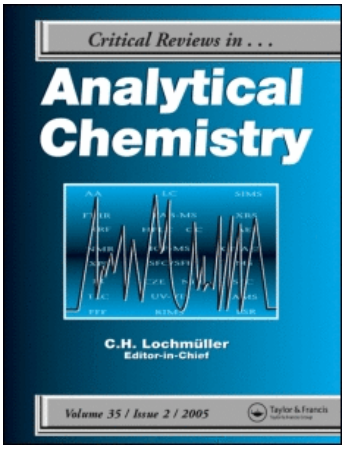


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# Model-Based Analysis for Kinetic and Equilibrium Investigations

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# Model-Based Analysis for Kinetic and Equilibrium Investigations

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**Equilibrium and kinetic data usually can be described quantitatively by a chemical model that is based on the law of mass action. In such instances parameters of interest like rate and equilibrium constants and, depending on the nature of the data, also spectral information can be determined by model-based analysis of the appropriate data sets. In this contribution the essential aspects of the complete process are discussed, these include data acquisition, modelling of the concentration profiles and the actual fitting algorithms which are identical for both types of investigation. An overview of recent developments like globalisation of the analysis and attempts to analyse industrially relevant data incorporating corrections for non-ideal behaviour are also given.**



## INTRODUCTION

In any data analysis we deal with a set of measured data from which we try to extract useful information. Within the context of kinetic and equilibrium investigations there are two fundamentally different ways of extracting information: model-based analyses and model-free analyses.

In model based analysis, the goal is to extract from the measurement the basic parameters of the process, i.e., the rate constants in kinetics and the equilibrium constants in equilibrium investigations. Consider rate constants in kinetics. Such constants are of great value as they allow predictions of chemical behaviour under different yet unexplored conditions. They also allow comparisons with other systems and of course there are tremendous numbers of published rate and equilibrium constants. Depending on the nature of the data additional information can also be extracted, e.g., if absorption spectra are acquired during the process it is possible to determine molar absorption spectra of all reacting species, even if they only exist as transitory minor species. Such spectra allow structural analysis which is not easily achieved otherwise.

In model-free analyses [Anna de Juan and Romà Tauler, Multivariate Curve Resolution (MCR) from 2000: Progress in Concepts and Applications, *Critical Reviews in Analytical Chemistry* 36(3-4) (2006):163–176.] the analysis is restricted to the determination of response curves, usually concentration profiles of the active species and their spectral shapes.

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In this contribution, we will demonstrate that many aspects of model-based data fitting are identical for kinetic and equilibrium studies. Thus, we aim at providing a unified view of data fitting that encompasses very different kinds of data and processes. Naturally, we will not ignore the differences between the two kinds of processes.

There are three distinct components in a model based analysis (a) the data, (b) the chemical model used to describe the chemical process and (c) the fitting of the parameters (e.g., rate constants) that are pertinent to the model.

## THE DATA

In order to be able to fit a model to a measured set of data, more data are required than the minimum number needed to determine the parameters of the model. For example, two (x,y) data pairs are sufficient to define a straight line. However such a line is calculated and not fitted; only if three or more data pairs are used to define a straight line can we speak of fitting a line. With modern computerised instruments, there are usually many more data available than parameters that need to be fitted. Not only does this result in parameters that are much better defined, there is also statistical information available about the parameters and, very importantly, there is an indication about the validity of the chosen model.

A complete set of data comprises two components: the independent variables, such as the reaction time in a kinetic experiment; and the dependent variables, such as absorption readings acquired as a function of time. In an equilibrium investigation, the independent variable typically is the volume of added reagent

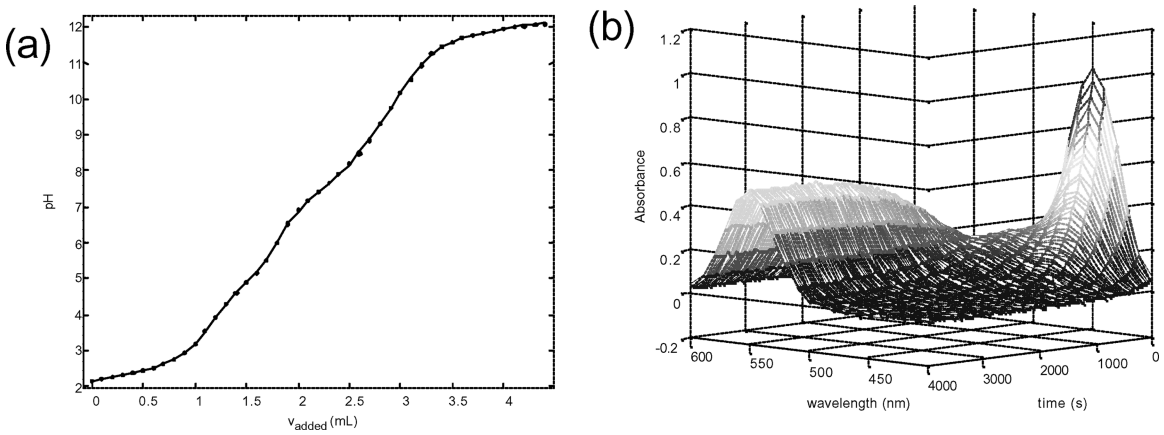


FIG. 1
(a) A potentiometric titration as an example of a monovariate data set; (b) A series of absorption spectra measured as a function of time as an example of a multivariate data set.

and the dependent variable is the pH reading or a measured spectrum as a function of the reagent addition.

Sometimes it is useful to distinguish between mono-variate and multi-variate data. However, it needs to be stressed that mono-variate can be regarded as a special case of multi-variate data. For monovariate data, the complete data set can be arranged in two vectors: a vector $\mathbf{x}$[1] of $n$ elements for the dependant variable, e.g. time in kinetics, and a vector $\mathbf{y}_{\mathbf{meas}}$ with the same number of elements for the dependant variable, e.g. the absorptivity at a particular wavelength, or the conductivity, or the pH or any other signal. The convention is to store the data in column vectors $\mathbf{x}$ and $\mathbf{y}_{\mathbf{meas}}$. If $n$ measurements were taken the two vectors comprise $n$ elements. A typical mono-variate data set is represented in Figure 1(a), which displays a potentiometric pH titration where the pH of a solution is measured as a function of the addition of the reagent, usually a strong base.

With modern instrumentation, multivariate data are readily available. The typical example is the diode array spectrophotometer which delivers a complete absorption spectrum of a solution at one particular point in the process under investigation. There are other examples, such as the IR spectrum provided by a Fourier-transform infrared instrument or an NMR spectrum. For each value of the independent variable (e.g., time) a complete spectrum is acquired and the convention is to store them as rows in a matrix $\mathbf{Y}_{\mathbf{meas}}$. The matrix $\mathbf{Y}_{\mathbf{meas}}$ then has the dimensions $n \times l$ where $n$ is the number of experimental points and $l$ the number of wavelengths (or equivalent) at which the spectra were taken. Figure 1(b) displays a data matrix of a multiwavelength kinetic investigation.

## THE EXPERIMENT

The experiments for kinetic and equilibrium studies are necessarily different. We will discuss the two separately.

[1]Note; we use bold lower case characters for vectors, bold upper case characters for matrices, italic characters for scalars and chemical species.

### Kinetics

In kinetics the experiment is conceptually much simpler than in equilibrium investigations (1, 2). The reaction is initiated by mixing the reactants and subsequently the signal, mono- or multi-variate, is recorded as a function of time. For slow reactions the mixing can be carried out manually, e.g., by mixing two reactant solutions in a cuvette, placing the cuvette into the light path of the spectrophotometer and initiating data acquisition. Using such manual manipulations, the spectra can be recorded within approximately 10 seconds after the mixing. Stopped-flow instruments replace manual mixing by pneumatically (or stepper-motor) driven syringes. In stopped-flow instruments, the mixing deadline is about 1 $\mu$sec, limited by the hydrodynamics of the solutions. In situ generation of one or several reagents by techniques such as pulse radiolysis or flash photolysis allows the investigation of faster reactions. Alternative techniques such as temperature-jump are not used much presently.

In kinetics the independent variables are the reaction times at which the data are acquired, they are conveniently collected in a column vector $\mathbf{t}$ (instead of the vector $\mathbf{x}$) of $n$ elements. Another independent variable is the set of initial concentrations of the reactants. These concentrations are conveniently stored in a row vector $\mathbf{c}_0$. Usually this vector is comprised of two to three initial concentrations, however, there is no theoretical limit to the complexity of the reaction investigated and thus to the number of initial concentrations. In Scheme 1, the sets of independent and dependent variables for kinetics and equilibrium studies are represented graphically.

### Equilibrium Investigations

Equilibrium investigations generally require more complex experiments (3). A titration is the preparation of a series of solutions which contain different total concentrations of the components that interact with each other to form the equilibrium species. The components are the simplest entities, e.g., metal

| | Independent variables, experimental conditions | Dependent variables, measure data |
|---|---|---|
| kinetics: | t $c_0$ | $Y_{meas}$ or $y_{meas}$ |
| equilibrium study: | $C_{tot}$ | |

SCH. 1

ions, $M$, and/or ligands, $L$, and/or protons, $H$. Their combination results in one or many different species, such as metal ligand complexes, $ML_x$, or protonated species such as $LH_y$, or protonated complexes such as $ML_xH_y$. The measurements comprise one or several data of each solution, e.g. absorption at one or many wavelengths in a spectrophotometric titration, or very typically the pH of the solution in a potentiometric titration. The potentiometric determination of the free metal concentration with ion-selective electrodes is conceptually very similar with pH measurements, but they are far less common; they are. It is also possible to acquire multiple types of data, e.g., spectra and pH of the solutions.

The independent variables in equilibrium studies are the total concentrations of the components. They are conveniently stored in a matrix $\mathbf{C}_{tot}$, whose columns are formed by the total concentrations of the components in the $n$ different solutions. Refer to Scheme 1 for a graphical representation.

The preparation of the required series of solutions is called a 'titration'. While these solutions can be prepared manually in a series of volumetric flasks, automatic computer controlled titrations are clearly superior. Precision is generally much better and dilution effects are straightforwardly accounted for in the computational analysis.

Titrations generally serve two different purposes: (a) they are used for quantitative analyses, determination of the concentration of an acid or a base, in analytical chemistry. Then, the determination of the equivalence or end-point is the main objective (4, 5); (b) in the context of this review, the task is the determination of equilibrium constants. However, these two objectives require only marginally different experimentation and very minor adaptation of the data analysis software.

Irrespective of the purpose of the titration, the appropriate reagent has to be added to the reactant(s). A metal-ligand complexation investigation in an aprotic solvent consists of the addition of ligand solution to a metal solution or vice versa, of course with the acquisition of an appropriate set of data. In aqueous solution most equilibria are pH dependent and in such cases the titration often involves the addition of a base solution (e.g. NaOH) to an acidified solution of the substrate. In the case of a protonation equilibrium, this acidified solution contains the protonated form of the acid-conjugate base couple, in a metal-ligand complexation study, the acidified solution contains the protonated ligand and the free metal.

There are several techniques to perform titrations (6, 7). We restrict the discussion to automatic, computer controlled titrations with an automatic burette and recording of signal (e.g. pH and/or spectrum). Figure 2(a) is a schematic representation of a computer controlled potentiometric pH titration. The computer, running the appropriate software, controls the burette and monitors the pH. Both external instruments are interfaced to the computer via an A/D-D/A board. In commercial set-ups this is

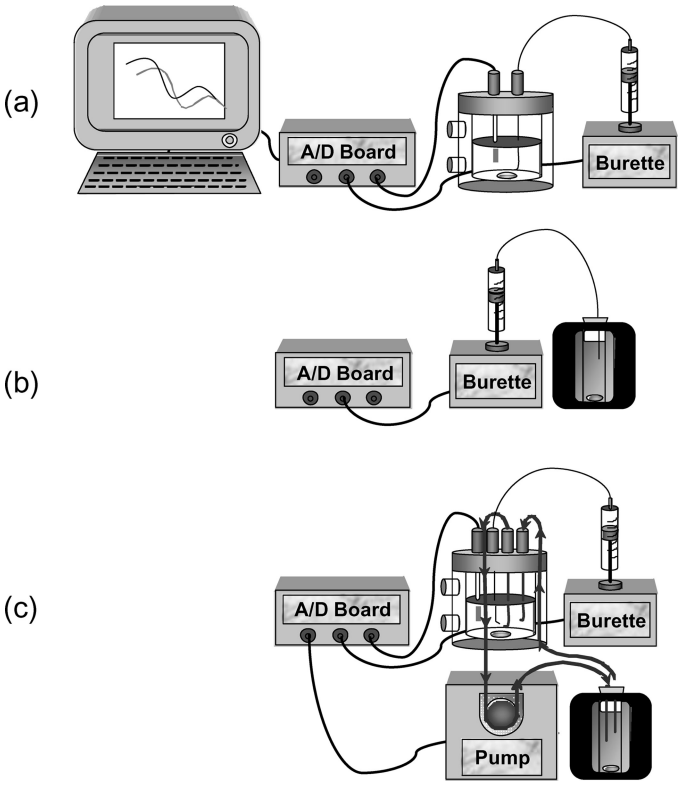


FIG. 2

Several typical computer controlled titration setups: (a) A potentiometric titration; (b) A spectrophotometric titration with solution mixing in the cuvette; (c) A spectrophotometric and potentiometric titration with external mixing and pumping of the solution through a flow-cell in the spectrophotometer.

usually incorporated into the computer, in research laboratories this is often done via external A/D boards. The sequence of events is: addition of reagent through the burette; mixing and waiting for the establishment of the equilibrium; acquisition of the data (pH). Figure 2(b) represents a spectrophotometric titration where the reagent is delivered directly into the cuvette placed in the spectrophotometer. Naturally, the solution has to be stirred. Minimal volumes of solutions are required in this mode. Figure 2(c) represents another alternative. Here the solutions are mixed in a standard titration vessel, then they are pumped through a flow cell situated in the spectrophotometer. Additional measurement of the pH is easily achieved by placing an electrode in the titration vessel. Minor variations on these arrangements include the possibility of having the pH electrode in the cuvette of set-up 2(b) or using a fibre-optic optrode to measure the absorption spectrum in the set-up in Figure 2(a). It is straightforward to adapt to other spectroscopic techniques such as NIR, IR or NMR.

As described earlier, the original data are collected in a vector $\mathbf{y}_{\mathbf{meas}}$ or a matrix $\mathbf{Y}_{\mathbf{meas}}$, together with the total concentrations of the components for each solution.

In traditional measurements, temperature and ionic strength need to be kept as constant as possible. Thermostating is easy, whereas maintaining constant ionic strength is difficult and can usually only be approximately achieved by adding an excess of an inert salt. It will be demonstrated later that is possible to allow variations in temperature and ionic strength if these changes are incorporated into the modelling computations.



## THE MODEL

### Computation of the Concentration Profiles

Apart from the actual measurement, the computations required for the numerical analysis also differ for kinetic and equilibrium studies. Nevertheless, several aspects between the two types of data are essentially identical.

The core of the model-based data fitting is the computation of the concentration profiles of all interacting species. The concentration profiles are commonly collected as columns of a matrix **C**. Each column of **C** contains the concentration of one particular species as a function of the progress of the reaction or titration. If there are *ns* species the matrix **C** has the dimensions $n \times ns$, where $n$ is the number of measurements (spectra or other data).

Naturally, the algorithms required for this task are different for the two types of processes considered here. The concentrations of all reacting species are computed for each solution in equilibrium studies and at each reaction time in kinetics. These computations are based on the independent variables defined by the experiment; they are the analytical or total concentrations of the components in the equilibria or the initial concentration together with the reaction time in kinetics. These concentrations together with the chemical model allow the determination of all the species concentrations. See Figure 3 for a graphical representation.

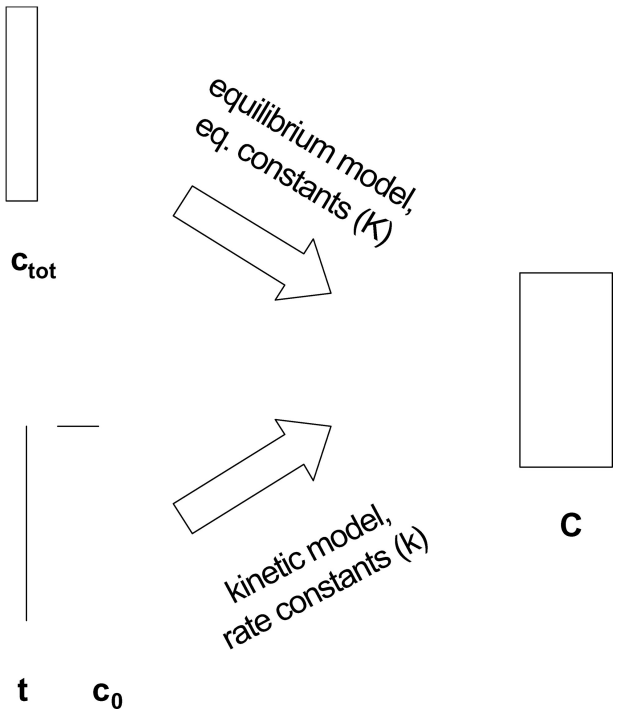


FIG. 3
The independent variables and the parameters are required for the computation of the matrix **C** of concentration profiles.

The main difference between software dealing with equilibrium and software dealing with kinetic investigations is this particular step, the computation of the concentration matrix based on the independent variables.

### Kinetics

In kinetics the model quantitatively describes the concentrations of all reacting species, it is defined by a reaction mechanism and the pertinent rate constants. An instructive example is a consecutive reaction scheme with two first order reaction steps as shown in Eq. [1].

$$X \xrightarrow{k_1} Y \xrightarrow{k_2} Z \qquad [1]$$

The mechanism defines a system of ordinary differential equations (ODE) (1, 2). For the above example the ODEs are [2].

$$\begin{aligned} \frac{\mathrm{d}[X]}{\mathrm{dt}} &= -k_1[X] \\ \frac{\mathrm{d}[Y]}{\mathrm{dt}} &= k_1[X] - k_2[Y] \\ \frac{\mathrm{d}[Z]}{\mathrm{dt}} &= k_2[Y] \end{aligned} \qquad [2]$$

This system of ODEs needs to be solved or integrated. If the reaction mechanism only includes first order reaction steps, as is the case for the preceding example, the integration can be

performed explicitly. For the initial concentration $[X]_0$, $[Y]_0 = 0$ and $k_1 \neq k_2$ integration of Eqs. [2] results in:

$$\begin{aligned}[X] &= [X]_0 e^{-k_1 t} \\ [Y] &= [X]_0 \frac{k_1}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) \\ [Z] &= [X]_0 - [X] - [Y]\end{aligned} \quad [3]$$

For the above example, the matrix **C** of concentration profiles will have three columns, one for each concentration profile for the species $X$, $Y$ and $Z$.

For most mechanisms there is no explicit solution, in such cases numerical integration is necessary (8–10). Numerical integration involves approximations of the concentrations based on the differential Eqs. [2]. Algorithms that are time efficient and accurate are rather difficult to develop and will not be further discussed here. Fortunately numerical integration routines are readily available (8). It is important to distinguish between stiff and non-stiff problems, the first require special stiff solvers, and the latter are best solved by standard algorithms of the Runge–Kutta type.



## Equilibrium Studies

For equilibrium studies the analysis is based on the law of mass action (11, 12). The equivalent of the reaction mechanism in kinetics, e.g., Eq. [1], is the collection of components and species that are formed from the components. For the example of a metal-ligand equilibrium study, in aqueous solution, the following species might be formed:

$$M, ML, ML_2, L, LH, LH_2, H \quad [4]$$

In this example the components include $M$, $L$ and $H$ while the species include the components as well as $ML$, $ML_2$, $LH$ and $LH_2$. In aqueous solution, Lewis bases (ligands) are generally also Brønstedt bases, and thus the protonation of the ligand has to be included. In aprotic solvents, protonation does not need to be taken into account.

The independent variables in a titration are the total concentrations of the components, in the example they are $[M]_{tot}$, $[L]_{tot}$ and $[H]_{tot}$. They are computed from the dilutions occurring during the titration.

The Newton–Raphson technique is generally used to compute all species concentrations for a given set of total component concentrations and all required equilibrium constants (11, 12).

The law of mass action applies to the formation of each species; in a general way, for the formation of the species $M_x L_y H_z$, we can write:

$$\beta_{xyz} = \frac{[M_x L_y H_z]}{[M]^x [L]^y [H]^z} \quad [5]$$

Such an equation is written for each species formed from the components. An additional set of equations can be written that defines the total concentrations of all components.

$$\begin{aligned}M_{tot} &= \sum x[M_x L_y H_z] = \sum x\, \beta_{xyz}[M]^x[L]^y[H]^z \\ L_{tot} &= \sum y[M_x L_y H_z] = \sum y\, \beta_{xyz}[M]^x[L]^y[H]^z \\ H_{tot} &= \sum z[M_x L_y H_z] = \sum z\, \beta_{xyz}[M]^x[L]^y[H]^z\end{aligned} \quad [6]$$

Thus there are as many equations as there are unknowns, and hence there is a unique solution to that system, it is the one realised in the actual chemical solution. The Newton–Raphson algorithm starts with a set of initial guesses for the free component concentrations, from these the other species concentrations are computed via Eqs. [5], then the total concentrations are computed by applying Eqs. [6]. These computed total concentrations are compared with the known total concentrations. Iterative refinement is based on a Taylor-series expansion of the difference between computed and known total concentrations. Of course alternative algorithms are possible, but they are generally much slower and not recommended.

The matrix **C** is composed from all species concentrations; for each physical solution a row of the species concentrations is formed. The dimensions of **C** are as in the case of kinetics, $n \times ns$. Each column of **C** contains the concentration profile of one particular species during the titration.

## Establishing the Connection Between Concentration Profiles and Measurement

The next step is the establishment of the relationship between the computed concentrations **C** and the actual measurement. This step is independent of the kind of process under investigation, i.e., it is the same for kinetic or equilibrium studies. It does, however, depend on the type of data that are collected.

The most common measurement technique is spectrophotometry in the UV-Vis region of the electromagnetic spectrum. Here Beer–Lambert's law governs the relationship between the concentrations and the absorption measurement. The relationship can be expressed in a matrix equation:

$$\mathbf{Y_{calc}} = \mathbf{C}\,\mathbf{A} \quad [7]$$

The product of the concentration matrix **C** and the matrix **A** of the molar absorptivities (molar absorption spectra organised as the rows of **A**) forms the computed matrix $\mathbf{Y}_{calc}$. The matrix **A** has dimensions $ns \times l$. If there are measurements at only one wavelength, the matrices $\mathbf{Y}_{calc}$ and **A** reduce to column vectors $\mathbf{y_{calc}}$ and **a**, otherwise the equations are identical.

There are several other spectroscopic techniques which also feature a linear dependence between concentration and signal and thus are governed by Beer–Lambert's law and for which the preceding equation applies. These techniques include spectroscopies in the NIR or IR (if recorded as absorptions rather than transmissions), circular dichroism (CD), NMR as long as the processes are slow on the NMR timescale (if this is not the case, e.g., protonation equilibria, the NMR spectra have to be analysed differently), and ESR.

Important alternatives to spectrophotometry include potentiometric methods that determine specific concentrations such as pH measurements or any other ion-specific electrodes. All potentiometric methods deliver a signal that is proportional to the logarithm of the concentration. In a potentiometric pH titration the measurement $\mathbf{y_{calc}}$ is:

$$\mathbf{y_{calc}} = -\log(\mathbf{C}_{:,\mathbf{H}}) \quad [8]$$

We adapt a Matlab-based notation where $\mathbf{C}_{:,\mathbf{H}}$ signifies the column of $\mathbf{C}$ which contains the proton concentration.

For slow kinetics, it is possible to perform chromatographic analyses of samples taken during the reaction. These measurements directly deliver concentrations of one or several reacting components (13).

## FITTING

The task of the fitting algorithm is to determine the set of parameters for which the fit is optimal. Before we turn to the actual fitting algorithm, we need to define clearly what the parameters are that are fitted and what the optimal solution is (14–18).

### The Parameters

Any variable that is required to compute $\mathbf{Y_{calc}}$ is potentially a parameter that can be fitted. Obvious parameters are the rate constants in a kinetic study or the equilibrium constants in an equilibrium study, but also the molar absorptivities of the different species in a spectrophotometric investigation are parameters. In fact, the complete matrix $\mathbf{A}$ of Eq. [7] is made up from unknown parameters. There are also less obvious parameters that can be fitted, e.g., the initial concentrations of the starting materials in kinetics, or the initial concentration of any of the reagents in a titration. In such instances, the data are analysed in terms of concentrations rather than the equilibrium constants. This of course is the more common mode in analytical chemistry. The parameters then are one or several of the initial concentrations of the solutions used for the titration and which define the matrix $\mathbf{C}_{\text{tot}}$ of total concentrations. The standardisation of a NaOH solution as determined by a titration with a primary standard acid, such as potassium hydrogen phthalate, is best seen as the fitting of the base concentration.

It is theoretically possible to use kinetic data in a similar way for quantitative analytical purposes. For example, the curvature of the concentration profiles of a second order reaction does contain information about initial concentrations. However, the influence is very subtle and the concentrations are only very poorly defined and as such it is not a technique for concentration determination that can be recommended.

The list of potential parameters is long and it is tempting but dangerous to fit too many of them. If badly defined or strongly correlating parameters are fitted, the resulting fitted parameters will be very poorly defined. This is manifest to some extent by the error margins, but not all fitting techniques supply them and then the problem easily remains unnoticed. Many parameters are completely correlated, e.g., in a first-order reaction $A \rightarrow B$, the initial concentration $[A]_0$ is completely correlated with the molar absorption spectrum of $A$. Hence, it is not possible to fit both.

Equations [7] and [8], along with any other type of measurement, can be written in a generalised way, indicating that the model and its parameters define the calculated data:

$$\mathbf{Y_{calc}} = \text{f(parameters, model)} \quad [9]$$

The longer the list of parameters the more difficult the fit and the more likely there are strong correlations.

Consider Eq. [7]; all elements of the matrix $\mathbf{A}$ are parameters. To fit them all appears to be an overwhelming task. In fact, if they are all treated in the same way as the other parameters (i.e. rate or equilibrium constants), hardly any fitting algorithm could cope with the task. It is absolutely crucial to recognise that the matrix $\mathbf{A}$ is composed of linear parameters which can be computed explicitly. For any matrix $\mathbf{C}$ as defined by the rate or equilibrium constants, the best corresponding matrix $\mathbf{A}$ can be computed as

$$\hat{\mathbf{A}} = \mathbf{C}^{+}\mathbf{Y} \quad [10]$$

where $\mathbf{C}^{+}$ is the so called pseudo-inverse of the matrix $\mathbf{C}$. It can be calculated as $\mathbf{C}^{+} = (\mathbf{C^t C})^{-1}\mathbf{C^t}$ or by numerically more advanced and superior methods (8). This substitution of $\mathbf{A}$ by $\hat{\mathbf{A}}$ dramatically reduces the number of parameters to be fitted iteratively to those defining the matrix $\mathbf{C}$ (rate or equilibrium constants) and possibly a few extra parameters such as initial concentrations (19).

### The Residuals and the Sum of Squares

The task of the fitting algorithm is to find that, hopefully unique, set of parameters for which the measured data $\mathbf{Y_{meas}}$ and their calculated values $\mathbf{Y_{calc}}$ are as similar as possible. We need a properly defined measure for this similarity. The sum over all the squares of the differences between $\mathbf{Y_{meas}}$ and $\mathbf{Y_{calc}}$ is the almost universally accepted measure. The differences are called the residuals $\mathbf{R}$

$$\mathbf{R} = \mathbf{Y_{meas}} - \mathbf{Y_{calc}} = \mathbf{Y_{meas}} - \mathbf{C}\,\hat{\mathbf{A}} \quad [11]$$

It must be recognised that the residuals are defined as a function of the non-linear parameters only. Note that for univariate data $\mathbf{r}$, $\mathbf{y_{meas}}$ and $\mathbf{y_{calc}}$ are vectors instead of matrices, otherwise there are no differences.

The sum of squares, *ssq*, is defined as

$$ssq = \sum_i \sum_j R_{i,j}^2 \quad [12]$$

Again, for vectors of data, $\mathbf{r}$ is also a vector and the summation is over one dimension only.

While there are others, the minimisation of *ssq* is by far the most common approach. There are statistical reasons for such a choice, in fact for normally distributed noise, the minimisation of *ssq* results in the most likely outcome (8, 20). Even if the requirement of white noise is not met, *ssq* fitting usually results

in very good estimations for the true parameters. Probably more importantly, *ssq* based algorithms can be written very efficiently and thus are much faster than any of the alternatives. An example of an alternative to *ssq* fitting would be minimisation of the largest residual. This is possible, and in certain cases useful, but it is computationally much more demanding.

## Least-Squares Fitting, the Newton–Gauss Algorithms

All algorithms for the fitting of non-linear parameters are iterative (8, 14). Starting from an initial set of guessed values for the parameters, these are varied systematically until the minimal sum of squares is found. Direct search methods that apply brute force by covering the whole parameter space are conceptually very easy, but for any number of parameters larger than two or three they are unacceptably slow. More sophisticated algorithms such as the simplex algorithm are still fairly simple, but to achieve reasonable speed complex shrinking and growing of the simplex has to be implemented. Simplex algorithms are feasible for small numbers of variable parameters, such as five or smaller, but of course there are no absolute limits. The main disadvantage of the simplex algorithm is the lack of any information regarding the determinability of the parameters. It is easy to fit too many parameters and the algorithm will not give any indication about the usefulness of the results.

The Newton–Gauss algorithm, adapted for *ssq* minimisation is the method of choice (19, 20). It is fast, relatively straightforward to implement and it delivers estimates for the standard deviations of the fitted parameters. The Newton-Gauss algorithm for *ssq* minimisation requires the computation of the derivatives of the residuals with respect to the parameters. These derivatives are collected in the Jacobian **J**.

$$\mathbf{J} = \frac{\delta \mathbf{R}}{\delta \mathbf{par}} \qquad [13]$$

The parameters that are fitted are collected in the vector **par**. The computation of **J** is relatively time consuming but the reward is the speed of convergence which is quadratic close to the minimum. It is always possible to approximate **J** numerically:

$$\frac{\partial \mathbf{R}}{\partial par_i} \cong \frac{\mathbf{R}(\mathbf{p} + \Delta par_i) - \mathbf{R}(\mathbf{par})}{\Delta par_i} \qquad [14]$$

In Eq. [14], $\mathbf{p} + \Delta par_i$ is a new parameter vector with only the $i$-th parameter $par_i$ shifted by the small amount $\Delta par_i$. Typically $\Delta par_i$ is calculated as $1 \times 10^{-4}\ par_i$.

If the matrix **C** can be computed explicitly it is usually also possible to compute the derivatives of the matrix **C** with respect to the parameters $\frac{\delta \mathbf{C}}{\delta \mathbf{par}}$. In such instances it is also possible to explicitly define **J** as a function of $\frac{\delta \mathbf{C}}{\delta \mathbf{par}}$ (19). These instances however are rare and often not worth the extra complexity of programming.

We repeat, in the case of spectrophotometric data, particularly multiwavelength measurements, it is absolutely essential to implement the elimination of the linear parameters as indicated in Eq. [10]. The parameter vector **par** only contains the non-linear parameters. It would go beyond the aims of this review to describe the Newton-Gauss algorithm in any detail. The important aspects are as follows:

The iterative refinement of the parameters is given by the following formula. The shift vector $\Delta\mathbf{par}$ is computed and added to the vector **par**.

$$\Delta \mathbf{par} = -\mathbf{J}^{+}\mathbf{R} = -(\mathbf{J}^{\mathbf{t}}\mathbf{J})^{-\mathbf{1}}\mathbf{J}^{\mathbf{t}}\mathbf{R} \qquad [15]$$

Usually this results in convergence and the minimum in *ssq* is reached in a few iterations. The test for convergence is performed by comparing the calculated *ssq* with the value from the previous iteration. If improvement is below a certain threshold, i.e. the shift in the parameters resulted in no further improvement of the *ssq* value, then the process is terminated and the results are reported. In the case of divergence (increase in *ssq* from one the next iteration) the well-proven Marquardt-Levenberg algorithm is invoked (8, 19, 20). The pseudo-inverse $\mathbf{J}^{+}$ is calculated as $\mathbf{J}^{+} = (\mathbf{J}^{\mathbf{t}}\mathbf{J})^{-\mathbf{1}}\mathbf{J}^{\mathbf{t}}$ and the Marquardt parameter is added to the diagonal elements of $(\mathbf{J}^{\mathbf{t}}\mathbf{J})$ prior to inversion. Increasing the Marquardt parameter shortens the shift vector and directs it to the direction of steepest descent.

The availability of estimates for the standard deviations of the fitted parameters is a crucial advantage of the Newton-Gauss algorithm. The relevant information is contained in the inverse of the Hesse matrix $(\mathbf{J}^{\mathbf{t}}\mathbf{J})^{-1}$ (20). The standard error $\sigma_{par_i}$ in the fitted parameters $par_i$ can be estimated from the expression

$$\sigma_{par_i} = \sigma_r \sqrt{d_{i,i}} \qquad [16]$$

where $d_{i,i}$ is the $i$-th diagonal element of the inverted Hesse matrix $(\mathbf{J}^{\mathbf{t}}\mathbf{J})^{-1}$. $\sigma_r$ represents the estimated standard deviation of the measurement error in $\mathbf{Y}_{\mathbf{meas}}$ or $\mathbf{y}_{\mathbf{meas}}$.

$$\sigma_r = \sqrt{\frac{ssq}{df}} \qquad [17]$$

The denominator represents the degree of freedom, *df*, which is defined as the number of experimental values $m$ (elements of **Y** or **y**), subtracted by the number of optimised parameters $np$.

$$df = m - np \qquad [18]$$

As mentioned, convergence of the Newton-Gauss algorithm is quadratic near the minimum, however, far from the minimum convergence cannot be guaranteed and can be slow. For completely new investigations it can be difficult to guess sufficiently good initial values for the parameters. Genetic algorithms (GA) have been developed that efficiently cover the whole parameter space (21, 22). Convergence is notoriously slow near the minimum and the obvious strategy is to run a GA-based program to localise the vicinity of the minimum and subsequently switch to a fast Newton-Gauss method to reach the exact minimum.

## Published Software Packages

There are a few software packages available for the fitting or equilibrium and kinetic data. Well established are the QUAD

familiy of programs (15, 23), which includes MINIQUAD, SUPERQUAD and HYPERQUAD. SPECFIT is another well-used package which includes analysis tools for spectrophotometric titrations and kinetics (24, 25). Less widely spread are a few packages for spectrophotmetric titrations (26–28). The most recent kinetic package (Pro-kll) is described in (29, 30).

## RECENT DEVELOPMENTS

There are several published algorithms that incorporate some, or most, of the aspects discussed so far. These methods could be called standard. There are a few more recent and not yet generally accepted developments, which are discussed next.

### The Singular Value Decomposition

Modern diode-array spectrophotometers deliver absorption readings at typically 1024 wavelengths. Thus, the number of columns in the matrices $\mathbf{Y_{meas}}$, $\mathbf{A}$ and $\mathbf{R}$ is substantial and consequently the number of individual absorption measurements can be very large. It is possible to reduce the number of columns in $\mathbf{Y_{meas}}$, $\mathbf{A}$ and $\mathbf{R}$ and thus the number of elements in these matrices dramatically.

According to the singular value decomposition (8), it is possible to decompose the matrix $\mathbf{Y_{meas}}$ as

$$\mathbf{Y_{meas}} = \mathbf{USV} \qquad [19]$$

There are many benefits that can be gained from this decomposition, it is beyond the scope of this review to discuss them in depth. Important aspects here are: (a) the decomposition is automatic, no operator input is required; (b) the diagonal matrix $\mathbf{S}$ contains the singular values; the number (*ne*) of significantly positive values corresponds to the number of absorbing chemical species in the process under investigation, it is also known as the chemical rank (31). Determination of this number prior to the fitting allows the estimation of the complexity of the process, as defined by the number of absorbing species; (c) the number *ne* of columns in $\mathbf{U}$ and rows in $\mathbf{V}$ are reduced to that number of species. Both matrices are orthonormal $\mathbf{U^tU} = \mathbf{VV^t} = \mathbf{I}$.

Combining Eqs. [11] and [19]

$$\mathbf{Y_{meas}} = \mathbf{C}\,\hat{\mathbf{A}} + \mathbf{R} = \mathbf{USV} \qquad [20]$$

and post-multiplication with $\mathbf{V^t}$

$$\mathbf{Y_{meas}V^t} = \mathbf{C}\,\hat{\mathbf{A}}\mathbf{V^t} + \mathbf{RV^t} = \mathbf{US}$$
$$\mathbf{Y'_{meas}} = \mathbf{C}\,\hat{\mathbf{A}}' + \mathbf{R}' \qquad [21]$$

results in the reduced size matrices $\mathbf{Y'_{meas}} = \mathbf{Y_{meas}V^t}$; $\hat{\mathbf{A}}' = \hat{\mathbf{A}}\mathbf{V^t}$ and $\mathbf{R}' = \mathbf{RV^t}$ which have only *ne* columns rather than the original 1024. Memory savings were crucial some 20 years ago (32), but nowadays where RAM is inexpensive this advantage is not crucial. Computation times are also improved, but not significantly.

An interesting application of the singular value decomposition has been published recently (33). Combining equations (20) and (21) we get

$$\mathbf{C}\hat{\mathbf{A}}' = \mathbf{US} - \mathbf{R}' \qquad [22]$$

Post-multiplication with $(\hat{\mathbf{A}}')^{-1}$ and replacing $\mathbf{S}(\hat{\mathbf{A}}')^{-1}$ and $\mathbf{R}(\hat{\mathbf{A}}')^{-1}$ with $\hat{\mathbf{A}}^*$ and $\mathbf{R}^*$, we get

$$\mathbf{C} = \mathbf{U}\hat{\mathbf{A}}^* - \mathbf{R}^* \qquad [23]$$

Due to the orthonormality of $\mathbf{U}$, the best $\hat{\mathbf{A}}^*$is computed as $\hat{\mathbf{A}}^* = \mathbf{U^tC}$. Thus

$$\mathbf{R}^* = \mathbf{U}\hat{\mathbf{A}}^* - \mathbf{C} = \mathbf{UU^tC} - \mathbf{C} = (\mathbf{UU^t} - \mathbf{I})\mathbf{C} \qquad [24]$$

The computation of the residuals as defined in Eq. [24] is faster than based on Eq. [11]. In essence this is due to the orthonormality of the matrix $\mathbf{U}$. An added advantage of this mode is that some instances of baseline problems are effectively eliminated (33). Under certain circumstances (certain models) it is also possible to fit the nonlinear parameters individually, i.e., instead of one multi-parameter fit several one-parameter fits are undertaken (34). Generally this is advantageous.

However, the computational advantages are minimal and the distortion of the relative weights of the residuals renders the statistical analysis more difficult. There are several implementations of the singular value decomposition using Eq. [24] instead of [11] in kinetics (35) and equilibrium studies (36).

### Second-Order Global Analyses

Proper investigation of complex processes often requires the acquisition of more than one set of data, as often it is not possible to find experimental conditions which result in data that adequately define all parameters of interest (37).

The traditional approach in such a situation is to establish experimental conditions where only a selection of all processes occurs. These data enable the researcher to determine the relevant parameters, equilibrium or rate constants as well as the absorption spectra of all species formed under these particular conditions. Additional experiments include the formation of additional species. These data sets are analysed in terms of the new parameters (and spectra) while the previously determined parameters are kept fixed at their now known values. This process can be continued to several levels. While there is nothing dramatically wrong with such an approach, it is easy to see that error propagation of the initially fitted parameters is unavoidable and if several levels of fitting/knowing from previous fits are operating, error propagation is essentially uncontrollable. Global analysis of the complete set of measurements is much more straightforward and transparent.

The concept is best explained by an example (28). The *Zn(II)/phenanthroline* system has been investigated by spectrophotometric titrations. Titration of a solution with concentrations $[Zn^{2+}] = 1 \times 10^{-5}$M and $[phen] = 3 \times 10^{-5}$M cannot be resolved due to linear dependences between the concentration profiles of the absorbing species $M$, $L$, $LH$, $ML$, $ML_2$ and $LH_3$. In fact it is impossible to determine all relevant equilibrium constants in one single titration. Global analysis of solutions with

M:L ratios 0:1, 1:1, 1:2 and 1:4 results in well defined equilibrium constants and absorption spectra of all species. As an additional bonus, global analyses of data taken under different conditions can result in significantly better determined constants as defined by their standard deviations. The advantages of globalised analyses are also well documented in model-free methods (38).

### Incorporation of Non-Ideal Behaviour such as Non-Constant Temperature or Ionic Strength

In academic research it is traditional to control external variables, such as temperature, as closely as possible. Rate and equilibrium constants are both temperature dependent and analyses of data acquired under constant temperature are clearly simpler and thus easier to analyse. Such control is impossible in industrial situations that prevents the application of model-based analyses in such situations.

In particular for kinetics the investigation of the temperature dependence of the rate constants reveals important information about the reaction via the availability of activation energies, defined either via the Arrhenius or the Eyring equations (1, 2). It has been shown that incorporation of variable temperature into the fitting algorithm is feasible. All that is required are some changes to the differential equations (2) using the activation parameters and the temperature to define the $k_i$. Analysis of non-constant temperature kinetics delivers the important activation parameters as well as simplifying the experimental procedure as external temperature control is not necessary and activation parameters can be determined form only one data set (39–41).

The determination of activation parameters allows the prediction of the kinetics of a reaction at any temperature (of course within the range in which the activation parameters and the reaction mechanism do not change).

In a similar line the ionic strength has to be maintained as constant as possible. The law of mass action states that the activities, not the concentrations, of the interacting species need to be entered into Eqs. [2] and [5]. Activity coefficients are strongly dependent on the ionic strength and thus in reactions where ions interact it is very likely that the ionic strength varies during the process. Therefore traditionally a large excess of an inert salt has been added to the reaction in order to maintain constant ionic strength. In a few important cases, the dependence of the rate or equilibrium constant has been determined, allowing extrapolation to zero ionic strength, thus defining ideal thermodynamic parameters. To our knowledge there are no publications where changing ionic strength has been incorporated into the fitting algorithm, thus avoiding the necessity of addition of inert salt. There are, however, reports on the modelling of kinetics (9) and on equilibrium computations (42) at variable ionic strength.

### Equilibria in Kinetics

An apparently similar, but from the computational analysis point of view fundamentally different, problem arises in pH dependent kinetics. In most reactions in aqueous solution the pH plays an important role. To enable relatively straightforward analysis of the kinetics, the solutions have to be buffered to a specific pH. Clear disadvantages are that the buffer action is never perfect and, more importantly, buffer anions and cations can interfere with the process under investigation. In particular, buffer anions are Lewis bases and thus potential ligands and therefore metals will interact with the buffer and their chemistry is consequently influenced (43). To circumvent this dilemma it has been proposed to include the protonation equilibria into the computations that model the chemical reaction and in this way eliminate the necessity of buffers altogether (29).

### Combination of Calorimetry and Spectroscopy in Kinetics

Spectroscopy is by far the most important method for the investigation of kinetics and equilibria. Calorimetry is a traditional tool for the investigation of equilibria (12), but only gained importance in kinetics very recently (44–46). Calorimetric data, acquired in parallel with spectroscopic measurements, increases the information content in a completely new dimension and adds valuable additional information. In particular for industrial process control, calorimetry is crucial for the safety of the setup. Global analyses of very different types of measurements are novel and require careful statistical analysis.

## CONCLUSIONS

Model-based data fitting is a powerful instrument in the toolbox of the chemist. With today's software robust analysis of large collections of data, based on rather complex models is common and often straightforward. There are a few improvements that would enhance the power of existing packages: (a) Statistical analysis of the results that goes beyond the delivery of standard deviations for the fitted parameters. While these are good estimates, many researchers found them of limited reliability. Bootstrap methods should deliver more robust results. (b) The determination of the correct model is difficult and requires much input from the researcher. Model-fitting procedures are reasonably well advanced and usually proceeds routinely and essentially automatic. However, in order to determine the best model the researcher has to try different alternatives and make a decision based on the statistical information given by the program and the chemical understanding available for the system under investigation. We may expect more immediate progress on the first issue rather than on the second.

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