Introduction

CrV is a very important environmental pollutant and a well-known occupational contaminant. Although it is not doubted that CrVI induces cancer, there is still a discussion regarding the species most probably responsible for cell damage and the mechanism(s) involved. CrV itself cannot react with DNA in vitro or with isolated nuclei. On the other hand, when reducing agents are present in the medium, it causes an extensive diversity of DNA damage, which includes damage to Cr-DNA complex, DNA-protein crosslinks, and apurinic–apyrimidinic sites as well as oxidative damage. The formation of CrV and CrIV intermediates during the oxidation of a variety of organic compounds by CrV has been observed and their involvement in Cr-induced cancers has aroused much curiosity in the chemistry and biochemistry of chromium. In fact, the detection by continuous-wave electron paramagnetic resonance (CW-EPR) or electron spin resonance (ESR) of a long-lived CrV species is focused on the probable role(s) performed by CrV species in carcinogenesis brought about by CrVI. A similar situation has arisen with CrIV. In the course of the examination of the interactions of aldohexoses and carboxylic acids with CrVI, we were able to demonstrate the interactions of CrVI, CrV and CrIV with different sugars present in biological systems.

tert-2-Hydroxy acid, quinic acid (QA), [(1R,3R,4R,5R)-1,3,4,5-tetrahydroxy-cyclohexanecarboxylic acid] (Fig. 1) is a natural cyclic polyol compound found in plums, peaches, pears, apples, quinia bark, Eucalyptus globulus, carrot and tobacco leaves, coffee beans and other vegetables. Additionally, QA is related to the acidity of coffee. QA is an important biological substrate, because it is important in the cellular synthesis of aromatic compounds, and it is also a multipurpose chiral starting material for new pharmaceuticals.

Considering its structure, QA is a perfect ligand for studying the redox reactions of Cr in vitro with biologically significant donor groups. The hydroxyl-substituted cyclohexane ring in QA can act as a cellular carbohydrate. Functional groups such as diols (e.g., ascorbic acid, ribose, D-glucose and their derivatives) and 2-hydroxy acids (e.g., citric, malic, and lactic acids) can be substituted by different regions of QA (tert-2-hydroxycacid moiety and cis-diol [O(3),O(4)] and trans-diol [O(4),O(5)] groups), each of which is a potential chelating agent for CrIV. It has been established that CrIV/CrV can be produced intracellularly during the reduction of CrVI besides the oxidation of CrIII in the presence of activated oxygen generated throughout enzymatic reactions. Additionally, the lifetimes of identified CrIV complexes under biological conditions (pH ~ 7) are measured in minutes or seconds as well as in a slightly acidic medium (pH ~ 4.5–5.5) similar to that occurring in the cellular uptake of insoluble chromates by phagocytosis. QA makes it possible to

![Fig. 1. The structure of QA.](https://example.com/fig1.png)
perform intramolecular competition experiments between different functional groups (vic-diol versus tert-2-hydroxy acid) or different orientations of the same functional group (trans versus cis diol). Lay et al. studied QA by CW-EPR in the presence of CrV to determine and understand the structures of the complexes formed between both species. However, no kinetic studies have been carried out on QA/Cr systems, which are necessary to obtain information about what is known about this substrate and its relation with chromium species and to propose a mechanism for the reaction between CrVI and QA. It is also important to measure the half-lives of intermediate species as well as their interaction with QA because of their possible presence in the intracellular environment and the potential for damage that they represent.

Experimental section

Materials

QA (Sigma, p.a.), potassium dichromate (Mallinckrodt), perchloric acid (Baker, A.C.S.), sodium hydroxide (Cicarelli, p.a.), H2SO4 (Fluka, puriss. p.a. (HPLC)), methanol, oxalic acid (Biopack, p.a.), HCl (Cicarelli, p.a.), argon (99.9%), acrylamide (Merck, 99.0%), ethba = 2-ethyl-2-hydroxybutanoic acid (Aldrich, 99.0%), diphenylpicrylhydrazyl (dpip) (Aldrich, p.a.), Zn (Sigma-Aldrich, 99.9%), HgCl2 (Merck, 99.8%), Cr(C11O4)3·6H2O (Sigma-Aldrich, p.a.) and [Fe(NH4)2(SO4)]2 (Cicarelli, p.a.) were used without further purification. Sodium perchlorate monohydrate (Fluka, 98.0%), oxygen (99.9%), Zn (Cicarelli, p.a.), and HgCl2 (Cicarelli, p.a.) were also used. 4-(2-Hydroxyethyl)-1-piperazinethanesulfonic acid (Hepes) buffer (Sigma Ultra, 99.5%) was added to adjust the pH of solutions to 7.05. Aqueous solutions were prepared in Milli-Q water (18.2 MΩ cm⁻¹).

For experiments performed in the pH range of 1–5, the pH of the solutions was adjusted by the addition of 0.5 M HClO4 or hydrate (Fluka, 98.0%), oxygen (99.99%), Zn (Cicarelli, p.a.), and performance liquid chromatography (HPLC). Chromatograms of oxygen used in the kinetic measurements was tested by high-performance liquid chromatography (HPLC). Chromatograms of the eluent at 33 °C were obtained using a KNK-500A chromatograph equipped with a 7125 HPLC pump. The analysis was carried out on an Aminex HPX-87H HPLC column (300 × 7.8 mm, Bio-Rad Laboratories) using 9.0 × 10⁻³ N H2SO4 with a flow rate of 0.6 mL min⁻¹ as the eluent at 33 °C. The effluent was monitored with a UV detector (ProStar 325 UV-vis detector, λ = 220 nm). QA was incubated at 33 °C in the conditions of the kinetic experiments. At different times, aliquots were taken, diluted with the eluent medium and filtered using a nylon filter with 0.2 μm pores (Nalgene) prior to injection.

Polymerization test

The polymerization of acrylamide was investigated during the reaction of QA with CrVI by employing a specific test for the generation of free radicals. A solution of CrVI (0.19 mL, 0.53 M) was added to 1.0 mL of a reaction mixture containing QA (1.0 mmol) and acrylamide (3.66 mmol). When [CrVI] was negligible, 5.0 mL of cold methanol (0 °C) was added to the mixture, and a white polymer precipitated. Control experiments showed that no polymerization of acrylamide occurred under the experimental conditions with either CrVI or QA alone. The reaction of oxalic acid with CrVI was employed as a positive control. A solution of CrVI (1.0 mL, 0.53 M) was added to 1.0 mL of a mixture containing oxalic acid (2.0 mmol) and acrylamide (7.3 mmol). After the disappearance of CrVI, 10.0 mL of cold methanol (0 °C) was added to the reaction mixture, and the immediate appearance of a white polymer was observed. Possible reactions of CrV and CrIV with acrylamide were investigated using Na[CrO4(ehba)] Cl and [CrVO(ehbaH)2]. No precipitation occurred upon mixing CrV or CrIV complexes with acrylamide under the conditions used in the CrVI/QA reaction.

Generation of CrII

As was previously described in the main text, aqueous CrO2⁺ species can be generated in situ by rapid oxidation of Cr²⁺ using oxalic acid. To generate the required Cr²⁺, it is necessary to employ a highly reducing medium. The procedure involves a Zn/Hg amalgam and a strong flow of hydrogen. The Zn/Hg amalgam was prepared in a 5 mL balloon by stirring a mixture of Zn (~5.0 g, previously washed with 3.0 M HCl for 5 min) and HgCl2 (0.3 M in 1.0 M HCl) for 30 min. Afterwards, excess HgCl2 was eliminated, and the resulting amalgam was washed three times with 1.0 M HClO4 and finally with distilled water. An appropriate volume of HClO4 and distilled water was added to the amalgam in the balloon to obtain pH of 1.0 in a final volume of 3.5 mL. Finally, the balloon was closed with a rubber septum cap and stirred while being bubbled with H2 for at least 45 min to ensure a reducing medium. Then, 200 μL of 6.0 mM Cr(ClO4)3 was injected while keeping the H2 bubbling and stirring constant. After 1.0 h, Cr(ClO4)3 was quantitatively reduced to Cr²⁺. The value of [Cr²⁺] was determined by treating an aliquot of the reaction mixture with an aqueous solution of [Co(NH3)6]Cl2 under anaerobic atmosphere (Ar); the mixture was then poured into concentrated HCl, and the CoCl4⁻ content was determined by measuring the absorbance of [CoCl4]²⁻ at 692 nm.
**In situ generation of oxo-CrIV (CrO2^2+)**

For *in situ* generation of CrO2^2+, a deoxygenated solution of Cr2+ was injected into an acidic aqueous solution of QA, which was saturated with O_2 (1.26 mM). At very low Cr2+/O_2 ratios (≤0.05), CrO2^2+ was quantitatively formed, whereas at intermediate Cr2+/O_2 ratios (≤0.15), the reaction afforded mixtures of CrO2^+ and CrO2^2+. In a typical experiment, 100 μL of 6.0 mM Cr2+ was injected into a septum-capped spectrophotometric quartz cell with a path length of 1.0 cm, which was filled with 2.3 mL of an O_2-saturated solution containing 0.25–6.0 mM QA and appropriate concentrations of HClO_4 and NaClO_4 ([H^+] = 0.1–0.6 M, I = 1.0 M) at 15 °C. Under these experimental conditions, the reaction between Cr2+ and O_2 rapidly produced 0.07 mM CrO2^+ (average yield of 28% based on total [Cr2+]). After CrO2^2+ was formed, it reacted with QA to yield Cr2+, which was then quantitatively transformed to a superoxo-CrIII ion (CrO2^2+) by reacting with the remaining oxygen ([Cr2+]/O_2 ratio <0.05) with no autocatalytic consumption of CrO2^2+ by Cr2+.25

**Quantification of CrO2^2+**

The concentration of CrO2^2+ generated by the reaction of Cr2+ with O2 was determined by injecting 100 μL of 6.0 mM Cr2+ into 2.3 mL of an O_2-saturated solution of 0.1 M ethba buffer (pH 3.0) at 15 °C. Immediately after mixing, the solution turned pink, and the absorbance of [CrO2^2+](ethba)_2^2+ at 512 nm (ε = 2380 M⁻¹ cm⁻¹) was measured.37

**Spectrophotometric measurements**

All kinetic measurements were performed by monitoring the absorbance changes with a Jasco V-550 spectrophotometer with fully thermostated cell compartments (±0.2 °C). The reactions were followed under pseudo-first-order conditions using an excess of QA with respect to Cr. The reactant solutions were thermostated prior to the experiment and transferred into a quartz cell with a path length of 1.0 cm immediately after mixing. All kinetic data were fitted using routines in the Origin 6.0 package.

Table 1 lists key wavelengths for different Cr species analyzed in this study.

**Chromate esters**

Chromate esters were investigated by UV-vis spectrophotometry in the region of 250–600 nm, in which they exhibited characteristic absorption bands. Reactions were performed at pH of 7.05 (Hepes buffer), at which the redox reaction is slow enough to enable observations of the formation of esters. The instrument was zeroed using an arrangement in which the reference and sample beams passed through matching cells, and both contained 5.0 × 10⁻⁴ M CrVI in 0.1 M Hepes buffer with a pH of 7.05. The solution in the sample cell was replaced with a reaction solution containing 3.0 × 10⁻⁴ M CrVI and 0.25–0.013 M QA in Hepes buffer at pH = 7.05 and T = 24 °C. The spectra obtained after mixing showed a characteristic absorption at 460 nm. Each mixture was monitored for 5 minutes, and no variations were observed in the shape and intensity of the spectra.

**Time evolution of the QA/CrVI reaction**

Time-dependent UV-vis spectra were recorded for two different reaction mixtures. The first mixture containing 0.3 M QA, 0.1 M HClO_4, and 6.0 × 10⁻⁴ M CrVI with I = 1.0 M at 33 °C was monitored between 200 and 800 nm every 4 minutes until total consumption of CrVI to determine the presence of the isosbestic point. The second reaction mixture containing 0.3 M QA, 0.1 M HClO_4, and 6.0 × 10⁻³ M CrVI with I = 1.0 M at 33 °C was monitored between 450 and 900 nm every 4 minutes until total consumption of CrVI to determine whether the final redox product of the reaction was CrIV or CrIII ligand. In this reaction, the time evolution of the QA/CrVI mixture was monitored by following changes in the absorption band at 570 nm. The concentration of CrVI used in this experiment was 10 times higher because CrIII species have a very low ε value at 570 nm. The rate constants obtained at this wavelength were in agreement with those calculated from the data recorded at 350 nm in these experimental conditions.

**QA/CrVI reactions**

The disappearance of CrVI in the reaction mixtures at 33 °C was followed by monitoring the absorbance at 350 nm until at least 80% conversion. The initial concentration of CrVI was 6.0 × 10⁻³ M, whereas the QA concentration was varied from 0.03 M to 0.12 M. In these kinetic measurements, I was kept constant at 1.0 M, whereas [HClO_4] was varied from 0.1 to 0.5 M at various [QA] values. The rate constants (k_6 and k_9) were deduced from multiple determinations and were within ±10% of each other. The rate constants obtained at 350 nm were used to fit the absorbance changes at 420–440 nm. The goodness of fit at these wavelengths was used to corroborate the rate expressions used to determine the rate constants for 350 nm. The first-order dependence of the rate upon [CrVI] was confirmed by a set of experiments, where [CrVI] was varied between 0.3 and 0.6 mM, but T, [QA] and ionic strength were kept constant.

**Detection of superoxo-CrIII ions during the reaction of QA with CrVI**

The fact that CrIV is involved in the oxidation mechanism of several alcohols by CrIV and CrVI in HClO_4 was demonstrated by its conversion to CrO2^2+ upon reacting with dioxygen.2526 The possible formation of CrO2^2+ in QA/CrVI mixtures was investigated by periodic UV-vis scanning (220–500 nm) of solutions of 0.09 M QA, 0.06 mM CrVI and 1.0 M HClO_4 saturated with oxygen ([O_2] = 1.26 mM) at 25 °C. Periodic scanning of the reaction mixture showed that the CrVI band at 350 nm decreased in intensity, whereas new peaks appeared at 290 nm and 245 nm. When the
value of $[\text{Cr}^{IV}]$ was negligible. 0.30 mM Fe$^{2+}$ was added to bring about the following reaction (eqn (1)):

$$\text{CrO}_2^{2+} + 3\text{Fe}^{2+} + 4\text{H}^+ \rightarrow \text{Cr}^{3+} + 3\text{Fe}^{3+} + 2\text{H}_2\text{O} \quad (1)$$

The spectrum of the reaction mixture was subtracted from the corresponding spectrum recorded prior to the addition of Fe$^{2+}$. The presence of a negative difference in absorbance around 290 nm between these spectra was consistent with the presence of Cr$^{2+}$.

In addition, the same reaction was conducted under strictly anaerobic conditions (Ar). In this case, the absence of oxygen implied that no Cr$^{IV}$ would be generated and also no CrO$_2^{2-}$ would be formed. The characteristic absorption bands at 245 and 290 nm were not present.

**QA/Cr$^{IV}$ reactions**

The oxidation of QA by CrO$_2^{2-}$ was studied under pseudo-first-order conditions with an excess of QA with respect to Cr$^{IV}$ and monitored using a spectrophotometer by following CrO$_2^{2-}$ as a final redox product. Kinetic data were recorded spectrophotometrically following the formation of CrO$_2^{2-}$ at 290 nm ($\varepsilon = 3000 \text{ M}^{-1} \text{ cm}^{-1}$) by employing a septum-capped spectrophotometer cell with a path length of 1.0 cm, which was filled with 2.3 mL of an O$_2$-saturated solution of the organic substrate. At this wavelength, neither the substrate QA nor the oxidized products exhibited absorption. All mixtures of QA/CrO$_2^{2-}$ showed an increase in two absorption bands (245 and 290 nm) with a relative intensity of $\text{Abs}_{245}/\text{Abs}_{290} = 2.2$, which is characteristic of CrO$_2^{2-}$. Kinetic measurements were performed at [Cr$^{IV}$] = 0.07 mM, $I = 1.0 \text{ M}$, $[\text{O}_2] = 1.26 \text{ mM}$ and 15 °C. The range of QA concentrations used was 1.0–60 mM, and no disproportionation reaction of CrO$_2^{2-}$ was observed. The range of $[\text{HClO}_4]$ used was 0.10–60 M. The experimental pseudo-first-order rate constants ($k_{4exp}$), which were determined from nonlinear least-square fits of absorbance data for 290 nm, were the averages of at least five determinations and were within ±10% of each other. The data used to calculate the kinetic constant, $k_{4exp}$, corresponded to 80% of the exponential growth in the experimental values. The first-order dependence of the rate upon [Cr$^{IV}$] was confirmed by a set of experiments, where [Cr$^{IV}$]$_0$ was varied between $3.0 \times 10^{-5}$ and $6.0 \times 10^{-5}$ M, but $T$, [QA], and $I$ were kept constant.

**EPR measurements**

EPR spectra were obtained with a Bruker Elexsys 500 spectrometer operated at X-band frequencies (∼9 GHz). Microwaves were generated 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 at 1024 points at 18 ± 1 °C using a microwave power of 202 mW, a modulation amplitude of 2.0 G, a time constant of 20 ms, a sweep width of 120 G and a conversion time of 40 ms. Also, $g$-values were determined by reference to dpph ($g = 2.0036$) as an external standard reference. The power values used in the EPR experiments did not exceed 10 mW to avoid signal saturation. In the EPR measurements, the speed and number of scans were fixed to reduce the time taken for each measurement; this was done to avoid fluctuations in the EPR signals during scanning of the samples.

**Results**

**Reaction time of the QA/Cr$^{VI}$ mixture**

The UV-vis absorption spectrum of the QA/Cr$^{VI}$ reaction mixture in an acidic medium (HClO$_4$) showed the characteristic behavior of Cr$^{VI}$ in an acidic medium$^{25–27}$ with a shoulder at 420/450 nm and a band at 350 nm (Fig. 2). The absorbance at 350 nm decayed, and the absorbance at the shoulder first increased and then decayed over time; also, there was an increase in the absorbance at 570 nm (Fig. 2A).

The absence of an isosbestic point (highlighted area in Fig. 2A) indicates that there are more than one competing redox reactions involving intermediate chromium species, which must be present in appreciable concentrations during the reduction of Cr$^{VI}$ to Cr$^{III}$.

As the reaction proceeded, two d–d bands were detected in the electronic absorption spectrum at $\lambda_{max} = 406$ nm ($\varepsilon = 40 \text{ M}^{-1} \text{ cm}^{-1}$) and 573 nm ($\varepsilon = 18 \text{ M}^{-1} \text{ cm}^{-1}$) (Fig. S1†). These bands were assigned to the $A_{2g} \rightarrow T_{1g}$ and $A_{2g} \rightarrow T_{2g}$ octahedral transitions of Cr$^{III}$ in $O_h$ symmetry.$^{28}$ After 6 h, significant changes were observed in these two bands; for example, the band at 539 nm ($\varepsilon = 90 \text{ M}^{-1} \text{ cm}^{-1}$) shifted to 571 nm ($\varepsilon = 15 \text{ M}^{-1} \text{ cm}^{-1}$) (Fig. 2B). This observation suggested that the absorption at 571 nm originated from Cr$^V$ and Cr$^{III}$–QA complex species. The Cr$^{III}$–QA complex formed initially was slowly hydrolysed to Cr$^{III}$[O$_4$].

**Detection of Cr$^{VI}$ esters**

Differential UV-vis spectra of QA/Cr$^{VI}$ mixtures were used to study the formation of Cr$^{VI}$ esters. An absorption band ($\lambda_{max} = 470$ nm) was observed (Fig. 3), which was consistent with literature data.$^{29}$ At pH values near neutrality, the redox reaction of QA/Cr$^{VI}$ occurred extremely slowly, and the reduction of Cr$^{VI}$ was negligible. In consequence, the ester formation and electron transfer reactions could be clearly distinguished.
the total absorbance to determine the real contribution of the interesting to determine how much each species contributes to 0.10 M, $[\text{QA}] = 0.25$ M (black), $0.013$ M (grey), $[\text{Cr}^{IV}] = 5.0 \times 10^{-4}$ M, $[\text{Hepes}] = 0.10$ M, $T = 24.0$ °C, and pH = 7.05.

Detection of $\text{Cr}^{II}$

The involvement of $\text{Cr}^{II}$ species in the oxidation of different organic alcohols by $\text{Cr}^{IV}/\text{Cr}^{VI}$ in acidic media has previously been established by the formation of superoxo-$\text{Cr}^{III}$ ($\text{CrO}_2^{2+}$) by a reaction with dioxygen.\textsuperscript{\text{14,26,27,37,55,62}} Periodic scanning of the $\text{QA}/\text{Cr}^{VI}$ reaction mixture in 1.0 M $\text{HClO}_4$ with a high oxygen concentration and a very low $\text{Cr}^{VI}$ concentration (please see experimental conditions) reveals two bands at 290 and 245 nm, which are characteristic of $\text{CrO}_2^{2+}$ (Fig. 4). This observation confirms the formation of $\text{Cr}^{II}$ and can be considered to be indirect evidence of the participation of $\text{Cr}^{IV}$ in the $\text{QA}/\text{Cr}^{VI}$ redox mechanism, as has been observed with other saccharides.\textsuperscript{\text{14,25-27,37}}

Taking into account the spectra shown in Fig. 4, the absorbance at 245 nm and 290 nm at any time in these experimental conditions is due to the contributions of $\text{CrO}_2^{2+}$ and $\text{Cr}^{VI}$. It is interesting to determine how much each species contributes to the total absorbance to determine the real contribution of the redox reaction of $\text{Cr}^{IV}$ to the total oxidation mechanism. To do this, the contribution of $\text{CrO}_2^{2+}$ ions at the absorbance at 245 nm is calculated according to eqn (2):

$$\text{Abs}_{245} (\text{CrO}_2^{2+}) = \text{Abs}_{245} - \epsilon_1 \times \epsilon_2$$

$$\text{Abs}_{350} (\text{CrO}_2^{2+}) = \epsilon_1 \times \epsilon_2$$

Fig. 3 Differential UV-vis spectra of $\text{QA}/\text{Cr}^{VI}$ mixtures at pH of 7.05: $[\text{QA}] = 0.25$ M (black), $0.013$ M (grey), $[\text{Cr}^{IV}] = 5.0 \times 10^{-4}$ M, $[\text{Hepes}] = 0.10$ M, $T = 24.0$ °C, and pH = 7.05.

here, $\epsilon_1$ and $\epsilon_2$ are the molar absorption coefficients of $\text{Cr}^{VI}$ at 350 nm and 245 nm, respectively. Under our experimental conditions, the molar absorptivity values were $\epsilon_1 = 1550$ M$^{-1}$ cm$^{-1}$ and $\epsilon_2 = 1900$ M$^{-1}$ cm$^{-1}$. The inset in Fig. 4 indicates time evolution of $[\text{CrO}_2^{2+}]$.

Considering that the molar absorption coefficient of $\text{CrO}_2^{2+}$ at 245 nm is 7000 M$^{-1}$ cm$^{-1}$,\textsuperscript{\text{28}} the maximum concentration of $\text{CrO}_2^{2+}$ was $3.48 \times 10^{-5}$ M (yield, 49.7%). The yield of $\text{CrO}_2^{2+}$ should approximate to 100% if the reaction takes place entirely via the $\text{Cr}^{VI} \rightarrow \text{Cr}^{IV} \rightarrow \text{Cr}^{III}$ pathway. The fact that the percentage yield of $\text{CrO}_2^{2+}$ reached only 50% of the expected theoretical value suggested that only half of $\text{Cr}^{VI}$ reacted with $\text{QA}$ via a pathway involving $\text{Cr}^{IV}$.

At 350 nm, the absorbance was insignificant; 0.30 mM $\text{Fe}^{II}$ was added, and a new spectrum was recorded. The latter spectrum was subtracted from the spectrum recorded prior to the addition of $\text{Fe}^{II}$. The difference spectrum displayed a negative absorbance around 290 nm, which is consistent with the formation of $\text{CrO}_2^{2+}$, as has been observed with other saccharides (Fig. S2).\textsuperscript{\text{18}}

When the $\text{QA}/\text{Cr}^{VI}$ reaction was conducted in the same experimental conditions but in a strictly anaerobic medium (Ar), the spectrum showed the disappearance of the characteristic bands of $\text{CrO}_2^{2+}$ at 245 and 290 nm due to anaerobic conditions (Fig. 5). This fact also confirmed the identity of $\text{Cr}^{III}$ species.

Rate studies

Reaction of $\text{QA}/\text{Cr}^{IV}$

As was reported in the experimental section, $\text{Cr}^{IV}$ was generated in situ through the reaction between $\text{Cr}^{III}$ and $\text{O}_2$ in appropriate experimental conditions. The $\text{QA}/\text{Cr}^{IV}$ reaction under acidic media and $\text{O}_2$-saturated conditions produced $\text{Cr}^{III}$. Since neither the substrate nor the oxidized products absorb at 290 nm, this reaction can be indirectly observed by measuring the increment in absorbance at this wavelength, which corresponds to the formation of $\text{CrO}_2^{2+}$. Typical sequential electronic spectra, which show the characteristic bands of $\text{CrO}_2^{2+}$ at 245 and 290 nm, are shown in Fig. 6A. The intensity ratio between the absorption bands at 245 and 290 nm was 2.2, which confirmed

Fig. 4 Time evolution of the $\text{QA}/\text{Cr}^{VI}$ mixture saturated with dioxygen. $[\text{H}^+] = 0.10$ M, $I = 1.0$ M, $[\text{Cr}^{IV}]_0 = 0.07$ mM, $[\text{QA}] = 0.015$ M, and $T = 25.0$ °C, and $[\text{O}_2] = 1.26$ mM. The first trace was recorded at $t = 0$ min, and the time interval between each trace was 2.0 min. Inset: evolution of $[\text{CrO}_2^{2+}]$ with time in black (experimental data) and grey (calculated data).

Fig. 5 Time evolution of the anaerobic $\text{QA}/\text{Cr}^{VI}$ mixture. $[\text{H}^+] = 0.10$ M, $I = 1.0$ M, $[\text{Cr}^{IV}]_0 = 0.07$ mM, $[\text{QA}] = 0.015$ M, and $T = 25.0$ °C in the absence of oxygen. The first trace was recorded at $t = 0$ min, and the time interval between each trace was 2.0 min.
the presence of CrO$_2^{2+}$. As occurred with other substrates studied previously, the monotonic growth at 290 nm was found to follow first-order kinetics (Fig. 6B). A series of experiments using constant values of temperature, [QA] and ionic strength ($I$) and [Cr$^{IV}$]$_0$ in the range of 3.0–6.0 × 10$^{-5}$ M were used to confirm the first-order dependence of the rate upon [Cr$^{IV}$] (data not shown). The experimental rate constant, $k_{4exp}$, was calculated by applying a nonlinear least-square fit to the absorbance/time data using 80% of the exponential growth in the experimental values, according to eqn (3):

$$\text{Abs} = \text{Abs}_0 + (\text{Abs}_0 - \text{Abs}_\infty) e^{-(k_{4exp})t}$$  \hspace{1cm} (3)

here, $\text{Abs}_0$ and $\text{Abs}_\infty$ correspond to the initial absorbance and the absorbance at infinite time. It is known that Cr$^{IV}$ can disproportionate into Cr$^{III}$ and Cr$^{V}$ with an inverse dependence on [H$^+$] and through second-order kinetics on [Cr$^{IV}$]$^6$. This must be prevented because Cr$^{VI}$ absorbs at 290 nm, as was previously established, which interferes with the determination of CrO$_2^{2+}$. At a very low concentration and in the absence of QA, we observed a typical spectrum of Cr$^{VI}$ with the distinctive band at 350 nm (data not shown). For this reason, the experimental conditions were selected to avoid the disproportionation reaction of Cr$^{VI}$. Fitting of the time-dependent absorbance data for 290 nm using eqn (3) is shown in Fig. 6B.

**Table 2** Observed pseudo-first-order rate constants ($k_{4exp}$) for different concentrations of [HClO$_4$] and [QA]$^6$

| [HClO$_4$]/M | [QA]/mM | 0.10   | 0.20   | 0.30   | 0.40   | 0.60   |
|--------------|---------|--------|--------|--------|--------|--------|
| 1.00         | 0.070 ± 0.007 | 0.044 ± 0.004 | 0.035 ± 0.004 | —      | —      | —      |
| 2.00         | 0.142 ± 0.014 | 0.088 ± 0.009 | 0.066 ± 0.007 | 0.060 ± 0.006 | 0.05 ± 0.005 |
| 3.00         | 0.177 ± 0.018 | 0.110 ± 0.011 | 0.086 ± 0.009 | 0.076 ± 0.008 | —      | —      |
| 4.00         | 0.212 ± 0.021 | 0.132 ± 0.013 | 0.104 ± 0.010 | 0.090 ± 0.009 | 0.074 ± 0.007 |
| 5.00         | 0.138 ± 0.014 | 0.120 ± 0.012 | 0.100 ± 0.010 | —      | —      | —      |
| 6.00         | 0.173 ± 0.017 | 0.150 ± 0.015 | 0.124 ± 0.012 | —      | —      | —      |

$^a$ T = 15.0 °C, [Cr$^{IV}$]$_0$ = 0.070 mM, and $I$ = 1.0 M. Mean values from multiple determinations. The rate constants were obtained using the Origin 6.0 program.

Oxidation of QA by Cr$^{VI}$

As was previously observed with other substrates, for the QA/Cr$^{IV}$ mixture, the curves of absorbance at 350 nm vs. time displayed a monotonic decreasing behavior that cannot be described using a single-exponential decay. An acceptable
description of the kinetic profiles could be obtained by using a set of consecutive first-order reactions, as presented in Scheme 1.

Considering the superposition of the absorbance of CrV throughout the redox reaction, the absorbance at 350 nm is given by eqn (7):

$$\text{Abs}_{350} = \varepsilon^V \text{CrVI} + \varepsilon^V \text{CrV}$$

Combining eqn (6) with rate expressions that result from considering first-order reactions gives the following expression (eqn (8)):

$$\text{Abs}_{350} = \text{Abs}_0 e^{-2k_6 t} + k_6 e^{-k_5 t} \text{CrVI} \left( e^{-2k_5 t} - e^{-2k_6 t} \right) (2k_6 - k_5)$$

where $\varepsilon^V$ is the molar absorptivity of oxo-CrV-QA at 350 nm ($\varepsilon^V = (1.7 \pm 0.50) \times 10^3 \text{ M}^{-1} \text{cm}^{-1}$), and the parameters $k_5$ and $k_6$ are the rates of disappearance of CrV and CrVI, respectively. A nonlinear iterative computer fit using eqn (8) was used to estimate both parameters $k_5$ and $k_6$. Table 3 shows the calculated values of $k_5$ and $k_6$ for different concentrations of QA and HClO$_4$. It is important to note that in eqn (8), $k_6$ appears twice in the denominator and in the exponential terms and only once in the numerator of the pre-exponential term, which is consistent with the reaction in Scheme 1, in which half of CrVI is converted to CrIII via CrV intermediates. In the range of proton concentrations employed in this study, plots of $k_6$ vs. [QA] gave good straight lines (Fig. 8A). Values of $k_6$ were determined using these experimental data by eqn (9). The bimolecular rate constant, $k_{6H}$, exhibited variation with [H$^+$], which can be described as quadratic dependence, as shown in Fig. 8B. This behavior can be described using eqn (10). Consequently, by combining eqn (9) and (10), $k_6$ can be calculated as indicated by eqn (11).

$$k_{6\exp} = k_{6H}[\text{QA}]$$

$$k_6 = (k_6^1 + [\text{H}^+]^2)[\text{QA}]$$

Here, $k_6^1 = (1.31 \pm 0.01) \times 10^{-2} \text{ s}^{-1} \text{ M}^{-3}$ and $k_6^2 = (3.05 \pm 0.09) \times 10^{-1} \text{ s}^{-1} \text{ M}^{-3}$ (Fig. 8B).

Plots of $k_{6\exp}$ vs. [QA] for a constant value of [H$^+$] revealed linear dependence, as can be seen in Fig. 9A. Using these experimental data and eqn (12), the bimolecular rate constant $k_{6H}$ could be calculated. A quadratic dependence was revealed by plotting $k_{6H}$ vs. [H$^+$] (Fig. 9B). The corresponding $k_{6H}$ values were calculated using eqn (13).

$$k_{6\exp} = k_{6\exp}[\text{QA}]$$

$$k_{6H} = k_6^1 + k_6^2 [\text{H}^+]^2$$

$$k_5 = (k_5^1 + k_5^2 [\text{H}^+]^2)[\text{QA}]$$

Table 3 Observed pseudo-first-order rate constants ($k_{6\exp}$ and $k_{6\exp}$) for different values of [HClO$_4$] and [QA]$^a$

| [QA]/M | [HClO$_4$]/M | 0.10 | 0.20 | 0.30 | 0.40 | 0.50 |
|-------|--------------|------|------|------|------|------|
| 0.030  | 0.48 ± 0.05  | 0.73 ± 0.07 | 1.27 ± 0.13 | 1.80 ± 0.18 | 2.30 ± 0.23 |
| 0.045  | 0.60 ± 0.06  | 1.30 ± 0.13 | 1.90 ± 0.19 | 2.50 ± 0.25 | 3.90 ± 0.39 |
| 0.080  | 1.19 ± 0.12  | 2.10 ± 0.21 | 3.36 ± 0.34 | 4.86 ± 0.49 | 6.85 ± 0.69 |
| 0.100  | 1.40 ± 0.14  | 2.80 ± 0.28 | 4.22 ± 0.42 | 5.70 ± 0.57 | 8.00 ± 0.80 |
| 0.120  | 1.70 ± 0.17  | 3.10 ± 0.31 | 4.90 ± 0.49 | 6.80 ± 0.68 | 9.80 ± 0.98 |

$^a$ $T = 33.0 \degree C, [\text{CrVI}]_0 = 6.0 \times 10^{-4} \text{ M}$, and $I = 1.0 \text{ M}$. $^b$ Mean values from multiple determinations.
According to Fig. 9B, $k^2 = (2.22 \pm 0.3) \times 10^{-3} \, \text{s}^{-1} \, \text{M}^{-1}$ and $k^3 = (7.01 \pm 0.19) \times 10^{-2} \, \text{s}^{-1} \, \text{M}^{-3}$. For the disappearance of CrVI and CrV, the rate constants are specified by eqn (11) and (14), respectively.

By using CW-EPR spectroscopy, the rate constants $k_5$ and $k_6$ could be independently obtained. The EPR peak-to-peak height for CrV in the reaction of 0.25 M QA and 2.5 $\times$ 10$^{-3}$ M CrVI in 0.5 M HClO$_4$ increased and decayed at 18 °C (Fig. 10). A higher modulation amplitude (2.0 G) was used to avoid superhyperfine coupling. Eqn (15) was used to fit the CW-EPR data. This equation was derived by considering consecutive first-order reactions.

$$h = A k_6(e^{-(k_5)t} - e^{-2k_6t})(2k_6 - k_5)$$

Eqn (15) describes the total absorbance at 570 nm at any time.

$$A_{570} = e^V[CrV] + e^{III}[Cr^{III} \text{-ligand}]$$

Eqn (17) describes the total absorbance at 570 nm at any time.

The values of both rate constants $k_6$ and $k_5$ determined by CW-EPR were in accordance with those obtained using eqn (11) and (14), considering the experimental error and the difference in temperature. The good fitting of the data shown in Fig. 10 and that of the kinetic measurements at 350 nm (Fig. 8 and 9) indicated that the use of two different spectroscopic techniques, namely, UV/vis and CW-EPR could confirm the suggested consecutive first-order reactions shown in Scheme 1. According to the previously determined $k_6$ values, it can be inferred that CrIV is involved in a fast kinetic step and CrV persists in the QA/CrVI mixture.

Kinetic studies at 570 nm were also carried out for QA/CrVI mixtures. In these experiments, it must be considered that oxo-CrV-QA complexes are not the only absorptive species present, and CrIV, CrIII and CrIII-ligand species also absorb at this wavelength. Additionally, and according to our previous results, CrIV did not accumulate and did not contribute to the absorption at 570 nm. The absorbance at 570 nm vs. time is shown in Fig. 11. First, the absorbance increased quickly (~500 seconds) to high values, which could be associated with the formation of CrV and CrIII-ligand complexes. After this maximum, the absorbance decayed in an exponential way. This was probably due to the decay of CrV species and the hydrolysis of CrIII-ligand complexes. The experimental data were fitted using eqn (16), derived from Scheme 1, which considers CrIII-ligand to be the final redox species in the mixture; this enabled us to confirm or reject the previous assumption.

$$A_{570} = e^V[CrV] + e^{III}[Cr^{III} \text{-ligand}]$$
The kinetic profile simulated using $k_5$ and $k_6$ shows comparable values for the time of the maximum intensity ($t_{\text{max}} = 475$ s) of Abs$_{570}$ and the calculated time (465 s) at which $[\text{CrVI}]_{\text{max}}$ occurred in the reaction (more than 40% of total Cr) (Fig. 12). These results indicate that the Cr$^V$ intermediate species can be responsible for the behavior of the absorbance at 570 nm during the first period, whereas the next portion of the data corresponds to the slow decomposition of Cr$^\text{III}$ species into Cr$^\text{III}$(aq). Moreover, as can be seen from Fig. 12, Cr$^I$ still remains in the mixture after Cr$^\text{VI}$ is completely consumed.

**Discussion**

**Oxidation of QA by Cr$^\text{VI}$**

The reduction of Cr$^\text{VI}$ by QA exhibits strong pH dependence. The reaction is slow at pH > 1, and Cr$^\text{VI}$ is quickly consumed when $[\text{H}^+] > 0.5$ M. Therefore, the $[\text{H}^+]$ range of 0.1–0.05 M was preferred to perform the kinetic study of this reaction. When $[\text{H}^+]$ was 0.1 M, the time-dependent UV/vis spectra of the QA/Cr$^\text{VI}$ mixture (Fig. 2A) showed two relevant points; the absorbance (a) decayed over time at 350 nm and 420–470 nm and (b) increased without an isosbestic point at 570 nm. As was previously pointed out, the absence of an isosbestic point indicates that more than one reaction occurs throughout the reduction of Cr$^\text{VI}$ to Cr$^\text{III}$, and several chromium species are present in considerable amounts.

**Kinetics analysis**

The presence of Cr$^\text{IV}$ and/or Cr$^\text{V}$ intermediates during the reduction of Cr$^\text{VI}$ has been previously observed for dinuclear substrates. The detection of organic radicals and CrO$_2^{2+}$ in the QA/Cr$^\text{VI}$ mixture along with the observation of relatively long-lived oxo-Cr$^\text{V}$ species jointly indicate that Cr$^\text{IV}$/Cr$^\text{V}$ intermediates were produced in the reaction of QA with Cr$^\text{VI}$; this also strongly suggests that this redox process follows one- and two-electron pathways.

Considering the presence of the two detected chromium intermediates, namely, Cr$^\text{IV}$ and Cr$^\text{V}$ and that Cr$^\text{IV}$ reacts faster than Cr$^\text{V}$, it is necessary to determine whether both or only one of them must be considered during the analysis of experimental kinetic data. A comparison of the corresponding oxidation rates for Cr$^\text{IV}$, Cr$^\text{V}$ and Cr$^\text{VI}$ (eqn (18), (19) and (20)) can be made by employing $k_5$ values obtained from eqn (6), (14) and (11), respectively, in the following conditions: $[\text{QA}] = 0.03$ M, $[\text{H}^+] = 0.1$ M, and $[\text{Cr}^\text{V}] = [\text{Cr}^\text{VI}] = 6.0 \times 10^{-4}$ M.

\[
\begin{align*}
\nu_4 &= k_4[\text{Cr}^\text{IV}] = (k_8 + k_9[H^+]^{-1})[\text{QA}][\text{Cr}^\text{IV}] \\
\nu_5 &= k_5[\text{Cr}^\text{V}] = (k_4 + k_5[H^+]^3)[\text{QA}][\text{Cr}^\text{V}] \\
\nu_6 &= k_6[\text{Cr}^\text{VI}] = (k_6 + k_7[H^+]^3)[\text{QA}][\text{Cr}^\text{VI}] 
\end{align*}
\]

The calculated values of the rates were $\nu_4 = 1.3 \times 10^{-6}$ M s$^{-1}$ > $\nu_5 = 2.3 \times 10^{-7}$ M s$^{-1}$ > $\nu_6 = 5.3 \times 10^{-8}$ M s$^{-1}$, and the ratios between these values were (a) $\nu_4/\nu_5 \approx 25/1$, (b) $\nu_4/\nu_6 \approx 5/1$ and (c) $\nu_5/\nu_6 \approx 6/1$. These calculated data confirmed that Cr$^\text{IV}$ reacted faster than Cr$^\text{V}$ and Cr$^\text{VI}$ species, suggesting that although Cr$^\text{IV}$ was formed during the oxidation of QA with Cr$^\text{VI}$, it did not accumulate and cannot be considered for the fitting of experimental kinetic data. The rate values calculated for $\nu_4$ and $\nu_5$ confirmed the kinetic profiles represented in Fig. 12, which indicated that Cr$^\text{V}$ was still present when there was no remaining Cr$^\text{VI}$; this suggested that this intermediate species reacted more slowly than Cr$^\text{VI}$ with QA. Consequently, at any wavelength, the time dependence of the absorption data for the reaction can be fitted using the sequence proposed in Scheme 1. Moreover, eqn (15) can be used to fit the data for CW-EPR peak-to-peak height vs. time (Fig. 10). The first-order rate constants determined in this way agreed perfectly with those determined from the UV/vis spectroscopy data (eqn (11) and (14)).

At this point, and considering all the previously reported results, we are able to propose and discuss a novel insight into the possible mechanism for the reaction of QA with Cr$^\text{VI}$ (Scheme 2).

**Proposed mechanism**

According to the literature, at $[\text{Cr}^\text{V}]$ and $[\text{H}^+]$ used in these kinetic studies, Cr$^\text{VI}$ occurs as HCrO$_4^-$ and Cr$^\text{V}$ is oxidized faster than Cr$^\text{VI}$. Therefore, the first step in the mechanism is the formation of the Cr$^\text{VI}$ ester, where QA acts as a bidentate ligand (eqn A, Scheme 2). The next step in Scheme 2 is slow and comprises intramolecular two-electron transfer among molecules of the active Cr$^\text{VI}$ ester to yield Cr$^\text{IV}$ and Sox (eqn B1 and B2). Considering that this is an acidic substrate, it can be postulated, similar to that with other substrates, that both protonated and deprotonated forms of the Cr$^\text{VI}$ ester can be oxidized in two
different reactions. The first reaction B1 is independent of protons, and the second reaction B2 involves two protons. The theoretical rate law for the consumption of CrVI, mathematically derived from eqn A and B in Scheme 2, is represented by eqn (21). [CrVI]T denotes the total concentration of CrVI in the mixture and takes into consideration the concentrations of the ester and aqua-chromium forms.

\[-d[CrVI]/dt = (k_{q} + k_{v}[H^+])K^{VI}[QA][CrVI]/(1 + K^{VI}[QA]) \quad (21)\]

If $K^{VI}[QA] << 1$, as can be expected considering other previously studied substrates,26,27,28 eqn (21) becomes eqn (22), where $k_{q}K^{VI} = k_{q}^{VI}$ and $k_{v}[H^+] = k_{v}^{VI}$, which agrees with the experimental rate law (eqn (20)).

\[-d[CrVI]/dt = (k_{q} + k_{v}[H^+]^{2})K^{VI}[QA][CrVI]/(1 + K^{VI}[QA]) \quad (22)\]

Once CrIV is formed, it participates in the oxidation mechanism in two steps, with one- or two-electron reductions of CrIV by QA (eqn C and E). In eqn C, CrIV reacts in the presence of excess QA to generate CrIII and the QA radical (QA). This step is sustained by the polymerization of acrylamide when it is added to the QA/CrIV mixture. The alternative route for the reduction of CrIV by QA results in the formation of CrIII and Sox, which is supported by the detection of CrO2+ (product of the reaction of CrIII with O2). As was postulated for CrVI, QA exists in equilibrium between its protonated and unprotonated forms (eqn D, Scheme 2), both of which can react with CrIV in a fast reaction to yield CrIII and Sox (eqn E1 and E2). These are also two electron intramolecular transfers; one is independent of protons (E1) and the other involves one proton (E2). Once again, the theoretical rate law for the consumption of CrIV, which was mathematically derived from eqn D and E in Scheme 2, is described by eqn (23). [QA]T represents the concentrations of the protonated and unprotonated forms of QA, and $K_{a}$ is the acidity constant of QA.

\[-d[CrIV]/dt = (k_{q}^{I} + k_{v}[H^+]^{-1})[CrIV]/[QA] \quad (23)\]

If $k_{q}^{VI} = k_{q}^{I}$ and $k_{v}^{VI} = k_{v}^{I}$, this agrees with the experimental rate law (eqn (18)).

According to our results, CrIV does not accumulate in the mixture because it is involved in fast steps. The concentration of CrO2+ should be the same as [CrIV]0 if the reaction takes place entirely via the CrIV → CrV → CrIII pathway.55 Our experimental data indicate that the yield of CrO2+ increases as the value of [CrIV]0 decreases and reaches a limiting value of 49.7% (Fig. 4), indicating that nearly half of CrIV reacts via a route that does not involve CrIV. QA' and CrIV', which are formed as shown in eqn C and E1 and E2, respectively, rapidly react with CrIV to produce CrV and Sox in the first case (eqn F) and CrIV and CrIII in the second case (eqn I) ($k = 2.0 \times 10^{5}$ M$^{-1}$ s$^{-1}$).56,57 Both species can also be quickly trapped by O2 to give Sox (eqn G) and Cr(III) (eqn H). At this point, it can be seen that CrIV is formed via two alternative routes, namely, eqn F and I, which are both fast reactions involving one-electron transfers. Once formed, CrIV further oxidizes QA via a two-electron process to generate Sox and CrIII as the final products (eqn J, Scheme 2). As was previously demonstrated by the calculated rate values and as shown in Fig. 12, the reaction of QA with CrV is slower than that of QA with CrVI because after all the initial CrVI is consumed, CrV still remains in the mixture in measurable quantities. Based on the selectivity for the oxidation products of QA, the kinetic results and the CW-EPR results for the oxo-CrV–QA complexes,49 we proposed a fast reaction between CrV and QA that produces an oxo-CrV–(QA)$_2$ bichelate ($k_{q}^{VI}$, $k_{v}^{VI}$), which finally produces Sox and CrIII via two different routes, namely, acid-dependent and acid-independent steps (eqn J). The theoretical rate law deduced for the disappearance of the CrVI species (eqn (24)) is derived from eqn J in Scheme 2. [CrVI]T represents the total concentration of CrVI in the mixture.

\[-d[CrIV]/dt = k_{q}^{VI}K_{a}[CrIV]/(1 + K_{a}[QA]) \quad (24)\]

If $k_{q}^{VI}K_{a}[QA] >> k_{v}^{VI}$, as can be expected considering other previously studied substrates,26,27,28 eqn (24) becomes eqn (25), where $k_{q}^{VI} = k_{v}^{VI} = k_{v}^{II}$, which agrees with the experimental rate law (eqn (19)).

\[-d[CrIV]/dt = (k_{a}^{III} + k_{v}^{II}[H^+]^{2})[QA][CrIV]/(1 + K^{VI}[QA]) \quad (25)\]

Conclusions

The reaction of QA with CrVI strongly depends on pH. The oxidation of QA by CrVI generates Sox and a QA–Cr(III)$_{ac}$ complex, which is then slowly hydrolyzed to form Cr(III)$_{ac}$ complex. Kinetic studies strongly support the hypothesis that the redox reaction proceeds via a combined mechanism, which involves CrIV → CrV → CrIII and CrVI → CrV → CrIII pathways, as has been previously demonstrated with other substrates.26,27,28 The mechanism is supported by the observation of CrO2+ and CrV and free radicals as reaction intermediates. The bimolecular rate constant for QA/CrIV reaction is much higher than that with CrVI or CrIV', which proves that CrIV does not accumulate in the QA/CrIV reaction mixture. The detection of free radicals and the relatively long half lifetimes of CrIV species are some of the reasons for warnings against the use of CrIV in industry and other human activities because of its possible role in the oxidation of several substrates that are ubiquitous in nature.

Conflicts of interest

There are no conflicts to declare.

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