

## Pro-Kineticist II – 2<sup>nd</sup> Order Global Analysis Software

**Pro-Kineticist II (Pro-K II)** provides the latest global analysis capability for the elucidation of reaction mechanisms and their associated parameters from kinetic spectrophotometric data. Fitting may be conducted on single or multi-wavelength kinetics (1<sup>st</sup> order analysis) and to multiple data sets acquired at different starting concentrations (2<sup>nd</sup> order analysis).

### Key Feature Summary

- Second order globalisation now allows the analysis of concentration dependant kinetic data
- Greatly enhanced determination of rates and spectra of reagents and reaction intermediates
- Mixed data types: simultaneous analysis of fluorescence and absorbance (and CD) data to further aid the elucidation of reaction rates and mechanisms
- New support for rapid equilibrium and protonation steps in the reaction model
- Comprehensive visualisation tools for inspecting results
- Full data simulation tools
- Compatible with Windows 9x/2000/Me/NT/XP

### 2<sup>nd</sup> Order Global Analysis

Pro-K II allows the simultaneous analysis of multiple datasets gathered at different sets of initial conditions in what we term a 2<sup>nd</sup> order global analysis. This significantly increases the ability of the software to resolve rates and intermediate spectra of more complex reaction mechanisms without the extra provisions necessary for Pro-K, our original global analysis package. The simplest example is the 2<sup>nd</sup> order reaction:  $A+B \rightarrow C$ . Pro-K cannot directly determine the spectra of A and B as they are linearly dependant; it is necessary to fix the spectrum of one of the reactants. However, by performing the reaction at two (or more) starting concentrations of A and B and submitting the data to Pro-K II, the spectra and the rate can be routinely obtained. A more complex example which, by virtue of moving to 2<sup>nd</sup> order analysis can now be successfully resolved is:  $A+B \rightleftharpoons C \rightleftharpoons D$ , where all spectra and all forward and reverse rates can now be determined using Pro-K II.

### Rapid Equilibrium Feature

Pro-K II now incorporates the ability to model rapid equilibria by allowing the incorporation of association constants into the fitting mechanism. These may be either fixed or fitted. Since rapid protonation steps can be included in this way, pH dependant data sets can now be exploited in the global analysis. The association constant is typically provided as a pK, and in addition to the chemistry of interest, any buffers used can also be fully modeled during the fit. These are typically provided with a fixed (known) pK. However, any pH changes due to protonation changes are fully modeled and buffering in some cases is now superfluous.

The ability to fully model pH changes and their influence on the underlying chemistry in one step reduces the need for traditional buffering. This has potential advantages:

1. Removal of the need for preparation of buffer solutions at different pH, which can be time consuming.
2. Exclusion of buffers that could interfere with the reaction under investigation.
3. Exclusion of buffers that can absorb at the wavelength under investigation.
4. It is no longer necessary to extrapolate the total buffer concentration to zero, which would normally be required for properly defined thermodynamic constants.

*It is no longer necessary to experiment on strongly buffered environments; pH changes can now be accounted for through the inclusion of all relevant equilibria.*

## Simultaneous Analysis of Two Types of Measurement

Pro-K II enables absorbance and fluorescence (or CD) data sets to be analysed globally to a common underlying mechanism. This is useful if different species are detected by different events in the mechanism such that the complete reaction model can only be fitted when all data sets are present simultaneously to determine all spectra and rates involved.

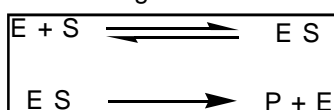
## Simulation Tool

A powerful simulation feature is incorporated into Pro-K II allowing the user to experiment with reaction models and rate and spectral parameters. Data can then be synthesized with added noise to explore the analysis (fitting) capabilities of Pro-K II, as well as to predict the experimental behaviour or sensitivity of a reaction to adjustments in particular parameters.

The simulation tool can also be used to familiarise the user with all of the features incorporated into the package without the need for real data.

## Example 1—Simulation and Fitting of the Michaelis-Menten Mechanism

The mechanism for a Michaelis-Menten reaction is given below:



This example utilises synthetic data created using the simulation feature of Pro-K II. The model is entered into the Simulation window, and the key parameters for each data set provided, including rates, spectral features and initial concentrations. Individual data sets are created for each set of starting reagent concentrations. The saved data can then be re-loaded and the analysis module used to fit the data to the theoretical model. This process is used to demonstrate the effectiveness of the minimisation algorithm when provided with such data (synthetic or real), a theoretical mechanism and only estimates of the true underlying rates and other parameters. Convergence of the fit to the correct original simulation parameters is a useful indication that similar fits to real data sets will also produce correct results.

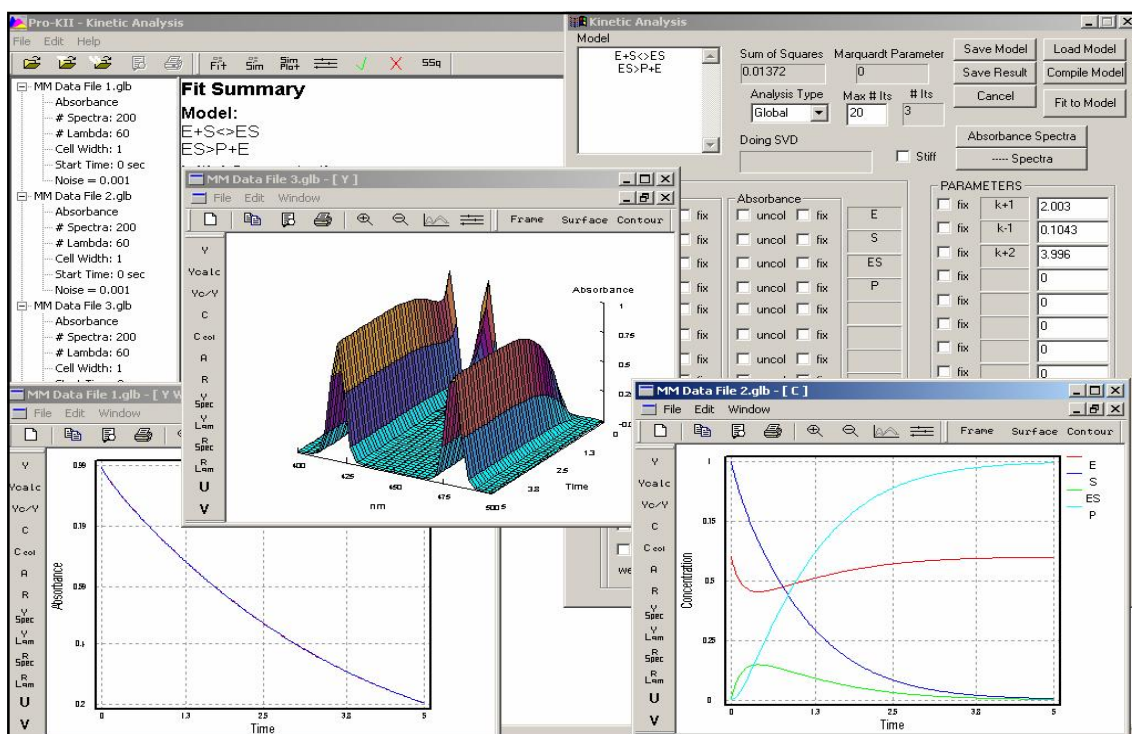
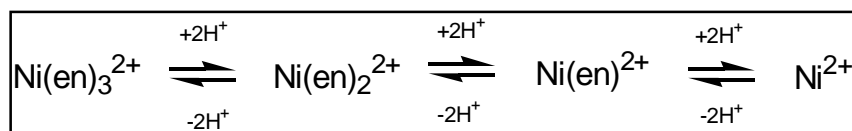


Figure 1— Pro-K II document interface

The concentration profile window (MM Data file 2, Figure 1) shows the change in concentration of all the reagents and intermediates associated with the reaction. The original 3D data window (MM Data file 3, Figure 1) gives the absorbance of each coloured species over a range of wavelengths as a function of time. The 2D wavelength plot (MM Data file 1, Figure 1) gives the absorbance at a specific wavelength (or over a range of wavelengths) as a function of time.

## Example 2—Fitting Rates of dissociation for Ni(ethylenediamine)<sub>3</sub><sup>2+</sup> Complex

Ni(en)<sub>3</sub><sup>2+</sup> degrades in presence of acid according to the following mechanism:



The model incorporated into Pro-K II for this reaction is displayed in the kinetic analysis window (Figure 2). Data obtained at various concentrations, including pH are submitted into Pro-K II (Ni-en Data Files 1-4), the model is compiled and the initial concentration for each species entered. Any known constants are added and fixed, and the data fitted. The change in proton concentration throughout the reaction is fully modelled by inclusion of the relevant association constants into the parameters section of the kinetic analysis window, and fixed prior to fitting.

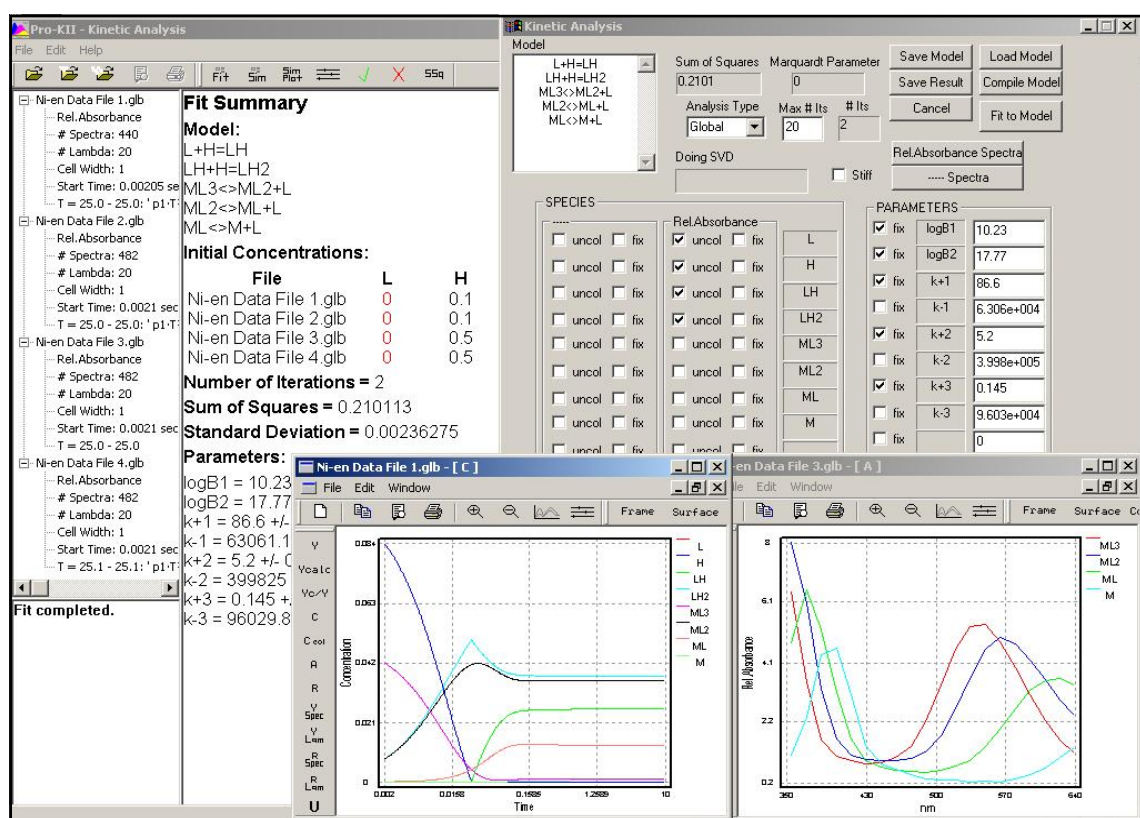


Figure 2 -Typical Pro-K II document interface

Once the fit is complete, a window for each data set can be opened allowing the user to view the spectra for each coloured species over a range of wavelengths, residuals, concentration profiles and fitted spectra, along with the number of coloured species present in the reaction. Ni-en Data file 1 (Figure 2) gives the concentration of each species as a function of time, using a log time base. Ni-en Data file 3 (Figure 2) shows the spectrum of each coloured species present in the reaction. These views and others can be shown for each set of conditions to give the user maximum flexibility in data analysis.

This example and others, along with the relevant data files are included in the Help file incorporated into Pro-K II.

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