COMBINED KINETIC RESPONSE OF TWO SUBSTRATES WITH SIMILAR REACTIVE PATTERNS

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ABSTRACT. The behaviour towards simultaneous oxidation by Cr(VI) in acidic environment of mixtures consisting of two structurally closely related thioethers (S-methylcysteine and DL-methionine) was tested, at 0.085 mol·l^{-1} H$^+$ and 298 ± 0.1 K. The kinetic and mechanistic patterns of the two substrates when oxidized separately were already known and very similar. Based on that knowledge, the individual kinetic parameters (rate and equilibrium constants) were first computed. Theoretical predictive models for the value of the observed rate constant of a mixture, based on the kinetic data from non-mixtures, are compared with experimental findings and subsequently an empirical calibration surface obtained from all experiments is discussed. Due to differences in the kinetic parameters of the two reactions, in all models the obtained surfaces are asymmetrical, and this opens the possibility to univocally determine the concentrations of both the thioethers without separation.

Keywords: chromium VI oxidation, DL-methionine, S-methylcysteine, combined kinetic response, kinetic modeling

INTRODUCTION

Identification and quantification of analytes from mixed samples can be quite challenging, particularly when dealing with complex mixtures. The classical approach to the matter involves laborious steps of separating the components of the sample. It comes as no surprise that methods are sought, which could eliminate part or even all of these separation steps.

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Within the overall spectrum of analytical methods, the kinetic ones have become increasingly interesting [1-4]. Besides their economic feasibility, one of their chief appeals resides precisely in the possibility of simultaneous determination of more analytical species without prior separation, although some pre-treatment of the sample (mineralising, filtering, concentrating etc.), may still be needed.

In our group, a subject of continued interest has been deciphering the kinetics and mechanism of sulphur compounds oxidation by various high valence transitional metal ions, in acidic environments. Much of our effort was directed to the oxidations by Cr(VI) of thiolic type substrates such as thiols, thioacids, thioethers [5-9]. Same area studies by other groups are also found in the literature [5, 10-12] and some general patterns are now well established. Among them there is a first and often fast step in which one Cr(VI) and one or more thio compound molecules form a condensation intermediate (adduct). This subsequently decomposes in an electron transfer step that can be mono-, bi- or even trielectronic and it may or may not involve an additional thio compound molecule. In acidic media, both these steps may be assisted by H$^+$ ions. The electron transfer is more often than not rate-determining. Following it, new types of intermediates are formed. They react in further steps and eventually lead to the final products: Cr(III) complexes and disulphides (S-S) or sulphoxides (-SO) respectively. These steps have sufficiently high rate constants to exert no influence on the overall rate.

With this knowledge, a new point of focus has become the study of mixtures of thio compounds. For this paper, bi-component mixtures were chosen, having as constituents two structurally closely related thioethers: DL-methionine (MET) and S-methylcysteine (MTC).

We have already reported on the kinetics and mechanisms of their individual reactions with Cr(VI) in aqueous acidic media [8,9]. Those studies benefited from very similar approaches in terms of the experimental setup (batch measurements, spectrophotometry as the tool, 350 nm as the monitoring wavelength, HClO$_4$ as the source for hydrogen ions, Cr(VI) as the limiting reactant, and comparable thioether and acid concentrations). The notable differences were in the work temperature and ionic strength.
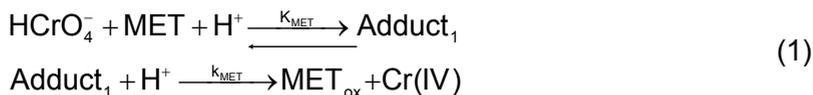
The kinetic and mechanistic features found for the two substrates are also mostly similar and are summarized below.

The thioether to Cr(VI) stoichiometric ratio was found to be 1.5 to 1 in both cases, indicating sulphoxides as the final reaction products. Also for both, four hydrogen ions are required for each reacted Cr(VI).
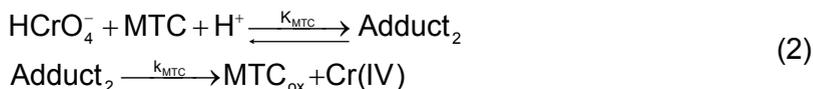
In terms of the reaction mechanism, one common feature is the initial formation of an adduct of 1 to 1 ratio between the Cr(VI) and the thio compound, in a hydrogen ion assisted equilibrium step. Another is the lack of

involvement of a second thio compound molecule in the decomposition of the adduct, which takes place with the transfer of two electrons and is the rate determining step in the case of either substrate. However, the involvement of a hydrogen ion in the rate determining step is noticed in the case of DL-methionine, but not in the case of S-methylcysteine.

Up until and including the rate determining step, the simplified reaction schemes that can be written for the two substrates are, therefore:

$$HCrO_4^- + MET + H^+ \xrightleftharpoons{K_{MET}} Adduct_1$$
$$Adduct_1 + H^+ \xrightarrow{k_{MET}} MET_{ox} + Cr(IV)$$

(1)

and:

$$HCrO_4^- + MTC + H^+ \xrightleftharpoons{K_{MTC}} Adduct_2$$
$$Adduct_2 \xrightarrow{k_{MTC}} MTC_{ox} + Cr(IV)$$

(2)

The rate laws for the two reactions, based on the above schemes and also taking into account the fact that the total Cr(VI) (HCrO$_4^-$ and the corresponding adduct) is experimentally monitored, are:

$$-\frac{d[Cr^{VI}]_{total}}{dt} = \frac{k_{MET}K_{MET}[MET][H^+]^2}{1 + K_{MET}[MET][H^+]}[Cr^{VI}]_{total}$$

(3)

in the case of DL-methionine, and:

$$-\frac{d[Cr^{VI}]_{total}}{dt} = \frac{k_{MTC}K_{MTC}[MTC][H^+]}{1 + K_{MTC}[MTC][H^+]}[Cr^{VI}]_{total}$$

(4)

in the case of S-methylcysteine, respectively.

The aim of this study is to assess the possibility of using the simultaneous oxidation by Cr(VI) of the two substrates with very similar reactive patterns to univocally determine both their concentrations in a mixture, without separation. This is viewed as a first and necessary step for future development of kinetic methods of analysis for multi-component mixtures of substrates with similar kinetic behaviour.

The chosen route to this end was finding suitable models to describe the oxidative behaviour of the mixtures, be they theoretical predictions derived from the kinetic data of the individual components, or empirical surfaces obtained from direct measurements of the mixtures themselves.

Also reported here is some additional insight on the individual oxidations of the two substrates acquired in the process.

RESULTS AND DISCUSSION

Before being able to draw reliable conclusions about the behaviour in the mixture of the two thioethers, the knowledge of their individual rate and equilibrium constants is important. Since these values depend on reaction conditions such as temperature or ionic strength, the figures from the literature could be of use only if the same conditions were to be employed for the mixtures. Clearly this was only partly achievable, since precisely those parameters were different for the two cases.

We decided for 298 K and constant 0.085 $mol \cdot l^{-1}$ $HClO_4$, the same as employed in the mechanistic study on DL-methionine. Unlike there, the ionic strength was here considered equal to the perchloric acid concentration and no further adjustment by inert salt addition was deemed necessary. Indeed, the 850-fold excess (212.5 with stoichiometry) of the acid over the Cr(VI) ensured that the contribution brought by the salt of the latter was insignificant. Also, the acid was 3 to 56 times more concentrated than the thioethers together. Since they only partly dissociate (pKa's of 2.28 and 9.21 for MET and 2.44 and 8.75 for MTC respectively [13,14]), and their acidic dissociation is further hindered in the presence of $HClO_4$, their contribution to the total acidity and to the ionic strength, was always 1.6 % or less and could be neglected as well. With the concentration of the perchloric acid being kept the same throughout the measurements, we concluded that the constancy of the total ionic strength was sufficiently well achieved.

Further support for this decision was found by analyzing the effect of the ionic strength on the two individual reactions. Some data acquired under the same conditions as in the previously published studies and listed in Table 1 were of help in this regard. The numbers given are averages of two to three separate measurements. The errors, here and elsewhere in the paper correspond to the standard error. The individual pseudo-first order constants were obtained by the means of the least square method, from plots of the type $ln(A_{350nm}-A_{\infty,350nm})$ *vs.* time, which showed good linearity (R between 0.9916 and 0.9999) over conversion regions from 5 to 90 %.

It can be seen that little change in the apparent rate constants is found in the sufficiently large range of ionic strengths covered by these measurements. The small differences are probably due to the ionic equilibria that involve the reaction participants prior to the rate determining step of the reaction (secondary salt effect). Such effects are often found to accompany the acid/base catalyzed reactions in solution.

The main group of recorded data refers to measurements of the mixtures, collected under the chosen work conditions. Also, for each varied substrate, a set at zero concentration of the other was obtained. Table 2 lists the average results of three individual runs performed in each case.

Table 1. Average k_{obs} values as a function of the ionic strength for MTC and MET oxidations by Cr(VI) (NaClO$_4$ added for MTC and NaNO$_3$ for MET).

Experimental conditions:			
10^{-4} mol·l^{-1} Cr(VI); 0.012 mol·l^{-1} **MTC**; 0.257 mol·l^{-1} HClO$_4$; 333.0 ± 0.1 K;		10^{-4} mol·l^{-1} Cr(VI); 0.0075 mol·l^{-1} **MET**; 0.085 mol·l^{-1} HClO$_4$; 298.0 ± 0.1 K	
j (mol·l^{-1})	$k_{obs,MTC}$ (10^{-3} s^{-1})	j (mol·l^{-1})	$k_{obs,MET}$ (10^{-3} s^{-1})
0.257	7.95 ± 0.15	0.085	1.35 ± 0.02
0.437	8.70 ± 0.50	0.170	1.28 ± 0.04
0.642	8.25 ± 0.55	0.255	1.31 ± 0.03
0.848	9.23 ± 0.58	0.340	1.23 ± 0.03
1.028	9.60 ± 0.10	0.426	1.17 ± 0.05

Table 2. Average pseudo-first order rate constants for different sets of MET and MTC concentrations, at 298 K, 10^{-4} mol·l^{-1} Cr(VI) and 0.085 mol·l^{-1} H$^+$ (HClO$_4$).

[MET] / 10^{-3}·mol·l^{-1}	\[MTC\] / 10^{-3}·mol·l^{-1}									
	0	1.5	3.0	4.5	6.0	7.5	9.0	12	15	18
	k_{obs} (exp) / 10^{-3} s^{-1}									
0		0.38 ± 0.01	0.74 ± 0.01	0.90 ± 0.03	1.21 ± 0.01	1.49 ± 0.05	1.59 ± 0.02	1.85 ± 0.03	2.32 ± 0.10	
1.5	0.32 ± 0.01	0.78 ± 0.02	0.99 ± 0.03	1.02 ± 0.02	1.27 ± 0.01	1.47 ± 0.06				
3.0	0.66 ± 0.01	1.06 ± 0.01	1.28 ± 0.03	1.31 ± 0.04	1.45 ± 0.05	1.52 ± 0.07	1.79 ± 0.06	2.17 ± 0.11		
4.5	0.86 ± 0.01	1.23 ± 0.02	1.25 ± 0.02	1.44 ± 0.01	1.88 ± 0.05	1.82 ± 0.01				
6.0	1.15 ± 0.01	1.45 ± 0.02	1.55 ± 0.03	1.69 ± 0.01	1.89 ± 0.03	2.01 ± 0.01	2.00 ± 0.10	2.14 ± 0.10		2.75 ± 0.05
7.5	1.30 ± 0.02	1.50 ± 0.03	1.73 ± 0.01	1.91 ± 0.01	1.97 ± 0.01	2.10 ± 0.05				
9.0	1.85 ± 0.04		1.94 ± 0.09		2.21 ± 0.04		2.46 ± 0.20	2.75 ± 0.09		
12	2.22 ± 0.09		2.32 ± 0.04		2.69 ± 0.14		2.55 ± 0.16	2.43 ± 0.10		
15	2.67 ± 0.02									
18	2.95 ± 0.11				3.06 ± 0.11					

Individual kinetic response. Kinetic parameters

Figure 1 illustrates the non-linear behaviour of the rate constants when the substrates are oxidized individually, with a slight tendency of levelling of. This feature corresponds to the previous findings [8,9].
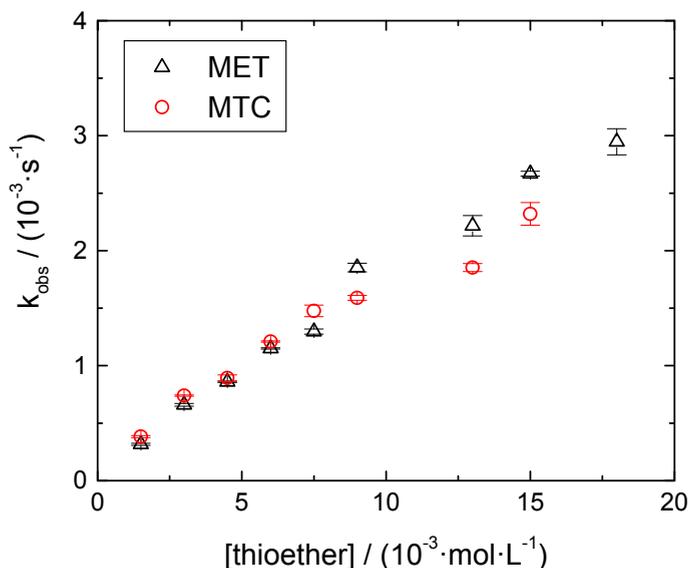
Figure 1. The non-linear increase of the rate constants with the concentration, for the two series of measurements of individual thioethers.

Based on equations 3 and 4, the data in Figure 1 were used to obtain plots of the form $1/k_{obs}$ *vs.* $1/[thioether]$. They were expected to be linear and indeed they were, indicating that the previously given mechanisms hold. The corresponding linear equations are:

$$\frac{1}{k_{obs,MET}} = \frac{1}{k_{MET}K_{MET}[H^+]^2}\frac{1}{[MET]} + \frac{1}{k_{MET}[H^+]} \tag{5}$$

$$\frac{1}{k_{obs,MET}} = (4.59 \pm 0.07)\frac{1}{[MET]} + (84 \pm 21); \quad R = 0.9967 \tag{5'}$$

and:

$$\frac{1}{k_{obs,MTC}} = \frac{1}{k_{MTC}K_{MTC}[H^+]}\frac{1}{[MTC]} + \frac{1}{k_{MTC}}; \tag{6}$$

$$\frac{1}{k_{obs,MTC}} = (3.57 \pm 0.07)\frac{1}{[MTC]} + (229 \pm 20); \quad R = 0.9960 \qquad (6')$$

The parameters in the equations 5' and 6' were determined using all the individual rate constants obtained from the experimental runs, rather than the averages listed in Table 2. From these, the respective rate and equilibrium constants for the two substrates under the current reaction conditions were computed. The extracted values are listed in Table 3, as compared with the literature ones.

Table 3. Computed values for the rate and equilibrium constants of the two thioethers, at 298 K, except for those marked with *, that correspond to 333 K.

	MET		MTC	
	k_{MET} ($l \cdot mol^{-1} \cdot s^{-1}$)	K_{MET} ($l^2 \cdot mol^{-2}$)	k_{MTC} (s^{-1})	K_{MTC} ($l^2 \cdot mol^{-2}$)
Litt. [8,9]	0.131 ± 0.023	224 ± 45	*0.097 ± 0.028	*29.0 ± 7.5
This work	0.141 ± 0.035	214 ± 56	0.0044 ± 0.0004	756 ± 80

For DL-methionine the numbers are in very good agreement within the limits of the experimental errors. This comes to support further the finding that the ionic strength has little influence on the rate of this reaction.

In the case of S-methylcysteine, the comparison is not possible unless the activation energy for the electron transfer process and the free enthalpy for the intermediate formation equilibrium are known. Instead, the k_{MTC} values in Table 3 were used to estimate the activation parameters (E_a, lnA, $\Delta H^{\neq}$, $\Delta S^{\neq}$) for the electron transfer step. These are: 73 ± 10 kJ·mol^{-1}, 24 ± 8.8, 70 ± 10 kJ·mol^{-1} and -54 ± 33 J·mol^{-1}·K^{-1} respectively.

Kinetic response to simultaneous oxidation

With the individual reaction constants established, the evaluation of the mixtures is now possible, with the objective of developing a method to predict the concentrations of the two thioethers in an unknown mixture, based on the experimentally determined array of data. The observed rate constants found for the various mixtures and given in Table 2 are shown as well in the 3D representation of Figure 2.
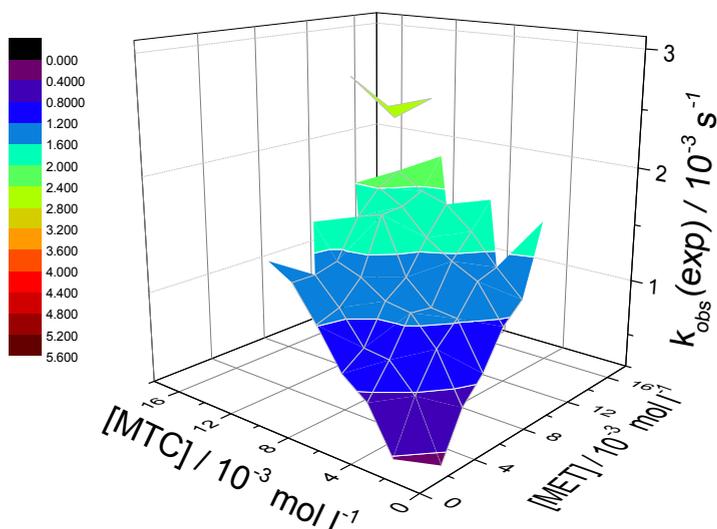
Figure 2. 3D surface plot of the experimental rate constants
obtained for mixtures of MTC and MET.

Theoretical predictive models

One way to approach the task is by looking for a theoretical predictive model based on the available information for the individual compounds. Since no second molecule of thio compound is expected to participate in the rate determining step or before, the two substrates could be assumed to react completely independently of each other. Therefore, the simple addition of the two terms in equations 3 and 4 was considered first. By using the values of the rate and equilibrium constants for the single-thioether experiments, it was possible to create a prediction, k_{obs}(calc), as a function of the two concentrations [MET] and [MTC] (equation 7).

$$k_{obs}(calc) = \frac{a_1[MET]}{1 + a_2[MET]} + \frac{a_3[MTC]}{1 + a_4[MTC]}$$

(7)

Here the parameters a_1 to a_4 are given as $a_1 = k_{MET} \cdot K_{MET} \cdot [H^+]^2$, $a_2 = K_{MET} \cdot [H^+]$, $a_3 = k_{MTC} \cdot K_{MTC} \cdot [H^+]$ and $a_4 = K_{MTC} \cdot [H^+]$, respectively.

A 3D plot corresponding to this model is shown in Figure 3 (a) together with the difference between the predicted values and the average of the experimental ones (Figure 3 (b)), expressed as a percentage of the experimental value. The latter has been defined as:

$$\Delta k_{obs} = \frac{k_{obs}(exp) - k_{obs}(calc)}{k_{obs}(exp)} \cdot 100\% \qquad (8)$$
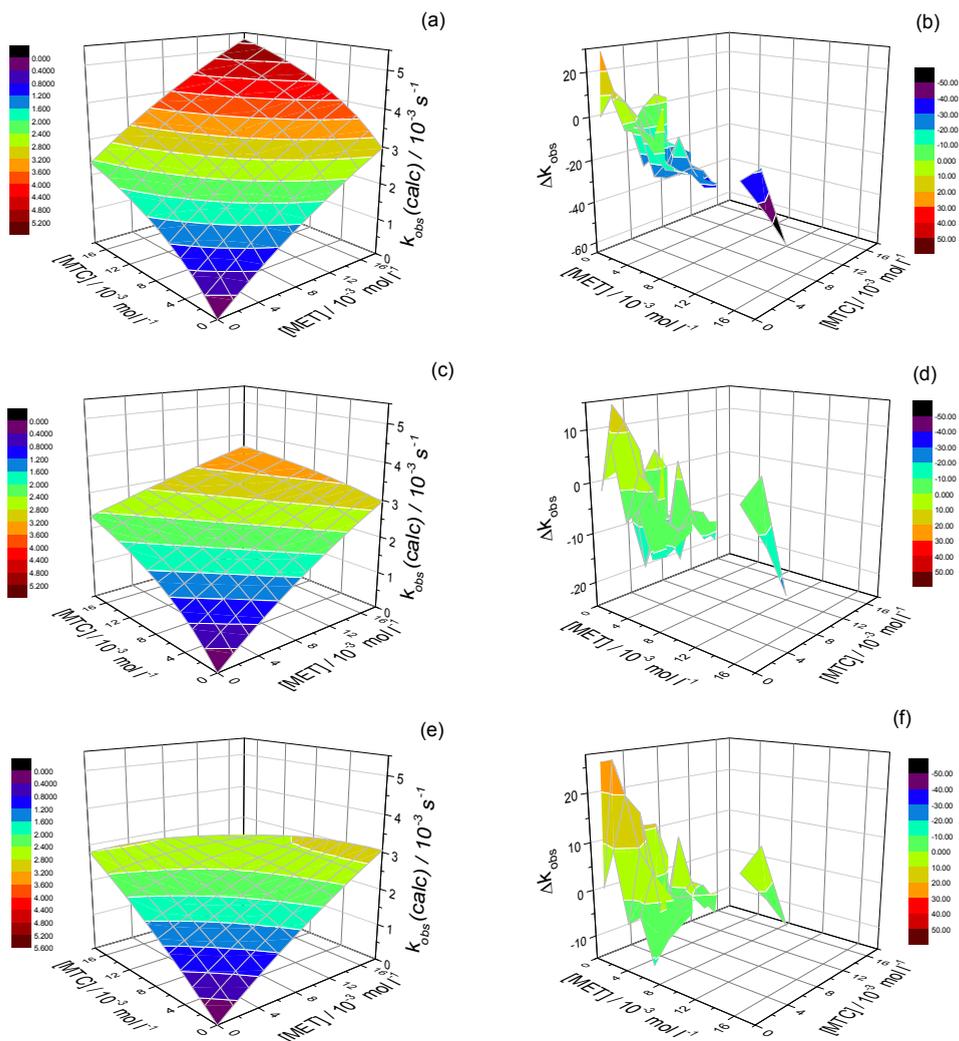
Figure 3. Surface plots for the theoretical models based on the parallel reactions rate law according to equations 7 (a) and 10 (c), as well as for the empirical model based on equation 11 (e). Corresponding plots of the percent differences between each model and the experiment (the data in Figure 2) are shown in (b), (d) and (f).

To provide a better visualisation, the plots of Δk_{obs} have been rotated about the z-axis of the coordinate system. It is clear that as both concentrations increase, the model overestimates the value of $k_{obs}(exp)$ to a higher and higher degree. In other words, the experimentally investigated mixtures show overall rate constants which are significantly smaller than the simple addition model would predict.

Indeed, it comes as no great surprise that this model should not work adequately, since it does not take properly into account the fact that the analytical signal from which the pseudo-first order rate constants are obtained reflects in fact the overall consumption of all the Cr(VI) species.

Therefore, a second, improved theoretical model was obtained by writing the mass balance of the total Cr(VI) (equation 9) and solving the rate law equation for the two parallel processes while also taking this into account.

$$[Cr^{VI}]_{total} = [HCrO_4^-] + [Adduct_1] + [Adduct_2] \tag{9}$$

Note that this follows the same approach as the one used when deriving the equations 3 and 4, from which the electron transfer and equilibrium constants of the two individual processes were computed. With this, we obtained equation 10, where the parameters a_1 to a_4 are the same as in equation 7.

$$k_{obs}(calc) = \frac{a_1[MET] + a_3[MTC]}{1 + a_2[MET] + a_4[MTC]} \tag{10}$$

As it is seen from the corresponding representations in Figure 3 (c and d), this approach delivers a much better estimate of the experimental constant. At very low concentrations, the model underestimates somewhat the results of the experiments, whereas at very high concentrations the opposite is true. However, especially at high concentrations the discrepancy between experiment and calculation is much less than in the previous model.

Of course, since the predicted k_{obs} values are based only on the two sets of data describing the individual oxidations of the thioethers, an increased number of measurements from which to obtain more accurate estimates of the rate and equilibrium constants would benefit the predictions.

Empirical model

In addition to the theoretical predictive models, a more empirical approach to analysing the mixture exists. By choosing a mathematical equation which describes the experimental data adequately, it is possible to create a 3D calibration surface which allows the prediction of the rate constant

for any given mixture by interpolation, provided that the concentrations used for this mixture are within the ranges of those originally used to determine the calibration surface. The equation that was used in the present case with the better results is given below (equation 11) and is an adaptation of equation 7 by the addition of a concentration cross-term. Note that the terms in an empirical equation are not required to have a physical meaning, but are simply meant to describe the data as accurate as possible.

$$k_{obs}(calc) = \frac{b_1[MET]}{1+b_2[MET]} + \frac{b_3[MTC]}{1+b_4[MTC]} + \frac{b_5[MET][MTC]}{1+b_6[MET][MTC]}$$

(11)

The coefficients b_1 to b_6 were calculated using all the experimental data in MATLAB, were there used the averages of the individual values. The calculated surface is shown in Figure 3 (e) and the corresponding values of the coefficients are listed in Table 4.

Table 4. The values of coefficients b_1 to b_6 in equation 11.

Coefficient	b_1 $l\ mol^{-1}\ s^{-1}$	b_2 $l\ mol^{-1}$	b_3 $l\ mol^{-1}\ s^{-1}$	b_4 $l\ mol^{-1}$	b_5 $l^2\ mol^{-2}\ s^{-1}$	b_6 $l^2\ mol^{-2}$
Value	0.213	14.165	0.194	13.973	-10.633	-19.981

In comparison to the two theoretical models, the empirical equation produces a particularly good fit at all but the lowest concentration (Figure 3 (f)). This is not surprising as it is based on a larger sample of data points than the theoretical models. In turn, one disadvantage of such empirical models is that they are strictly valid within the limits of the region covered by the experiments which generated them.

Application of the models to unknown mixtures

Once an acceptable surface is obtained, it may be used to investigate mixtures of the two thioethers where the concentrations of the individual components are unknown. A single measurement would not be sufficient to find both their concentrations, as the $k_{obs}(exp)$ determined would correspond to an infinite number of concentration pairs along the level curve which corresponds to that particular value on the surface.

However, with the inclusion of a single additional measurement, the unknown concentrations can be determined, on the condition that the change in concentrations between the two measured mixtures is known. Two possible ways of achieving this are adding to the original mixture a

predetermined amount of one of the two components or diluting the mixture, for instance to half of the original concentrations, with an appropriate solution (*e.g.* a buffer or a solution of equal acidity).

Once the k_{obs}(exp) of this second mixture is known, a second infinite group of concentration pairs corresponding to this value is obtained (a second level curve). Since the surface is asymmetric in the two concentration parameters, something which is common for all three discussed models, as well as nonlinear, no pair of level curves can be "parallel". Thus, there must be a unique solution that fits the change in k_{obs}(exp) at a given change in the concentrations.

It should be noted that for particularly large values of k_{obs}(exp) for the original mixture, it may be more appropriate to use the predictive model based on equation 10, with a physical meaning, rather than the better fitting empirical model, as there is a possibility that at least one of the compounds has a concentration which lies outside of the limits of the calibration surface.

CONCLUSIONS

As far as the insight into the individual reaction mechanisms of the two substrates goes, in the light of the new measurements the original findings do hold. Moreover, the new rate and equilibrium constants computed for DL-methionine are in very good agreement with the previously determined ones in the same temperature and acidity conditions, but different ionic strength. This is in line with the findings that the ionic strength does not have any strong effect upon the reaction rates of the two substrates in the investigated region. For S-methylcysteine, by pairing the results in this work with the literature data, it was possible to give an estimation of the thermodynamic parameters.

In mixture, the two thioethers are oxidized in parallel. Two versions of theoretical predictive models were tested against the experimental data. They were developed based on the rate law equation derived for the system of two parallel reactions and on the kinetic parameters obtained for the individual oxidations of the two substrates from the measurements of non-mixtures. Of the two models, the version that reflects the evolution of the total chromium VI concentration through the experimentally monitored parameter – the absorbance – is giving the best results, but they can only be as good as the accuracy of the estimated rate and equilibrium constants for the two individual thioethers oxidations are.

An alternative, empirical model was tested with somewhat better results. This was expected, due to the fact that its construction was based on all the available data for mixtures and non-mixtures together. However, its

application for any data belonging to unknown mixtures is limited to those that fall within the range covered by the experiments.

Even though the mechanistic patterns of the oxidations of the two substrates are quite similar, due to the differences in the individual rate constants for the two reactions, either type of model generates asymmetrical surfaces. From these, the univocal determination of the concentrations of the two thioethers is possible without separation, from only two measurements, if at least one of the species is varied by a known amount.

EXPERIMENTAL SECTION

All the reagents were of analytical purity (Merck, Aldrich) and used as received. The solutions were always prepared in demineralised and tetradistilled water (Infusion Solutions Laboratory, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca). Fresh batches of stock solutions of the two thioethers were prepared before each set of measurements and checked spectrophotometrically. The concentration of $HClO_4$ stock solution was verified by titration with NaOH solution of known factor. The aqueous stock solution of Cr(VI) was always $4 \cdot 10^{-4}$ mol·l^{-1}.

In the reaction mixture, the oxidant (chromium VI) was always the reactant in the lowest concentration (the limiting component; 10^{-4} mol·l^{-1}). The addition of an inert mineral acid ($HClO_4$) served to provide acidic conditions. The concentrations of the thioethers and hydrogen ions were always chosen so that pseudo-first order behaviour with respect to the chromium species was ensured. This meant a 10-fold or higher excess over the chromium VI for the thioethers and always a 212.5-fold one for the acid (taking into account the respective of 1.5 to 1 and 4 to 1 stoichiometric ratios). The total chromium concentration and acidity level were chosen such that they fell in the appropriate range, allowing that, prior to reaction, virtually all Cr(VI) was in the $HCrO_4^-$ form [15-17]).

Spectrophotometry was employed as the main analytical tool for monitoring the reaction progress, since Cr(VI) has a well-defined absorption maximum at 350 nm, where none of the thioethers absorb. A Jasco V-530 UV/VIS spectrophotometer ("Jasco", Japan), equipped with automatic data acquisition software was used. The temperature was kept at 298.0 ± 0.1 K, controlled by the means of a Lauda – M16 ("Lauda", Germany) thermostat directly connected to the cell holder.

Prior to use, all the stock solutions were kept inside the water tank of the thermostat, at the work temperature. The reaction was initiated by fast injection of 4 ml of $4 \cdot 10^{-4}$ mol·l^{-1} Cr(VI) solution over a 12 ml mixture of the other reactants already placed in a 5 cm path length quartz cuvette and the

acquisition program was simultaneously started. The mixing time did not exceed 0.5 s. Data was collected every 1 or 2 seconds, for a total of 1200 s or 2400 s respectively. The residual absorbance (A_∞) was measured after 24 hours from the start of the reaction. Three replicate measurements were made for each set of experimental conditions.

The Excel (Microsoft), Origin (OriginLab Corporation, USA) and MATLAB (MathWorks) programs were utilized to process the data. The pseudo-first order constants were computed by the method of least squares.

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