ReactLab™ REDOX

Reaction Modeling for Redox Reactions

USER MANUAL

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ABOUT THIS MANUAL

This manual is in two parts. Part 1 comprises a comprehensive description of the program architecture and functionality and provides a systematic guide to using it. Part 2 consists of two worked examples demonstrating specific analysis case studies using pre-prepared workbooks. All these are provided in the Excel examples folder.

CONTACT INFORMATION AND SUPPORT

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PART 1: REFERENCE GUIDE

INTRODUCTION

ReactLab™ REDOX provides tools for the fitting of the reduction potential(s) for one- and two-step redox processes. A typical measurement consists of a collection of absorption spectra acquired as a function of the solution potentials (a spectroelectrochemical titration).

All raw data, model entry and the output of results are organised in Excel Workbooks, it is therefore necessary for Excel to be installed. The use of Excel provides a familiar spread sheet format for all experimental data and results, and allows the independent application of Excel tools and features for further processing, in particular graphical presentations.

When a workbook is saved it contains all information and settings associated with the current analysis session as well as the numerical data and results.

The program requires Macros to be enabled (see Troubleshooting) and the Excel workbooks must retain a strict format, as is provided in the examples and templates.

Analysis of the data with ReactLab™ REDOX is performed within the Excel workbook, based on complex calculations and the Excel Solver. Additional graphical output is also organised within the workbook.

In Part 1 the basic principles of ReactLab™ REDOX are explained, in Part 2 we will discuss in some detail the analysis of two representative examples.

EXCEL TEMPLATES and WORKBOOKS

Two ReactLab™ REDOX workbook templates are supplied in the downloaded package, one for a 1-step reduction and one for a 2-step reduction. In the same directory a fully worked example is given for cindoxin, a cytochrome P450 analogue (thanks to the authors of ‘Cloning, Expression and Purification of Cindoxin, an Unusual Fmn-Containing Cytochrome P450 Redox Partner’; David B. Hawkes, Kate E. Sessor, Paul V. Bernhardt, and James J. De Voss; ChemBioChem 2010, 11, 1107–1114). We will use this example, cindoxin.xlsx, in this Section of the manual.

The Excel workbook contains the following worksheets:

- **Data** The principle model and parameter entry interface and the location of the experimental data
- **Results** Location for fitted concentration profiles and calculated spectra for all redox-active species
- **Fit Plots** Location for plots comparing measured and calculated spectra at specific wavelengths
- **Residuals** Location for the difference between measured and calculated absorbance data
- **About** Sheet with contact information and the workbook format version

The format of these sheets is important as ReactLab™ depends on everything being in particular locations. Figure 1 illustrates the organisation, model entry fields and data entry in the ‘Data’ worksheet which is the principle sheet of the workbook requiring the majority
of user interaction. The various fields in this sheet will be described later on in this document.

![Figure 1: The ‘Data’ worksheet in cindoxin.xlsx illustrating a 2-step redox model; it contains spectra, wavelength range, total concentration and potential readings.](image)

The template ‘Data’ worksheet is prepared for the entry of the data, i.e. total concentration of the active species, recorded potential and absorption spectra for all solutions. See Figure 2 for the template, ready for the addition of the experimental information.

![Figure 2: The ‘Data’ worksheet in the template Redox_2_Template.xlsx.](image)

**Important:** The workbooks provided have certain sheets that are ‘protected’, ‘Results’, ‘Fit_Plots’, ‘Residuals’ and ‘About’. The cells of these sheets are locked to prevent any overwriting of formulae; however the graphs can still be edited and manipulated so that the user can tailor each graph to their needs. The ‘Data’ sheet is unprotected so that the user
can enter data, as such care needs to be taken to not change any cells with formula as these are important for the calculations.

To get a practical demonstration of ReactLab™ REDOX capabilities quickly refer to the Example workbooks and the corresponding descriptions in Part 2 of this manual. What follows here is a systematic overview of the whole program.

**REDOX DATA, SPECIES AND COMPONENT CONCENTRATIONS**

When working with new measurements start with an empty workbook template. We advise making a copy of *Redox_1_Template.xlsx* and *Redox_2_Template.xlsx* for this purpose. The first step is to populate the ‘Data’ worksheet with a new measurement matrix $Y$. In a typical measurement, the absorption spectrum together with the potential reading is recorded for each solution. The potential is typically imposed externally using an Optically Transparent Thin Layer Electrochemical (OTTLE) Cell and the spectrum recorded after equilibration. An alternative procedure is an actual titration with a non-absorbing oxidant or reductant where the spectrum, as well as the potential, is recorded after each addition once equilibration is complete. Such a titration will result in dilution and the total concentration of the active compound $A$ will decrease over the course of the titration.

In the example of Figure 1, a measurement performed using the OTTLE cell, the concentration of $A$ is constant and are collected in columns B18, B19, ... The potential readings (in volt) $E_{j}$ are placed in the column C18, C19, ..., and the wavelength vector $\lambda \rightarrow$ is placed in the row starting at D17, E17 ... The data are placed in an array corresponding to the intersection of the potential reading and wavelength coordinates. Data entry is supported by the shading of the relevant cells. The total number of spectra (no. of spectra) and the number of wavelengths (no. of $\lambda$'s) are calculated automatically from the data range pasted into the worksheet.

*Important: When using Excel 1997-2003 compatible workbooks the maximum number of wavelengths permitted is 253 (due to the column number restriction in this version of Excel). For Excel 2007 ReactLab™ can in principle handle >17000 wavelengths. Note therefore saving an xlsx workbook in xls format can lead to truncation of large data sets.*

There are two ReactLab REDOX spreadsheets, one for a 1-electron and one for a 2-electron redox process.

The reactions are written as reductions:

$$A + e^- \rightarrow E_{j}^{-} \rightarrow A^-$$

$$A + e^- \leftrightarrow E_{j} \rightarrow A^-$$

$$A^- + e^- \leftrightarrow E_{j} \rightarrow A^{2-}$$

**Scheme 1:** The Reduction equations for the 1-step and 2-step processes

$A$ is the redox active component in the reaction. In the 1-step process $A$ is reduced to form $A^-$. In the 2-step process $A^-$ is further reduced in a subsequent reaction to form $A^{2-}$. The species $A$, $A^-$ and $A^{2-}$ are formed according to the potentials of the redox couples ($E_1$ and $E_2$) in the scheme. Knowing $[A]_{tot}$ and the potential of the redox couple allows the individual species concentrations $[A]$, $[A^-]$ and $[A^{2-}]$ to be calculated automatically during fitting and
the results transferred to the ‘Results’ worksheet, columns C, D and E in Figure 6, for use in subsequent calculations.

The species concentration \([A]\), \([A^-]\) and \([A^{2-}]\) are computed for each solution such that they satisfy the conservation of mass principle

\[
[A]_{\text{tot}} = [A] + [A^-] + [A^{2-}]
\]

As well as the Nernst equations

\[
E = E_1 - 0.059 \log \frac{[A^-]}{[A]} = E_2 - 0.059 \log \frac{[A^{2-}]}{[A^-]}
\]

If \([A]_{\text{tot}}\) is constant throughout the measurement then the same concentration value needs to be entered in the column B18, B19, ..., for each measured spectrum. However, if \([A]_{\text{tot}}\) changes the respective concentrations need to be entered for each spectrum in B18, B19, ....

**MODEL ENTRY**

Before analysing data it is necessary to decide on a reaction model. If the process under investigation is a 1-step reduction, the template Redox_1_Template.xlsx is to be used and if it is a 2-step reduction the Redox_2_Template.xlsx is to be used. In both ‘Data’ worksheets the models are predefined as indicated in Figure 3. Note that each reaction step is entered individually, i.e. \(A + e^- = A^-\) and \(A^- + e^- = A^{2-}\), which allows for a one-to-one correspondence of each reaction step to its parameter value in the ‘E_0 (V)’ column.

**PARAMETER ENTRY**

Prior to fitting the data, the numerical parameter estimates or known values if they are available from other work, should be entered in the appropriate fields of the ‘Data’ sheet, G7 and G8 (Figure 3). The potential of the redox couples must be entered in Volt, e.g. -0.3 and -0.4 for the given example. The user must decide whether parameters will be either fixed (\[\quad\], kept constant) or fitted (\[✓\], optimised) using the tick boxes.

It is assumed that all species or oxidation states of \(A\) are absorbing and thus contributing to the measurement in the wavelength range covered by the measurement.

**FITTING THE MODEL TO DATA**

**Before Fitting**

Incorporating the data and the estimated redox potentials automatically results in the computation of the concentration profiles and absorption spectra in the ‘Results’ worksheet, see Figure 4.
Figure 4: Calculated concentration profiles and molar absorption spectra in the ‘Results’ worksheet

More immediately useful are the comparisons of measured and fitted profiles at five equidistant wavelengths covering the complete wavelength range; they are produced in the ‘Fit Plot’ worksheets. Assessing the validity of initial estimates for parameters is a useful precursor to fitting and Excel automatically updates the calculated data values when a value for $E^0$ is entered in the model. The graph shown in Figure 5 is a good indication of how consistent the measured and calculated data are before trying to fit the parameters to the data. If the discrepancy between measured and calculated data is excessive it is possible that a fit will not converge. Experiment with different initial guesses until they are reasonable and then begin the fit. Figure 5 demonstrates that for the $E^0$ values in Figure 3, the fits are reasonable and thus the fitting is likely to converge straightforwardly.

Figure 5: Comparison of the measured and calculated data in ‘Fit Plot’ giving an indication about the validity of the initial parameter estimates before executing a fit

Fitting

Selecting ‘Fit’ initiates the Solver algorithm which proceeds to minimise the residual square sum (or ssq) which is a measure of the difference between the real data and that
predicted by the current model and current parameter values. Ssq is displayed in cell G10 of the ‘Data’ worksheet. The selected parameters of the model are iteratively refined using Solver to adjust the species spectra, according to a least squares criterion.

Following convergence the final results are returned to the ‘Data’ Excel worksheet and the concentration and spectra matrices in the ‘Results’ worksheet are automatically updated. Any Excel graphs linked to these data ranges will be updated accordingly (Figure 6). The implementation and adaptation of such graphs is entirely at the discretion of the user.

### Table

<table>
<thead>
<tr>
<th>Reactants</th>
<th>Products</th>
<th>$E^0$ (V)</th>
<th>Fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>A + e^-</td>
<td>A</td>
<td>0.2000</td>
<td>✓</td>
</tr>
<tr>
<td>A + e^-</td>
<td>A^-</td>
<td>0.1269</td>
<td>✓</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ssq</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.6E-05</td>
</tr>
</tbody>
</table>

**Figure 6:** Fitted reduction potentials in the ‘Data’ worksheet and final concentration profiles and absorption spectra in the ‘Results’ worksheet
PART 2: EXAMPLES

Example 1: One-Step Reduction A + e- = A- (REDOX_1_Example)

The first worked example is based on data generously provided by Prof. Paul Berhardt, University of Queensland, Australia. The system under investigation is the redox couple

\[
\text{met-myoglobin(Fe(III))} + e^- \underset{E_1}{\overset{E_1}{\rightleftharpoons}} \text{deoxy-myoglobin(Fe(II))}
\]

For which the standard potential \( E_1 \) is to be determined

The Experiment

The experiment has been performed in a Spectroelectrochemical Transmission Cell, a liquid cell that encompasses an optically-transparent thin-layer electrode (OTTLE) structure that allows the application of a predetermined potential to the solution. Complete absorption spectra are recorded after equilibration as a function of the applied voltage

The experimental details are the following: the total concentration of \( A \), \( [A]_{\text{tot}} \) is 16 µM; spectra are measured after each pre-set voltage, ranging from -0.5 to +0.1 V; the cell thickness is 1mm; spectra are acquired in the wavelength range 300 to 645 nm in 0.4 nm intervals. This results in a total of 20 measured spectra at 956 wavelengths. The data set is represented in Figure 7. All solutions were buffered at pH 8, using a tris buffer.

![Measurement](image)

**Figure 7:** The measurement, spectra measured as a function of change in voltage

The Data

The data required for analysis by ReactLab™ REDOX consists of the following:

1. A collection (matrix) of spectra recorded as a function of voltage.
2. The vector of wavelengths at which absorption measurements were taken.
3. The total concentration \( [A]_{\text{tot}} \) in each solution.
4. The potential of the solution.
Figure 8: The data, arranged in the ‘Data’ worksheet

All experimental information is stored in the ‘Data’ worksheet. The vector of concentrations is stored in the column of cells B17:B36, under the heading \([\text{[A]}]_{[\text{tot}]};\) the vector with measured potentials in the cells C17:C36, under the heading ‘E’; the vector of wavelengths in the row of cells D16:AJW16. The matrix of data is stored the array D17:AJW36. The number of spectra and wavelengths are computed by excel, in cells J11 and J12. The yellow headings are pre-set in the worksheet. Figure 8 displays the relevant section of the ‘Data’ worksheet.

As this is a one-electron reduction we have used the REDOX_1_Template. The translation of the reaction equation into the spreadsheet is self-explanatory, see Figure 9.

The fitting of the normal potential is a non-linear process; fitting is a iterative process and has to be started from an initial estimate. Naturally, the better this initial estimate the faster and more reliably the best parameter can be computed. The initial estimate can be significantly wrong, however, if it is completely wrong the algorithm might collapse. The borders between significantly and completely wrong are not well defined as no general rules can be given. Trial and Error is the simple answer.

The initial estimate is entered as shown below in the example: \(E = -0.30\).

Figure 9: Initial parameter estimate for the normal potential \(E_1\)
After a value is entered in G7 the concentration profiles and the corresponding absorption spectra are automatically calculated. They appear in the ‘Results’ worksheet, Figure 10, and these can be graphed as normal excel plots. Note the residuals, i.e. the difference between the measured and calculated absorbances, are graphed in the ‘Residuals’, Figure 14, and ‘Fit Plot' worksheets, Figure 11.

**Figure 10**: Concentration profiles and absorption spectra in the ‘Results’ worksheet before fitting

**Figure 11**: The ‘Fit Plot’ worksheet displays the measured and calculated absorption curves for the present normal potential

The ‘Fit Plot’ worksheet is most useful for the evaluation of the quality of the guessed parameter. In this example it is obvious that a better value would be around -0.1 V. However, the present value is good enough for successful fitting.
Fitting

Obviously, the fits are not perfect with the starting estimate entered for the normal potential in this example. This is clear from the graph of the residuals, see ‘Residual’ worksheet, and also from the sum of squares, ssq, in the ‘Data’ worksheet in cell G9, ssq=6.15. Nevertheless, the fits are also not hopelessly wrong, and we can press the button in the ‘Data’ worksheet. Make sure the fit box is ticked in H7 of the spreadsheet. After successful fitting, the ‘Solver Results’ window appears, Figure 12

![Figure 12: The excel solver displays the above window to indicate successful fitting](image)

The result of the fit is automatically updated in the ‘Results’ worksheet which show the concentration profiles and molar absorption spectra as shown in Figure 13.

![Figure 13: Concentration profiles and absorption spectra in the ‘Results’ worksheet after fitting the redox couple value](image)

Additional statistical information is the sum of squares, now ssq=0.327, in cell G9 of the ‘Data’ worksheet, and the plot of the residuals, Figure 14.
Figure 14: Representation of the residuals

However, most instructive are the ‘Fit Plots’, they show best the quality of the fit. With the exception of the very short wavelengths, the fits are essentially perfect.

Figure 15: The ‘Fit Plots’ are the best indication for the quality of the fits after the the parameter(s) have been optimised
Example 2: Two-Step Reduction \( A + e^- \rightarrow A^- \) and \( A^- + e^- \rightarrow A^{2-} \) (REDOX_2_Example)

The Experiment

This experiment is aimed at determining the two normal potentials of the redox couples for the two step redox reaction:

\[
A + e^- \xrightleftharpoons{E_1} A^- \\
A^- + e^- \xrightleftharpoons{E_2} A^{2-}
\]

This is an artificial, computer generated data set. It mimics a titration with 100mL of a solution of \( 10^{-5} \) M \( A \) with a non-absorbing reductant; a total of 27 additions of 1mL results in 28 spectra. Consequently, the total concentration of \( A \) is continuously reduced. Spectra are acquired in the wavelength range 400 to 600 nm at 10 nm intervals. The absorption data set is represented in Figure 16. For each solution the potential has also been measured and recorded in the cell D18 downwards. Pseudo-random noise has been added by truncating the perfect calculated absorbances to 3 decimal places, e.g. 0.5432 is reduced to 0.543.

![Absorbance vs Wavelength](image.png)

**Figure 16:** The measurement, spectra measured as a function of progress of titration

The ‘Data’ worksheet

The data required for analysis by ReactLab™ REDOX consists of the following:

1. A collection (matrix) of spectra recorded as a function of voltage, they are entered into the cells D18 to X45.
2. The vector of wavelengths at which absorption measurements were taken, cells D17 to X17.
3. The total concentrations of the active component in each solution, cells B18 to B45.
4. The measured potential of the solutions, cells C18 to C45.
Figure 17: The data, arranged in the ‘Data’ worksheet

Figure 17 displays the relevant sections of the ‘Data’ worksheet. The entries ‘no. of spectra’ (28) and ‘no. of wavelengths’ (21) are computed automatically, the relevant cells are protected and should not be altered.

The model is defined by the choice of the spreadsheet, in this case the data were entered into the REDOX_2_Template as we deal with a 2-step redox process. There are two reaction steps, the reaction $A + e^– \rightarrow A^+$ followed by the subsequent reaction $A^+ + e^– \rightarrow A^{2+}$. The representation in the spreadsheet is self-explanatory, see Figure 18.

Initial Guesses for the Redox Potentials

The fitting of the redox couples is a non-linear process; fitting has to be started from a set of initial guesses. Naturally, the better these initial guesses are the faster and more reliably the best parameters can be computed. Initial guesses are allowed to be significantly wrong, however, if they are completely wrong the algorithm might collapse. The borders between significantly and completely wrong are not well defined as no general rules can be given as the parameters are strongly dependent on the data set and redox model. Trial and Error is the simple answer.

The initial guesses are entered as shown below in the example: $E_1 = 0.3$ and $E_2 = 0.1$ V.

Figure 18: Initial parameter guesses for $E_1$ and $E_2$

When values are entered in G7 and G8, the concentration profiles for the present set of constants, and the corresponding absorption spectra, are automatically calculated. They
appear in the ‘Results’ worksheet, Figure 19, and these can be graphed as normal excel plots.

![Concentration profiles and absorption spectra in the ‘Results’ worksheet before fitting the redox couple values](image)

**Figure 19:** Concentration profiles and absorption spectra in the ‘Results’ worksheet before fitting the redox couple values

The residuals, i.e. the difference between the measured and calculated absorbances, are graphed in the ‘Residuals’ worksheet. More useful are the plots that combine measured and calculated data in the ‘Fit Plots’ worksheet, see Figure 20 for the present set of initial guesses.

![Measured and Calculated Data](image)

**Figure 20:** The Fit Plots display measured and calculated data at five wavelengths.

Five evenly distributed wavelengths between the minimum and maximum wavelengths are preselected. It is possible to over-write the preselected wavelengths in cells E5 to I5, the plots are updated automatically, Figure 21.
**Fitting**

As indicated by the Fit Plots, the fits are not perfect with the starting guesses entered for the redox couples in this example. This is clear from the graph of the residuals, see ‘Residual’ worksheet, the ‘Fit Plots’ worksheet and also from the sum of squares, ssq=0.16, in the ‘Data’ worksheet in cell G10. Nevertheless, the fits are also not hopelessly wrong, and we can press the ‘Fit’ button in the ‘Data’ worksheet. Make sure the fit boxes are ticked in column H of the spreadsheet.

The result of the fit is automatically updated in the ‘Results’ worksheet which show the correct concentration profiles and molar absorption spectra as shown in Figure 22.

![Fit Plots](image)

**Figure 21:** The preselected five wavelengths (cells E5 to I5) for the ‘Fit Plots’ can be changed to any other values.

![Concentration profiles and absorption spectra](image)

**Figure 22:** Concentration profiles and absorption spectra in the ‘Results’ worksheet after fitting the redox couple values

The ‘Fit Plots’ are now essentially perfect, see Figure 23
**Figure 23:** After the fitting the Fit Plots are essentially perfect  

Additional statistical information is the sum of squares, now $ssq=3.63\times10^{-5}$, in the ‘Data’ worksheet, and the plot of the residuals, Figure 24 for a 3-D plot, which shows the ideal random distribution of the residuals.

**Figure 24:** 3-D Representation of the residuals
Troubleshooting

The following steps should be followed in case the program is giving an error message instead of fitting the measurement.

a) Make sure that Macros are enabled, as shown in Figure 25.

![Figure 25](image-url)

**Figure 25:** In order to run the Excel workbook Marcos must be enabled by clicking on the ‘Enable Content’ button identified above by the red arrow

b) Check whether Solver has been added into your Excel program. Follow the sequence of steps shown in Figure 26:

1. In an Excel Workbook click on ‘File’;
2. Click on ‘Options’;
3. Click on ‘Add-Ins’;
4. Check that ‘Excel Add-Ins’ is selected in the ‘Manage’ dropdown list and click on ‘Go...’;
5. Check the box for ‘Solver Add-in’ and click ‘OK’
Figure 26: Enabling Solver as an Add-in
If the workbook is not automatically updating when the values of $E^0$ are changed in the ‘Data’ sheet, make sure that updating is ‘Automatic’ in the Excel settings. To check these follow these steps:

1. In an Excel Workbook click on ‘File’ and then click on ‘Options’;
2. Click on ‘Formulas’;
3. Under the first heading ‘Workbook Calculation’ make sure that ‘Automatic’ is selected
4. Click ‘OK’

References
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Marcel Maeder, Yorck-Michael Neuhold. Practical Data Analysis in Chemistry, Elsevier 2007