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*María C. Maciel*

*Domingo A. Tarzia (Eds.)*

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# MAT

## SERIE A: CONFERENCIAS, SEMINARIOS Y TRABAJOS DE MATEMÁTICA

No. 14

### TERCERAS JORNADAS SOBRE ECUACIONES DIFERENCIALES, OPTIMIZACIÓN Y ANÁLISIS NUMÉRICO

**Maria Cristina Maciel - Domingo Alberto Tarzia (Eds.)**

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## AN ALTERNATIVE METHOD TO COMPUTE MICHAELIS-MENTEN PARAMETERS FROM NUTRIENT UPTAKE DATA

**Juan C. REGINATO<sup>1</sup> and Domingo A. TARZIA<sup>2</sup>**

- 1 Departamento de Física, Facultad de Ciencias Físico-Químicas y Naturales - Universidad Nacional de Río Cuarto, Ruta 36 Km 601, X5804BYA Río Cuarto, Argentina.  
E-mail: [jreginato@exa.unrc.edu.ar](mailto:jreginato@exa.unrc.edu.ar)
- 2 Departamento de Matemática-CONICET, FCE – Universidad Austral, Paraguay 1950, S2000FZF Rosario, Argentina. E-mail: [Domingo.Tarzia@fce.austral.edu.ar](mailto:Domingo.Tarzia@fce.austral.edu.ar)

**Abstract.** An alternative method to compute the Michaelis-Menten parameters for nutrient uptake kinetics is presented. The method uses the explicit integration in time of the differential equation obtained from the relationship between decreasing nutrient concentration in a given volume of solution culture and nutrient uptake by roots. Thus, the depletion curve  $C = C(t)$  is obtained. Then, by using a non linear least squares fit method we obtain good estimations of the initial first derivative (rate of nutrient uptake) and the initial second derivative of the concentration. With these values and the average concentration at large times, explicit formulas for  $J_m$  (maximum influx at infinity concentrations),  $K_M$  (the Michaelis-Menten constant) and  $E$  (the efflux) are obtained. The method is applied to the pioneer depletion curve obtained by Claassen and Barber. Results obtained by Claassen-Barber, linearization Hanes method, non linear fit method applied to  $C'(t)$  vs.  $C$  and our method are compared. The results shown that the error obtained by our method is significantly smaller for the parameter  $E$  in contrast to the results obtained by the other methods.

**Key words:** Depletion curves, solution culture, kinetic parameters.

### Introduction

One of the methods of generating nutrient uptake data is the pioneer nutrient depletion method of Claassen and Barber (1974). By this method, growing plants absorbing nutrient in a limited volume of nutrient solution decrease the nutrient concentrations over a short period of time. Aliquots are removed and analyzed for concentrations of the nutrient of interest. In a simple case, volume changes due to evapotranspiration and quantities of nutrients withdrawn with the aliquots are small to the point of being negligible. The slope of concentration with respect to time is the rate of nutrient uptake, i.e.  $dC/dt$ , and the rate of nutrient uptake can be measured for a range of concentrations in a single experiment as the nutrient concentration is drawn down by plant uptake.

By assuming Michaelis-Menten kinetics (Michaelis-Menten, 1913) with an additional term of efflux rate  $E$ , the rate of absorption of nutrient can be described by the differential equation

$$\frac{dC(t)}{dt} = -\frac{\ell}{V} \left[ \frac{J_m C}{K_M + C} - E \right] \quad (1)$$

where  $C$  is the concentration of nutrient in soil solution,  $\ell$  is root length,  $V$  is the volume of nutrient solution,  $J_m$  is the influx at infinity concentrations,  $K_M$  is the Michaelis-Menten constant and  $E$  is a constant efflux rate. This equation is generally applicable to this situation and the issue divides in two problems: to express Michaelis-Menten kinetics in an integral form as a function of time or to make a parameter optimization of the coefficients  $J_m$  and  $K_m$  in order to generate the best fit of the kinetic model to the experimental data. For the first case the Michaelis-Menten equations can be analytically integrated in order to give an explicit expression in  $C$  but not in  $t$ . This means that if  $C$  is measured, then with values of  $E$ ,  $J_m$ ,  $K_M$ ,

and  $C_0$ , the time  $t$  predicted by the Michaelis-Menten kinetics model to draw down concentrations to that value of  $C$  could be calculated. Alternatively, the Michaelis-Menten differential equation can be numerically integrated by Euler or Runge-Kutta's methods. Another methods used are the Hanes linearization (Hanes, 1932) method and the non linear least squares fit method applied to  $C'(t)$  vs.  $C$  (Levenberg, 1944; Marquardt, 1963) which are widely applied in biochemical sciences to estimate parameters of Michaelis-Menten kinetics.

### Our method

In order to solve the first case we consider the differential problem for the depletion curve (Claassen and Barber, 1974), which is given by:

$$\frac{dC(t)}{dt} = -A \left[ \frac{J_m C}{K_M + C} - E \right], \quad C(0) = C_0 \quad (2)$$

with  $A = \frac{\ell}{V}$ . By integration of the Cauchy differential problem (2) we obtain:

$$vt = G \left( \frac{(J_m - E)C(t) - EK_M}{(J_m - E)C_0 - EK_M} \right) = G \left[ 1 + \frac{(J_m - E)(C(t) - C_0)}{(J_m - E)C_0 - EK_M} \right] = G[1 - \eta(C_0 - C(t))] \quad (3)$$

where:

$$v = \frac{(J_m - E)^2 A}{(J_m - E)C_0 - EK_M}, \quad \eta = \frac{J_m - E}{(J_m - E)C_0 - EK_M} \quad (4)$$

and real function  $G$  is defined by:

$$G(x) = 1 - x - \mu \log x, \quad 0 < x < 1, \quad (5)$$

$$\mu = \frac{K_M J_m}{(J_m - E)C_0 - EK_M}$$

By using the following properties

$$G(0^+) = +\infty, \quad G(1) = 0, \quad G'(x) < 0, \quad 0 < x < 1 \quad (6)$$

and the real inverse function  $F(x) = G^{-1}(x)$ , defined for  $x > 0$ , which satisfies the properties

$$F(0^+) = 1, \quad F(+\infty) = 0, \quad F'(x) < 0, \quad \forall x > 0 \quad (7)$$

then we get the following explicit expression for the concentration  $C(t)$  versus time  $t$ :

$$C(t) = \frac{EJ_m}{J_m - E} + \left( C_0 - \frac{EK_M}{J_m - E} \right) F(vt), \quad t > 0 \quad (8)$$

Now, from the depletion curve  $C = C(t)$ , are obtained the following experimental data:

$$\begin{aligned} C'(0) &= -\alpha, & \alpha > 0 \\ C''(0) &= \beta \\ C(\infty) &= \gamma \end{aligned} \quad (9)$$

These data are related with the efflux  $E$ , the maximum influx at infinity concentrations  $J_m$  and the Michaelis-Menten  $K_M$  constant through the following analytical relations obtained from the expression for  $C(t)$  shown above, i.e.:

$$\begin{aligned} A \left[ \frac{J_m C_o}{C_o + K_M} - E \right] &= \alpha \\ \frac{A^2 K_M J_m}{(C_o + K_M)^3} \left[ (J_m - E) C_o - E K_M \right] &= \beta \\ \frac{E K_M}{J_m - E} &= \gamma \end{aligned} \quad (10)$$

Solving the system of three equations for the unknown  $E$ ,  $J_m$ ,  $K_M$  we obtain the following expressions for  $E$ ,  $J_m$  and  $K_M$ .

$$\begin{aligned} E &= \frac{\alpha}{A \left[ \frac{\beta C_o}{\alpha^2} \left( \frac{C_o}{\gamma} - 1 \right) - 1 \right]} \\ J_m &= \frac{\gamma \beta}{A \alpha} \frac{\left( \frac{C_o}{\gamma} - 1 \right)^2}{\left[ \frac{\beta C_o}{\alpha^2} \left( \frac{C_o}{\gamma} - 1 \right) - 1 \right] \left[ 1 - \frac{\beta \gamma}{\alpha^2} \left( \frac{C_o}{\gamma} - 1 \right) \right]} \\ K_M &= \gamma \frac{\left[ \frac{\beta C_o}{\alpha^2} \left( \frac{C_o}{\gamma} - 1 \right) - 1 \right]}{\left[ 1 - \frac{\beta \gamma}{\alpha^2} \left( \frac{C_o}{\gamma} - 1 \right) \right]} \end{aligned} \quad (11)$$

Moreover, from the above formulas obtained for  $E$ ,  $J_m$  and  $K_M$  as functions of the coefficients  $\alpha$ ,  $\beta$  and  $\gamma$  we deduce that a necessary and sufficient condition in order to have positive  $E$ ,  $J_m$  and  $K_M$  parameters is given by the following inequalities:

$$\frac{\gamma}{C_o} < \frac{\gamma \beta}{\alpha^2} \left( \frac{C_o}{\gamma} - 1 \right) < 1 \quad (12)$$

From this, we remark that coefficients  $\alpha$ ,  $\beta$  and  $\gamma$  are not independent. The independent dimensionless coefficients are:

$$\xi = \frac{C_o}{\gamma} > 1 \quad \text{and} \quad \eta = \frac{\alpha^2}{\beta \gamma} > 0 \quad (13)$$

which determine the admissible region given by:

$$\xi - 1 < \eta < \xi(\xi - 1) \quad (14)$$

Previously to apply our method, in order to obtain a more exact results is necessary a very good estimation of  $C'(0) = -\alpha$  and  $C''(0) = \beta$ . By using (3) we transform the expression (8) in an equivalent expression:

$$C(t) = C_o - \frac{1}{\eta} I(vt) \quad (15)$$

where  $I: [0, +\infty) \rightarrow [0, 1)$  is the inverse function of the  $H: [0, 1) \rightarrow [0, +\infty)$  defined by the expression

$$H(x) = x - \mu \log(1-x) \quad (16)$$

which has the following properties

$$H'(x) = 1 + \frac{\mu}{1-x} > 0, \quad H''(x) = \frac{\mu}{(1-x)^2} > 0, \quad H'''(x) = \frac{2\mu}{(1-x)^3}, \quad 0 \leq x < 1$$

$$H(0^+) = 0, \quad H(1^-) = +\infty, \quad H'(0^+) = 1 + \mu, \quad H''(0^+) = \mu, \quad H'''(0) = 2\mu \quad (17)$$

Moreover, function  $I(x)$  has the following properties:

$$I(0^+) = 0, \quad I(+\infty) = 1, \quad I'(0^+) = \frac{1}{1+\mu}, \quad I''(0^+) = -\frac{\mu}{(1+\mu)^3}, \quad I'''(0^+) = \frac{\mu(\mu-2)}{(1+\mu)^5}$$

Now, in order to fit the depletion curve  $C(t)$  vs.  $t$  we define a new function

$$\Gamma(t) = 1 - \frac{C(t)}{C_o} = \frac{1}{C_o \eta} I(vt) \cong \frac{1}{C_o} [P_1 t - P_2 t^2 + P_3 t^3] \quad (18)$$

where:

$$P_1 = \frac{v}{\eta(1+\mu)}, \quad P_2 = \frac{\mu v^2}{2\eta(1+\mu)^3}, \quad P_3 = \frac{\mu(2-\mu)v^3}{6\eta(1+\mu)^5} \quad (19)$$

The coefficients  $P_1$ ,  $P_2$ , and  $P_3$  are obtained from fitted curve  $\Gamma(t)$  versus  $t$ . Then, the coefficients  $v$ ,  $\eta$ , and  $\mu$  can be obtained analytically solving the system of three equations (19) which solution is given by:

$$v = \frac{9}{2} \frac{(P_1 P_3 + 2P_2^2)^2}{P_1 P_2 (3P_1 P_3 + 2P_2^2)}, \quad \eta = \frac{3}{2} \frac{P_1 P_3 + 2P_2^2}{P_1^2 P_2}, \quad \mu = \frac{4}{3} \frac{P_2^2}{3P_1 P_3 + 2P_2^2} \quad (20)$$

and therefore the coefficients  $\alpha$  and  $\beta$  are given by:

$$\alpha = \frac{v}{\eta(1+\mu)} = P_1, \quad \beta = \frac{\mu v^2}{\eta(1+\mu)^3} = 2P_2 \quad (21)$$

The coefficient  $\gamma$  is obtained by averaging the last four values of  $C(t)$ .

### Results and Discussion

By using the experimental data for concentrations ( $\text{mol cm}^{-3}$ ) from Claassen-Barber, 1974:  $y_0 = 0.1918 \times 10^{-6}$ ,  $y_1 = 0.1713 \times 10^{-6}$ ,  $y_2 = 0.1509 \times 10^{-6}$ ,  $y_3 = 0.1266 \times 10^{-6}$ ,  $h = 600$  sec., solution volume =  $2800 \text{ cm}^3$ , root length =  $28610 \text{ cm}$  (Claassen, N. 2000, personal communication) and

taking for large time the arithmetic mean of the last four values of the depletion curve of Claassen-Barber, we obtain  $22.81 \text{ pmoles cm}^{-1} \text{ s}^{-1}$  for  $J_m$ ,  $576.6 \text{ mmoles cm}^{-3}$  for  $K_M$  y  $0.1299 \text{ pmoles cm}^{-1} \text{ s}^{-1}$  for  $E$ . In Table I we show a comparison between the different results obtained by Claassen-Barber method, linearization method of Hanes, non linear fit method applied to  $C'(t)$  vs.  $C$  and our method.

Table 1.  $J_m$ ,  $K_M$ ,  $E$  parameters computed by Claassen-Barber, Hanes, Non linear least squares methods and our method as described in this paper

<i>Parameter</i>	<i>Claassen-Barber method</i> <sup>§</sup>	<i>Hanes method</i>		<i>Non linear least squares fit of C'(t) vs. C method</i> <sup>#</sup>		<i>Our method</i>	
			% Error		% Error		% Error
$J_m \text{ (pmoles cm}^{-1} \text{ s}^{-1}\text{)}$	4	10.54	21.1	11.7	3	22.81	16.7
$K_M \text{ (mmoles cm}^3\text{)}$	27.9	180	19.5	11.5	17.2	576.6	33.8
$E \text{ (pmoles cm}^{-1} \text{ s}^{-1}\text{)}$	0.18	0.274	61	0.143	156	0.1299	24.16
$k \text{ (x } 10^{-4} \text{ s}^{-1}\text{)}^*$	0.143	0.586	40.6	1.02	20.2	0.4019	50.5

§ Data obtained from Claassen-Barber paper

# Results obtained applying the non linear fit (Levenberg-Marquadt) of the software Microcal Origin 7.5

\*As defined by Cushman  $k = J_m/K_M$  (Cushman, 1979)

As it can be appreciated, the non linear least squares method gives us the best value for  $J_m$  and  $K_M$  parameters. Instead, our method predicts the best value for  $E$ . Moreover, in Figure 1 we show the different curves  $C(t)$  vs.  $t$  obtained by integrating the equation (1) with the results obtained by the four methods.

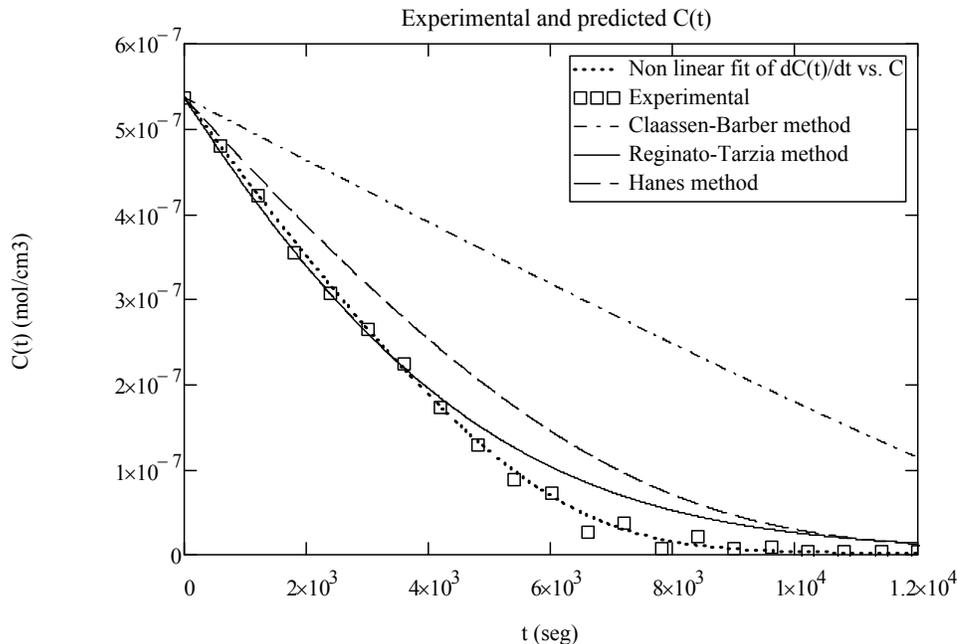


Figure 1. A comparison of the curves  $C(t)$  vs.  $t$  obtained by the four methods. Integration was performed with the mathematical package Mathcad

From the comparison between the experimental and predicted curves  $C(t)$  we observe that the best prediction is provided by the non linear least squares method and our method produces the second best prediction.

We have presented an alternative method to compute the Michaelis-Menten parameters of kinetics of nutrient uptake. The simplicity of the method is given by the utilization of few data (a good determination of initial first and second derivative, and the value of concentration at large times obtained from an explicit depletion curve) and the computation of  $J_m$  (maximum influx at infinity concentrations),  $K_M$  (the Michaelis-Menten constant) and  $E$  (the efflux) through explicit formulas. With respect to experimental data we obtain reasonable values for  $J_m$  and  $K_M$  although with greater errors than those obtained by the non linear least squares method. In the case of the efflux  $E$  we obtain a more exact result with respect to Hanes and non linear least squares methods. This last fact shows that the election of method to determine kinetic parameters not only depends of type de equations which parameters we desire to know but in addition of the algorithm used. The present method have been applied in the determination of kinetic parameters for potassium uptake by maize roots (Dekalb 762) growing in a typical Hapludol soil from Rio Cuarto, Cordoba. The results obtained were applied to validate a moving boundary model of nutrient uptake (Reginato et. al, 1999) and a model of coupled kinetics absorption of P and N by maize ( Bernardo, 2005).

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