated solutions (1.26 mM) and [Cr<sup>VI</sup>]<sub>0</sub> ≤ 0.06 mM, reactions **21** and **22**  
 24 can be neglected because R· and Cr<sup>II</sup> intermediates formed in reactions **19** and  
 25 **20** should be rapidly trapped by O<sub>2</sub>, **eqn 24** and **25** (Bakac, et al., 1995). The  
 26 proposed mechanism is in accordance with the observation that O<sub>2</sub> has no  
 27 kinetic effect on this reaction, because when [Cr<sup>VI</sup>]<sub>0</sub> ≥ 0.06 mM (as employed in  
 28 the kinetic measurements), both Cr<sup>II</sup> and R· react with Cr<sup>VI</sup> faster than they do  
 29 with O<sub>2</sub>, and therefore **eqn 24** and **25** could be neglected (Scott, et al., 1992;  
 30 Lay & Levina, 1998).

1 Detection of free radicals and  $\text{CrO}_2^{2+}$  in the  $\text{Cr}^{\text{VI}}$ /alginic acid reaction, provided  
2 information supporting that the reduction of  $\text{Cr}^{\text{IV}}$  by alginic acid took place  
3 through **eqn 19** and **20**.

4 The reaction of  $\text{Cr}^{\text{V}}$  with alginic acid was also much faster than  $\text{Cr}^{\text{VI}}$ /alginic acid.  
5 Only a few seconds were required to reduce  $\text{Cr}^{\text{V}}$  in acid solution, at 15°C. The  
6 redox reaction involves two-electrons. At higher pH, the redox reaction was  
7 slower, and oxo- $\text{Cr}^{\text{V}}$ -alginic acid species remain in solution for hours. At pH  
8 1.0-3.0, alginic acid is a good scavenger of oxo- $\text{Cr}^{\text{V}}$ , and the EPR parameter,  
9  $g_{\text{iso}}$  values observed, suggests that the donor sites of alginic acid involved in  
10 coordination to the metal are carboxylate and hydroxyl groups.

11 The overall reaction stoichiometry deduced from **eqn 14** to **23** of **Scheme 4**  
12 agrees with the experimental 0.60:1  $\text{CO}_2/\text{Cr}^{\text{VI}}$  ratio found in mixtures of  $\text{Cr}^{\text{VI}}$  and  
13 alginic acid.

14 The present results suggest that  $\text{Cr}^{\text{VI}}$  (as well as  $\text{Cr}^{\text{IV}}$  and  $\text{Cr}^{\text{V}}$ ) selectively  
15 oxidizes the free carboxylic groups of alginic acid through C—C bond break.  
16 Hassan and Co-workers had reported the oxidation of similar polysaccharides  
17 by this oxidant and they found that the oxidation processes led to the oxidation  
18 of the hydroxyl groups on  $\text{C}_2\text{-C}_3$  bonds to its corresponding keto forms without  
19 the occurrence of any bond cleavage (Zaafarany et al., 2009; Hassan et al.,  
20 2010; Hassan et al., 2013). The difference between our work and that of  
21 Hassan and Co-workers is due principally by differences in the experimental  
22 conditions. We work at higher temperature (60 °C) and lower  $[\text{H}^+]$  (lower than  
23 0.5M), whereas Hassan and Co-workers use lower temperatures (around 40  
24 °C) and higher  $[\text{H}^+]$  (higher than 2.0 M). Experimental conditions employed in  
25 our work increases the oxidant power of  $\text{HCrO}_4^-$  allowing the cleavage of C-C  
26 bond with the release of  $\text{CO}_2$ .

27 This pattern of selectivity distinguishes chromic oxidation of polyuronic acids  
28 from that observed by others oxidizer agents. Thus,  $\text{NaVO}_3$  oxidizes the  
29 terminal unit of the polymeric chain (Gessa et al., 1983),  $\text{KMnO}_4$  yields  
30 ketoderivatives upon oxidation of vicinal hydroxyl groups (Abdel-Hamid et al.,  
31 2003), and  $\text{NaIO}_4$  oxidizes vicinal hydroxyl groups to yields aldehyde groups  
32 (Gomez et al., 2007).

1

## 2 **4. Conclusions**

3 The reaction of alginic acid with  $\text{Cr}^{\text{VI}}$  strongly depends on pH. In acid media,  
4 redox reaction occurs and reactive  $\text{Cr}^{\text{V}}$ ,  $\text{Cr}^{\text{II}}$ , and  $\text{Cr}^{\text{IV}}$  intermediate species are  
5 generated in the redox process, together with  $\text{CO}_2$ , free radicals, and oxidized  
6 forms of alginic acid. At pH 1- 3, alginic acid slowly reduces  $\text{Cr}^{\text{VI}}$ , and stabilizes  
7  $\text{Cr}^{\text{V}}$  yielding long-lived oxo- $\text{Cr}^{\text{V}}$ - alginic acid species, that, if the medium  
8 becomes acid, rapidly affords redox processes. The Cr-binding sites of alginic  
9 acid are mainly the free carboxylic groups that can undergo redox processes  
10 with  $\text{Cr}^{\text{VI-IV}}$  in acid media or stabilize  $\text{Cr}^{\text{V}}$  together with hydroxo donor groups of  
11 the polysaccharide. The present results evidence that alginic acid can act as  
12 reducing agent of chromate acid (particularly at low pH), with formation of  
13 highly toxic free radicals,  $\text{CrO}^{2+}$  and  $\text{Cr}^{\text{V}}$  intermediate species, or can be  
14 involved in the transport of higher oxidation state of chromium at  $\text{pH}>2$ .

15

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**Highlights**

Alginic acid reduces hypervalent chromium to Cr<sup>III</sup>.

The mechanism combines Cr<sup>VI</sup>→Cr<sup>IV</sup>→Cr<sup>II</sup> and Cr<sup>VI</sup>→Cr<sup>IV</sup>→Cr<sup>III</sup> pathways.

Oxo-chromate(V)–alginic acid species are stable at pH 3- 5 for several hours.

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## Figure Captions

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

**Fig. 2** UV-vis differential spectra of alginate/ $\text{Cr}^{\text{VI}}$  mixtures at pH 6.0, showing the increasing band at 375 nm with increasing [alginate]. (a) [alginate] = 0.228 g/L, (b) [alginate] = 0.457 g/L.  $[\text{Cr}^{\text{VI}}] = 0.5\text{ mM}$ ,  $\mu = 0.50\text{ M}$ ,  $T = 25\text{ }^{\circ}\text{C}$ .

**Fig. 3** Experimental and simulated X-band EPR spectra of oxo- $\text{Cr}^{\text{V}}$ -alginate species.  $T = 15\text{ }^{\circ}\text{C}$ ; [alginate] = 4.57 g/L;  $[\text{Cr}^{\text{VI}}] = 1.0\text{ mM}$ ;  $\mu = 0.50\text{ M}$ ; pH = 2; Center Field = 3410 G ; Modulation amplitude = 2.0 G ; resolution = 1024 points ; Frequency = 9.4312 GHz. Spectra were collected after 1 hour of mixing reactants.

**Fig. 4**  $\text{CrO}_2^{2+}$  formation from the reaction between alginate and  $\text{Cr}^{\text{VI}}$ . [alginate] = 0.78 g/L,  $[\text{HClO}_4] = 3.0\text{ M}$ ,  $[\text{O}_2] = 1.26\text{ mM}$ ,  $[\text{Cr}^{\text{VI}}] = 0.0797\text{ mM}$ ,  $\mu = 3.0\text{ M}$ ,  $T = 25\text{ }^{\circ}\text{C}$ . Spectra were recorded every 3.0 min. Inset: differential spectrum after addition of  $\text{Fe}^{\text{II}}$ .

**Fig. 5** Formation of  $\text{CrO}_2^{2+}$  from the reaction 2.28 g/L alginate,  $[\text{H}^+] = 0.30\text{ M}$ ,  $[\text{O}_2] = 1.26\text{ mM}$ ,  $\mu = 0.50\text{ M}$ ,  $T = 25\text{ }^{\circ}\text{C}$ ,  $[\text{Cr}^{\text{VI}}] = 0.207\text{ mM}$ . Inset: absorbance at 290 vs. time. Fitted lines were calculated using eqn (4) and SigmaPlot 11.0 program.

Fig. 1

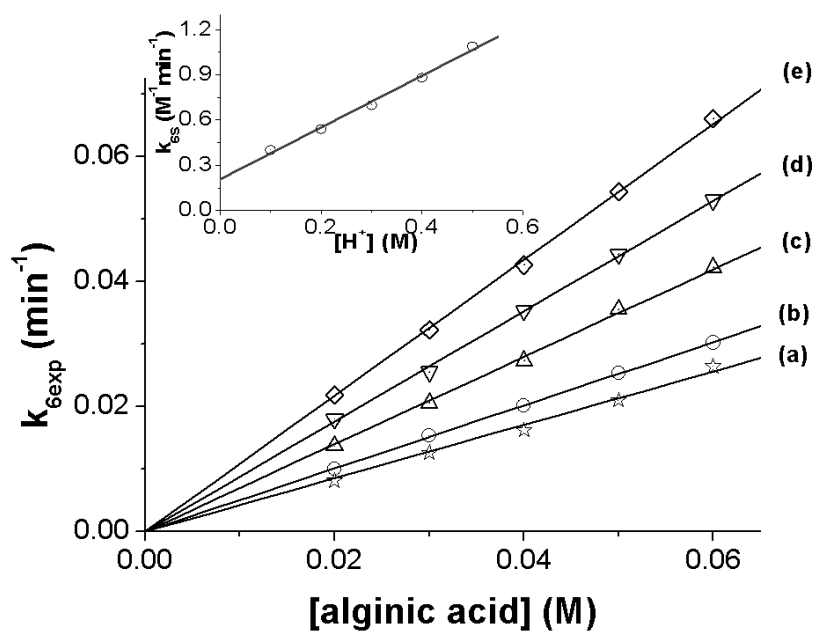
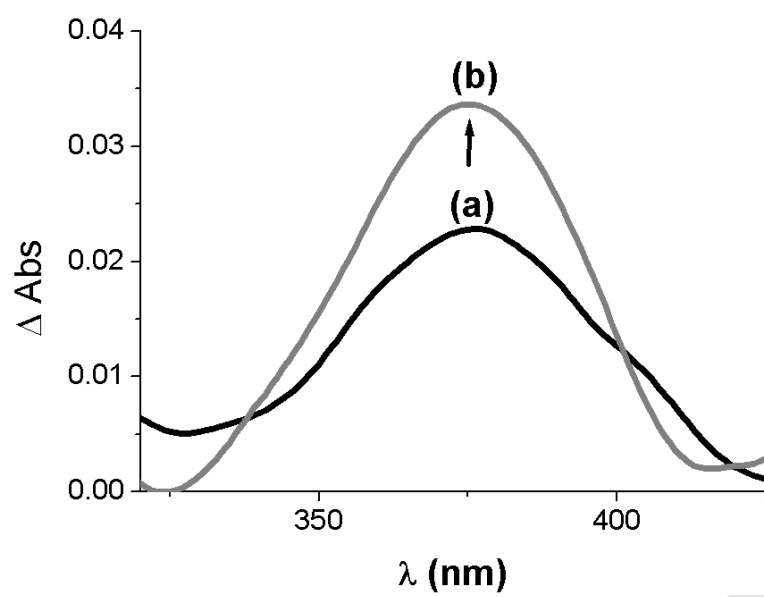
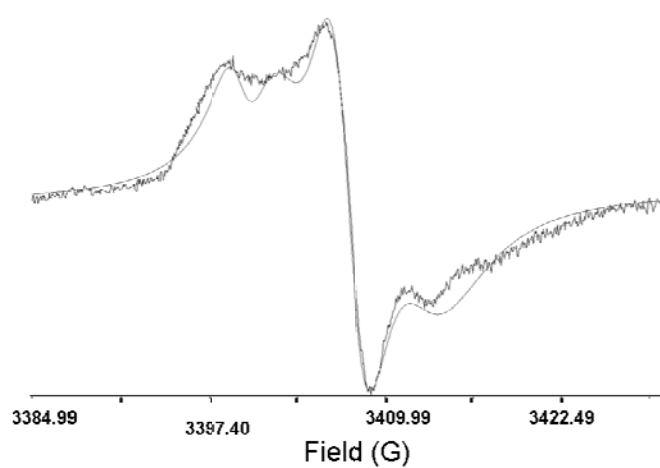


Fig. 2



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Fig. 3



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Fig. 4

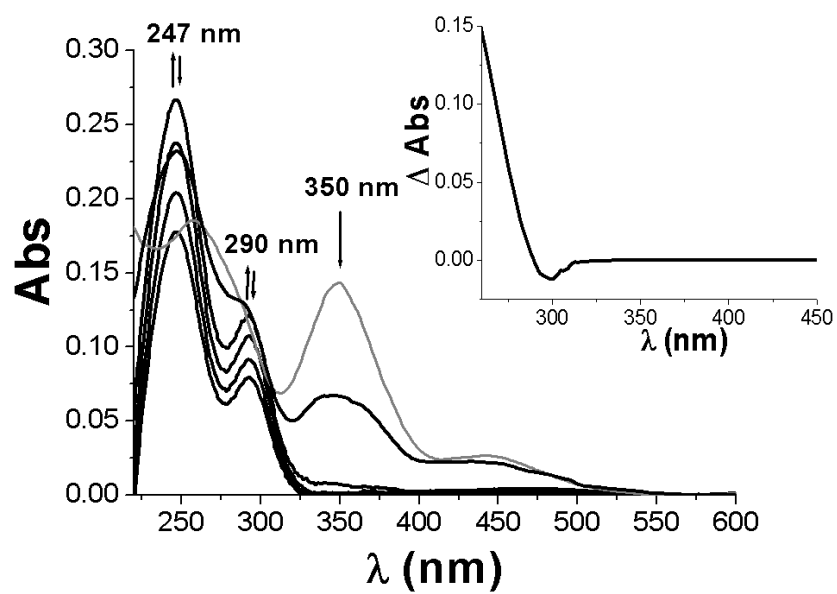
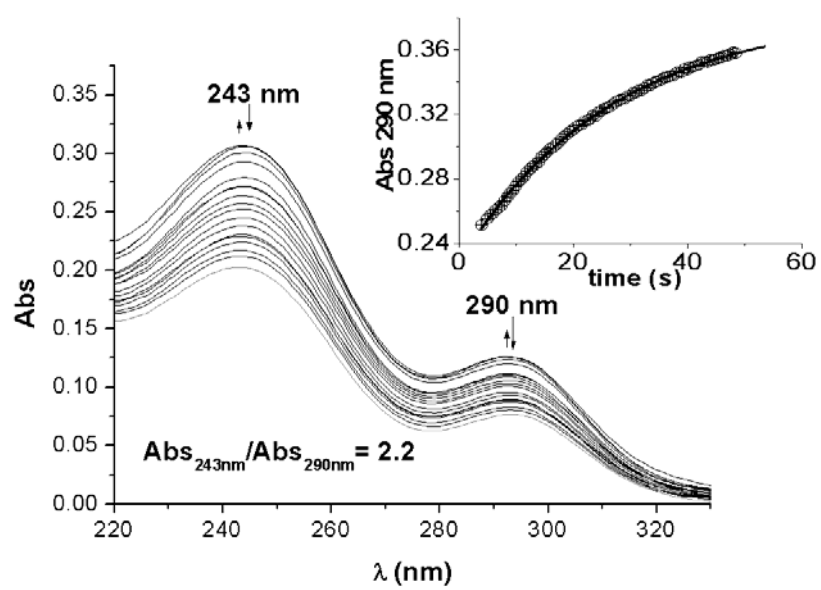
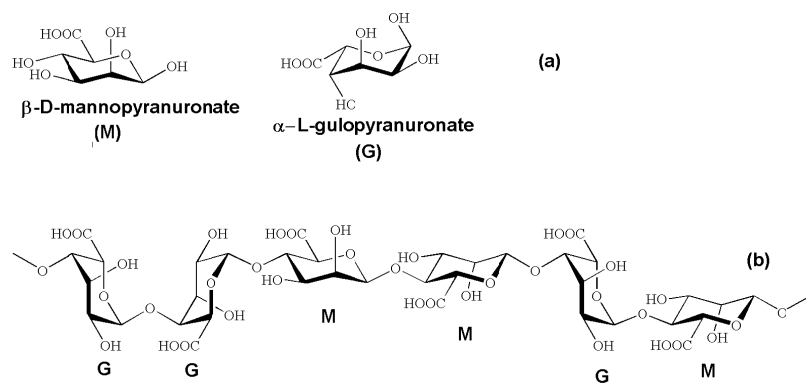


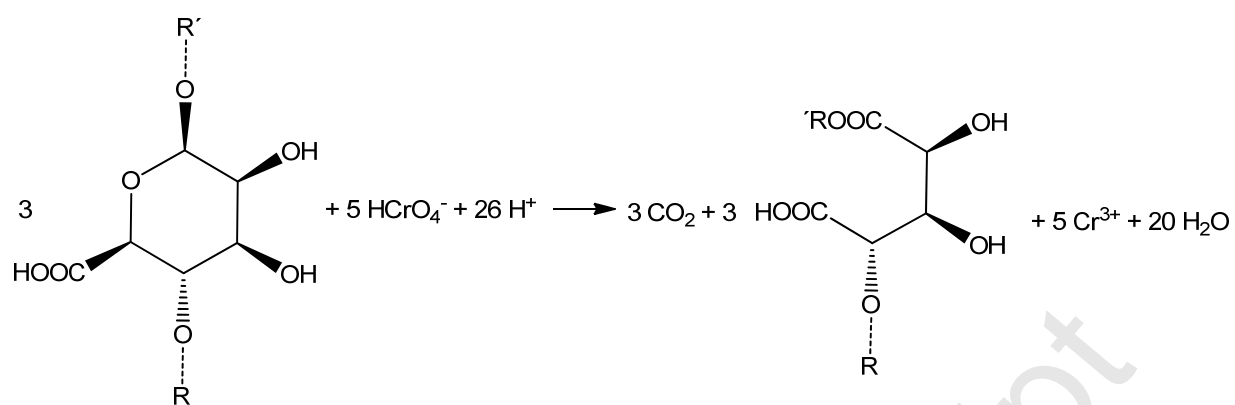
Fig. 5





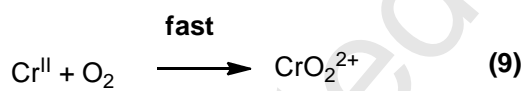
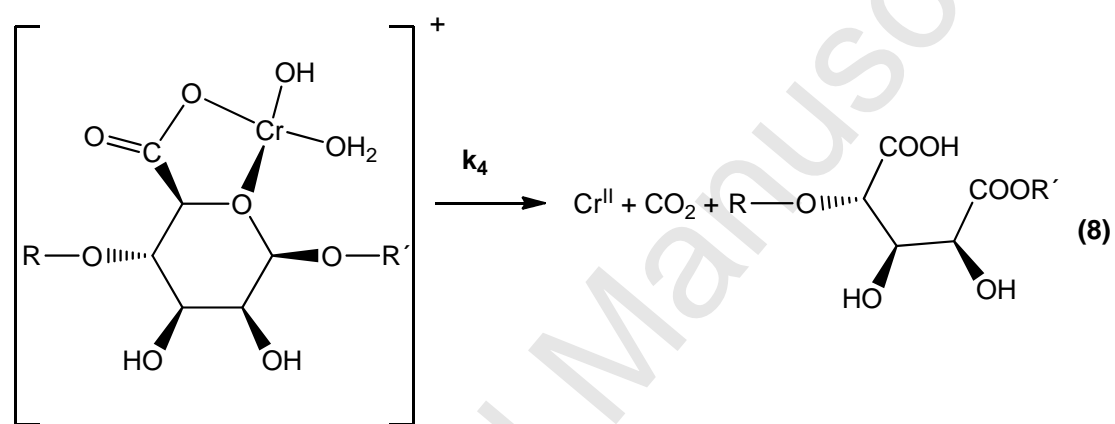
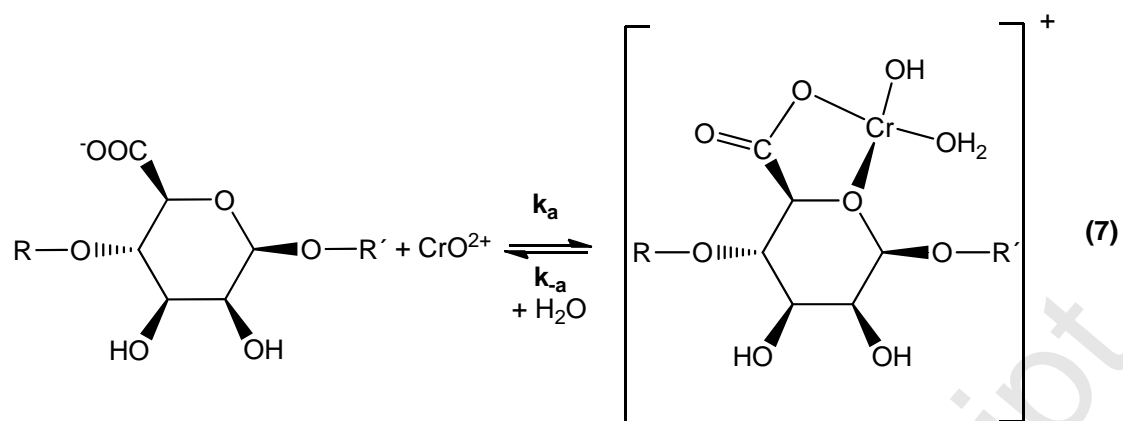


**Scheme 1: Structure of alginic acid (a) monomers (b) polymeric chain**

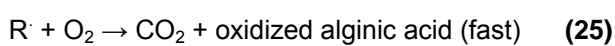
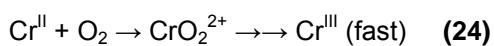
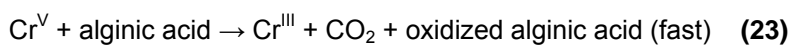
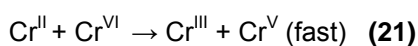
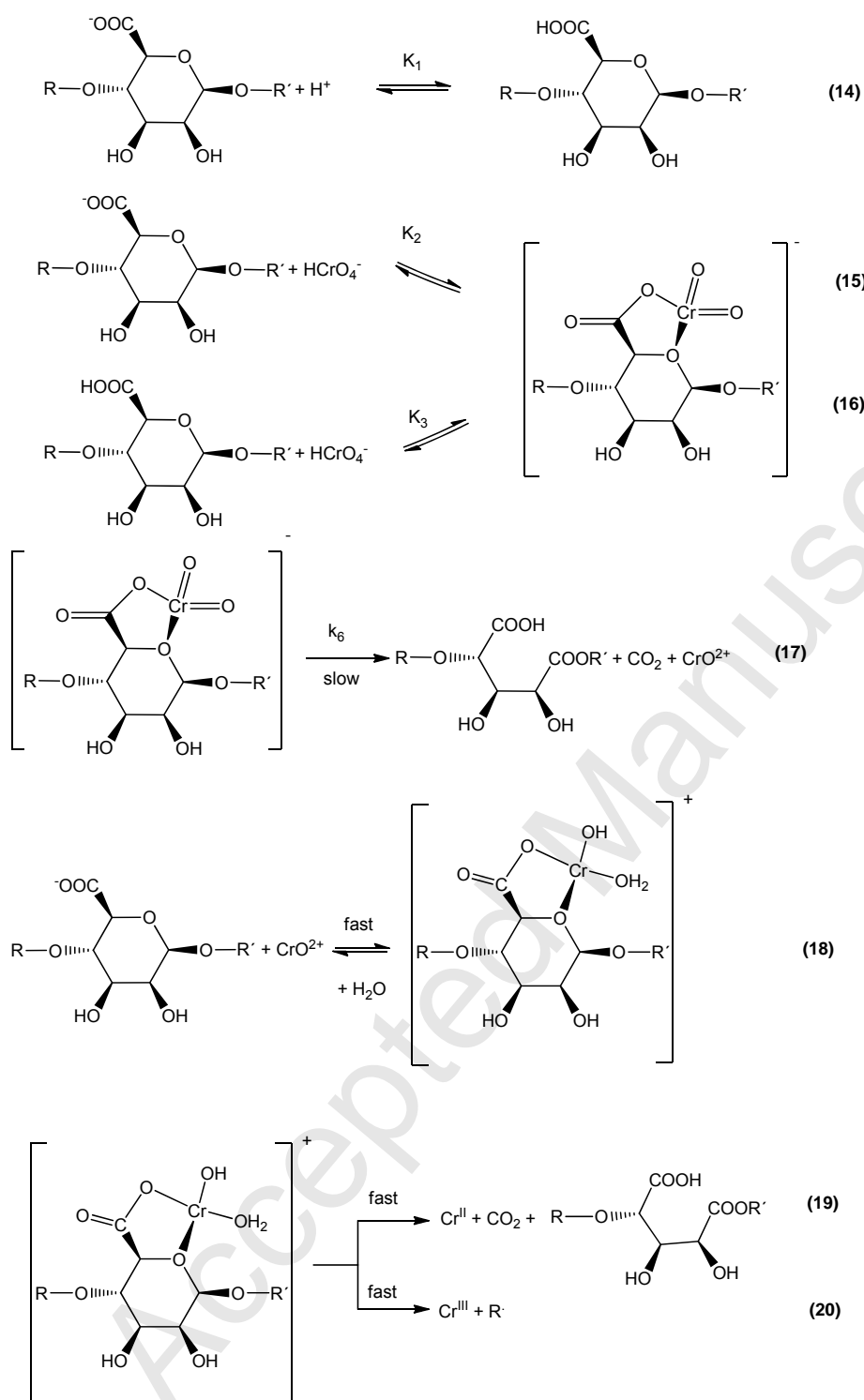


**Scheme 2: Stoichiometry of the redox reaction between alginic acid and  $\text{Cr}^{\text{VI}}$  in acid media**

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Scheme 3: Proposed mechanism for the oxidation of alginic acid by  $\text{CrO}_2^{2+}$



**Scheme 4: Proposed mechanism for the oxidation of alginate by Cr<sup>VI</sup>**