Estimating rate constants of chemical reactions using spectroscopy
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Chapter 5

Description of Datasets and Experimental Set-Up

5.1 Introduction
In this Chapter, four different experimental datasets used in this thesis are described. A short summary of the datasets can be found in the Appendix. Dataset 1 contains short-wavelength near-infrared (SW-NIR)\(^1\)\(^2\) measurements of a two-step epoxidation performed under pseudo-first order conditions. Dataset 2 contains ultraviolet-visible (UV-Vis)\(^1\)\(^2\) measurements of a two-step biochemical reaction also performed under pseudo-first order conditions at a certain pH. At a different pH the same reaction has been monitored using UV-Vis spectroscopy performed under pseudo-first order conditions and second order conditions, resulting in dataset 3 and dataset 4, respectively.

For dataset 2, 3 and 4, pure spectra of some of the reacting absorbing species involved in the reaction have been measured. This is described in this Chapter together with the experimental set-up, dataprocessing and the repeatability of experiments.

5.2 A two-step epoxidation reaction
5.2.1 Description of the reaction
The two-step consecutive epoxidation of 2,5-di-tert-butyl-1,4-benzoquinone using tert-butyl hydroperoxide and Triton B catalyst\(^3\)\(^4\) was monitored in time using SW-NIR spectroscopy. The reaction consists of the following two steps:\(^1\)

\[
U + V \xrightarrow{k_1} W + Z \\
W + V \xrightarrow{k_2} Y + Z
\]

\(^1\) In this thesis, reaction rate constants with Arabic subscript are second order reaction rate constants and reaction rate constants with numerical subscript are (pseudo-) first order reaction rate constants.
with second order reaction rate constants $k_a$ and $k_b$ both in $M^{-1}\text{min}^{-1}$. Species $U$, $V$, $W$, $Y$ and $Z$ are specified in Table 1.

**Table 1.** The species involved in the SW-NIR dataset.

<table>
<thead>
<tr>
<th>Species</th>
<th>2,5-di-tert-butyl-1,4-benzoquinone</th>
<th>tert-butyl hydroperoxide</th>
<th>2,5-di-tert-butyl mono-epoxide-1,4-benzoquinone</th>
<th>cis and trans 2,5-di-tert-butyl di-epoxide-1,4-benzoquinone</th>
<th>tert-butyl alcohol</th>
</tr>
</thead>
</table>

No distinction was made between the cis and trans product (species $Y$) of the second step of the reaction, because the spectral differences are negligible in SW-NIR.\(^4\)

The first and second reaction step are both second order reactions. However, if species $V$ is present in large excess, the first and second reaction step become both pseudo-first order reactions with pseudo-first order reaction rate constants $k_1$ (min\(^{-1}\)) and $k_2$ (min\(^{-1}\)), respectively. Hence, Equation (6)-(8), defined in Section 2.2 of Chapter 2, can be used in order to describe the concentration profiles of the reactant (species $U$), intermediate (species $W$) and main-product (species $Y$) of the reaction, respectively.

**5.2.2 Sample preparation**

A cuvette was filled with 0.264 g (1.2 mmol) of 2,5-di-tert-butyl-1,4-benzoquinone, as synthesized by the procedure described by Hairfield *et al.*\(^3\) A melting point of 152-153\(^\circ\)C was obtained for 2,5-di-tert-butyl-1,4-benzoquinone. The reported melting point is 152.5\(^\circ\)C.\(^5\) Next, 2,5-di-tert-butyl-1,4-benzoquinone was dissolved in 15 ml dioxane (Acros 99\%\%) and 1.55 ml (12 mmol) of a tert-butylhydroperoxide 70\% (Acros) solution in water and 13.21 ml ethanol (BDH Laboratory Supplies, pro analysis) were both added. After the target reaction temperature had been reached, data collection was started immediately after addition of 0.24 ml ice-cold Triton B catalyst (Acros, 40 wt. \% in methanol) in 0.50 ml ethanol and 0.60 ml dioxane. The excess of tert-butylhydroperoxide in moles was ten times 2,5-di-tert-butyl-1,4-benzoquinone. At this excess, pseudo-first order kinetics may be assumed.
5.2.3 Experimental set-up

The experimental set-up is shown in Figure 11. To measure SW-NIR spectra, a Hewlett Packard 8453 UV-Vis spectrophotometer with diode array detection was used. A quartz cuvette with 10.00 cm pathlength (Hellma Benelux) was used to obtain spectra of the reaction mixture. On both sides of the cuvette, a small unit of glass was glued to be able to thermostate the cuvette. The cuvette contained two stirring modules. Water was pumped from a constant temperature bath (Neslab) through the cooling units of the cuvette. A Pt-100, which is a thermocouple, connected to the constant temperature bath was used inside the cuvette to control the temperature. To obtain a temperature below room temperature an immersion cooler (Haake) was used to cool the water of the constant temperature bath.

![Diagram of experimental set-up](image)

Figure 11. The experimental set-up.

The cuvette was placed in a special constructed cell holder. The spectrophotometer and the cuvette were placed in a home-made air thermostated box, to avoid the influence of temperature fluctuations of the surroundings. The box temperature was controlled by a constant temperature bath (Haake). A Hewlett Packard Vectra XM2 Chemstation (IntelDX4-100 MHz with 16 MB RAM and a 800 MB harddisk) using the Hewlett Packard software was used to collect and store the data. The experimental conditions are listed in Table 2. Eight individual batch process runs were performed at identical conditions which are labeled dataset 1 in this thesis.
<table>
<thead>
<tr>
<th>Reaction Temperature</th>
<th>17 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Integration Time</td>
<td>1 s</td>
</tr>
<tr>
<td>Sampling Time</td>
<td>5 s</td>
</tr>
<tr>
<td>Total Run Time</td>
<td>1200 s</td>
</tr>
<tr>
<td>Wavelength Range</td>
<td>800-1100 nm</td>
</tr>
<tr>
<td>Wavelength interval</td>
<td>1.0 nm</td>
</tr>
<tr>
<td>Number of Measured Spectra</td>
<td>241</td>
</tr>
</tbody>
</table>

5.2.4 **Dataprocessing**

In order to remove offset and drift, second-derivative spectra were estimated using a Savitzky-Golay filter using a window size of 15 wavelengths. To stress the spectral features of the appearing and disappearing species, second derivative difference spectra were calculated after subtracting the fourth measured spectrum from all the other spectra remaining. The first three measured spectra were not used for dataprocessing because of the moderate reproducibility of these spectra. The small wavelength range 860-880 nm was used for dataprocessing. If this wavelength range is used the spectral features are only caused by the three species which were monitored (species $U$, $W$ and $Y$). The second-derivative difference spectra of one individual batch process run are shown in Figure 12. The first and last measured spectra are indicated. From Figure 12 it is obvious that a moderate signal to noise ratio is present.

![Figure 12. The second derivative difference spectra of one individual batch process run.](image)
In case of using the WCR algorithm, described in Section 2.6 of Chapter 2, the datamatrix with measured spectra of the reacting system was truncated into three singular values. For the two-way methods starting values of 0.30 min\(^{-1}\) and 0.05 min\(^{-1}\) were used for \(k_1\) and \(k_2\), respectively, based on the estimates of these reaction rate constants obtained by others.\(^3\)\(^4\)

Data processing was performed in the Matlab environment (Version 5.2. The Mathworks Inc.) on a Pentium 133 MHz Computer with 64 MB RAM and a 1.2 GB harddisk.

5.2.5 The repeatability of experiments
The repeatability, \(R_{\text{batch}}\), for batch process runs was calculated using Equation (50).\(^7\)

\[
R_{\text{batch}} = \frac{\sqrt{\sum_{i=1}^{I} \| X_i - \bar{X} \|^2}}{\| \bar{X} \|} \times 100\%
\]

where \(X_i\) is the spectral matrix for experiment \(i\) and \(\bar{X}\) is the averaged spectral matrix obtained from the \(I\) individual experiments.

The repeatability was equal to 23.28\% for dataset 1. This poor repeatability is mainly a consequence of the small differences in absorbances of the species and the error propagation due to taking the second derivative.

5.3 A two-step biochemical reaction
5.3.1 Description of the reaction
The two-step consecutive reaction of 3-chlorophenylhydrazonopropane dinitrile with 2-mercaptoethanol\(^8\)\(^9\) was monitored using UV-Vis spectroscopy. The reaction consists of the following two steps:

\[
U + V \xrightarrow{k_1} W \\
W \xrightarrow{k_2} Y + Z
\]

with second order reaction rate constant \(k_1\) (M\(^{-1}\)min\(^{-1}\)) and first order reaction rate constant \(k_2\) (min\(^{-1}\)). Species \(U, V, W, Y\) and \(Z\) are specified in Table 3.

If species \(V\) is present in large excess the first step of the reaction becomes pseudo-first order with pseudo-first order reaction rate constant \(k_1\) (min\(^{-1}\)). Hence, Equation (6)-(8) from Section 2.2 of Chapter 2. can be used in order to describe the
concentration profiles of $U$, $W$ and $Y$, respectively. A detailed reaction mechanism of the reaction described is given in Figure 13.

<table>
<thead>
<tr>
<th>Species</th>
<th>Reaction Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>$U$</td>
<td>3-chlorophenylhydrazono-propane dinitrile</td>
</tr>
<tr>
<td>$V$</td>
<td>2-mercaptoethanol</td>
</tr>
<tr>
<td>$W$</td>
<td>intermediate adduct</td>
</tr>
<tr>
<td>$Y$</td>
<td>3-chlorophenylhydrazono-cyanoacetamide</td>
</tr>
<tr>
<td>$Z$</td>
<td>ethylenesulphide</td>
</tr>
</tbody>
</table>

Figure 13. The reaction mechanism.

5.3.2 Sample preparation
All chemicals were used as received without further purification. An amount of 0.0529 g (0.2585 mmol) of species $U$ (Acros, 99%+) was dissolved in water using a minimum volume of 0.1 mol$^{-1}$ NaOH (Baker Chemicals, 98.8%) to give a stock solution of 1.034 mmol$^{-1}$. This stock solution was diluted into a working solution containing 51.71 µmol$^{-1}$ of species $U$, buffered with KH$_2$PO$_4$ (Acros, pro analysis 0.2 mol$^{-1}$, pH 4.4). The pH of the working solution was equal to 5.4. This working solution had to be prepared freshly every day. At this pH, species $U$ was
supersaturated and slowly crystallized from the solution after a couple of days. This was not observed by Bisby and Chau.\textsuperscript{8,9} The cuvette was filled with 2.5 ml of the working solution. When the temperature inside the cuvette had reached the target temperature, data collection was started upon addition of 10 μl of a $V$-solution, which contained 35.65 μmol of species $V$, by means of a pipette. This $V$-solution consisted of 250 μl pure $V$ (Acros, 99%) and 750 μl KH$_2$PO$_4$ buffer solution. However, if pure species $V$ is added it will take some time to mix with the reaction mixture. This mixing time can be reduced if species $V$ is already in the same buffer solution as the buffer solution used to create the reaction mixture. The excess of species $V$ in moles was 276 times species $U$.

The reaction described was also performed using an other pH of the working solution. At a pH of 5.2 of this solution, experiments were performed where the excess of species $V$ in moles was 265 times species $U$. The reaction was also investigated under second order conditions for three specific ratio's of initial concentration of both reactants (species $U$ and species $V$). The ratio's $C_{V,0}/C_{U,0}$ were chosen equal to 1:4, 1:5 and 1:6. The pH of the working solution in case of second order kinetic experiments was also equal to 5.2.

5.3.3 Experimental set-up

The experimental set-up used, was equal to the experimental set-up described in Section 5.2.3 of this Chapter. However, in this case a quartz cuvette with 1.00 cm pathlength was used to obtain spectra of the reaction mixture. The experimental conditions are given in Table 4 for pseudo-first order experiments.

For second-order experiments, the experimental conditions were equal to those listed in Table 4 apart from the sampling time and total run time, which were equal to 20s and 5400s, respectively.

In case of a pH of the working solution equal to 5.4, ten experiments were performed at identical pseudo-first order conditions which are labeled dataset 2 in this thesis. For a pH of 5.2 the pseudo-first order experiment was repeated 32 times which is labeled dataset 3 in this thesis. At the same pH, the second order experiment was repeated six times for three specific ratio’s of initial concentrations of both reactants. Hence, in total 18 second order experiments were performed which are labeled dataset 4 in this thesis. The spectra of one individual batch process run are shown in Figure 14 for pseudo-first order conditions and second order conditions using a pH of the working solution of 5.2 in both cases. The spectra obtained under pseudo-first order conditions are similar for a pH of 5.4 and a pH of 5.2. From Figure 14 it is

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Table 4. The experimental conditions.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Temperature</td>
<td>25 °C</td>
</tr>
<tr>
<td>Integration Time</td>
<td>1 s</td>
</tr>
<tr>
<td>Sampling Time</td>
<td>10 s</td>
</tr>
<tr>
<td>Total Run Time</td>
<td>2700 s</td>
</tr>
<tr>
<td>Wavelength Range</td>
<td>200-600 nm</td>
</tr>
<tr>
<td>Wavelength interval</td>
<td>1.0 nm</td>
</tr>
<tr>
<td>Number of Measured Spectra</td>
<td>271</td>
</tr>
</tbody>
</table>

Figure 14. The spectra of one individual batch process run performed under pseudo-first order and second order conditions.

It is obvious that in case of second order conditions, very small absorbances differences are obtained in time compared to the absorbance differences for pseudo-first order conditions. A reason for this, is that if second order conditions are chosen the first reaction step is very slow, which results in these small absorbance differences in time. Because of the slow first reaction step, the intermediate formed reacts fast into the product.

5.3.4 Pure spectra

In order to obtain the pure spectrum of the reactant, species $V$ was not added and 21 spectra were taken of the reactant for the wavelength range 300-500 nm. The averaged spectrum is an estimate of the pure individual spectrum of species $U$. In case of dataset 3 and dataset 4, 21 spectra of the reactant were measured and averaged for every individual batch process run. Hence, every individual batch process run is connected with an averaged pure spectrum of the reactant.

The pure spectrum of the product was obtained by taking 21 spectra of the reaction mixture after a reaction time of eight hours. This procedure was repeated two
times and every set of 21 spectra was averaged. Hence, two averaged pure spectra of the product are available. After eight hours, it is assumed that no species $U$ and species $W$ are left in the reaction mixture and hence there is approximately 100% conversion. Only species $V$, species $Y$ and species $Z$ are present. However, species $V$ and species $Z$ both have no absorbances in the wavelength range considered. Therefore, it can be assumed that the averaged measured spectrum of the reaction mixture represents the pure spectrum of product species $Y$. From the reaction rate constant estimates reported in Chapter 6, 7, 8 and 9 of this thesis, it is reasonable to assume that no reactant and intermediate are left after eight hours. The averaged measured pure spectrum of reactant and product are both shown in Figure 15. From this figure it is observed that the spectral overlap between both pure spectra shown is very large.

Figure 15. The measured pure spectrum of reactant and product.

5.3.5 Data processing

A spectrum of KH$_2$PO$_4$ buffer solution was used as blank. The wavelength range 300-500 nm was used for dataprocessing. Using this spectral range there are only spectral features caused by species $U$, $W$ and $Y$. At $\lambda < 300$ nm species $V$ absorbs and by-product $E$ shows an increasing absorbance. At $\lambda > 500$ nm there is no significant contribution of the reacting species to the absorbance. The first measured spectrum was not used for dataprocessing because of the moderate reproducibility of this spectrum. In case of using two-way methods for estimating reaction rate constants,
starting values of 0.30 min\(^{-1}\) and 0.05 min\(^{-1}\) were used for \(k_1\) and \(k_2\), respectively, based on the estimates obtained by others.\(^8\)\(^9\)

Data processing was performed in the Matlab environment (Version 5.2, The Mathworks Inc.) on a Pentium 133 MHZ Computer with 64 MB RAM and a 1.2 GB harddisk.

5.3.6 The repeatability of experiments
The repeatability was calculated for the UV-Vis datasets using Equation (50) from Section 5.2.5 of this Chapter. The repeatability was equal to 0.54\% and 1.60\% for dataset 2 and dataset 3, respectively. For dataset 4, three repeatability numbers were calculated, because there were three different ratio’s of initial concentrations of the reactants involved. For a ratio of 1:4, 1:5 en 1:6 the repeatability was equal to 1.28\%, 2.15\% and 1.15\%, respectively. Hence, it can be concluded that dataset 3 and dataset 4 have a similar repeatability. The repeatability of dataset 2 is excellent for spectroscopic measurements.

5.4 References