"Kinetics", user's guide

General

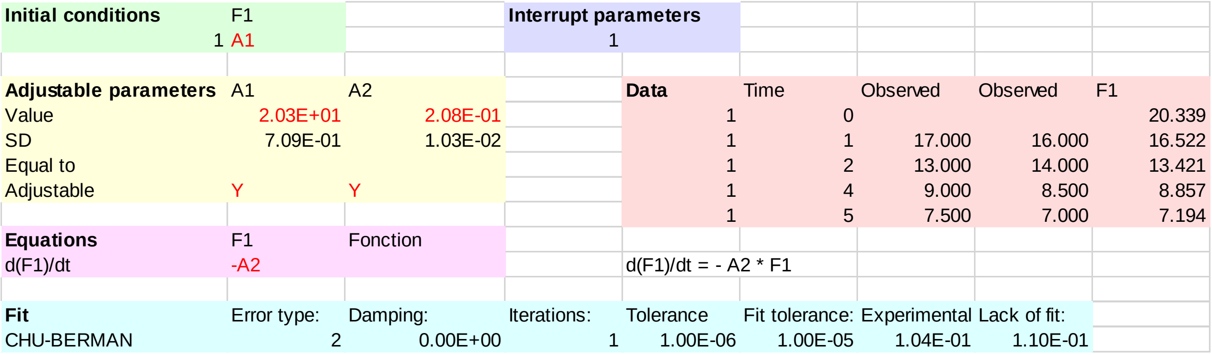
The program "Kinetics", an add-in for Microsoft Excel (Edition 2007 or later), aims to make accessible to all the modeling of molecular interaction kinetics and pharmacokinetics, these two questions being in fact very close mathematically: resolution of linear or nonlinear (e.g. bilinear or Michaelis-Menten type) systems of differential equations.

This program, like those of the same series ("Interactions", "Population kinetics"), incorporates some Equilex's concepts (Raguin et al. 2002): pre-established layout of data in an Excel worksheet, compilation of formulas, use of Excel for graphical displays, flexibility in an attempt to cover a maximum of experimental situations, use of an Excel worksheet as a dashboard to quickly see the whole problem, including the conditions of its resolution and the results. In particular, "Kinetics" allows the simultaneous handling of multiple experiments involving multiple measurements at each time point without the need for preliminary calculations that may introduce biases (noise subtraction of calculation of reports, etc.).

There is nothing sophisticated in the calculation routines, which are based on the "Numerical recipes in Pascal" toolkit of 1989. For the resolution of differential equation systems, "Kinetics" uses classic Runge-Kutta routines (see Wikipedia for more information on these methods) or the Chu-Berman routine, which was re-written from the algorithm published in the original article (Chu and Berman, 1974).This routine is especially effective when the solutions of differential equations are exponential (exact solution in this case) or are close to exponential functions (for example, linear combinations of exponentials). In the systems that we are dealing with, it is the routine to be used as a priority. It is also used in conversational SAAM, SAAM II and WinSAAM (Boston et al., 1981, Barrett et al., 1998, Novotny et al.2003).

An interface in Visual Basic ("Kinetics.xlam") forwards the information to a DLL ("Kinex_32.dll" or "Kinex_64.dll") written in Pascal that solves the problem and returns the solution to the interface that modifies the worksheet. Visual Basic 32-bits limiting the size of the structures and the volume of data transfer, it goes through the hard disk (saving and reading files). This does not impact the speed because the size of the data remains limited. The DLL integrates the calculation routines described above and a pseudo-compiler that transforms all literal expressions written in the worksheet. This pseudo-compiler is very effective, although it is probably not very orthodox from the computer-science point of view. Calculating the derivatives of adjustment error with respect to all adjustable parameters is performed through the repetition of the kinetics simulation. The program implements the algorithm of Levenberg-Marquardt (H.P. Gavin, people.Duke.edu/~hpgavin/ce281/LM.pdf).

The Excel sheet is organized in blocks separated by empty cells. The program recognizes the keywords: **"initial conditions"**, **"data"**, etc. written in the top left corner of each data block. "Kinetics" delimits the blocks on its own (this is why it is necessary to leave empty cells between the blocks) and gives them a background in pretty pastel colors.



Initial conditions	F1		**Interrupt parameters**					
1	A1		1					
Adjustable parameters	A1	A2		**Data**	Time	Observed	Observed	F1
Value	2.03E+01	2.08E-01		1	0			20.339
SD	7.09E-01	1.03E-02		1	1	17.000	16.000	16.522
Equal to				1	2	13.000	14.000	13.421
Adjustable	Y	Y		1	4	9.000	8.500	8.857
				1	5	7.500	7.000	7.194
Equations	F1	Fonction						
d(F1)/dt	-A2			d(F1)/dt = - A2 * F1				
Fit	Error type:	Damping:	Iterations:	Tolerance	Fit tolerance:	Experimental	Lack of fit:	
CHU-BERMAN	2	0.00E+00	1	1.00E-06	1.00E-05	1.04E-01	1.10E-01	

All blocks must exist in the sheet except the block "interrupt parameters" when the kinetics have only one step.

The purpose of the program is to solve differential equation systems dependent on the independent variable time (T). In the file "Kinetic analyses.xls", the worksheet "Monexponential" gives a very simple example that uses adjustable parameters in the definition of "outputs" (see below). The worksheet "ligand receptor interaction" shows how this works for molecular (non-linear) interaction and the sheet "multiple dosing" for a multiple-injection pharmacokinetics. Other examples are also given.

Most entries in the worksheet cells are compiled by the program. Almost all block entries (except interrupt numbers, times or data in the data block, of course) may contain formulas written in quasi-natural language (example A1 * (F1 + F2)). Current mathematical operations (plus Ln, for natural logarithm, and Ex for exponential), including parentheses and powers (^) may be used. All formulas are compiled at the beginning of the execution.

It is convenient to use a simple coding of the different variables of the system: Fi's are the solutions of the system of differential equations, Ai's are adjustable parameters and Pi's are parameters that can change at each interruption but are not adjustable parameters. This coding is used in this manual. Nevertheless, users can set the names of all parameters and all compartments as it pleases.

For the molecular species (or compartments) Fi, i can go from 1 to 30. For the Pj and Aj parameters, j can go from 1 to 50. Up to 30 interruptions are allowed. The program can calculate for each time point up to 40 different values. For each time point, up to 10 replicas (experimental data) may be entered. This number can range from 0 (simulation only) to 10 and there is no need to always have the same number of replicas. The number of data points, all interruptions combined, is limited to 1000.

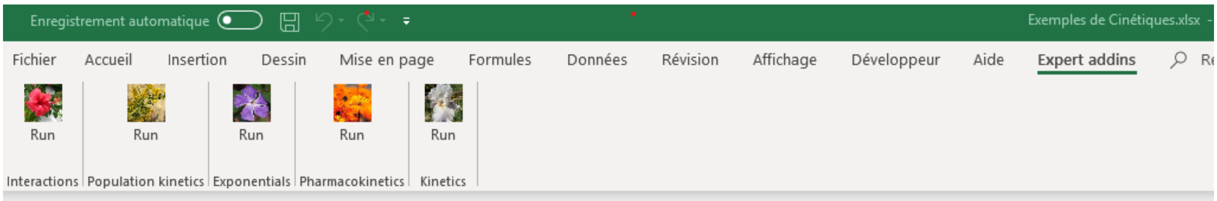
The time is explicitly represented as T. It can appear in formulas.

Caution: Excel interpret formulas entered in cells. For example – A1 can be understood by Excel as a reference to cell A1. In case of problems, one can either force the format of the cells to "text" or start the formula with an apostrophe: '-A1 will then be understood by Excel as text.

Installation

Kinetics is an add-in that needs to be installed in Excel: copy the files "Kinex_32.dll" and "Kinex_64.dll" in the C:\Windows folder and the file "Kinetics.xlam" in a folder of your choice. Sample files can be copied anywhere. Then the "Kinetics.xlam" add-in must be linked Excel: see Excel Help. This will show an "Expert addins" tab in the menu bar, if it did not already exist, and a tab called "Kinetics" in the Ribbon.

- Create a folder of your choice and copy the “Kinetics.xlam” file in it
- Copy “Kinex_32.dll” and “Kinex_32.dll” in the C:\Windows folder (needs administrator privileges)
- Open Excel, go to Excel Options (location depends on Excel version), then to Complements and link the “Kinetics.xlam” file as a complement
- A new “Expert addins” tab should show in the ribbon
- By clicking on the tab, a “Kinetics” command should show



Interruptions

Interruption is a notion introduced by SAAM II and WinSAAM. The aim is to allow the simulation of a system in which some parameters are changed at a given time, e.g. after a given incubation time or at the end of an infusion. This feature may also be used to simulate different experiments that depend on common adjustable parameters.

These interrupts are numbered consecutively from 1. Interrupt i occurs at a given moment immediately preceding the time indicated in the **"data"** block in the first line that begins with the number i. At that time, the initial conditions, given in the block **"initial conditions",** and the values of the Pj parameters given in the block **"interrupt parameters"** are changed. Note that these values can be formulas.

Initial conditions		F1	F2	F3
	1	2.00E+01	1.00E+01	0.00E+00
	2	0.00E+00	F2	F3

Interrupt par	P1
1	1.00E+00
2	1.00E+00

The interrupt number, 1, 2..., makes the link between the **"initial conditions", "interrupt parameters"** and **"data"** blocks. The time at which the interruption occurs is given in the **"data"** block.

In the **"initial conditions"** block, the values of the variables F1, F2, F3... are given to the different interruptions (the first one corresponds to time 0 of the experiment). These values, which are initial values, can be numeric or be functions of the fixed parameters and the Fi variables. Thus, if a variable Fi is unchanged at interrupt 2, Fi is written in the block to indicate that the value of Fi after the interruption is the same as before. In the example " ligand receptor interaction", this is the case for F2 and F3, while F1 is set to 0 at time 5 minutes. This system allows the modeling of washings, infusions, injections of another product or another dose, additions of a competitor...

The **"interrupt parameters"** block is used to describe the changes during interruptions. This block must have the same number of lines as the **"initial conditions"** block, since each line reflects an interruption (again interrupt 1 is the beginning of the kinetics, it can of course be the only one).

Data

The data block aggregates the inputs and outputs, that is, the observed data and the calculated results for each time point.

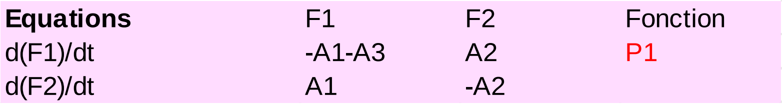
Data	Time	Observed	Observed	F1/A1	F1	F2/A5	F2	F3	F4	F1+F2
1	0.0			27.05%	1.000					1
1	0.7	27.0%	26.5%	26.14%	0.966	1.03%	0.0	0.7	0.0	0.98
1	1.6	24.5%	24.6%	25.04%	0.926	2.24%	0.0	1.6	0.0	0.96
1	2.0			24.62%	0.910	2.69%	0.0	1.9	0.0	0.95
1	2.5			24.06%	0.890	3.28%	0.1	2.4	0.1	0.94
1	2.9	23.5%	23.5%	23.65%	0.874	3.71%	0.1	2.7	0.1	0.93
1	3.0			23.53%	0.870	3.84%	0.1	2.8	0.1	0.93
1	6.0			20.70%	0.765	6.61%	0.1	5.2	0.4	0.87
1	12.0			16.55%	0.612	9.93%	0.2	9.3	1.2	0.77
1	18.0			13.72%	0.507	11.35%	0.2	12.7	2.2	0.69
1	19.2			13.26%	0.490	11.49%	0.2	13.3	2.4	0.67
1	21.0	12.7%	12.6%	12.62%	0.467	11.63%	0.2	14.2	2.8	0.65
1	24.0			11.71%	0.433	11.70%	0.2	15.5	3.3	0.62
1	48.0			7.27%	0.269	9.52%	0.2	23.6	7.5	0.42
1	92.9	3.5%	3.6%	3.58%	0.132	4.94%	0.1	32.2	12.6	0.21
1	124.2	2.2%	2.2%	2.22%	0.082	3.06%	0.0	35.5	14.6	0.13
1	360.0			0.06%	0.002	0.08%	0.0	40.7	17.7	0.00
1	720.0			0.00%	0.000	0.00%	0.0	40.9	17.8	0.00

The first column of the block corresponds to the interrupt number. The second column of the data block is the time. In this column, we can find identical times if they do not belong to the same interruption, but for a given interruption, they must be strictly increasing.

Then come the "outputs" that represent what you measure in the experiment or what you want to simulate. Experimentally measured data are listed in the data block in the "Observed" columns. There may be several "observed" columns. The output to calculate is defined in a column to the right of the experimental data by the analytical expression of the measurement, which may depend on product quantities F1, F2... in compartment 1, 2, etc. for each time point. Note that it is not necessary to have experimental (observed) values.

Equations

The system equations are given in the **"equations"** block.



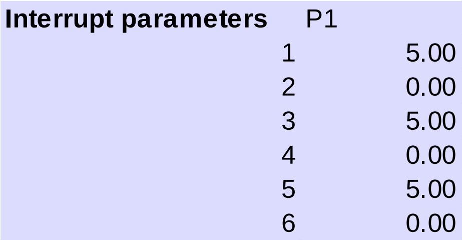
Equations	F1	F2	Fonction
d(F1)/dt	-A1-A3	A2	P1
d(F2)/dt	A1	-A2	

It must be understood that the columns (in number equal to the lines below the line of headings) represent a matrix M. The last column to the right (function or function) is a vector Y. The vector of the derivatives relative to the time dF/dt equals M.F + Y in matrix notation. In other words, for the species Fi, dFi/dt = Σ Mij FJ + Yi. In classical compartment models, components (Fi) are transferred between compartments j to i through linear exchanges. The "Chu-Berman " method is based on this matrix notation. The Yi function receives any contribution to dFi/dt that is not proportional to any Fj. For example, a constant will mimic an infusion in a compartment. Time can appear explicitly in these equations.

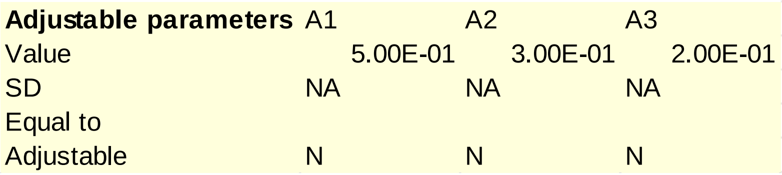
Settings

The parameters are of two types:

1. Interrupt parameters, Pj, which can change during the experiment at given times called interruptions. If these parameters are to be adjustable, it is sufficient to give, instead of a numerical value, the name of an adjustable parameter, Aj.
2. Adjustable parameters, Aj, that the program will estimate if there is enough experimental data.



Interrupt parameters		P1
	1	5.00
	2	0.00
	3	5.00
	4	0.00
	5	5.00
	6	0.00



Adjustable parameters	A1	A2	A3
Value	5.00E-01	3.00E-01	2.00E-01
SD	NA	NA	NA
Equal to			
Adjustable	N	N	N

The definitions of these two parameter types are given in the **"interrupt parameters"** and **"adjustable parameters"** blocks. For Pj, the first column gives the number of the interrupt, the others the name, Pj, of the parameter and its value at each interrupt (numeric or literal value, defining them according to the other data of the problem, Aj for example).

Initial numerical values must be given to all Adjustable parameters. The program will return a best fit estimation with an asymptotic estimation of SD. Complex systems may not be identifiable. The suggested pragmatic approach is to try fitting and in case the calculation does not converge, some Adjustable parameters can be set as not adjustable ("N" in the "Adjustable" line) or forced to be equal to a given function of other (adjustable or fixed) parameters given in the "Equal to" line. A forced parameter is not adjustable.

The fit block

The last block is the **"fit"** block. It is usually not necessary to change it, except for the number of iterations: if it is set to 0, only a simulation is performed, if it is set to 3, 5 or 10, there will be so many iterations to identify the adjustable parameters. The final fit is obtained when the program returns SD values for the adjustable parameters.

Another interesting choice is the method for solving the system of differential equations. CHU-BERMAN is a method especially developed for pharmacokinetics by Chu and Berman at NCI in the 1960's. It was the flagship method of SAAM and CONSAAM. In addition, one can use RUNGE-KUTTA 4 or 5. These are the classic methods of Runge and Kutta to the 4th or 5th order. In principle all must give the same result with speed differences according to the problems. They all depend on the "tolerance" parameter, which must not be too large nor too small (10^{-3} or 10^{-4}).

Fit	Error type:	Damping:	Iterations:	Tolerance	Fit tolerance:	Experimental	Lack of fit:
CHU-BERMAN	1	0.00E+00	0	1.00E-04	1.00E-03	0.00E+00	0.00E+00

Marquardt "Damping" may be necessary in some problems where there are strong correlations between adjustable parameters. "Tolerance" corresponds to the quality of Fi calculations. "Fit tolerance" corresponds to the quality of the final adjustment.

Execution

When the worksheet is ready, the program is started from the Excel Ribbon. A small window appears giving the choice between execution (Calculate) or display of a help (Help or Aide). This window disappears when the program runs and the Excel worksheet is refreshed after each iteration. The number of performed iterations and the elapsed time are displayed in the "status" bar (bottom left of the Excel worksheet). Nothing can be done in Excel until the small window has reappeared. At this time, you can restart the calculation, consult the help or close the window.

Conclusion

The idea was to develop a kinetic modeling platform made as flexible as possible through the use of formulas in the cells of an Excel worksheet and not black boxes that process only predefined systems. The examples provided give an idea of the possibilities.

Bugs may still be found. Please point them out, like any other questions about Kinetics, at: barbet@arronax-nantes.fr.

This software is free for use and distribution provided that these are not for profit, that the GIP ARRONAX is thanked in the communication or publication of any result obtained by using it and that the beneficiaries of the transfer commit to the previous conditions.

Sources can be obtained by express request to barbet@arronax-nantes.fr.

References

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