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THE FAST FORMATION OF AN INTERMEDIATE IN THE CHROMIUM (VI) REDUCTION BY THIOLACTIC ACID – A KINETIC APPROACH BY MEANS OF THE STOPPED-FLOW TECHNIQUE

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ABSTRACT. The reaction between thiolactic acid and Cr(VI) in aqueous acidic solution at 295 K is a quite fast multi-step process, beginning with the formation of a condensation species, in an equilibrium that involves both the main reactants and H^+ ions. The kinetics of this first step was studied by means of the stopped-flow and spectrophotometrical techniques. Reaction orders of one were found for both the thiolactic acid (RSH) and Cr(VI), showing a 1:1 combination ratio of the two in the intermediate. A fractional order was found in the case of the hydrogen ion, consistent with two parallel pathways: one assisted and one not assisted by H^+.

Keywords: *kinetics, thiolactic acid, chromium(VI), redox, stopped-flow*

INTRODUCTION

There has always been an interest in the mechanism of Cr(VI)-thiol reactions, recently mostly under physiological conditions (pH in and near the neutral region), since biological thiols (glutathione, cysteine etc.) play a role in the detoxification from heavy metal ions [1] and because chromium(VI) is a known occupational and environmental hazard, due to its allergenic, carcinogenic and mutagenic properties [2-4].

Contamination with Cr(VI) can happen by inhalation of dusts of chromate salts or by prolonged skin contact with either solid or solution, (mainly in some industry workers) making lung cancers and skin ulcerations the most common harmful effects. Accidental intake cannot be excluded, for instance by repeated consumption of dietary chromium(III) food supplements - widely used for their effects in helping weight loss - that may be contaminated with low levels of Cr(VI).

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The toxicity of Cr(VI) is attributed mainly to the Cr(V) and Cr(IV) species formed during its reduction to Cr(III) inside the cell [5-7]. Besides the aggressive oxidizing properties of these intermediate valence states, their lability to ligand substitution allows them to bind to DNA protein sites, where the further reduction to stable Cr(III) complexes ultimately results in DNA cleavage and alteration of the genetic information [5-10]. The biological thiols, along with ascorbic acid, are known to readily reduce Cr(VI) [7-12], *in vivo*, thus likely getting involved in the mechanism of cellular damaging.

Thiolactic acid comes to the proximity of the human body by being used in cleaning and cosmetic products (such as permanent cold hair waving creams). Also, in small amounts (up to 50 ppm in the finished product), it has been approved as a food flavouring agent [13], but there is still a call for more genotoxicity data. Since acidic medium has usually been found to enhance the rate of the Cr(VI) reduction, besides the flavouring properties, thiolactic acid could potentially help reduce the trace amounts of freshly ingested Cr(VI) already in the acidic environment of the stomach, diminishing its transfer to the cells.

The oxidations of thiols by Cr(VI) are known to take place *via* complex multi-step mechanisms which allow for a quite large variety of path choices for a particular pair of reactants to follow, often ending up in disulfides as the oxidation product [14-17]. An established feature of these mechanisms is their debut by an equilibrium process in which a condensation intermediate is formed, with the replacement of an oxygen ligand by the thiolic sulphur [18,19]. Its formation may also benefit from a catalytic assistance of hydrogen ions. The composition of such a complex may vary, based on the chemical and steric properties of the reactants.

This study reports on the kinetic aspects of the fast build-up of such a condensation intermediate in the reaction between thiolactic acid and Cr(VI) in aqueous solutions of perchloric acid ($HClO_4$), under controlled temperature and ionic strength (μ = 0.5 M ($NaClO_4$)).

RESULTS AND DISCUSSION

The oxidation of thiolactic acid (denoted RSH) by chromium(VI) in aqueous acidic ($HClO_4$) environment is overall a fairly fast process, that also consists of more than one stage, as tests at room temperature and the employment of solutions of the two reactants at concentrations in the range of 10^{-4} – 10^{-3} M have shown. This was observable by the changes in the colour of the reaction mixture, which very quickly turned from yellow to reddish-brown, and then somewhat slower, but still within seconds, to colourless. Given these features, flow methods and the spectrophotometrical detection technique were considered suitable for following the reaction progress, in particular the stage in which the formation of the intermediate takes place.

It is well known that Cr(VI) is subject to a number of equilibria in aqueous solutions, depending on both its own total concentration and the acidity of the solution [20,21]. In diluted solutions, mainly CrO_4^{2-}, $HCrO_4^-$ or $Cr_2O_7^{2-}$ species are encountered. In this work, the conditions were chosen so that the $HCrO_4^-$ form was dominant [20-22]. To limit the formation of the dimer, the total concentration of Cr(VI) could not be higher than $6 \cdot 10^{-4}$ M.

The UV-VIS region of the $HCrO_4^-$ electronic spectrum has its highest peak centred at 350 nm (molar absorptivity of 1560 M^{-1} cm^{-1} [23]), and a second, lower one, around 420 nm. The 350 nm wavelength was chosen to monitor the reaction progress, for two reasons.

First, the molar absorptivity, together with the path length of the mixing chamber of the stopped-flow apparatus (ℓ = 0.336 cm), were the factors deciding the lowest Cr(VI) concentration still detectable with a good signal to noise ratio. In this case, it was 10^{-4} M.

Second, some spectral data have shown that the reddish-brown intermediate displays a significant interfering absorbance around 420 nm, consistent with the expected batochromic shift in the charge-transfer maximum when a sulphur replaces an oxygen. The same spectra were inconclusive for 350 nm, so it was hoped that at this wavelength the intermediate does not absorb. However, this was proved to not be the case.

A curve describing the entire reaction progress at 350 nm is shown in figure 1. The obvious biphasic behaviour undoubtedly establishes both the formation of the intermediate, and the fact that it also absorbs at 350 nm. Its composition can be assessed based on the reaction orders of the participants in the elementary step of its formation, since for an elementary process the reaction orders accurately describe its molecularity.

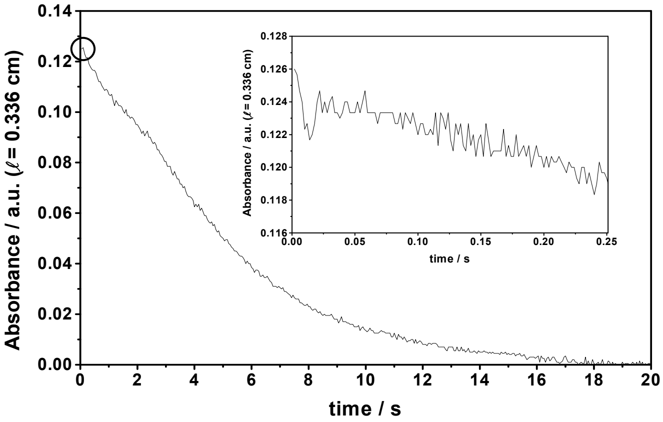


Figure 1. Experimental curve at 350 nm - long and short run (inset). Conditions: [Cr(VI)] = $4 \cdot 10^{-4}$ M, [RSH] = $8 \cdot 10^{-3}$ M, [H^+] = 0.1 M, T = 295 K, μ = 0.5 M ($NaClO_4$).

To determine these reaction orders, the initial rates method has been employed, in conjunction with the isolation method.

Under the range of conditions employed, the whole process takes 15 to 45 seconds. In order to obtain the initial rates, only the beginning (about the circled region in figure 1) is of interest, thus the measurements targeted this region (inset in figure 1). The points accounting for 1.5% or less of the total reaction served to determine the initial slopes of the time resolved absorbance curves by linear regression. In all cases, this corresponded to at least 150 data points.

Although the formation of the intermediate is considered to be an equilibrium process, in its initial stages it can reasonably be assumed that the amount of intermediate formed is so small that its decay by either the reverse step or the further decomposition to products is insignificant, and the build-up rate is dominant. Therefore, the rate law at zero reaction time can be expressed as in equation (1):

$$r_0 \approx r_1 = \left|\frac{d[HCrO_4^-]}{dt}\right|_{t\to 0} = k_1 \cdot [RSH]_0^a [H^+]_0^b [HCrO_4^-]_0^c \qquad (1)$$

where r_0 is the initial rate, r_1 and k_1 are the reaction rate and the rate coefficient for the forward step, and a, b and c are the reaction orders for the three possible reactants: RSH, H^+ and $HCrO_4^-$ respectively.

The initial rate can further be expressed in terms of absorbance. If the respective molar absorptivities of the reactant and the intermediate (denoted I) are $\varepsilon_{350,HCrO4-}$ and $\varepsilon_{350,I}$, and ℓ is the path length of the mixing chamber (0.336 cm), the total absorbance at 350 nm (A_{350}) is:

$$A_{350} = [HCrO_4^-] \cdot \varepsilon_{350,HCrO_4^-} \cdot \ell + [I] \cdot \varepsilon_{350,I} \cdot \ell \qquad (2)$$

Under the assumptions made, the concentration of the intermediate can be expressed as:

$$[I] = [HCrO_4^-]_0 - [HCrO_4^-] \qquad (3)$$

Combining equations 2 and 3 to obtain the expression of the concentration of $HCrO_4^-$, and substituting into equation 1, the following form for the reaction rate results (equation 4):

$$\frac{1}{(\varepsilon_{350,HCrO_4^-} - \varepsilon_{350,I}) \cdot \ell} \left|\frac{dA_{350}}{dt}\right|_{t\to 0} = k_1 \cdot [RSH]_0^a [H^+]_0^b [HCrO_4^-]_0^c \qquad (4)$$

Equation 5 (in which $\alpha = (\varepsilon_{350,HCrO4-} - \varepsilon_{350,I})\cdot\ell\cdot k_1$) is the logarithmic form of equation 4, and permits the determination of the reaction order (i.e. a, b or c) of a certain reactant, if all the terms of the sum are constant, except for the term involving that reactant (the isolation method).

$$\log\left|\frac{dA_{350}}{dt}\right| = \log(\alpha) + a\cdot\log([RSH]_0) + b\cdot\log([H^+]_0) + c\cdot\log([HCrO_4^-]_0) \quad (5)$$

As needed, three series of measurements were made. In each of them the concentration of one species (Cr(VI), RSH or H^+) was varied, with the other two kept constant. Always, Cr(VI) was the limiting reactant. Also, the excess of thiolactic acid and hydrogen ion were ensured to be large enough in each case, so that they could be considered invariable. The reaction order with respect to the species of which the concentration varied in each series of measurements can be determined as the slope of the appropriate double-logarithmic plot. Since the initial slopes (dA_{350}/dt) of the absorbance-time curves are directly proportional to the rates, they were used in their stead.

Details about the actual concentrations of the reactants in the three series of measurements, as well as the dA_{350}/dt values, are listed in table 1.

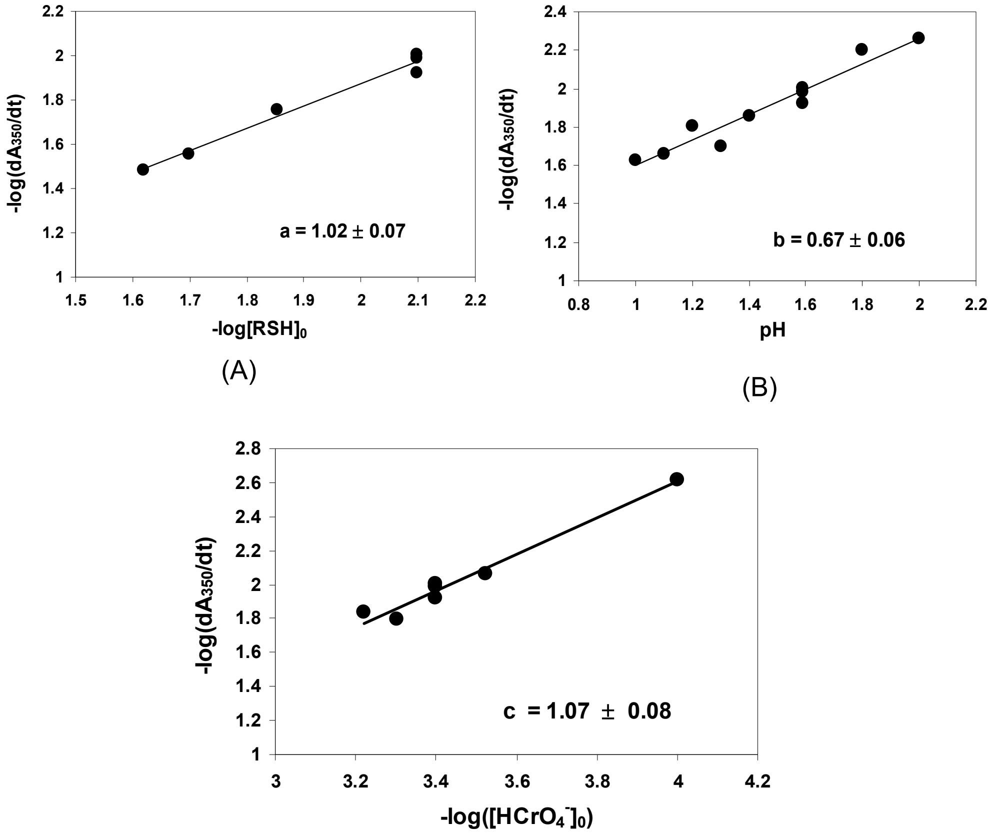
In a parallel approach, similar data were collected at 435 nm. The dA_{435}/dt values obtained are also presented in table 1, and will be discussed later in the paper.

Table 1. Initial slopes $|dA_{350}/dt|$ (directly proportional to the initial rates) computed from the experimental curves of absorbance *vs.* time, for the three series of measurements used to determine de reaction orders (T = 295 K, μ = 0.5 M).

$[H^+]_0$ (10^{-2} M)	$[RSH]_0$ (10^{-3} M)	$[HCrO_4^-]_0$ (10^{-3} M)	$\|dA_{350}/dt\|$ (10^{-3} s^{-1})	$\|dA_{435}/dt\|$ (10^{-3} s^{-1})
2.6	8.0	0.40	10.3 ± 0.2	54.5 ± 0.9
	14		17.6 ± 0.4	71 ± 2
	20		27.7 ± 0.7	140 ± 6
	24		32.9 ± 0.9	171 ± 7
2.6	8.0	0.10	24.0 ± 0.5	11.1 ± 0.7
		0.30	8.6 ± 0.2	32.4 ± 0.7
		0.40	12.0 ± 0.7	50.3 ± 0.7
		0.50	16.2 ± 0.3	56.2 ± 0.7
		0.60	14.6 ± 0.8	63.5 ± 0.8
10	8.0	0.40	23.6 ± 0.3	144 ± 7
8.0			21.9 ± 0.5	106 ± 5
6.3			15.8 ± 0.4	93 ± 2
5.0			20.0 ± 0.5	95 ± 2
4.0			13.9 ± 0.6	56 ± 1
2.6			9.9 ± 0.6	43 ± 1
1.6			6.3 ± 0.2	27.8 ± 0.7
1.0			5.5 ± 0.4	21.3 ± 0.6

The errors mentioned in the table and in the rest of the paper, are the standard errors of the parameter, as determined from the linear regression.

The corresponding double-logarithmic plots for the data at 350 nm and their results are presented in figure 2, for all three participants in the reaction process. The slopes of the plots, with their respective errors, are given on the figure 2 and represent the values of the three reaction orders (a, b and c).



(A)

(B)

(C)

Figure 2. Double-logarithmic plots for determining the reaction orders with respect to the RSH (A), H^+ (B) and Cr(VI) (C), for the formation of the intermediate (at 350 nm). Conditions like in table 1.

First order with each RSH and Cr(VI) (figure 2A and 2C) and a fractional order between zero and one with H^+ (figure 2B) were found. In mechanistic terms, this means that the intermediate has a 1:1 ratio RSH:Cr(VI) - namely that one oxygen ligand in $HOCrO_3^-$ is substituted by one thiolactic acid molecule - and its formation can happen either catalyzed by one proton or not. Therefore, the reaction can be described by one of the equations 6 or 7, respectively:

$$RSH + HOCrO_3^- \rightleftharpoons RSCrO_3^- + H_2O \qquad k_1^0,\ k_{-1}^0,\ K_1 \quad (6)$$

$$RSH + HOCrO_3^- + H^+ \rightleftharpoons RSCrO_3^- + H_2O + H^+ \qquad k_1^H,\ k_{-1}^H,\ K_1 \quad (7)$$

where k_1^0 and k_{-1}^0 stand for the reaction coefficients of the forward and reverse reactions of the non-catalysed path respectively, k_1^H and k_{-1}^H are their analogues for the H^+ assisted path and K_1 denotes the equilibrium constant (which is the same, regardless the path).

Beforehand and kinetically indistinguishable from each other, in the presence of H^+, either RSH or $HOCrO_3^-$ is involved in a protolytic equilibrium. If $HOCrO_3^-$ is the one that protonates, the species so formed is not the chromic acid (H_2CrO_4), but rather H_2OCrO_3. Indeed, by protonating the –OH ligand in the tetrahedral complex $HCrO_4^-$, the leaving group would become H_2O, much easier to be substituted by the thiolic sulphur. Therefore, this seems the likelier choice. The H^+ assisted path (equation 7) would in this case consist of two consecutive equilibria (equations 8 and 9):

$$HOCrO_3^- + H^+ \rightleftharpoons H_2OCrO_3 \qquad K_p \quad (8)$$

$$H_2OCrO_3 + RSH \rightleftharpoons RSCrO_3^- + H_2O + H^+ \qquad k_f,\ k_{-f} \quad (9)$$

where K_p is the equilibrium constant for the protonation of $HCrO_4^-$ and k_f, k_{-f} are the reaction constants for the forward and reverse elementary steps of the intermediate formation, with $K_p \cdot k_f = k_1^H$.

If the parallel reactions 6 and 7 are considered, the overall rate law that can be written for the build-up of the intermediate consists of two terms, as described in equation 10:

$$r_1 = k_1^H[RSH][H^+][HCrO_4^-] + k_1^0[RSH][HCrO_4^-] \qquad (10)$$

The values of the two rate constants (k_1^H and k_1^0) are not accessible, since they can only be computed if $\varepsilon_{350,HCrO4-}$ and $\varepsilon_{350,I}$ are both known, and the value for $\varepsilon_{350,I}$ could not be determined. Instead, it was possible to estimate the importance of the two terms in the sum, in relation to the H^+ concentration. For this purpose, the quantities $(dA_{350}/dt)/(\ell\cdot[RSH]\cdot[HCrO_4^-])$ were computed and plotted against the concentration of the hydrogen ion (figure 3).

The slope and the intercept of the plot presented in figure 3 correspond to the quantities $(\varepsilon_{350,HCrO4-} - \varepsilon_{350,I})\cdot k_1^H$ and $(\varepsilon_{350,HCrO4-} - \varepsilon_{350,I})\cdot k_1^0$. Their values, obtained by linear regression, are given on the figure. The errors of the two parameters (as computed from the plot only) were of 15% and 9.7% respectively. From the ratio between the intercept and the slope, $(2.8 \pm 0.7)\cdot 10^{-2}$ M, it can be estimated that for a concentration of H^+ corresponding to a pH of approximately 1.56, the H^+ catalysed and non-catalysed paths (the two terms in equation 10) contribute equally to the total rate. For higher H^+ concentration, the catalysed path becomes dominant, while for concentrations 10 times lower or less (pH 2.6 or higher), it will already become negligible.

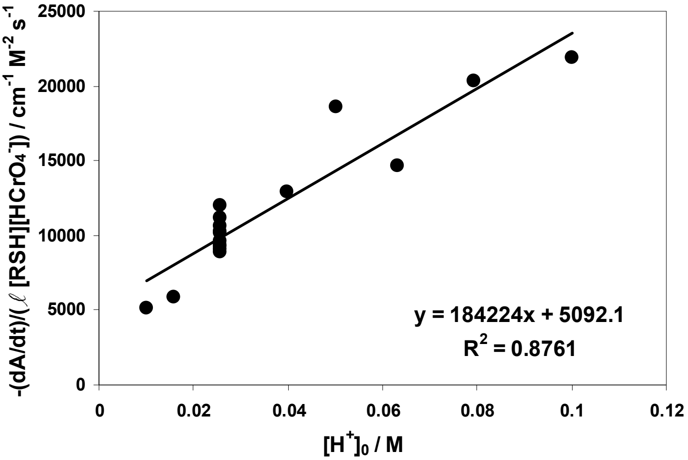


Figure 3. Global plot for determining the ratio of the rate constants (T = 293 K, μ = 0.5 M).

Some final considerations are worth mentioning.

First, it was established that both $HCrO_4^-$ and the Cr(VI)-RSH intermediate absorb at all wavelengths, meaning that there is no simpler spectrophotometrical approach for this reaction. It should be added that all the measurements were repeated at the wavelength of 435 nm (the exact wavelength of 420 nm could not be applied due to the lamp configuration of the experimental setup). At this wavelength, the experimental curves showed in the beginning a steep increase (consistent with the intermediate having here a higher absorbance than the reactant), reached a maximum and later decreased. The obtained values of $\left|dA_{435}/dt\right|_{t\to 0}$ have been included in table 1. The big differences in the numbers, as compared to those for 350 nm, are likely due to a much higher difference between the absorption coefficients $\varepsilon_{435,HCrO4-}$ and $\varepsilon_{435,I}$ at 435 nm. However the outcome was very similar, as far as the kinetic parameters are concerned. The obtained orders were 1.1 ± 0.1; 1.01 ± 0.05 and 0.81 ± 0.05 for the thiolactic acid, $HCrO_4^-$ and H^+ respectively, with a notable difference found only in the intercept to slope ratio ($(1.5 \pm 0.5)\cdot 10^{-2}$ at 435 nm). The results obtained at 350 nm are considered more reliable. Any decay of the intermediate that may happen within the time frame used to determine the slopes would affect more the slopes at 435 nm, where the absorption of the intermediate dominates.

Second, the whole reaction process is quite fast. The stopped-flow approach is aimed at gathering as accurate data as possible for the beginning of the reaction, but in this case it drastically limited the range of concentrations that could be spanned, partly due to the small dimensions of the mixing chamber. On one hand, concentrations of Cr(VI) lower than 10^{-4} M gave too noisy experimental curves. On the other hand, they needed to be lower than $6\cdot 10^{-4}$ M, in order to: 1) have the Cr(VI) solely in the form of $HCrO_4^-$, and 2) allow the use of the thiolactic acid and the mineral acid in high enough excess to ensure their constancy without the reaction becoming too fast to follow, even by the stopped-flow technique. In turn, this also limited the concentration range of these two other reactants.

CONCLUSIONS

In the reaction between thiolactic acid and Cr(VI) in aqueous acidic solution, a reaction intermediate is first built, in a fast step. First orders were found for each of the two reactants, meaning a 1:1 composition of the intermediate. The fractional reaction order (0.67 or 0.81) found with respect to H^+ shows that the formation of the intermediate happens both in the presence or the absence of protons. The $(k_1^H\cdot[H^+])$ term becomes more and more dominant for pH's lower than 1.56.

For a better kinetic description of the overall process and in particular of the second stage, dealing with the decay of the intermediate, the reaction conditions need to be changed, so that larger ranges of concentrations of the reactants can be spanned. This requires lower concentrations of Cr(VI), which would make the reaction slower, but also would require a longer path length for the spectrophotometrical cell. Hence, batch measurements could be considered for studying this system in greater detail.

EXPERIMENTAL SECTION

A custom-built multi-channel stopped-flow apparatus with spectrophotometrical detection and oscillographic recording has been utilized to collect the data. The mixing chamber of the apparatus has a path length of 0.336 cm. The working wavelength was 350 nm or 435 nm and the temperature 295 K. For each set of conditions, the experiments were repeated four times, using the same batches of solutions. To minimize the noise, the obtained curves were first mediated and the averaged curve was further processed to obtain the kinetic data.

The solutions of the two main reactants were prepared in identical environments (the same concentrations of $HClO_4$ and $NaClO_4$). The total ionic strength was 0.5 M.

Initial Cr(VI) concentrations between 10^{-4} and $6\cdot10^{-4}$ M were used. Due to the small path of the mixing chamber, an appropriate signal could not be obtained for lower concentrations. The concentrations for the thiolactic acid ($8\cdot10^{-3}$ to $2.4\cdot10^{-2}$ M) and hydrogen ion (0.01 to 0.1 M) were chosen accordingly, to ensure a significant excess over the Cr(VI).

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