DIXIEME COLLOQUE SUR LE TRAITEMENT DU
SIGNAL ET SES APPLICATIONS

NICE du 20 au 24 MAI 1985.

APPLICATION OF THE PADE-LAPLACE METHOD TO MULTIEXPONENTIAL DECAYS : ANALYSIS OF CHEMICAL RELAXATION SIGNALS

J. AUBARD*, P. LEVOIR*, A. DENIS+, P. CLAVERIE≠

* Institut de Topologie et de Dynamique des Systèmes de l'Université Paris VII associé au C.N.R.S., 1 rue Guy de la Brosse, 75005 PARIS (France).

+ Laboratoire de Chimie Quantique de l'Université Paris VI, Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie, 75005 PARIS (France).

≠ Dynamique des Interactions Moléculaires (E.R. du C.N.R.S. n° 271), Université Paris VI, Tour 22, 4 place Jussieu, 75230 PARIS Cedex 05 (France).

RESUME

De nombreux phénomènes en Chimie, Physique et Biologie donnent lieu à des décroissances exponentielles $V(t)=\sum_{k=1}^{n} V_k^o \exp(-t/\tau_k)$, où n est le nombre de composantes, V_k^o et τ_k désignant respectivement les amplitudes et les temps de relaxation.

L'analyse de tels signaux multiexponentiels est difficile, étant donné le caractère non linéaire du problème. Aussi de nombreuses méthodes numériques ont-elles été élaborées pour analyser de tels signaux. La plupart des procédés utilisent la méthode des moindres carrés. Le succès d'une méthode donnée dépend de facteurs tels que les rapports des temps de relaxation, les rapports des amplitudes, et le niveau de bruit. Les procédés ci-dessus peuvent conduire à des résultats incorrects, notamment dans le cas de signaux très faibles brouillés par du bruit. Nous avons donc appliqué à ce problème une méthode originale, dénommée Padé-Laplace, parce qu'elle introduit la transformée de Laplace du signal et représente cette transformée au moyen d'approximants de Padé. Convenablement utilisée, cette méthode fournit des valeurs correctes des temps de relaxation et des amplitudes, et contrairement à la plupart des méthodes antérieures, elle ne requiert pas d'hypothèse a priori concernant le nombre de composantes.

Des signaux de relaxation rapide, correspondant aux équilibres des formes tautomères de la cytosine, ont été obtenus au moyen d'une technique très sensible pour l'étude des phénomènes de relaxation (saut de température micro-ondes), et ont été analysés avec la méthode de Padé-Laplace. Nous mettons en évidence, pour ces signaux, les capacités de la méthode à prendre en compte les difficultés expérimentales (bruit déterministe ou bruit blanc, nombre de points échantillonés, choix de la ligne de base...), en évitant ainsi l'apparition d'instabilités ou d'artefacts.

Nous décrivons aussi brièvement une application de la méthode de Padé-Laplace à un problème neurobiologique (collaboration avec E. Yeramian), à savoir l'analyse en sommes d'exponentielles de la fonction de corrélation du courant ionique à travers les canaux des membranes nerveuses. Le nombre de tels canaux travaillant de manière coopérative dans une unité fonctionnelle correspond au nombre de composantes exponentielles, lequel peut être efficacement déterminé par l'analyse de Padé-Laplace.

SUMMARY

Many phenomena in Chemistry, Physics and Biology occur as a single or multiple exponential decays, $V(t)=\sum_{k=1}^{n} V_k^o \exp(-t/\tau_k)$ where n is the number of individual decays, V_k^o and τ_k are amplitudes and decay times respectively. The analysis of such a multiexponential signal is known to be difficult due to its non linear nature and to the high degree of correlation among the parameters.

Consequently, a number of numerical methods have been developped for the analysis of exponential decays. Most techniques use the well known least-squares method, to obtain the best fit between the data and the corresponding calculated decay function. The success of a given method depends on factors such as ratios between decay times, ratios between amplitudes and the noise level. Clearly the above procedure of analysis can lead to erroneous results, especially in the case of very weak signals blurred by noise. We therefore applied to this problem an original method, referred to as Padé-Laplace since it introduces the Laplace transform of the signal and represents this transform through Padé approximants. When suitably applied, the method not only gives correctly the relaxation times and the amplitudes of the components, but, in contrast with most previous methods, it does not require an "a priori" assumption concerning the number of components.

Fast relaxation signals from tautomeric equilibria in cytosine, obtained with a very sensitive transient relaxation technique, the microwave temperature jump apparatus, were analyzed by the Padé-Laplace method. We demonstrate, on these specific signals, the capabilities of the method to take into account experimental difficulties (namely deterministic or white noise, number of sampling points, choice of baseline...), thus avoiding unstabilities and/or artifacts in the results.

As a further illustration, we shall also brieffly describe an application of the Padé-Laplce method to a neurobiological problem (collaboration with E. Yeramian), namely the analysis into a sum of exponentials of the correlation function of the ionic current through channels of nerve membranes. The number of such channels clustered together and working cooper tively into a functional unit corresponds to the number of exponential components, which may be efficiently determined by the Padé-Laplace analysis.

APPLICATION OF THE PADE-LAPLACE METHOD TO MULTIEXPONENTIAL DECAYS : ANALYSIS OF CHEMICAL RELAXATION SIGNALS

I. Synopsis of the theory of the Padé-Laplace method

The Padé-Laplace method is a special case of the Padé-integral transform method [1]. As shown in paper [1], the use of a suitable integral transform provides an efficient way for the representation of a given function in terms of components of some prescribed type. The essential idea is that the components will correspond to the singularities (poles) of the integral transform.

Now, the signal under investigations is a special case of a large class of functions f(t)

$$(1)\quad f(t)=\sum_{k=1}^{n} C_k \exp(\mu_k t),\quad t \geq 0$$

where the $\mu_k=\lambda_k+i\omega_k$ are complex numbers.

As is well known,when the μ_k are purely imaginary, the problem of the detection of components in (1) is easily performed by means of Fourier analysis through the Fourier transform. When the μ_k are not purely imaginary the most appropriate integral transform is the unilateral Laplace transform :

$$(2)\quad Lf(p)=\int_0^{\infty} e^{-pt} f(t)dt, \text{ with p complex number.}$$

Applying this transform to the equation (1), one obtains

$$(3)\quad Lf(p)=\sum_{k=1}^{n} \frac{C_k}{p-\mu_k}$$

with Re p > $\sup_k(\mathrm{Re}\,\mu_k)$, this condition ensuring the convergence of the Laplace transform as well as of its derivatives. Then Lf(p) is an analytic function defined by eq.(2) in a right half complex plane.When f(t) is only known from sampled data $f(t_j)$ ($t_j = j\,T$,with T the sampling interval), the Laplace transform must be evaluated from some numerical integration , but it remains analytic in the variable p, in the previously defined region. The detection problem is theoretically solved since, in eq.(3), the μ_k appear as the poles of the function Lf(p) while the C_k amplitudes are the corresponding residues. But, since Lf(p) converges only in a region which does not contain the poles μ_k it is not possible to detect these poles by direct numerical integration : in order to detect them, it is necessary to consider the continuation of the analytic function Lf(p) in the whole complex plane.

An optimal way to perform the continuation of Lf (p) is, firstly, to evaluate, at some point p_o suitably chosen in the convergence half-plane, the Taylor series S of Lf(p) :

$$(4)\quad S=\sum_{r=0}^{\infty}\left(\frac{1}{r!}\frac{d^r Lf}{dp^r}\Big|_{p=p_o}\right)p'^r=\sum_{r=0}^{\infty} c_r p'^r,\ \text{with } p'=p-p_o$$

and secondly, to resort to the Padé approximants method [2] to effectively perform the continuation of Lf(p) in the whole complex plane. A Padé approximant , usually noted [N/M], is a rational fraction,

$$(5)\quad [N/M]=A_N(p')/B_M(p')=\sum_{s=0}^{N} a_s p'^s \Big/ \sum_{v=0}^{M} b_v p'^v,\ \text{with } b_o=1$$

satisfying the formal identity

$$(6)\quad \sum_{r=0}^{\infty} c_r p'^r = \sum_{s=0}^{N} a_s p'^s \Big/ \sum_{v=0}^{M} b_v p'^v + O(p'^{N+M+1})$$

The expression (6) leads to a system of linear equations wich allows to determine the set of coefficients $\{a_s\}$ and $\{b_v\}$. Once the $\{a_s\}$ and $\{b_v\}$ coefficients are available, the [N/M] Padé approximants may be rewritten in terms of the roots $\{\gamma'_s\}$ and $\{\beta'_v\}$ of the polynominals $A_N(p')$ and $B_M(p')$. By returning to the original variable $p=p' + p_o$ and by setting $\gamma_s=p_o+\gamma'_s$ and $\beta_v=p_o+\beta'_v$, eq.(5) now writes :

$$(7)\quad [N/M]=(a_N/b_M)\prod_{s=1}^{N}(p-\gamma_s)/\prod_{v=1}^{M}(p-\beta_v)$$

Now, we remark that the expression (3) of Lf(p) is nothing but the result of the decomposition into partial fractions of a rational function $P_{n-1}(p)/Q_n(p)$ which may be written, in a form analogous to the expression (7):

$$(8)\quad Lf(p)=(a'_{n-1}/b'_n)\prod_{u=1}^{n-1}(p-\alpha_u)/\prod_{k=1}^{n}(p-\mu_k)$$

From this fact it becomes obvious that the construction of the set of the (N-1/N) Padé approximants is sufficient : moreover, from a theorem about the representation of a rational function by Padé approximants [2], it follows that all Padé approximants [N-1/N] with N > n must reduce to the [n-1/n] Padé approximant which will then represent exactly the rational function Lf (p) :

$$(9)\quad Lf(p)=(a'_{n-1}/b'_n)\prod_{u=1}^{n-1}(p-\alpha_u)/\prod_{k=1}^{n}(p-\mu_k)$$
$$\equiv (a_{n-1}/b_n)\prod_{s=1}^{n-1}(p-\gamma_s)/\prod_{v=1}^{n}(p-\beta_v)$$

Thus, from equation (9), it appears clearly that the exponents μ_k of the given signal f(t) are the roots β_v of the polynomial denominator of the [n-1/n] Padé approximant ; the number n of exponential components in f(t) being directly given by the degree of this polynominal denominator of [n-1/n]. Thus, this method does not require any a priori assumption about the number of components prior to computation.

APPLICATION OF THE PADE-LAPLACE METHOD TO MULTIEXPONENTIAL DECAYS :
ANALYSIS OF CHEMICAL RELAXATION SIGNALS

II. Application of the Padé-Laplace method

II.1. Analysis of chemical relaxation signals

Chemical relaxation methods are the best tool available today to study very fast chemical or biological equilibria in solutions. The basic principle of these methods [3] is to perturb a chemical (biological) system at equilibrium by a sudden change of some external parameter such as temperature, pressure, electric-field... Following the rapid variation of the reactants' concentrations to their new equilibrium value allows the determination of the reaction rates. In the most general case, where we consider a complex reaction involving several consecutive steps in equilibrium, the concentration change of any reactants following the perturbation can be described by a set of n differential equations [3]:

$$(10)\quad dx_i/dt = \sum_{k=1}^{n} a_{ik} x_k$$

where x_i is the deviation of the species i from its equilibrium concentration and the a_{ik} are functions of the rate constants and the equilibrium concentrations. The solution of eqs. (10) is evidently of the general form

$$(11)\quad f(t) = \sum_{k=1}^{n} C_k \exp(-t/\tau_k)$$

where C_k and τ_k are the amplitudes and relaxation times respectively.

In transient chemical relaxation techniques, such as temperature-jump (T-jump), the output signal, which is recorded in the form of equation (11) is often characterized by a very small amplitude blurred by a strong noise level. Despite the improvements of the experimental techniques [4,5] in numerous studies, particularly those devoted to the dynamics of very one-sided fast chemical reactions, the analysis of the relaxation signal into its exponential components could not be accurately performed with the usual methods, such as least-square fitting. We thus applied to this problem the Padé-Laplace method [1,6], whose characteristics have been briefly summarized in the preceding section. In the following we use this new method to analyze fast relaxation signals, from biological tautomeric equilibria in cytosine, obtained with a very fast T-jump relaxation technique.

The N(1)H$\rightleftharpoons$N(3)H tautomeric equilibrium in cytosine, a DNA nucleic base, is very one-sided in aqueous solution [7]. Detecting the very low proportion (0.25%) of the short-lived N(3)H amino-oxo species (a few microseconds) requires a very fast and highly sensitive relaxation method such as the microwave T-jump apparatus [5].

This system consists of a magnetron, operating at 9.3 GHz, which delivers up to 50 pulses per second ; with water as a solvent, dielectric losses cause temperature rises of 1.5°C for each pulse of 1.5µs duration. The fast variations of the reactants concentration, which follow the repetitive heating, where measured whith a spectrophotometric detection using light-pipe technology (rise time ca. 1µs, 10^{-5} Optical Density (O.D.) units sensitivity). Periodical relaxation signals delivered by the photomultiplier were recorded in the digital mode via a high speed A/D converter and were summed in the memory of a PDP 11 computer. The Figure 1 shows a typical relaxation signal (curve 1), recorded in the near U.V. at λ = 295 nm and obtained after 3000 accumulations in only 100 s recording time (30Hz repetition rate). This signal is composed of a very fast rise in O.D. probably arising from a solvation step (10^{-8} to 10^{-9}s), which is filtered by the detection system ; it is followed by a slower variation which corresponds to the rapid interconversion between the N(1)H and N(3)H species. Since the signal is blurred by an important noise its analysis in term of sum of exponentials is not very easy.

As mentioned in the preceding section, the Padé-Laplace analysis of a signal consists first in evaluating through numerical integration the value of the Laplace transform and a certain number of its derivatives at some point p_0 and second in representing Lf(p) through Padé approximants. Before performing these numerical integrations the procedure requires to search for the baseline of the signal in order to get it free of DC offset. In the example shown in the figure 1 below,

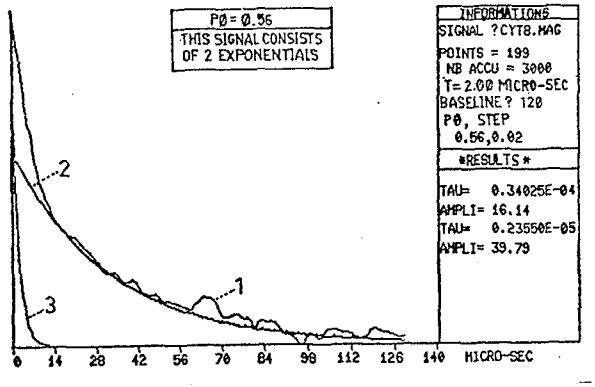

Figure 1.
Relaxation signal of aqueous cytosine detected at λ = 295 nm (curve 1). This signal was accumulated 3 000 times and sampled over 200 points with a 2µs sampling period. The results of the Padé-Laplace analysis are given in the frame ; curves (2) and (3) represent the computed slow and fast components in the experimental signal (1).

we select the last 120 points for the baseline : consequently the results displayed in the right frame of this figure were obtained from numerical integration over the 80 remaining points of this signal. These results were obtained for a value of p_0=0.56 and show a good statility over a rather large interval of p_0(0.45 to 0.65). It should be noted that we have checked that the fast exponent found in the signal by the Padé-Laplace analysis (τ_1=2.4µs) agrees well with the exponential response of the apparatus, whereas the slow relaxation time (τ_2=34µs) is the expected value for an intermolecular proton transfer which is know to be a diffusion controlled process [7].
Another example of application of the Padé-Laplace method has been recently completed, and we briefly describe it below in order to further demonstrate the capabilities of this new signal analysis method in many research areas.

II.2 A neurobiological problem : Analysis of the correlation function of the transmembrane current (collaboration with E.YERAMIAN (**))

The problem at hand concerns the analysis of the ionic currents flowing through the channels of the nerve membrane. According to the theoretical model of ref.[8], we consider independent functional subunits, each of them consisting of n elementary channels (non-independent). Each subunit current is modelled by an integer-valued Markov process m(t) (number of elementary channels open at time t). The question arises whether the elementary channels actually work independently (n=1) or not (n>1). In order to get an answer, we study the two-time properties of the total current $I(t)=\Sigma m_\alpha(t)$, and noticeably its correlation function $B(\tau)=<I(t)\ I(t+\tau)>=N<m(t)\ m(T+\tau)>=N\ B_{mm}(\tau)$ ($B_{mm}(\tau)$ denoting the correlation function pertaining to one subunit). It is indeed easily shown [8] that :

$$(12)\quad B_{mm}(\tau) - <m>^2 = \sum_{k=1}^{n} C_k \exp(\mu_k \tau)$$

where the n exponents μ_k are the non-zero eigenvalues of the transition probability matrix, which is of order (n+1), with one zero eigenvalue corresponding to the stationary distribution (more generally, each two-time probability $P_{ij}(\tau)$ is also a sum of exponentials). Therefore the problem lends itself quite naturally to the application of the Padé-Laplace method. Note that a correct determination of the number n of exponential components is essential here, since it precisely gives the number of channels working in a dependent way in each subunit . Figure 2 gives the results of Padé-Laplace analysis (with p_0=0.2) for one the correlation functions obtained from the experimental recordings of I(t) (a very weak damped oscillatory component has been omitted for simplicity). We may put aside the "fast component" $A_3\exp(\mu_3 t)$ because it corresponds to a kinetics of "brief openings", which is irrelevant as concerns the slower kinetics of normal openings, which is the one of interest for the study of coupling between elementary channels. Then we are left with the components 1 and 2, which have exponents of the same order of magnitude, and they clearly suggest a dependent behaviour within subunits containing n=2 channels. We also performed the Padé-Laplace analysis for the two-time probabilities $P_{01}(\tau)$ and $P_{12}(\tau)$. The exponents μ_1 and μ_2, found in the correlation function, were actually retrieved (weak damped oscillatory components were also detected, but their frequencies suggest that they could be artifacts due to the electric power source). Thus the Padé-Laplace analysis appears able to bring an affirmative answer to the initial question concerning the coupling between elementary channels (and it suggest the value n=2 for the number of these coupled channels inside each functional subunit).

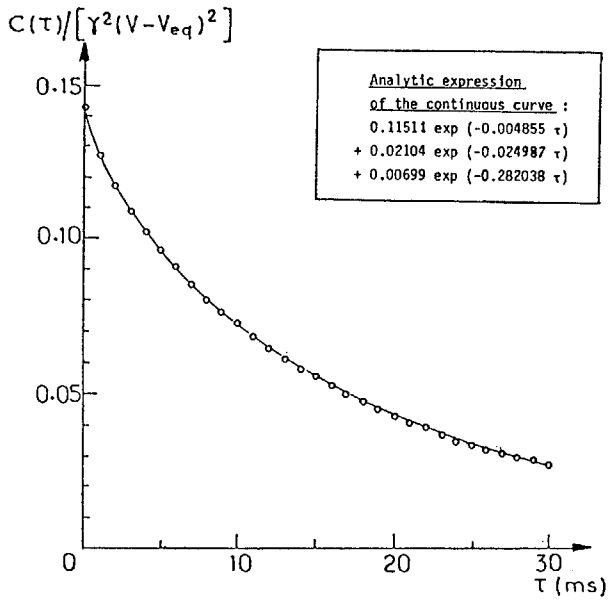

Figure 2.
Correlation function values of the transmembrane current (circles) and its analytic representation (continuous curve) obtained through the Padé-Laplace method. The correlation function values were calculated from the sampled experimental recordings of the transmembrane current ; the explicit analytical expression
$A_1 \exp(\mu_1 t) + A_2 \exp(\mu_2 t) + A_3 \exp(\mu_3 t)$
of the continuous curve is given in the frame.

APPLICATION OF THE PADE-LAPLACE METHOD TO MULTIEXPONENTIAL DECAYS :
ANALYSIS OF CHEMICAL RELAXATION SIGNALS

REFERENCES

(**) Unité de Physico-chimie des Macromolécules Biologiques, Institut Pasteur, 25 rue du Docteur Roux, 75724 Paris Cedex 15 (France).

[1] P. Claverie and A. Denis, "The Representation of functions through the Combined use of Integral Transform and Padé Approximants : Applications to the Analysis of Functions as Sums of Exponentials" submitted to SIAM J. Appl. Math.

[2] G. Baker, Jr., J. Math. Anal. Appl. 43, 498 (1973).

[3] M. Eigen and L. De Maeyer, "Techniques of Organic Chemistry", Vol. VIII, part 2, p. 895, Wiley-Interscience (1963).

[4] J. Aubard, J.J. Meyer and J.E. Dubois, Chem. Instrum., 48, 695 (1977).

[5] J. Aubard, J.M. Nozeran, J. J. Meyer, P. Levoir and J.E. Dubois, Rev. Sci. Instrum., 50, 52 (1979).

[6] J. Aubard, P. Levoir, A. Denis and P. Claverie, "Analysis of Multiexponential Signals by a Method Based on the Combination of Laplace Transform and Padé Approximants." (accepted for publication) Biophys. J. (1985).

[7] M. Dreyfus, O. Bensaude, G. Dodin and J.E. Dubois, J. Amer. Chem. Soc., 98, 6338 (1976).

[8] D. Colquhoun and A.G. Hawkes, Proc. Roy. Soc. (London) B 199, 231 (1977).