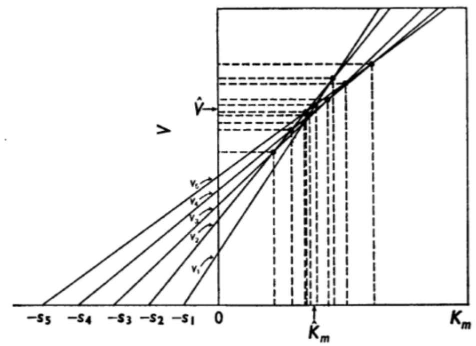
Validación estadística del método "Direct linear plot" aplicado a la inhibición acompetitiva por sustrato

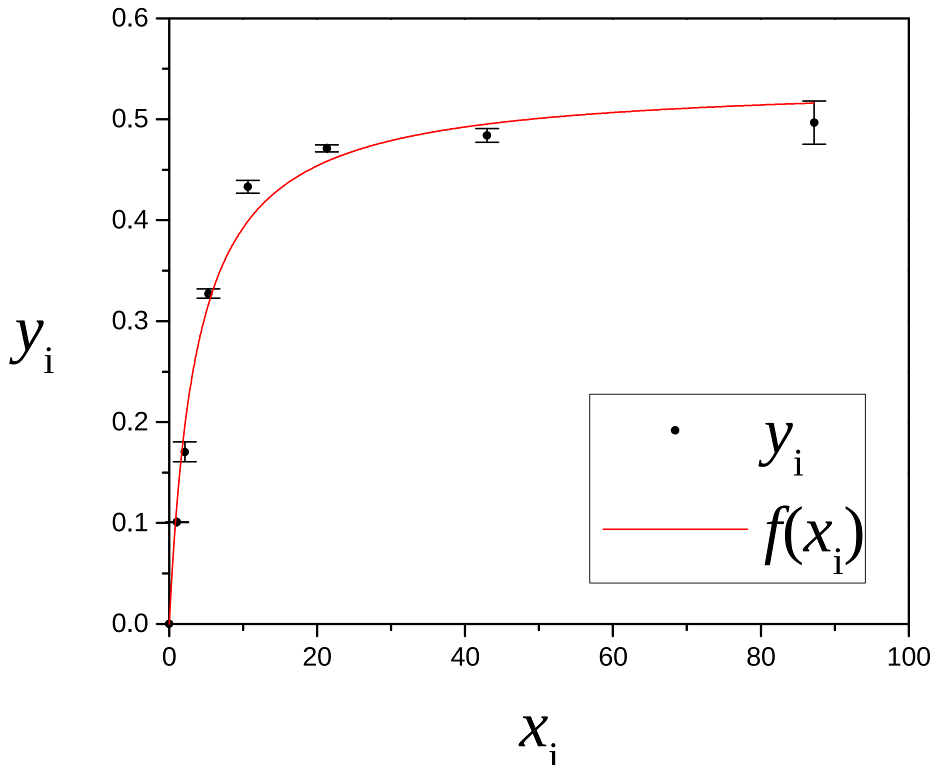


Pedro Valencia. Sebastián Flores

USM 2015

Determinación de parámetros cinéticos

Mínimos cuadrados:



$$S_r = \sum_{i=1}^{n} \left[y_i - f(x_i) \right]^2$$

Determinación de parámetros cinéticos

Durante el ajuste de un modelo se asume:

1. Los errores en las observaciones están sujetas a una distribución normal (Gaussiana).

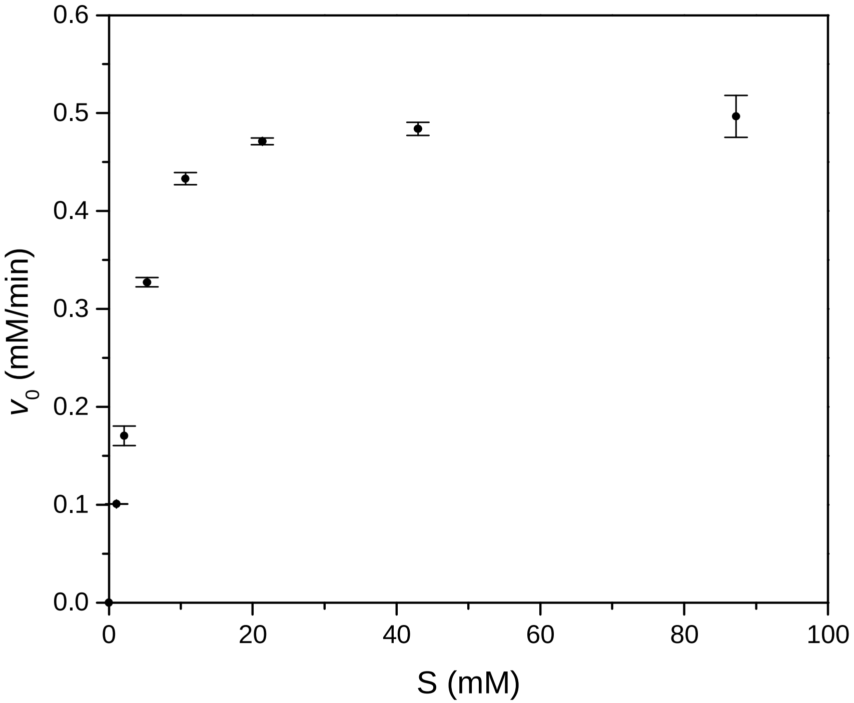
2. La variable independiente es exactamente conocida.

3. Las observaciones poseen la misma desviación estándar ó su función es conocida.

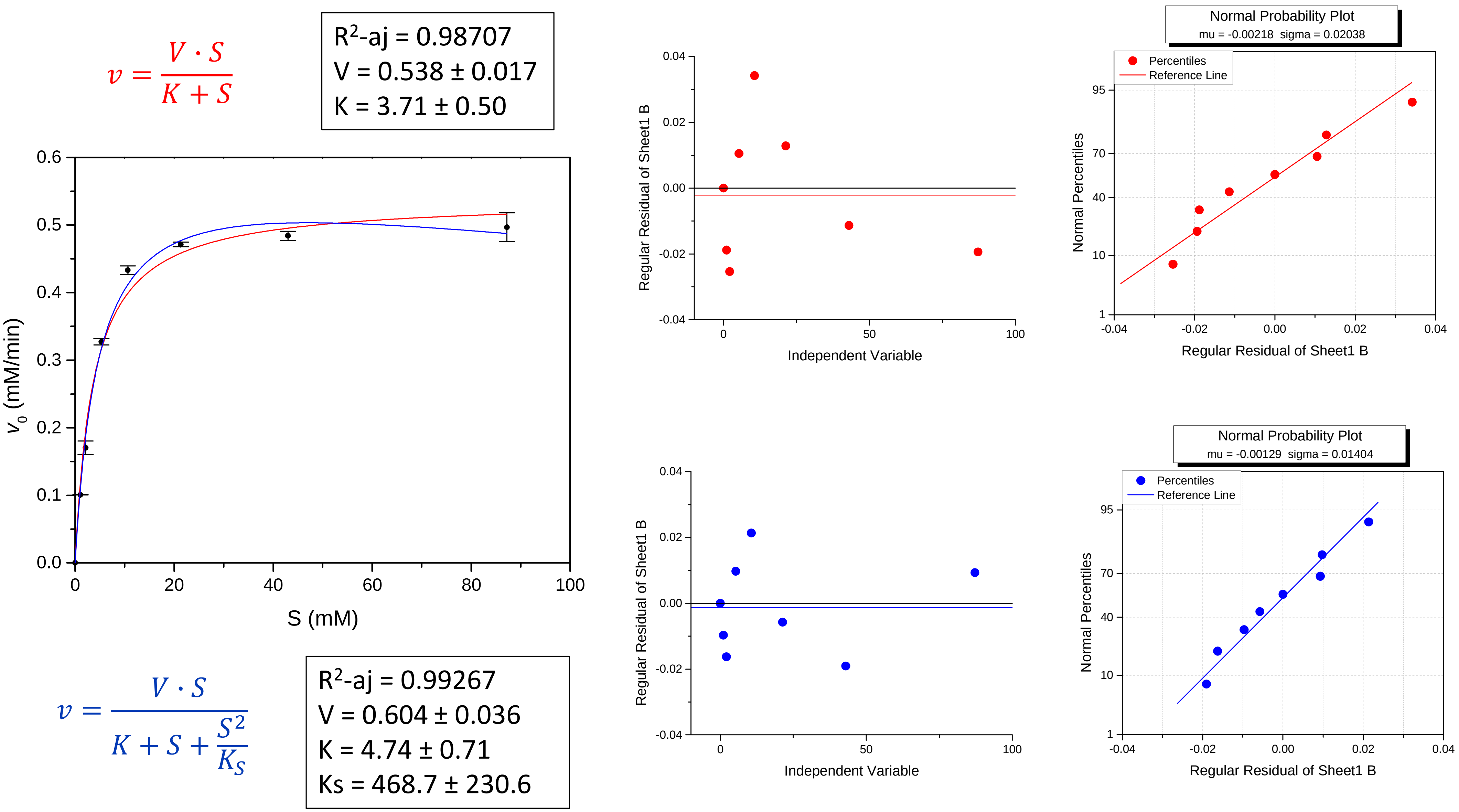
4. Los errores no se correlacionan.

5. No hay error sistemático: El modelo escogido es el correcto.

$$v = \frac{V \cdot S}{K + S}$$



$$v = \frac{V \cdot S}{K + S + \frac{S^2}{K_S}}$$



$v = \frac{V \cdot S}{K + S}$
R^2-aj = 0.98707
V = 0.538 ± 0.017
K = 3.71 ± 0.50
v_0 (mM/min)
S (mM)
$v = \frac{V \cdot S}{K + S + \frac{S^2}{K_S}}$
R^2-aj = 0.99267
V = 0.604 ± 0.036
K = 4.74 ± 0.71
Ks = 468.7 ± 230.6
Regular Residual of Sheet1 B
Independent Variable
Normal Probability Plot
mu = -0.00218 sigma = 0.02038
Percentiles
Reference Line
Normal Percentiles
Regular Residual of Sheet1 B
Regular Residual of Sheet1 B
Independent Variable
Normal Probability Plot
mu = -0.00129 sigma = 0.01404
Percentiles
Reference Line
Normal Percentiles
Regular Residual of Sheet1 B

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The Direct Linear Plot

A NEW GRAPHICAL PROCEDURE FOR ESTIMATING ENZYME KINETIC PARAMETERS

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A new plot is described for analysing the results of kinetic experiments in which the Michaelis–Menten equation is obeyed. Observations are plotted as lines in parameter space, instead of points in observation space. With appropriate modifications the plot is applicable to most problems of interest to the enzyme kineticist. It has the following advantages over traditional methods of plotting kinetic results: it is very simple to construct, because it is composed entirely of straight lines and requires no calculation or mathematical tables; the kinetic constants are read off the plot directly, again without calculation; it may be used during the course of an experiment to judge the success of the experiment, and to modify the experimental design; it provides clear and accurate information about the quality of the observations, and identifies aberrant observations; it provides a clear indication of the precision of the kinetic constants; constructed with care, it provides unbiased estimates of the kinetic constants, the same as those provided by a computer program; it may be used to simulate results for illustrative purposes very rapidly and simply.

Direct linear plot

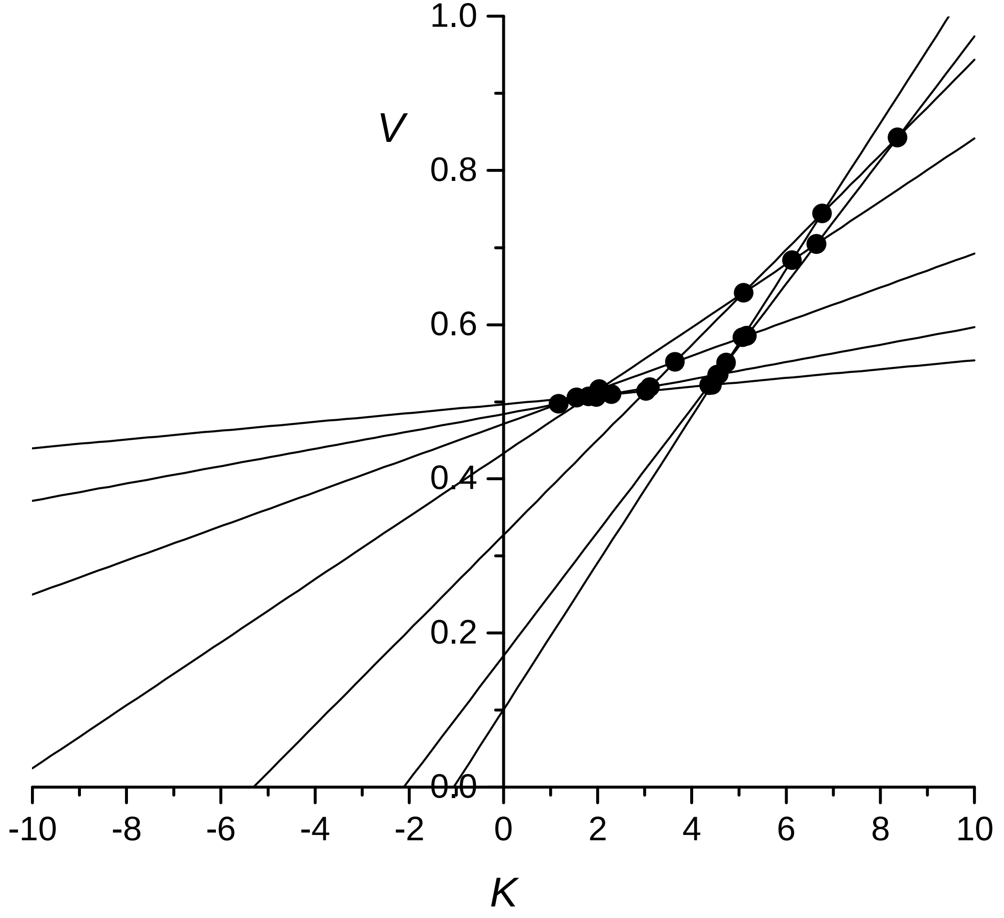
S	*v*
87.2	0.4967
43.0	0.4840
21.3	0.4712
10.6	0.4331
5.31	0.3273
2.12	0.1704
1.06	0.1008

$$v_i = \frac{V \cdot S_i}{K + S_i}$$

$$V = \frac{S_i - S_j}{\frac{S_i}{v_i} - \frac{S_j}{v_j}} \qquad K = \frac{v_i - v_j}{\frac{v_i}{S_i} - \frac{v_j}{S_j}}$$

$$\text{Combinaciones} = \frac{1}{2}n(n-1)$$

Direct linear plot



K	V
1.17323214	0.49715432
1.55242023	0.50554274
1.97004431	0.50612216
1.80875079	0.50700283
2.29376356	0.50976551
3.02952282	0.51395647
2.03347225	0.51618461
3.11071007	0.51895996
4.36923548	0.52158761
4.42849888	0.52192518
4.53613598	0.53500263
4.57003129	0.53538411
4.72923077	0.55052308
3.64366397	0.55180537
5.07590048	0.5834894
5.16347933	0.58542683
5.09616727	0.6413217
6.12694382	0.68343768
6.64745763	0.70470508
6.76670296	0.74427515
8.3660666	0.84284233

K ← 4.53613598 | 0.53500263 → *V*

Direct linear plot aplicado a Modelo Inhibición por Sustrato

S (mM)	*v* (mM/min)
0.025	0.036
0.050	0.052
0.100	0.095
0.250	0.134
0.500	0.168
1.00	0.187
10	0.192
50	0.186
100	0.175
200	0.163
300	0.146
400	0.139
600	0.115

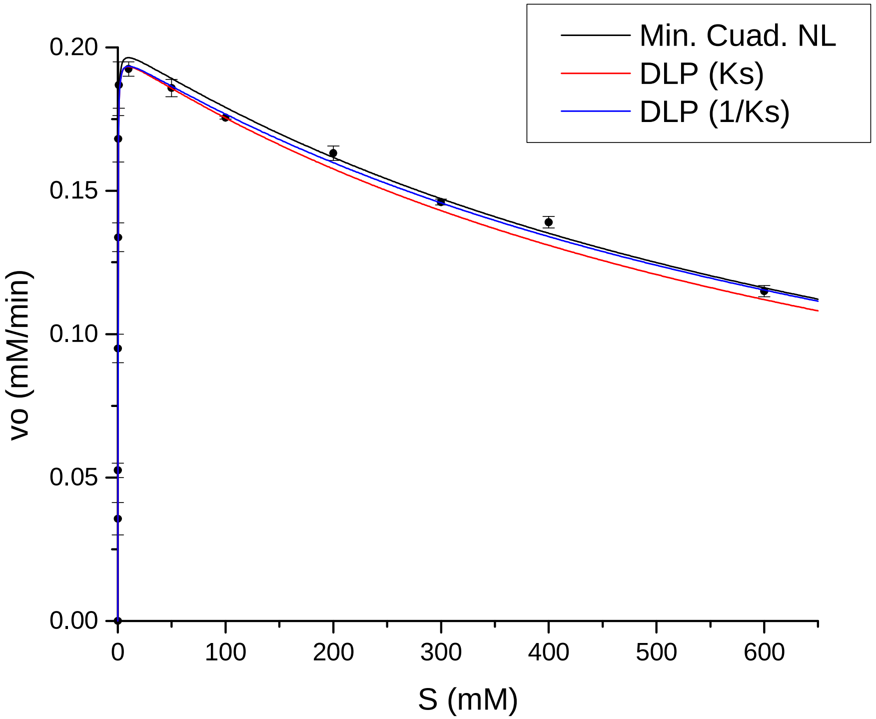
286 combinaciones

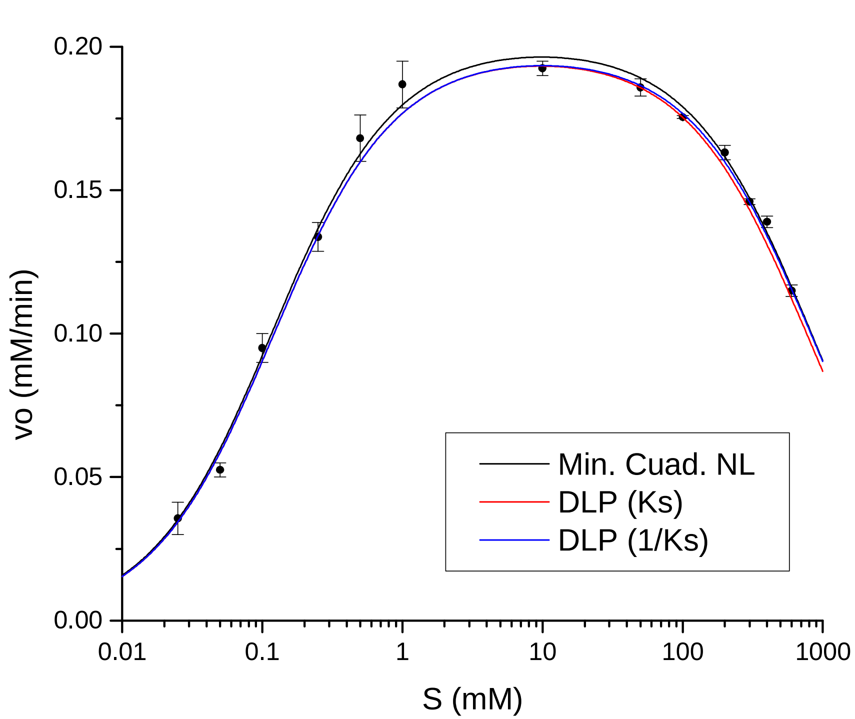
$$v_i = \frac{V \cdot S_i}{K + S_i + \frac{S_i^2}{K_S}} \quad \Rightarrow \quad V - \frac{v_i}{S_i} K - v_i S_i \frac{1}{K_S} = v_i$$

$$\begin{bmatrix} 1 & -{}^{v_1}/_{S_1} & -v_1 S_1 \\ 1 & -{}^{v_2}/_{S_2} & -v_2 S_2 \\ 1 & -{}^{v_3}/_{S_3} & -v_3 S_3 \end{bmatrix} \begin{bmatrix} V \\ K \\ K_S^{-1} \end{bmatrix} = \begin{bmatrix} v_1 \\ v_2 \\ v_3 \end{bmatrix}$$

Método\Parámetro	V	K	K_S
Mín. Cuad. NL	0.201	0.118	821
Mediana	0.198	0.119	838 (782)

Direct linear plot aplicado a Modelo Inhibición por Sustrato





$$v_i = \frac{V \cdot S_i}{K + S_i + \frac{S_i^2}{K_S}}$$

Direct linear plot

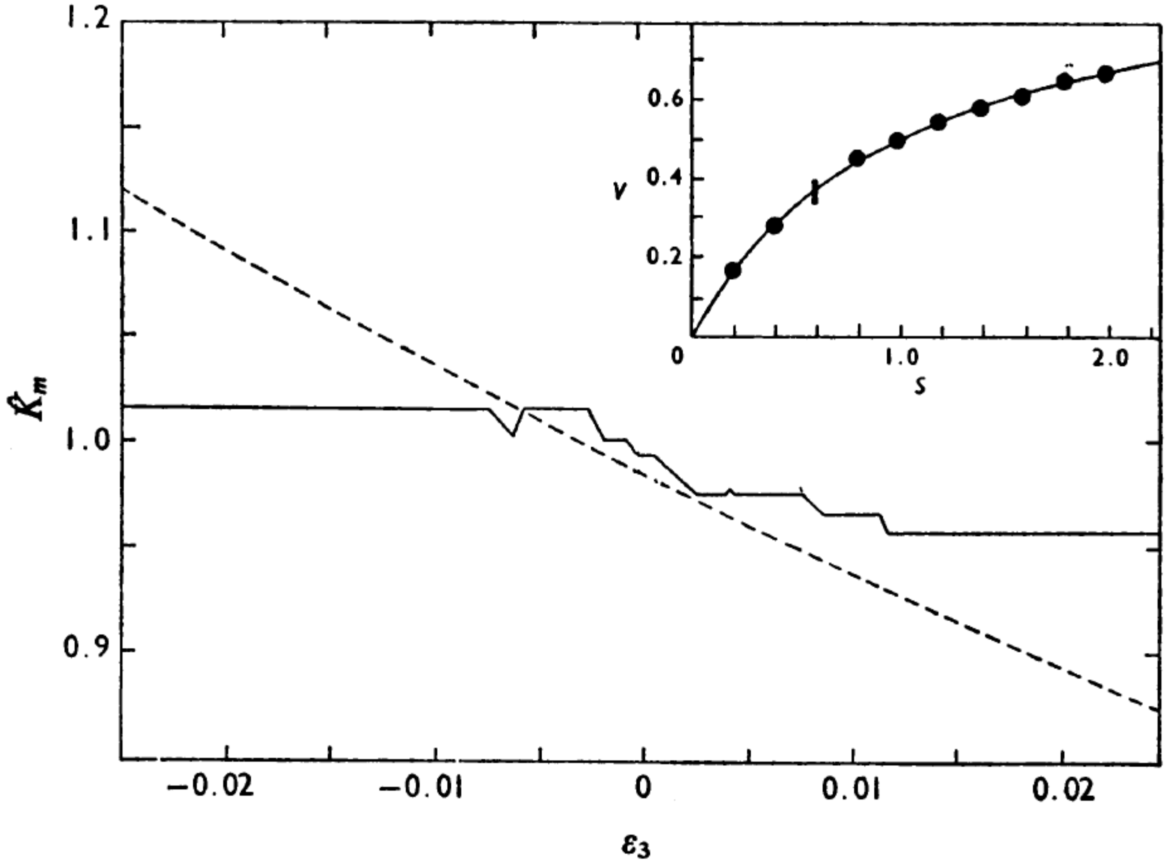


Fig. 2. *Variation of $\hat{K}_m$ with individual errors*

Direct linear plot aplicado a Modelo Inhibición por Sustrato

Objetivo

Validar estadísticamente el uso del método DLP aplicado al modelo de inhibición por sustrato

Actividades

1. **Implementar algoritmo computacional para métodos mínimos cuadrados y DLP.**
2. **Diseñar datos simulados.**
3. **Validar método con datos simulados.**
4. **Validar método con datos experimentales.**