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A NEW GRAPHICAL METHOD TO SOLVING HOMOGENOUS DIFFERENTIAL EQUATION SYSTEMS USED IN PHARMACOKINETIC ANALYSIS

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We have proposed an alternative method on the basis of flow graph principles for solving the linear homogenous differential equation systems with constant coefficients used in pharmacokinetic analysis, no matter the number of the unknowns. The classical method for solving these systems using flow graphs entails Laplace transforms before depicting a flow graph and the reverse transformation after using the Mason's rules for calculus. Our method, based also on the flow graph theory, avoids the complicated calculus using Laplace transforms and is more direct than the classical one. Both methods provide the analytical solutions, eliminating the error propagation occurring in the numerical analysis. The numerical analysis and the alternative flow graph method were applied to a well known pharmacokinetic model (parent drug and metabolite kinetics, pre- and systemic metabolism, distribution) and the corresponding pharmacokinetic parameters compared.

INTRODUCTION

In the pharmacokinetic (PK) analysis, there appear numerous cases in which the drug absorption and disposition are quite complex and therefore cannot be characterized by classical pharmacokinetic models. For example, the case of monitoring the drug and its metabolite is a complex one because there is involved a presystemic metabolization to form also active substances and distribution processes.[1-3]

In that case one has to write a linear system of differential equations describing each kinetic process and to use them for finding the corresponding pharmacokinetic parameters.

The differential equations are easy to write and implement in specialized software, this being the main advantage of the numerical analysis procedure.[4] However, this technique has also some limitations. Numerical solutions are always approximations, and this may cause errors, e.g. in the estimation of derivatives (as required for many fitting algorithms). Another disadvantage of using differential equations is that the process to reach convergence is rather slower as compared to the one with analytical solution. This may be important when we deal with a great amount of data, especially in population PK analysis.

Analytical solutions provide the calculation of pharmacokinetic parameters with more accuracy, which is indeed an advantage. The analytical solutions could be obtained for linear differential equations systems by using the classical integration,[5] the operator method, secular equation and eigenvalues method, constant variation method[6, 7] and flow graph method.[8-12]

By means of our flow graph method for these complex pharmacokinetic models the analytical solution can obtain directly by inspection the graphical representation of the model.

This method is limited only to the linear homogenous and no homogenous differential equation systems.

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METHODOLOGY

1. Pharmacokinetic data and model

In order to verify and compare the two methods of data analysis, namely numerical integration and our method using flow graphs, real pharmacokinetic data were used. The data were collected from a bioequivalence study of fluoxetine, when both parent drug and its active metabolite (norfluoxetine) were determined from time to time.[13-14]

To describe the absorption and disposition of fluoxetine to norfluoxetine, a well known pharmacokinetic model has been considered (Fig. 1). This model involves a first-order kinetic process for absorption of fluoxetine and bicompartmental distribution. Fluoxetine can be transformed into norfluoxetine by both presystemic and systemic metabolism. Fluoxetine and norfluoxetine can be also eliminated from the human body by either metabolic or non-metabolic paths. As the intravenous data for fluoxetine and norfluoxetine were not available, the distribution volume for both drugs was considered equal. A lag time for absorption was also considered (Tlag).

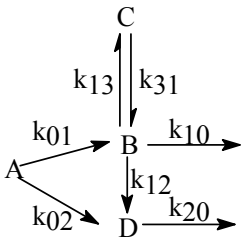

Fig. 1 – Pharmacokinetic model for fluoxetine and norfluoxetine.

The significance of the notations in Fig. 1 is as follows: *A* stands fluoxetine at administration place, *B* stands for fluoxetine in central compartment, *C* for fluoxetine in peripheral compartment and *D* for norfluoxetine in central compartment; the rate coefficients are: k_{01}-absorption rate constant for fluoxetine, k_{02} and k_{12} presystemic and systemic metabolism rate constant of fluoxetine to norfluoxetine, k_{13} and k_{31} the distribution rate constants for fluoxetine, k_{10} and k_{20} the elimination rate constants for fluoxetine and norfluoxetine. The initial dose of administered fluoxetine is denoted by X_0.

All the pharmacokinetic calculations (using either differential or analytical equations) were made using WinNonlin software.[4]

2. Analysis using the alternative flow graphs method

General presentation of flow graph method

A flow graph is a diagram derived from a set of simultaneous linear algebraic equations or a linear differential equations system, which are written (in this case) starting from a set of elementary chemical reactions on mass transfer included into a mechanism. The flow graph is used to represent the evolution of a physical system and to obtain the relationships between the system variables.

A flow graph consists of a network in which nodes (or vertices) are connected by directed *edges* (or branches). Each node represents a mechanism species, and each edge connecting two nodes acts as a signal multiplier. This multiplication factor, named *transmittance*, can be obtained from the mechanism kinetic constants. An arrow placed on the edge indicates the direction of the signal flow and the multiplication factor is indicated along the edge.[15-16] The signal flow graph depicts the flow of signals from one point of the graph to another (a path or a way) and gives the relationships between the signals;[8] a path should not go out any node of the graph more than once (only one outgoing edge) and every inner node of the graph has to be touched. A forward path is a path starting from an input node (or source, a node which has only the outgoing edges) or from one – continue path – or more – discontinue path – mixed nodes (a node which has incoming and outgoing edges) to one or more output nodes (a node which has only the incoming edges).[8,16] The product of the transmittaces of a forward path is named the gain of the path (FWG). The gain of the flow graph is the sum of the all forward path gains.[8-12]

In the case of solving a linear differential equations system, we associate for every determinant of the system a flow graph. The value of determinant is the gain of the associated flow graph. The flow graph associated with the secular determinant[16] is named the consumption flow graph and is derived directly from mechanistic model; on based on the gain of the consumption flow graph, one obtains the eigenvalues (γ_i) and the consumption determinants (Δ_c) which represent the products of differences of the eigenvalues. The flow graphs associated with the determinants obtained by replacing the columns of the secular determinant with the column of free coefficients (the formation determinants), are named the formation flow graphs[17] and they are depicted for

every species starting from the consumption flow graph.

The general mathematical solutions of the linear differential equations systems are the sum of exponential functions[5-8] (e.g., $C_B = B_1 \cdot \exp(-\gamma_1 t) + B_2 \cdot \exp(-\gamma_2 t) + B_3 \cdot \exp(-\gamma_3 t)$). In our approach, the pre-exponential coefficients (in this example, B_i) represent the ratio of the corresponding formation and consumption determinants, (in accordance with Cramer's rule[18]).

This new graphical method has not required writing the equations system but only the pharmacokinetic model.

Solving the pharmacokinetic model by using the new graphical method

In this approach a new mechanism equivalent to the model from Fig. 1 is drawn considering that every substance is transforming in a final product with a constant rate, even it is zero (see Fig. 2a). The consumption flow graph[17] is the image of the new mechanism with the γ decreased from every output transmittance (see Fig. 2b). Its name suggests that the system evolution brings about a loss (consumption) in all variable values and a gain of the output values (the final products I_1, I_2, I_3 and I_4) in how the arrows indicate the direction of the flux from all mixed nodes to the output nodes.

The formation flow graph[17] for a species is constructed considering the species of interest as being a target one (the final product, an output node) and adding a new node (the input node or the source which represents the initial conditions). They are named like this because indicate a formation and a gain of the chosen variable starting from an input node (the source *S*).

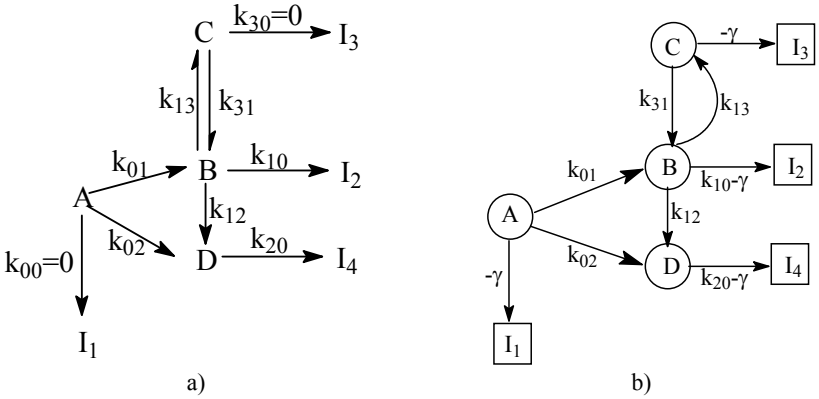

Fig. 2 – The equivalent mechanism (a) and consumption flow graph (b).

One has identified in totally 15 forward paths (FW, see the Fig. 2b) and by adding its gains (FWG), one obtains the gain of the consumption flow graph (CG); five of them are presented below (the others are obtained in the same manner by replacing one by one the transmittances of the node A, k_{01}, k_{02} and -γ):

$$\begin{aligned} FWG1 &= k_{01}k_{12}(k_{20}-\gamma)k_{31} \\ FWG2 &= k_{01}k_{12}(k_{20}-\gamma)(-\gamma) \\ FWG3 &= k_{01}k_{13}(k_{20}-\gamma)(-\gamma) \\ FWG4 &= k_{01}(k_{10}-\gamma)(k_{20}-\gamma)k_{31} \\ FWG5 &= k_{01}(k_{10}-\gamma)(k_{20}-\gamma)(-\gamma) \end{aligned} \quad (1)$$

The gain of consumption flow graph, according to the definition, is equal with the value of the secular determinant, Δ:

$$\Delta = CG = \sum_{i=1}^{15} FWGi = (k_{20}-\gamma)(k_{01}+k_{02}-\gamma)[(-\gamma)(k_{12}+k_{13}+k_{10}-\gamma)+k_{31}(k_{12}+k_{10}-\gamma)] = 0 \quad (2)$$

$$\Delta = (k_{01}+k_{02}-\gamma)[\gamma^2 - \gamma(k_{12}+k_{13}+k_{14}+k_{10}+k_{31})+k_{31}(k_{12}+k_{10})](k_{20}-\gamma) = 0$$

From the above equation it can be found the exponential factors and the expressions of consumption determinants (Δ_c):

$\gamma_1 = k_{01}+k_{02}$; γ_2, γ_3 (the solutions of the square equation), and $\gamma_4 = k_{20}$.

$$\text{Also, } \Delta_c(\gamma_i) = \prod_{\substack{j=1 \\ i \neq j}}^{n} (\gamma_j - \gamma_i), \text{ where } n \text{ is number of inner nodes.} \quad (3)$$

The formation flow graph for B (Fig. 3) is depicted from the consumption flow graph, considering the species of interest being a target one (a final product); the output edges of B species are rejected and a new input node is added (source S which represents the initial conditions). From the Fig. 2b, it is clear that no connections emerge from the node D to the node B, thus the D species will not appear in the formation flow graph of B species.

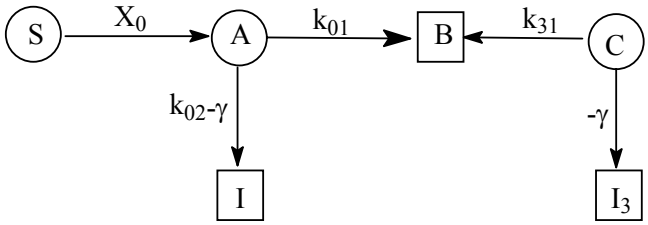

Fig. 3 – The formation flow graph for B species.

Now node I denotes the output node of A species whose transmittance is the sum of all its outgoing edge transmittances when the node D is missing.

For calculating the gain of the above formation graph one has to choose only the forward paths, which connect the input node with the target species $B^{8\text{-}12}$ (e.g., the discontinue forward path $X_0(k_{02}\text{-}\gamma)k_{31}$ doesn't directly link the node *S* with *B* species). The formation determinant is then:

$$\Delta_{Bi} = X_0 \cdot k_{01} \cdot k_{31} + X_0 \cdot k_{01} \cdot (-\gamma_i) = X_0 \cdot k_{01} \cdot (k_{31} - \gamma_i) \quad i = 1,2,3; \quad (4)$$

The final solution is presented below:

$$C_B = \frac{X_0 k_{01}(k_{31} - \gamma_1)e^{-\gamma_1 t}}{(\gamma_2 - \gamma_1)(\gamma_3 - \gamma_1)} + \frac{X_0 k_{01}(k_{31} - \gamma_2)e^{-\gamma_2 t}}{(\gamma_1 - \gamma_2)(\gamma_3 - \gamma_2)} + \frac{X_0 k_{01}(k_{31} - \gamma_3)e^{-\gamma_4 t}}{(\gamma_1 - \gamma_3)(\gamma_2 - \gamma_3)} \quad (5)$$

The formation flow graph for *D* is:

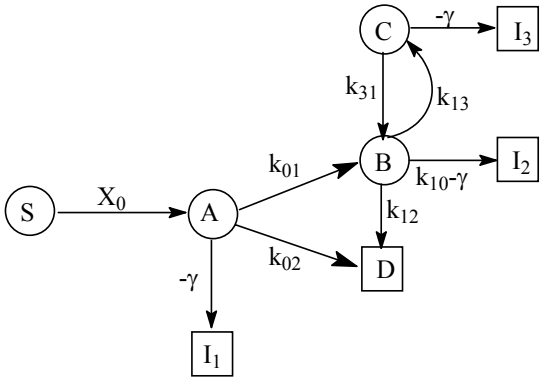

Fig. 4 – The formation flow graph for D species.

The formation determinants and the solution for *D* species are:

$$\begin{aligned} \Delta_{Di} &= X_0 \cdot k_{01} \cdot k_{12} \cdot (k_{31} - \gamma_i) + X_0 \cdot k_{02} \cdot [(k_{31} - \gamma_i)(k_{10} + k_{12} - \gamma_i) + k_{13} \cdot (-\gamma)] \\ &= X_0 \cdot k_{01} \cdot k_{12} \cdot (k_{31} - \gamma_i) + X_0 \cdot k_{02} \cdot [\gamma_i^2 - \gamma_i(k_{10} + k_{12} + k_{13} + k_{31}) + k_{31} \cdot (k_{10} + k_{12})] \\ i &= 1,2,3,4; \end{aligned} \quad (6)$$

$$C_D = \frac{X_0 \cdot (k_{01} \cdot k_{12} \cdot (k_{31} - \gamma_1) + k_{02} \cdot [\gamma_1^2 - \gamma_1(k_{10} + k_{12} + k_{13} + k_{31}) + k_{31} \cdot (k_{10} + k_{12})]) \cdot e^{-\gamma_1 t}}{(\gamma_2 - \gamma_1)(\gamma_3 - \gamma_1)(\gamma_4 - \gamma_1)} + \frac{X_0 \cdot k_{01} \cdot k_{12} \cdot (k_{31} - \gamma_2) \cdot e^{-\gamma_2 t}}{(\gamma_1 - \gamma_2)(\gamma_3 - \gamma_2)(\gamma_4 - \gamma_2)}$$
$$+ \frac{X_0 \cdot k_{01} \cdot k_{12} \cdot (k_{31} - \gamma_3) \cdot e^{-\gamma_3 t}}{(\gamma_1 - \gamma_3)(\gamma_2 - \gamma_3)(\gamma_4 - \gamma_3)} + \frac{X_0 \cdot (k_{01} \cdot k_{12} \cdot (k_{31} - \gamma_4) + k_{02} \cdot [\gamma_4^2 - \gamma_4(k_{10} + k_{12} + k_{13} + k_{31}) + k_{31} \cdot (k_{10} + k_{12})]) \cdot e^{-\gamma_4 t}}{(\gamma_1 - \gamma_4)(\gamma_2 - \gamma_4)(\gamma_3 - \gamma_4)} \quad (7)$$

A computer program was executed in the Java language. It recognizes all the forward paths and calculates the gains of the consumption and formation flow graphs.[19] If the polynomial degree from the equation (2) is inferior or equal with 4 one can find the symbolic expressions of the eigenvalues (γ_i), but if it is superior to 4 the numerical eigenvalues are calculated in the same time with the fitting of the real experimental data.

RESULTS

Equation (5) and respectively equation (7) represent the analytical solutions for fluoxetine (B) and norfluoxetine (D) in central compartment. A fitting plot of data using the considered pharmacokinetic model is presented in Fig. 5 (analytical solutions used). It can be noticed that the agreement with the experimental data is very good.

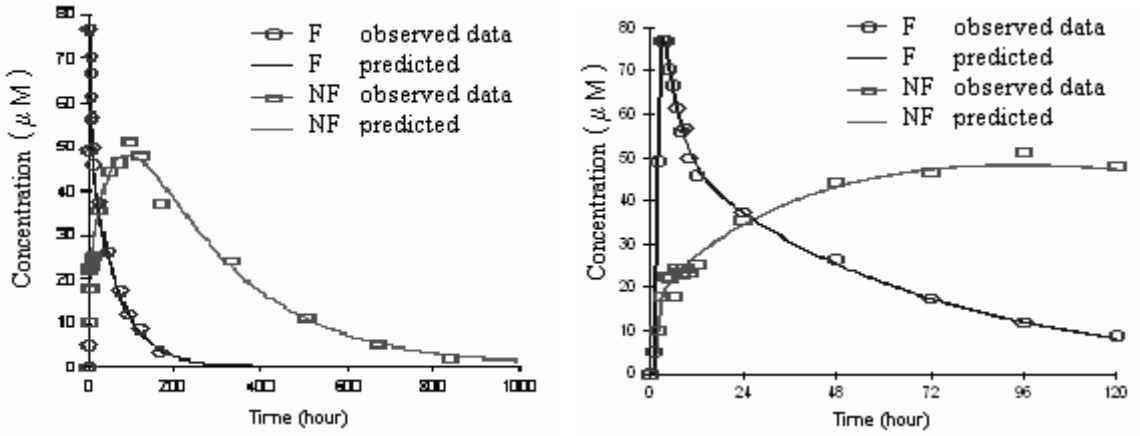

Fig. 5 – Fitting plot of pharmacokinetic model to the data: (a) the overall view and (b) detailed view for the first 120 hours. F-fluoxetine, NF-norfluoxetine.

The values of pharmacokinetic parameters of fluoxetine and norfluoxetine (for one set of data), obtained using numerical integration method and analytical equations are presented in Table 1.

Table 1

Comparison between pharmacokinetic parameters of fluoxetine and norfluoxetine obtained by using numerical integration method and analytical solutions

		Numerical solutions	Analytical solutions	
Parameter	Unit	Value	Value	Difference (%)
k_{10}	hr^{-1}	0.00990	0.00974	-1.6
k_{20}	hr^{-1}	0.00440	0.00440	0.0
k_{12}	hr^{-1}	0.01730	0.01734	0.2
k_{13}	hr^{-1}	0.09803	0.09489	-3.2
k_{31}	hr^{-1}	0.15444	0.15100	-2.2
k_{01}	hr^{-1}	1.77290	1.52228	-14.1
k_{02}	hr^{-1}	0.27225	0.27828	2.2
Volume	L	1155.92	1161.32	0.5
Tlag	hr	1.55830	1.56184	0.2

Examining the values obtained for calculated pharmacokinetic parameters in Table 1, there appear some differences within 0% and 14.1%. The latest seems to be an important one taking in account that we deal with small amounts of pharmaceutical substances. In this sense every little deviation becomes significant and forces us to use the most exact solutions, which provide the right simulation parameters. Because the analytical solution is the gold standard in this field, and it does not propagate the errors as numerical solution does, the first will be chosen in the process of simulation and the correct parameters will be those obtained through the implementation of the analytical solutions.

CONCLUSIONS

Using the proposed method one can obtain the analytical solution for complex pharmacokinetic models (if there are involved only first order reaction or could be reduced at these) directly by inspecting the graphical representation of the model.

The new graphical method is an alternative to the classical flow graph method which uses the Laplace transforms, because, for the last, the complexity of reaching the analytical solutions limits the use of these.

Analytical solutions provide calculation of pharmacokinetic parameters with more accuracy, which is indeed an advantage, in comparison with numerical solutions, which are always approximations and for this, they may cause errors and their propagation. Another disadvantage of using differential equations is the slower process for reaching the convergence. This may be important when we deal with a great amount of data, especially in population PK analysis.

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