STUDIA UBB CHEMIA, LVIII, 4, 2013 (p. 81-99)
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Dedicated to Professor Liviu Literat
On the occasion of his 85th birthday

ON THE OXIDATION OF GLUTATHIONE BY CHROMIUM (VI), IN AQUEOUS SOLUTIONS OF PERCHLORIC ACID

DANA-MARIA SABOU[a,*] AND IOAN BÂLDEA[a]

ABSTRACT. The oxidation of glutathione by chromium(VI) has been studied under pseudo-first order conditions at 293 K, in mildly acidic environment of controlled ionic strength (0.5 M, $NaClO_4$). The process was monitored spectrophotometrically and showed evidence of two stages. Both stages have been evaluated kinetically, the reaction orders were found and some of the rate constants were computed. The involvement of paramagnetic intermediates has been investigated. In agreement with the experimental findings, a reaction mechanism has been suggested, that showed changing features under the range of acidities employed.

Keywords: *redox, chromium VI, glutathione, kinetics*

INTRODUCTION

Chromium (VI) has been for a long time, and continues to be, widely used as a mild oxidant, for a variety of inorganic and organic substrates, not only in laboratories, but also at an industrial scale. The interest in clarifying the features of its reaction mechanisms is, therefore, understandable. A new boost has been given to the matter by the discovery of its ulcerating properties upon prolonged skin contact [1] and, more importantly, the carcinogenic effects of its dust upon inhalation, ultimately leading to lung cancers [2-4]. The toxicity of Cr(VI) contaminated water has also been periodically under assessment. While early results concluded it rather harmless, it has been recently proven positively carcinogenic upon either high dosage or long time ingestion [5]. It is believed that the responsibility lies with the mutagenic potential of the more active, lower valence states Cr(V) and Cr(IV) [6-8], formed during its

[a] *Babeş-Bolyai University, Faculty of Chemistry and Chemical Engineering, Str. Arany János, No. 11, RO-400028, Cluj-Napoca, Romania, * Corresponding author: dsabou@chem.ubbcluj.ro*

reduction to Cr(III) by cellular constituents, amongst which ascorbic acid and glutathione are considered the most active ones [9, 10]. To this day, little has been established without doubt concerning the chemistry of the pathways leading to DNA alterations, and similar studies sometimes seem to have reached contradictory conclusions [11]. The outcome is that such findings have prompted re-examinations of the chemistry of chromium(VI) reduction by compounds containing alcoholic or thiolic moieties, in various environments. In this context, the reaction with glutathione received much attention, with most of the work focused on its behaviour in solutions of neutral (close to physiological) pH.

It is well known that in aqueous media, Cr(VI) is involved in a number of protolytic and hydrolytic equilibria [12, 13] that control its speciation. While at pH's of 7 and higher CrO_4^{2-} is the dominant species in the solution, in acidic environment this is protonated to $HCrO_4^-$ and eventually to H_2CrO_4, which becomes noticeable at pH's below 2 [13]. Also, in acidic medium, the condensation of two molecules of $HCrO_4^-$ with the formation of the dimer $Cr_2O_7^{2-}$ becomes important at higher concentration of total Cr(VI) and increasing ionic strength. The dimer can also be involved in protolytic equilibria. However, it is possible to choose conditions so that the $HCrO_4^-$ is the dominant species. Moreover, $HCrO_4^-$ can undergo such condensation with virtually any partner able to provide an H^+, making any acid a good candidate [14]. The further reactivity of the condensed species is dependent on the acid [15-17]. At lower total Cr(VI) concentrations, such condensations are much more likely to happen than the dimerization. The readiness with which the condensed species is formed and thus its amount, depends on the acid involved. Perchloric acid is considered to be the least prone to such condensation, thus, in diluted aqueous perchloric solutions, Cr(VI) exists as $HCrO_4^-$.

The wealth of work which has been done on the subject of chromium (VI) redox reactions in various media, with inorganic and organic substrates, including thiols, has been concluded in establishing a few features that are generally agreed upon. Their inner-sphere type mechanism is one such feature. They start with a reversible step in which the bonding of the thiol to chromium takes place, much like any other condensation with an acid. A condensation complex is formed, which subsequently decomposes by electron transfer. The number of the electrons simultaneously transferred in this step, the molecularity, the rate-determining stage of the whole process and sometimes the type of the reaction products, are features that differ from one reductant to another in similar environments. The same is valid for any individual reducing agent if the reaction conditions change.

This study deals with the reduction of Cr(VI) by glutathione in mildly acidic aqueous solutions of $HClO_4$, providing both kinetic and extra kinetic data concerning the reaction. Thereby, it becomes possible to obtain more detailed insight on the reaction mechanism in this region of acidity.

RESULTS AND DISCUSSION

In this work, chromium (VI) was always used as the limiting reactant, while the glutathione and the hydrogen ion providing species were usually in large excess and the temperature and ionic strength were controlled (commonly 293 K and 0.5 M ($NaClO_4$) respectively). From literature data on the equilibrium constants, describing the speciation equilibria of Cr(VI) [12, 13, 18], it was computed that the dominant Cr(VI) species was always $HCrO_4^-$ (in most cases around 98÷99 %, with *ca.* 86.8 % and 89 % as the lowest values, under the least favorable sets of conditions).

Stoichiometry

To determine the stoichiometry, three reaction mixtures containing glutathione (abbreviated as GSH) and Cr(VI) in an initial molar ratio of 20 to 1 were prepared and the reaction was allowed to finish. The remaining glutathione was titrated iodometrically. The results showed an average of 3.2 ± 0.2 moles of glutathione to be consumed for each mole of Cr(VI). Therefore, the reaction yields mainly the disulphide GSSG. This is in agreement with many literature data, concerning thiols in general [19-24] and glutathione in particular [25-29]. The reaction stoichiometry can be written as below:

$$6\,GSH + 2\,HCrO_4^- + 8\,H^+ \longrightarrow 3\,GSSG + 2\,Cr(III) + 8\,H_2O \qquad (1)$$

Intermediates

UV-VIS Spectrophotometry

Upon mixing of the two reactants, a rapid change in colour of the solution can be observed within a time scale of seconds, followed by a much slower fading of this colour (tens to hundreds of minutes). This behaviour is explained by the occurrence of an intermediate [25, 28, 30-33]. McAuley and Olatunji [30] have calculated a spectrum showing a transient species with an absorption band in the region 375 ÷ 550 nm. We have also previously reported spectral evidence [14] for the formation, of a species with an absorption maximum at 435 nm, under conditions similar to those of this study. Such notable shift towards red, as compared to the 350 nm maximum of $HCrO_4^-$, is consistent with the replacement of a Cr-O bond in $HCrO_4^-$ by a Cr-S one, and supports the formation of a GS-Cr(VI) thioesther. The binding of the glutathione to Cr(VI) through the cysteinyl thiolate group has been confirmed by Raman spectroscopy [34].

Some controversy existed in the literature about the assignment of the 400-500 nm absorption band to the GS-Cr(VI) thioester. This was sparked by the observation of an additional absorption band when working at pH 5.2÷5.9 [32], with a peak at 373 nm and higher molar absorptivity than that

of the CrO_4^{2-} (the dominant Cr(VI) species at this pH), with the maximum at 372 nm [35]. It was proposed that the thioester absorbed at 373 nm and some Cr(V) or Cr(IV) [28, 32] was responsible for the band at 400-500 nm. However, such a small shift (of only 1 nm) is uncharacteristic when sulphur replaces an oxygen in the chromium complex [14], but rather specific for the reactions of hydroxy compounds, which bind to Cr *via* the hydroxylic oxygen. In these, the bond type is not changed and only the charge distribution within the complex is affected. Moreover, under our conditions (pH 1÷2) the 373 nm band is not present; on the contrary, a minimum of absorbance is noticed in that region [14]. Since $HCrO_4^-$, the dominant species in our case, is known to readily form condensation complexes, the species exhibiting the absorption band at 435 nm, that features in the spectra we recorded, should be the GS-Cr(VI) thioester. It is the band around 373 nm, occurring at the higher pH's, that should rather be attributed to a different kind of intermediate.

Combining absorption spectroscopy and EPR spectroscopy results, Lay and Levina [25] have later reported a spectrum for the thioester intermediate that was identical for pH's 2.9 and 7.4 and in perfect agreement with our spectral data. They attributed the high absorbance around 370 nm to a mixture of Cr(V) or potentially Cr(IV) intermediates and of CrO_4^{2-} (as one product of the Cr(V) disproportionation, at pH 8) [36].

ESR Spectroscopy

We made use of the ESR technique to observe the possible formation of Cr(V) species under the conditions of our study. For comparison with literature data, we worked at 277 K and spanned a larger range of pH's. Figure 1 A) shows comparatively the signals found in each case, two minutes after mixing the reactants, while B), C) and D) illustrate for some of them their time dependence.

It is evident that more than one type of Cr(V) intermediates may occur in the reaction mixture, and the extent to which each of them is formed, appears to be a pH-dependent feature. It could also be argued that after two minutes the reaction has reached different stages at the different acidities, hence some of the signals may have already vanished. However, the time evolution for each pH shows no growing signals, but only the decay of the already present ones.

While the recorded spectrum at pH 11 shows no notable features, for pH 8, two of the signals reported by Levina and Lay [36] with g_{iso}=1.996 and g_{iso}=1.986, are observable in figure 1 (A) and (D), with the latter being longer lived. Two signals similar to those reported earlier by Brauer and Wetterhahn (g_{iso}=1.986 and g_{iso}=1.972) [32] are seen at pH 5, and are quite stable (even at room temperature, where the measurement was repeated) but they almost vanish at pH 3. On the contrary, three signals (g_{iso} of 1.983,

1.980 and 1.978) comparatively weak at pH 5, and near-noise at pH 8 gain some importance at pH 3. At pH 1 no Cr(V) intermediate could be observed under the chosen settings and only a very broad Cr(III) signal was seen, indicating that the reaction had most likely already finished.

These findings show that there are several alternative pathways for the reaction, that proceed *via* Cr(V) intermediates. The differences in the Cr(V) species have to do with the involvement of hydrogen ions.

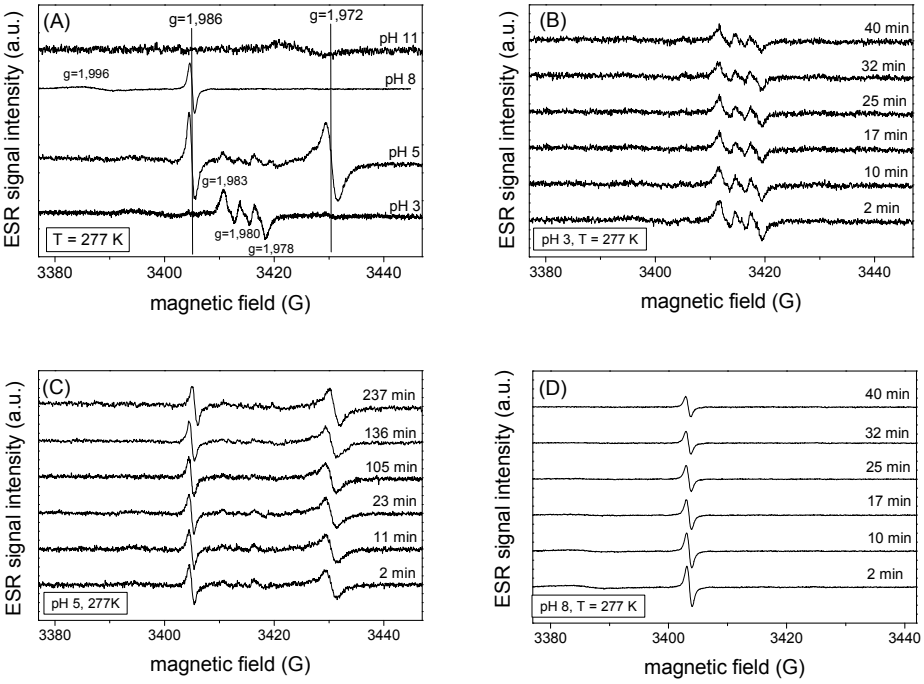

Figure 1. ESR spectral evidence for the formation of Cr(V)-glutathione complexes at various acidities during the oxidation of glutathione by chromium (VI).

Kinetics

At 435 nm, the shape of the kinetic curves clearly shows the two phases of the process, as illustrated in figure 2. The notable difference in their rates allowed us to further study them separately.

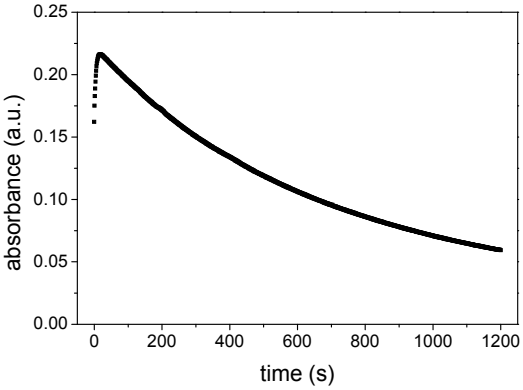

Figure 2. A kinetic curve at 435 nm for the reaction of Cr(VI) with glutathione in aqueous perchloric solution ($[Cr(VI)]_0=1.3\cdot10^{-4}$ M, $[GSH]_0=1.6\cdot10^{-2}$ M, $[H^+]_0= 3.0\cdot10^{-2}$ M ($HClO_4$), μ=0.5 M ($NaClO_4$), T = 293 K)

The formation of the Cr(VI)-GSH intermediate complex

As indicated by the batochromic shift in absorbance, the formation of the intermediate involves the binding of glutathione to the Cr(VI) *via* the thiolic moiety. This is a ligand substitution, hence a reversible step. In acidic environment, some involvement of the hydrogen ion can also be expected.

The reaction orders for the formation of the intermediate were determined using the initial rates method. A stopped-flow arrangement was used to monitor the beginning part of the reaction and the initial rates (expressed as dA/dt) were computed from the slopes of the curves absorbance-time for less than 0.1% total conversion (0.4 s or less). Three series of measurements were made. In each of them the concentration of only one species was varied while the others were kept constant. Table 1 lists the results obtained. (The errors, in the table 1 and throughout the paper, unless stated otherwise, are the standard errors of the parameter as determined from the linear regression.)

For such small conversions, the amount of intermediate formed is insignificant and the reverse reaction negligible. Therefore, the initial rate law can be written as:

$$\alpha \cdot r_{0,435} = \left|\frac{dA_{435}}{dt}\right|_{t\to 0} = \alpha \cdot k_1 \cdot [GSH]_0^a [H^+]_0^b [HCrO_4^-]_0^c \qquad (2)$$

where $r_{0,435}$ and A_{435} are the initial rate and the absorbance at 435 nm, α is a constant containing the path length of the mixing chamber (ℓ = 0.336 cm) and the molar absorptivity of $HCrO_4^-$ at the used wavelength ($\varepsilon_{435,HCrO4-}$), k_1 is the rate coefficient for the forward step, while a, b and c are the respective reaction orders for the three possible reactants.

Table 1. Initial slopes dA/dt (directly proportional to the initial rates) computed from the experimental curves of absorbance *vs.* time (μ = 0.5 M ($NaClO_4$), T = 293 K)

$[H^+]_0$ (10^{-2} M)	$[GSH]_0$ (10^{-3} M)	$[HCrO_4^-]_0$ (10^{-3} M)	dA/dt (10^{-3} s^{-1})
2.6	1.2	0.40	3.2 ± 0.5
	2.0		9.3 ± 0.3
	4.0		12.5 ± 0.2
	8.0		30 ± 3
	15		53 ± 5
	20		70 ± 10
2.6	4.0	0.20	7.1 ± 0.3
		0.60	19.0 ± 0.3
		0.80	25.7 ± 0.3
		1.0	37.9 ± 0.3
		1.2	41.4 ± 0.3
6.2	4.0	0.40	35 ± 2
4.9			29 ± 1
3.8			24 ± 1
3.1			19 ± 1
2.4			11 ± 2
0.97			4.4 ± 0.4
0.62			5.4 ± 0.4

With each series of measurements, the reaction order with respect to the varied species can be determined from the slopes of the appropriate double logarithmic plot of equation 3, as it is shown by figure 3.

$$\log\frac{dA_{435}}{dt} = \log\alpha + a\cdot\log([GSH]_0) + b\cdot\log([H^+]_0) + c\cdot\log([HCrO_4^-]_0) \qquad (3)$$

The three different plots are all linear and of slopes approximately unity.

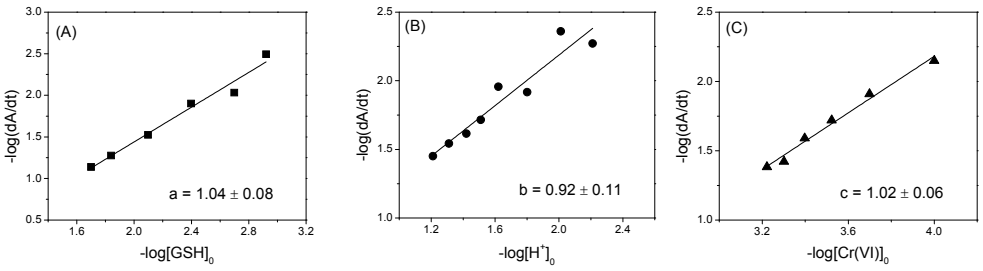

Figure 3. Double-logarithmic plots for determining the three reaction orders (GSH: (A), H^+: (B) and Cr(VI): (C)) for the intermediate formation. Conditions like in table 1.

In mechanistic terms, this means that the intermediate has a 1:1 ratio GSH:Cr(VI) and its formation is catalyzed by one proton. Therefore, the step of forming the intermediate can be described by the equation 4:

$$GSH + HOCrO_3^- + H^+ \rightleftharpoons GSCrO_3^- + H_2O + H^+ \qquad k_1, k_{-1} \quad (4)$$

where k_1 and k_{-1} stand for the reaction coefficients of the forward and reverse processes respectively. A simple third order rate law can be written for the build-up of the intermediate (the forward step):

$$r_1 = k_1[GSH][H^+][HCrO_4^-] \quad (5)$$

Furthermore, a value for the k_1 can be determined based on eq. 5, from the initial rates, which can be computed if the molar absorptivity of the reactant at 435 nm ($\varepsilon_{435,HCrO4-}$) is known. To estimate the latter, we used the set of measurements where Cr(VI) was varied, to plot the initial absorbance values against the initial Cr(VI) concentration. We obtained a good line, with the slope 231±12 M^{-1} cm^{-1}, equal to $\varepsilon_{435,HCrO4-}$.

Using the data of all three sets of measurements, the plot of the initial reaction rates against the product of the three reactant concentrations was linear (figure 4), with a zero intercept within the error ((3±10)·10^{-6} s^{-1}). The slope of the line was (4.6±0.1)·10^3 M^{-2} s^{-1}, and represents the third order rate constant k_1 at T= 293 K and μ = 0.5 M.

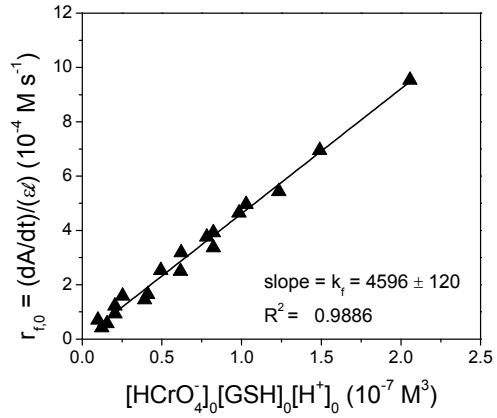

Figure 4. Dependence of the initial rate for the intermediate formation on the product of the three initial concentrations of the reactants (T = 293 K, μ = 0.5 M).

Decay of the GS-Cr(VI) intermediate by electron transfer

As established, the decay of the intermediate is much slower than its formation. It is assumed that this process takes place with the transfer of one or more electrons and therefore is the slower step, as this usually involves more important rearrangements in the chromium complex.

Classical batch measurements were appropriate to monitor this stage of the process. In these, Cr(VI) was, once again, the limiting reactant and its concentration was always $1.33 \cdot 10^{-4}$ M. The concentrations of the glutathione and hydrogen ion were always in large enough excess to be considered practically constant throughout the reaction. The illustrative curve in figure 2 is part of this group of batch measurements.

Under the excess settings, the assumption of two consecutive pseudo-first order steps (where R stands for reactant, I for intermediate and P for a generic product) can be applied to the studied system (equation 6):

$$R \xrightarrow{k_{1obs}} I \xrightarrow{k_{2obs}} P \tag{6}$$

The observed first order for both steps is with respect to the Cr(VI) species (R or I) and the observed rate coefficients k_{1obs} and k_{2obs} are functions of the excess concentrations of the other reactants involved.

As already stated, much of the total absorbance at 435 nm appears to be brought about by the intermediate. However, some contribution from the weaker band of the $HCrO_4^-$ in this region, or from the even weaker one of the Cr(III) product should be considered. Taking into account this and the rate law for the two steps first-order series (formation and decay of the intermediate), the following equation for the evolution of the absorbance in time is valid [19, 28]:

$$A - A_\infty = [R]_0 \ell \{\varepsilon_R + \frac{k_{2obs}\varepsilon_P - k_{1obs}\varepsilon_I}{k_{1obs} - k_{2obs}}\} e^{-k_{1obs}t} + [R]_0 \ell \{\frac{k_{1obs}(\varepsilon_I - \varepsilon_P)}{k_{1obs} - k_{2obs}}\} e^{-k_{2obs}t} \tag{7}$$

or its simpler form, obtained by combining all pre-exponential factors into some constants (γ_1 and γ_2):

$$A - A_\infty = \gamma_1 e^{-k_{1obs}t} + \gamma_2 e^{-k_{2obs}t} \tag{8}$$

If $k_{1obs} >> k_{2obs}$, equation (8) reaches a limit form for late stages, where the first exponential vanishes, and γ_2 and k_{2obs} can be determined, respectively, based on equation (9):

$$\ln(A - A_\infty) = \ln \gamma_2 - k_{2obs}t \tag{9}$$

For the early stages of the process, equation (8) can be re-arranged into equation (10), to yield γ_1 and k_{1obs} :

$$\ln\left(A - A_\infty - \gamma_2 e^{-k_{2obs}t}\right) = \ln \gamma_1 - k_{1obs}t \tag{10}$$

Equation (9) was used to process our data. Indeed, for later stages, the plots $\ln(A-A_\infty) = f(t)$ became linear (R^2 between 0.9989 and 0.9999 at conversions higher than 70%, based on absorbance). The slopes of the linear parts represent k_{2obs}, and are given in table 2, for one series of measurements at constant acidity and varying the GSH concentration, and for three series where the H^+ was varied at constant GSH.

Table 2. Mean values (3-5 measurements) for the observed rate constants of the second stage in the reduction of Cr(VI) by glutathione, at T = 293 K, and μ = 0.5 M, $[Cr(VI)]_0 = 1.33 \cdot 10^{-4}$ M. The listed errors are the standard errors of the means.

	$[H^+]_0$ (M)		[GSH] (M)		
	$3.0 \cdot 10^{-2}$		$6.7 \cdot 10^{-3}$	$3.3 \cdot 10^{-3}$	$1.3 \cdot 10^{-3}$
$[GSH]_0$ (10^{-3} M)	k_{2obs} (10^{-4} s^{-1})	$[H^+]$ (10^{-2} M)	k_{2obs} (10^{-4} s^{-1})	k_{2obs} (10^{-4} s^{-1})	k_{2obs} (10^{-4} s^{-1})
1.3	2.22 ± 0.02	9.9	18 ± 1	13.05 ± 0.02	9.1 ± 0.2
2.7	3.5 ± 0.1	8.0	14.2 ± 0.1	9.86 ± 0.03	6.8 ± 0.1
3.3	3.86 ± 0.07	6.3	11.6 ± 0.1	7.52 ± 0.01	4.95 ± 0.09
5.3	6.2 ± 0.1	5.0	9.6 ± 0.1	6.13 ± 0.02	3.85 ± 0.06
6.7	7.21 ± 0.05	3.9	8.20 ± 0.07	5.08 ± 0.02	2.83 ± 0.05
8.0	8.1 ± 0.3	3.0	7.21 ± 0.05	3.86 ± 0.07	2.22 ± 0.04
10	10.2 ± 0.1	1.6	6.34 ± 0.08	3.28 ± 0.02	1.56 ± 0.03
12	12.3 ± 0.3	0.51	5.80 ± 0.02	2.60 ± 0.03	0.96 ± 0.06
13	13.2 ± 0.2	0.30	5.9 ± 0.1	2.56 ± 0.07	0.85 ± 0.03
16	16.1 ± 0.7	0.13	6.0 ± 0.2	2.81 ± 0.08	0.83 ± 0.03

When a plot of the apparent first-order rate constants of the redox process against the varied concentration of GSH is made, it yields a good straight line, with a positive intercept and a slope as given in figure 5 A.

Moreover, if the rate of the decomposition is written as:

$$r_2 = k_2[GSH]_0^{a'}[H^+]_0^{b'}[GSCrO_3^-] \quad (11)$$

than $$k_{2obs} = k_2[GSH]_0^{a'}[H^+]_0^{b'} \quad (12)$$

and a plot of $\log(k_{2obs}) = f(\log[GSH]_0)$ is expected to be linear, with a slope equal to the order a'. In this way, a'= 0.87±0.02 was found (figure 5 B).

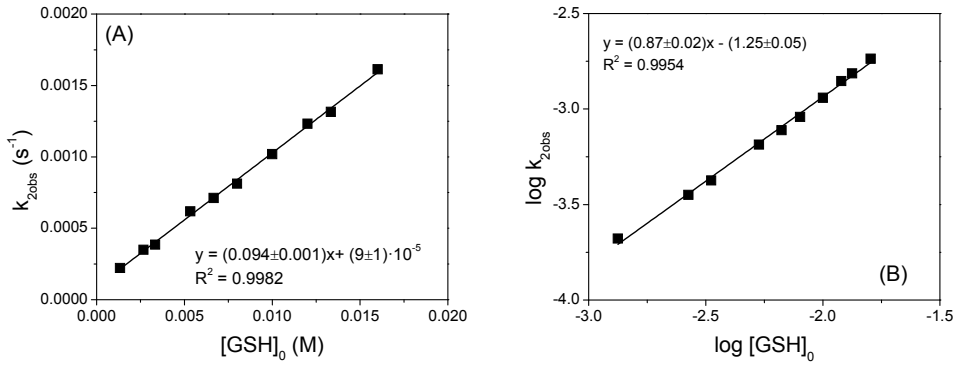

Figure 5. Dependence of the pseudo-first order constants k_{2obs} for the decay of the intermediate on the glutathione concentration (A) and the double logarithmic plot to find the reaction order a', based on equation 12 (B). Conditions like in table 2.

Combining the two observations, it can be assumed that two steps could be operative for the decomposition of the $GSCrO_3^-$ complex: one being a monomolecular decomposition (order zero with GSH) and the other a bimolecular process in which a second molecule of GSH intervenes (order one with GSH).

Through similar treatment of the corresponding k_{2obs} values at constant [GSH] and variable [H^+], dependences like those in figure 6 were found in the double logarithmic plot (A) and in the plot of k_{2obs} against [H^+] (B). Figure 6 (C) shows a comparative plot of k_{2obs} against $[H^+]^2$.

Two patterns of behaviour can be identified.

a) The double logarithmic plot has a part of slope nearly zero, between pH 1.8 and 2.9. From table 2, if comparing the three k_{2obs} values corresponding to the lowest acidities, for each set of data at constant glutathione concentration they can nearly be considered equal within the errors. This means that at low acidities, the hydrogen ion is not involved in the electron transfer decay of the intermediate. Bose et al. [28] have also noticed no significant pH dependence of the rate constants in the pH range 1.8÷3.3.

The non-zero intercepts in figure 6 (B) also support the existence of the H^+ independent path, which should, however, involve the glutathione, since the intercept values vary with the concentration of GSH used.

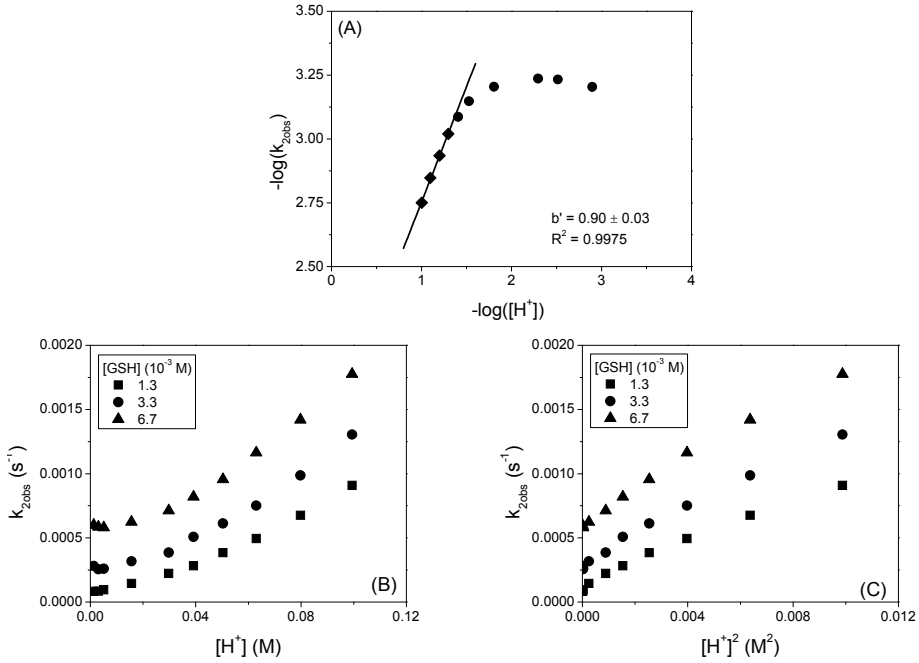

Figure 6. Example of a double logarithmic plot (A) and dependence of the pseudo-first order coefficients k_{2obs} on the first (B) or second (C) power of the H^+ concentration.

b) Focusing on the region of the higher acidities, although the double logarithmic plot suggests a first order with H^+, the curved dependence of k_{2obs} versus $[H^+]$ obtained (figure 6 B) indicates an order higher than first. However, a second-order representation as in figure 6 (C) does not result in a straight line either. This can only be reasoned by the occurrence of more parallel paths, which involve one or two protons respectively. Examining the three curves in figure 6 (B), they appear to increase with slightly different gradients, suggesting that at least one of the hydrogen ion assisted paths depends on the GSH concentration too.

To gain some more insight on the matter, more plots of the k_{2obs} values against the glutathione concentration were made, using the values in table 2 for the nine sets of three rate constants at different acidities, in comparison with those already shown in figure 5 A. They all gave straight lines (figure 7). The intercepts increased with the increase in acidity. At low acidity, the slopes were statistically equal, but progressing towards higher acidities ($3.9 \cdot 10^{-2} \div 9.9 \cdot 10^{-2}$ M), they increased monotonously.

As expected, for the three lowest acidities, the points almost overlapped. They were used all together to compute a regression line ($R^2 = 0.9978$). The intercept was slightly negative, approximately zero within the error. Comparing this with the positive intercepts found for the lines at higher acidities, it follows that a monomolecular decomposition within the intermediate complex alone (zero order with both GSH and H^+) is not very likely; such a path requires hydrogen ion assistance and thus becomes important at higher acidities.

On the other hand, the slope of the line was $(9.4 \pm 0.2) \cdot 10^{-2}$ $M^{-1}s^{-1}$. It represents the rate constant for the bimolecular decomposition of the intermediate, assisted by a second glutathione molecule, but not by the hydrogen ion (first order with the intermediate and glutathione, zero order with H^+). As it can be observed, the value is identical to the one obtained from the slope of the plot in figure 5 A, suggesting that at the acidity of $3.0 \cdot 10^{-2}$ M the GSH dependent path not involving hydrogen ions is still dominant. For even higher acidities, however, the increasing value of the slopes proves that some other GSH dependent paths, influenced by the H^+, gain importance.

Regarding the GSH dependent and independent paths, it was of interest to figure out if any distinction could be made between the number of hydrogen ions involved in each of them. For this, we tried plots of the slopes and the intercepts respectively, against various powers of $[H^+]$. In both cases the best fit was obtained when plotting against a fractional order between one and two (straight lines, with $R^2 = 0.9909$ and 0.9987 respectively, when plotting against $[H^+]^{1.5}$). Also, in the case of the slopes, the plot had a positive intercept (statistically different from zero), showing that the bi-molecular path of zero order in H^+ is still present. Therefore, no less than five parallel paths need to be considered at high acidities.

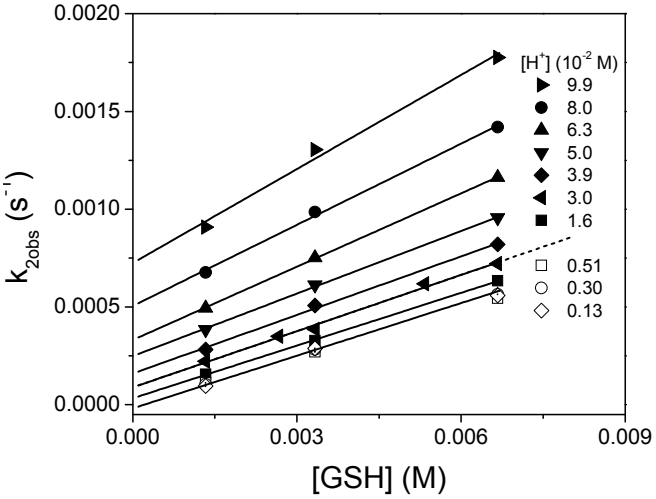

Figure 7. Plots of the k_{2obs} values against the glutathione concentration at various acidities. Conditions like in table 2.

Temperature and ionic strength effects on the decay of the intermediate

Measurements were made in order to determine the effects of the temperature and ionic strength on the electron transfer step. The pseudo-first order coefficients obtained are listed in table 3.

Table 3. Pseudo-first order rate constants k_{2obs} illustrating the effects of temperature (at μ=0.5 M) and ionic strength (at T = 293 K) on the decay of the intermediate. ($[Cr(VI)]_0 = 1.3 \cdot 10^{-4}$ M; $[GSH]_0 = 6.7 \cdot 10^{-3}$ M; $[H^+]_0 = 3.0 \cdot 10^{-2}$ M)

T (K)	k_{2obs} (10^{-4} s^{-1})	μ (M)	k_{2obs} (10^{-4} s^{-1})
293	7.09	0.05	8.74±0.01
298	9.66	0.1	8.37±0.05
303	12.74	0.3	7.91±0.05
308	16.84	0.5	7.39±0.04
313	21.09	0.7	7.41±0.05
323	32.71	1.0	7.56±0.03

Given the many parallel pathways, it was not possible to determine each of their individual rate constants and only an apparent activation energy was found. The Arrhenius plot using the k_{2obs} values at the six different temperatures showed very good linearity and from the slope a value of $E_{a,obs}$ = 40.1±0.9 kJ $mole^{-1}$ was computed.

The ionic strength effect was checked in the range 0.05 to 1 M. Some slight decrease of the pseudo-first order rate constant k_{2obs} of the increase of the ionic strength was observable, suggesting that species with opposite charge are involved in the reaction.

Reaction mechanism and rate law

The mechanism of the glutathione oxidation by Cr(VI) in acidic media is complex. Taking into account the features emerging from the interpretation of the kinetic and non-kinetic data, some considerations can be formulated.

The first step of the process is the reversible, hydrogen ion assisted formation of the 1:1 Cr(VI):GSH intermediate, described by the equation 4. This can be assumed to happen in two bi-molecular steps, with the first a rapid proton transfer to the leaving group in the Cr(VI) species.

$$HOCrO_3^- + H^+ \rightleftharpoons H_2OCrO_3 \qquad K_p \qquad (15)$$

$$H_2OCrO_3 + GSH \rightleftharpoons GSCrO_3^- + H_2O + H^+ \qquad K_f \qquad (16)$$

The subsequent step is rate determining and consists on the decay of the intermediate by electron transfer. In theory, the alternative paths 17 to 22 are all possible.

$$GSCrO_3^- \longrightarrow Cr(V) + GS^\bullet \qquad k_{21} \qquad (17)$$

$$GSCrO_3^- + H^+ \longrightarrow Cr(V) + GS^\bullet \qquad k_{22} \qquad (18)$$

$$GSCrO_3^- + 2H^+ \longrightarrow Cr(V) + GS^\bullet \qquad k_{23} \qquad (19)$$

$$GSCrO_3^- + GSH \longrightarrow Cr(IV) + GSSG \qquad k_{24} \qquad (20)$$

$$GSCrO_3^- + GSH + H^+ \longrightarrow Cr(IV) + GSSG \qquad k_{25} \qquad (21)$$

$$GSCrO_3^- + GSH + 2H^+ \longrightarrow Cr(IV) + GSSG \qquad k_{26} \qquad (22)$$

However, from our results, step 17 seems to be the least likely and has been disregarded, while the others are of different importance, depending on the acidity.

A particular situation was found for the lower acidities within the investigated range ($1.58 \cdot 10^{-2} \div 0.13 \cdot 10^{-2}$ M), where the decay of the intermediate showed no dependence on the hydrogen ion concentration, and a first order dependence on the glutathione concentration.

Therefore, only the intermediate formation (equation 4), together with the bimolecular step (20) have to be considered for the rate law. The rate law for the second stage of the reaction can be written as:

$$r_2 = k_{24}[GSH][GSCrO_3^-] \qquad (23)$$

The shape of the kinetic curves suggests that the intermediate is formed in a fast pre-equilibrium, therefore its concentration can be expressed as:

$$[GSCrO_3^-] = K_{11}[GSH][HCrO_4^-] \quad (24)$$

where K_{11} is the equilibrium constant for the overall process of the intermediate formation ($K_{11} = k_1/k_{-1} = K_p \cdot K_f$). The hydrogen ion concentration does not appear in this equation, since it acts as a catalyst.

If the balance of all absorbing chromium (VI) species is taken into account, its total concentration ($[Cr(VI)]_t$) is given by equation 25:

$$[Cr(VI)]_t = [HCrO_4^-] + [GSCrO_3^-] \quad (25)$$

Substituting in equation 24, the concentration of the intermediate will be:

$$[GSCrO_3^-] = \frac{K_{11}[GSH]}{1 + K_{11}[GSH]}[Cr(VI)]_t \quad (26)$$

and the expression of rate law becomes:

$$r_2 = -\frac{d[Cr(VI)]_t}{dt} = k_{24}\frac{K_{11}[GSH]^2}{1 + K_{11}[GSH]}[Cr(VI)]_t = k_{2obs}[Cr(VI)]_t \quad (27)$$

This rate law is identical to the one found by Wetterhahn [26] at pH 7.4.

Using the expression of k_{2obs} in (27), a linear equation can be derived:

$$\frac{[GSH]^2}{k_{2obs}} = \frac{1}{k_{24}K_{11}} + \frac{1}{k_{24}}[GSH] \quad (28)$$

A plot of $[GSH]^2/k_{2obs}$ *vs.* [GSH] using only the data at the low acidities is linear with the equation $[GSH]^2/k_{2obs}$ = 0.007±0.001 + (10.4±0.2)[GSH] (R^2 = 0.9962). From the slope, k_{24} can be computed, giving $(9.7\pm0.2)\cdot10^{-2}$ M^{-1} s^{-1}. (This is the same as found above $(9.4\pm0.2)\cdot10^{-2}$ M^{-1} s^{-1} from the plots *vs.* [GSH] of either the same k_{2obs} values, or of those at $[H^+] = 3.0\cdot10^{-2}$ M). Combining the slope and the intercept, a value of 1526±275 M^{-1} was computed for the equilibrium constant K_{11}. It fits well with the range of values characteristic for the formation of thioesters [14]. Although obtained from few data, this is close to the value of 1550±100 M^{-1} found by McAuley and Olatunji [30], under similar conditions. From K_{11}, together with the value of k_1 determined earlier, a k_{-1} of 3.0±0.6 M^{-1} s^{-1} could be estimated.

At the higher acidities, our results show that an alternative, glutathione independent path becomes available for the decomposition of the intermediate. McAuley and Olatunji [27] suggested that two protons are assisting, and gave a mechanism composed of steps 4, 19 and 20. Their conclusions were

based on fewer measured points, therefore the curved rather than linear dependences of the k_{2obs} on the $[H^+]^2$ might have been missed. It is reasonable to assume that the protons would also be involved in the bi-equivalent processes, as found for other systems [37, 38], and based on our observations such paths cannot be dismissed. Therefore, the sequence we propose is formed of steps (4) and (18) to (22), when the complex rate law takes the form:

$$-\frac{d[Cr(VI)]_t}{dt} = \left\{ k_{22}[H^+] + k_{23}[H^+]^2 + \left(k_{24} + k_{25}[H^+] + k_{26}[H^+]^2\right)[GSH] \right\} \cdot \cdot \frac{K_{11}[GSH]}{1 + K_{11}[GSH]}[Cr(VI)]_t \quad (29)$$

The paths (18), (19), (21) and (22) all involve some species of opposite charge. This in agreement with the effect of the ionic strength, which has been checked for one acidity within this range ($3.0 \cdot 10^{-2}$ M).

According to the stoichiometry, the final products of the reaction are Cr(III) and the disulphide. To complete the process, after the first electron transfer, Cr(V) or Cr(IV) intermediates react in further steps. Cr(V) could react with the excess GSH to form Cr(III) in a bi-equivalent step, or even with glutathiyl radical to reduce to Cr(IV). Cr(IV) can also react with a glutathiyl radical and reduce to Cr(III), or with another molecule of Cr(VI) to form Cr(V), although this is expected to be favoured when Cr(VI) is in excess. Two glutathiyl radicals can readily combine in a rapid step to yield the glutathione-disulphide.

The Cr(V) complexes observed in the ESR spectra at low acidities can be the result of one such step. On the other hand, a bi-molecular one-equivalent path for the electron transfer decomposition of the intermediate can also be envisioned (equation 30), leading to the disulphide radical $GSSG^{\bullet -}$:

$$GSCrO_3^- + GSH + H^+ \longrightarrow Cr(V) + GSSG^{\bullet -} \qquad k'_{24} \qquad (30)$$

This would be kinetically indistinguishable, leading to the same rate law, and could also explain the formation of Cr(V) in neutral media. Disulphide radicals were proved to exist [39] and through molecular orbital calculations were found to be likely more stable than the thiyl radicals.

CONCLUSIONS

The reaction between glutathione and chromium(VI) has been investigated under mildly acidic conditions. The process showed evidence of two distinct stages; a formation of an intermediate in a fast reversible step, followed by a much slower decay. A third order rate law was found for the intermediate formation, first order with both the main reactants and also the hydrogen ion. This step has been characterized by both rate constants and the equilibrium constant.

It was found that different mechanistic paths had to be considered for the decomposition of the intermediate, depending on the acidity. At low hydrogen ion concentrations (pH > 1.8), a bi-molecular and possibly bi-equivalent route involving a second molecule of glutathione was found to be dominant. For this, the value of the rate constant was computed.

At higher acidities, one-equivalent and bi-equivalent steps were proved to coexist, both of them involving either one or two hydrogen ions.

EXPERIMENTAL SECTION

The chemicals used were all of certified analytical reagent grade, purchased from commercial sources and used without further purification. The solutions were prepared in demineralized and tetra-distilled water. Stock solutions of $HClO_4$ and $NaClO_4$ were prepared and standardized by titration with NaOH solutions. Aliquots of the $NaClO_4$ solution were passed over the cationic resin Virolyte C-100 (Victoria, Romania) in the H-form, and subsequently the resulting acid was titrated. The solution of glutathione was freshly prepared and standardized iodometrically before each set of runs.

For the slow stage of the overall process, batch experiments were designed, in which the reaction was followed spectrophotometrically, at 435 nm. Glutathione was added in excess (10 to 120-fold), and also the hydrogen ion carrying species (10 to 750-fold). The ionic strength was adjusted by adding $NaClO_4$. The apparatus used was a double-beam Jasco V-530 spectrophotometer interfaced with a DTK computer and equipped with a cell holder connected to a FALC SB15 digital thermostat, allowing the control of temperature within ± 0.1 degrees. A quartz cuvette with 5 cm path length was used. The reaction process was initiated by injecting thermostated chromic acid solution directly into the cell, over a mixture of the other reactants. The mixing time did not exceed 0.5 s.

To study the fast stage of the overall process (the formation of the intermediate), some other experiments were carried out, making use of the stopped-flow technique. A home-built stopped-flow apparatus with spectrophotometrical detection and oscillographic recording has been utilized. Again, Cr(VI) was the limiting reactant ($1 \cdot 10^{-4}$ to $6 \cdot 10^{-4}$M), with the glutathione ($8 \cdot 10^{-3}$ to $2.4 \cdot 10^{-2}$ M) and hydrogen ion (0.01 to 0.1 M) in large excess. For each set of conditions four replicates were made using the same batches of solutions and the curves mediated, to minimize the noise.

ESR measurements were recorded using a Bruker ELEXSYS 500-Series spectrometer equipped with a Bruker liquid nitrogen temperature control system. The microwave frequency was 9.5 GHz (X-band) and the modulation frequency was 100 kHz. A modulation amplitude of 1 G was chosen in order to get the greatest possible signal intensity without suffering from overmodulation, which could otherwise have lead to distorted line shapes and positions. From

literature [32] a microwave power of 20mW and a temperature of 277 K were adopted. The remaining parameters were chosen according to the specifics of each individual sample.

ACKNOWLEDGMENTS

The authors would like to thank Prof. Dr. Günter Grampp, from the Institute of Physical and Theoretical Chemistry, Graz University of Technology, Austria, for providing the ESR and stopped-flow facilities and assistance with the ESR measurements.

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