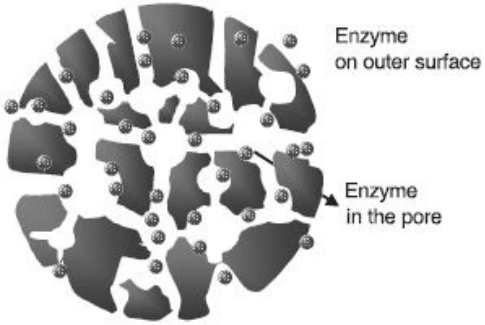
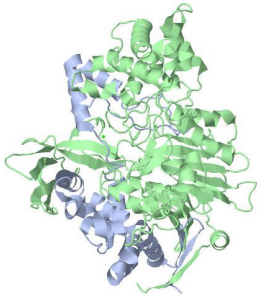
Determinación de parámetros cinéticos de catalizadores de enzimáticos



Pedro Valencia, Diego Gajardo

USM 2017

Catálisis enzimática



S → **P**

Ventajas:

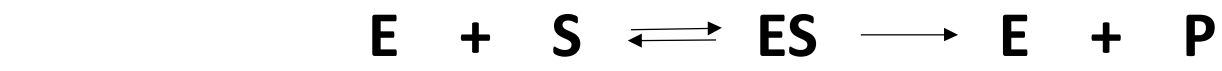
- **Alta velocidad de reacción.**
- **Elevada selectividad.**

Desventajas:

- **Alto costo.**
- **Termolábiles.**

Catálisis enzimática

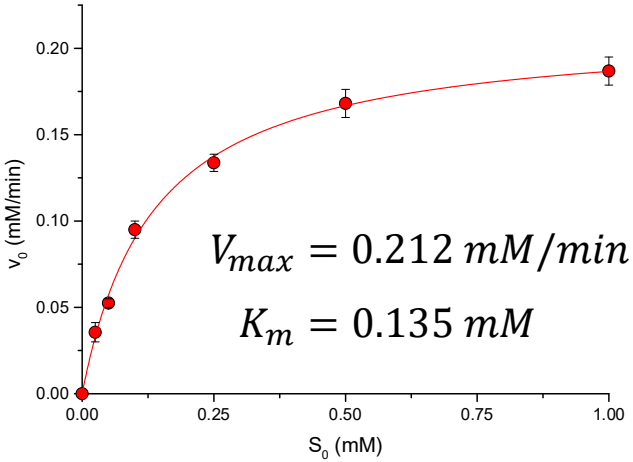
Ecuación de Michaelis-Menten



$$v = \frac{V_{\max} S}{K_m + S}$$

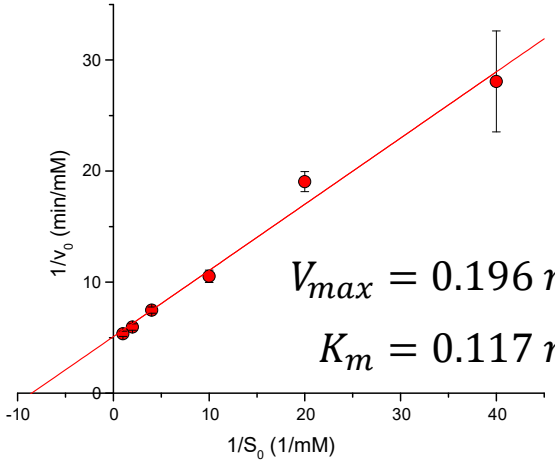
Catálisis enzimática

Ecuación de Michaelis-Menten: Estimación de V_{max} y K_m



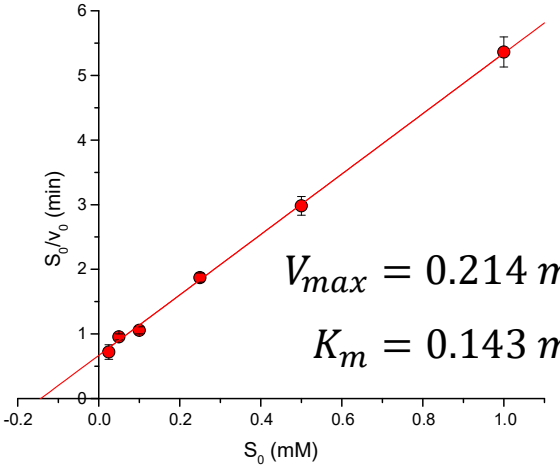
$V_{max} = 0.212\ mM/min$

$K_m = 0.135\ mM$



$V_{max} = 0.196\ mM/min$

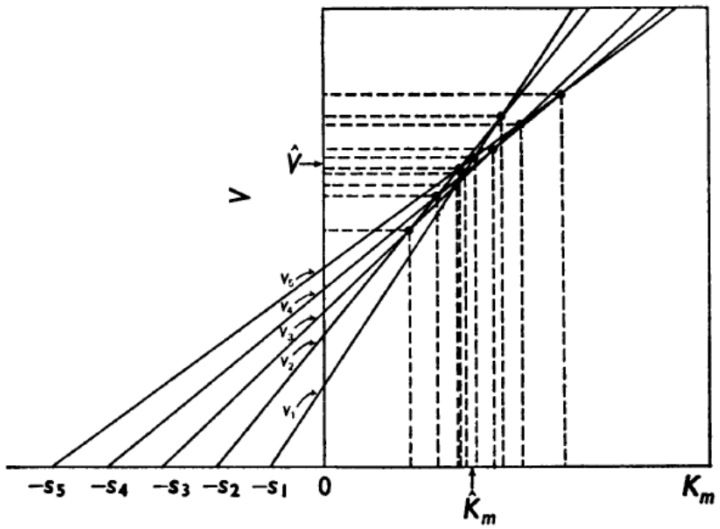
$K_m = 0.117\ mM$



$V_{max} = 0.214\ mM/min$

$K_m = 0.143\ mM$

Método de la Mediana



Biochem. J. (1974) **139**, 715–720
Printed in Great Britain

The Direct Linear Plot

A NEW GRAPHICAL PROCEDURE FOR ESTIMATING ENZYME KINETIC PARAMETERS

By ROBERT EISENTHAL*
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and ATHEL CORNISH-BOWDEN
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(*Received* 19 *December* 1973)

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

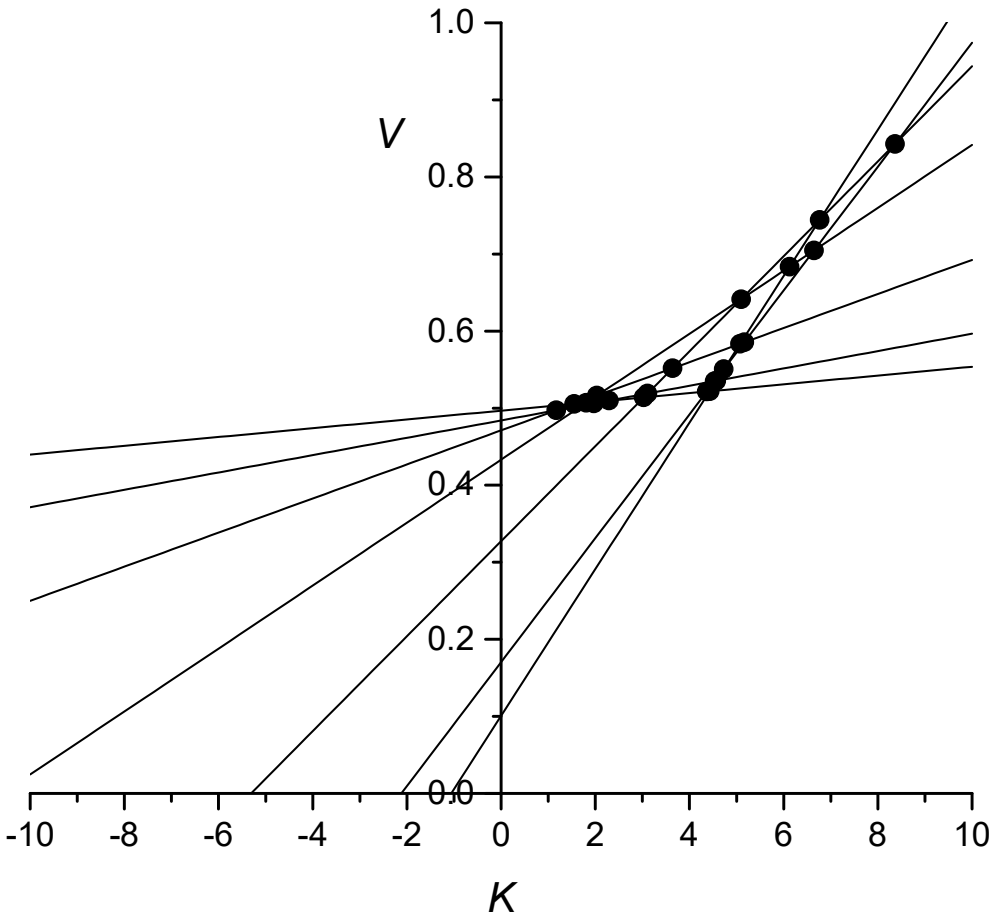
S_0	v_0
87.2	0.4967
43.0	0.4840
21.3	0.4712
10.6	0.4331
5.31	0.3273
2.12	0.1704
1.06	0.1008

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

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

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

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 ← → *V*

Biochem. J. (1986) 234, 21–29 (Printed in Great Britain)

Robust regression of enzyme kinetic data

Athel CORNISH-BOWDEN* and Laszlo ENDRENYI†

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A method described previously [Cornish-Bowden & Endrenyi (1981) Biochem. J. **193**, 1005–1008] for fitting theoretical equations to enzyme kinetic data without prior knowledge of weights or error distribution has been tested by computer simulation. With the equations for various kinds of linear inhibition as an example, the method performed well under all of the conditions examined, giving results that were often much better than those given by widely used least-squares alternatives, and were never appreciably worse. Although equations for two-substrate kinetics were not explicitly tested, the results for inhibition equations can be generalized to include two-substrate equations because the two are formally equivalent for simulation purposes. As a check on the results with inhibition equations the method was also tested for fitting bell-shaped pH–activity profiles and gave correspondingly good results.

the median method performed about equally well in the Michaelis–Menten case. However, the median method cannot readily be generalized to equations of more than two parameters, whereas the new method can in principle be applied to any equation normally analysed by linear or non-linear regression. The corresponding computer

BIOTECHNOLOGY AND BIOENGINEERING, VOL. 62, NO. 4, FEBRUARY 20, 1999

A Method of Graphically Analyzing Substrate-Inhibition Kinetics

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Received 11 December 1997; accepted 31 July 1998

Abstract: A model of substrate inhibition for enzyme catalysis was extended to describe the kinetics of photosynthetic production of ethylene by a recombinant cya-

where [S] is the substrate concentration, and V_{max}, K_s (this notation will be used throughout this article for different

and V_{max}, and the median values of these estimates are the best-fit values. A disadvantage of this plot is that it cannot be extended directly to fitting equations containing more than two parameters, such as Eq. (1). However, we can estimate $[S]_{max}$ first and rewrite the equation into

Yoshino and Murakami. *SpringerPlus (2015)4:292*
DOI 10.1186/s40064-015-1082-8

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Analysis of the substrate inhibition of complete and partial types

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Department of Biochemistry,
Aichi Medical University
School of Medicine.

Abstract

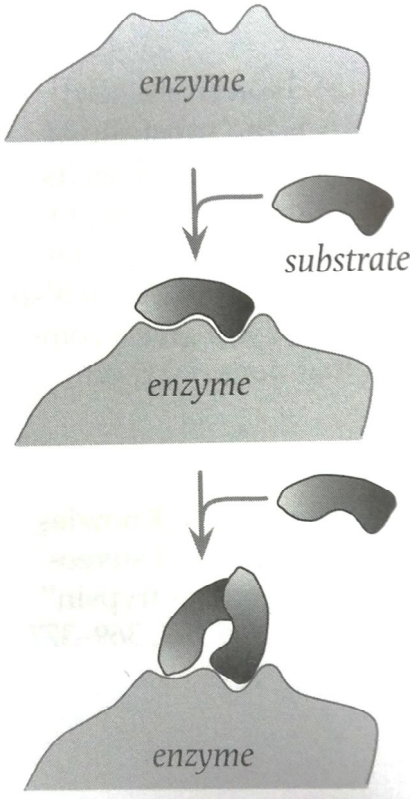
A simple graphical method was described for determining the kinetic parameters of substrate inhibition of complete and partial types. The method consists of plotting

strate inhibition. However, various kinetic methods for analyzing inhibition mechanism (Segel 1973; Cornish-Bowden 1974a, b; Eisenthal and Cornish-Bowden 1974; Baici 1981; Yoshino 1987) cannot be applied to substrate inhibition. In this report we describe a new graphical method for direct determination of the inhibition constants and the reaction

Inhibición Acompetitiva por Sustrato

$$E + S \rightleftharpoons ES \longrightarrow E + P$$

$$ES + S \rightleftharpoons ESS$$



$$v = \frac{V_{max} \cdot S}{K_m + S + \frac{S^2}{K_S}}$$

Journal of Theoretical Biology 418 (2017) 122–128

Contents lists available at ScienceDirect

Journal of Theoretical Biology

journal homepage: www.elsevier.com/locate/yjtbi

Application of the median method to estimate the kinetic constants of the substrate uncompetitive inhibition equation

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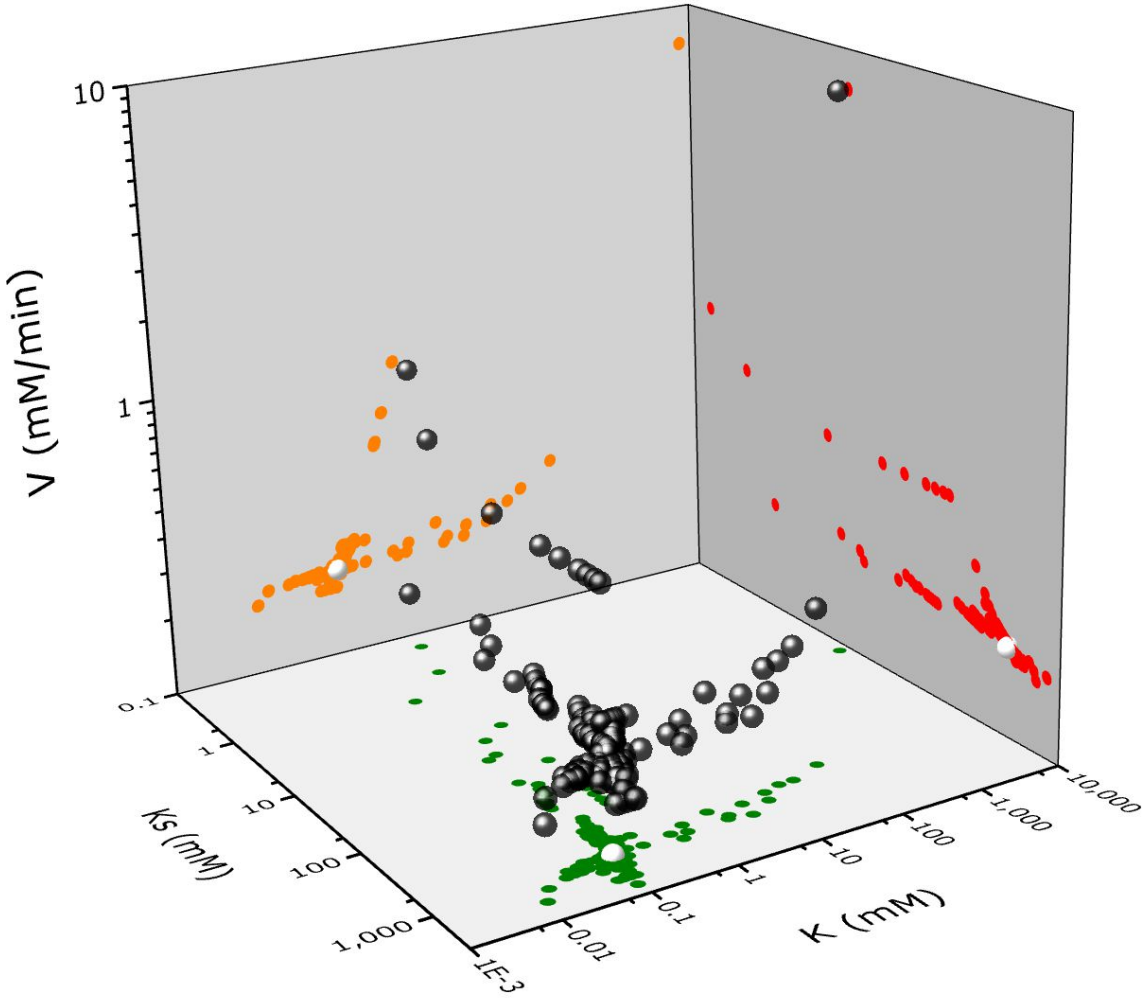
ARTICLE INFO

Keywords:
Direct linear plot
Median method
Substrate inhibition
Kinetic constants estimation

ABSTRACT

In 1974, Eisenthal and Cornish-Bowden published the direct linear plot method, which used the median to estimate the V_{max} and K_m from a set of initial rates as a function of substrate concentrations. The robustness of this non-parametric method was clearly demonstrated by comparing it with the least-squares method. The authors commented that the method cannot readily be generalized to equations of more than two parameters. Unfortunately, this comment has been misread by other authors. Comments such as "this method cannot be extended directly to equations with more than two parameters" were found in some publications. In addition, recently, the most drastic comment was published: "this method cannot be applied for the analysis of substrate inhibition." Given all of these presumptions, we have been motivated to publish a demonstration of the contrary: the median method can be applied to more than two-parameter equations, using as an example, the substrate uncompetitive inhibition equation. A computer algorithm was written to evaluate the effect of simulated experimental error of the initial rates on the estimation of V_{max}, K_m and K_S. The error was assigned to different points of the experimental design. Four different K_S/K_m ratios were analyzed with the values 10, 100, 1000 and 10,000. The results indicated that the least-squares method was slightly better than the median method in terms of accuracy and variance. However, the presence of outliers affected the estimation of kinetic constants using the least-squares method more severely than the median method. The estimation of K_S using the median method to estimate $1/K_S$ was much better than the direct estimation of K_S, causing a negative effect of non-linearity of K_S in the kinetic equation. Considering that the median method is free from the assumptions of the least-squares method and the arbitrary assumptions implicit in the linearization methods to estimate the kinetic constants V_{max}, K_m and K_S from the substrate uncompetitive inhibition equation, the median method is highly superior to all published methods, including the non-linear regression by least squares. We concluded that the median method can be applied to the substrate uncompetitive inhibition equation and other equations with more than two parameters. In addition, as we can project, the median method is the most reliable and robust method for the estimation of kinetic parameters from enzyme kinetic models.

Direct linear plot aplicado a Modelo Inhibición por Sustrato



Direct linear plot aplicado a Modelo Inhibición por Sustrato

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

$$V - \frac{v_i}{S_i} K - v_i S_i \frac{1}{K_S} = v_i$$

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

Direct linear plot aplicado a Modelo Inhibición por Sustrato

S_0 (mM)	v_0 (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

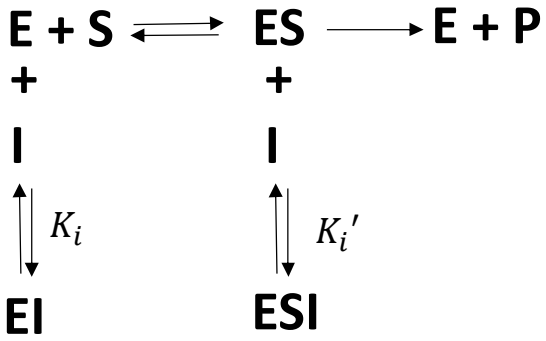
$$\begin{bmatrix} 1 & -{}^{v_1}/_{S_1} & -v_1 S_1 \\ 1 & -{}^{v_2}/_{S_2} & -v_2 S_2 \\ 1 & -{}^{v_3}/_{S_3} & -v_3 S_3 \end{bmatrix} \begin{bmatrix} V \\ K \\ K_S^{-1} \end{bmatrix} = \begin{bmatrix} v_1 \\ v_2 \\ v_3 \end{bmatrix}$$

$$\text{Combinaciones} = \frac{1}{6} n(n-1)(n-2)$$

Método\Parámetro	V	K	K_S
MMC	0.201	0.118	821
Mediana	0.198	0.119	837 (782)

Direct linear plot

Problema 1. Determinación de constantes cinéticas (V_{max}, K_m, K_i, K_i')



Inhibición Competitiva

$$v = \frac{V_{max} \cdot S}{K_m \left(1 + \frac{I}{K_i}\right) + S}$$

Inhibición Acompetitiva

$$v = \frac{V_{max} \cdot S}{K_m + S\left(1 + \frac{I}{K_i'}\right)}$$

Inhibición Mixta

$$v = \frac{V_{max} \cdot S}{K_m \left(1 + \frac{I}{K_i}\right) + S\left(1 + \frac{I}{K_i'}\right)}$$

Direct linear plot aplicado a modelos de inhibición por producto

$$v_i = \frac{V_{max} S_i}{K_m + S_i + \frac{K_m}{K_p} P_i} \quad \text{donde} \quad P_i = S_0 - S_i$$

$$v_i K_m - S_i V_{max} + \frac{v_i P_i K_m}{K_p} = -v_i S_i$$

Direct linear plot aplicado a modelos de inhibición por producto

Resolver el siguiente sistema de ecuaciones:

$$v_i K_m - S_i V_{max} + \frac{v_i P_i K_m}{K_p} = -\mathrm{v_i S_i} \qquad i = 1, 2, 3$$

Direct linear plot aplicado a modelos de inhibición por producto

Problema 1. Determinación de constantes cinéticas (V_{max}, K_m, K_p)

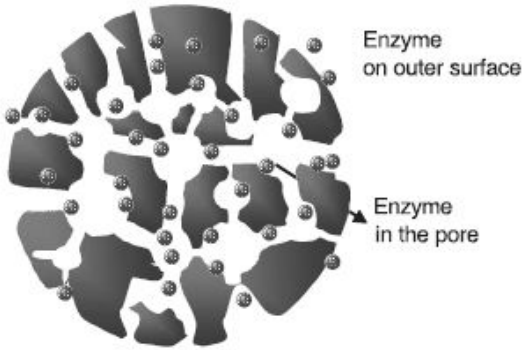
Objetivo

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

Actividades

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

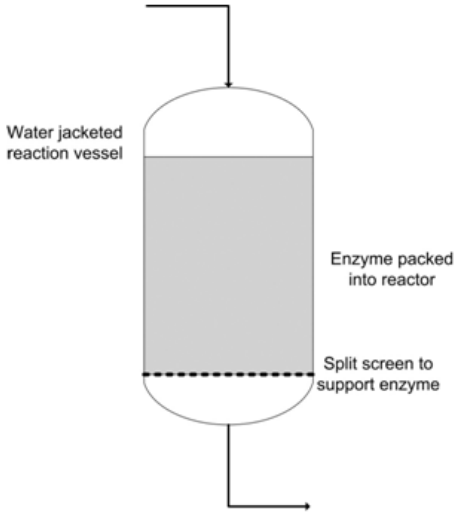
Catálisis Heterogénea



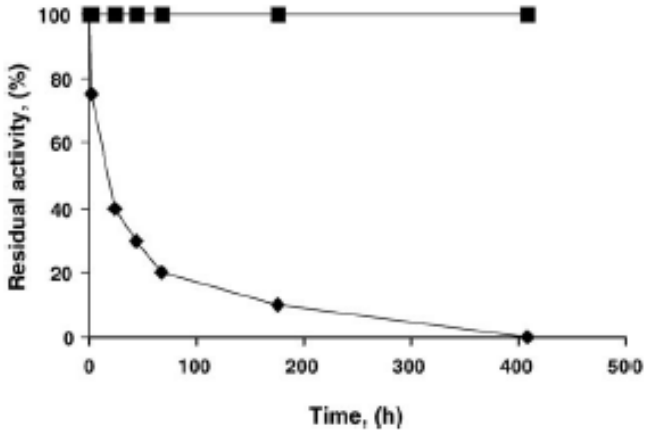
Inmovilización de enzimas

¿Por qué inmovilizar?

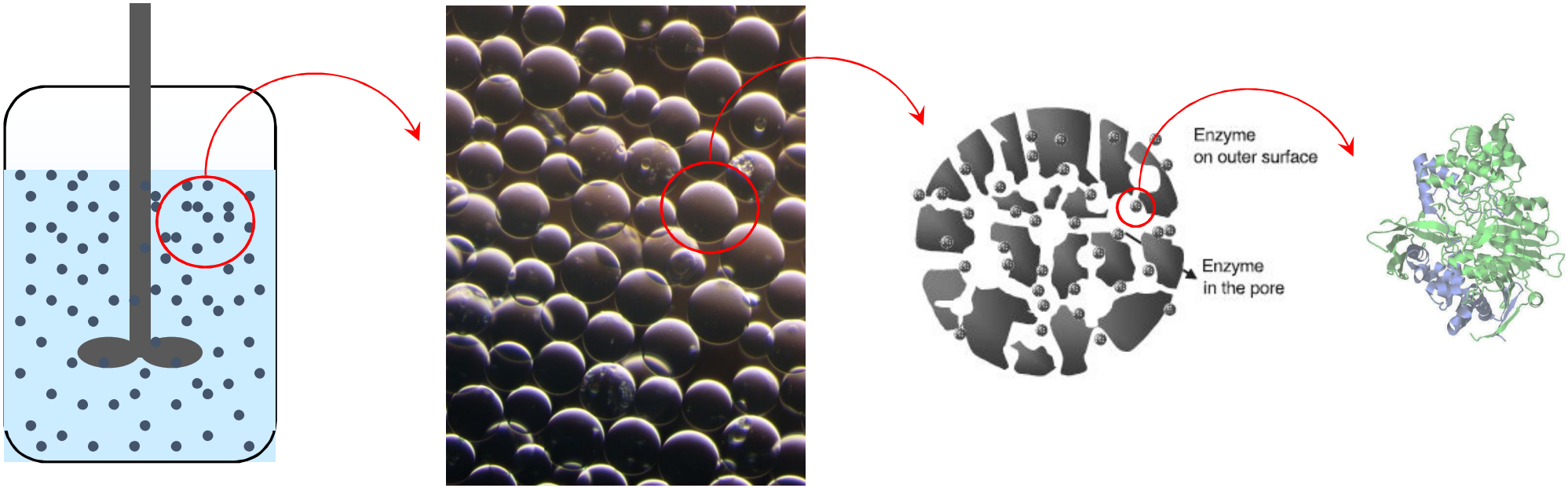
Reutilización



Estabilización



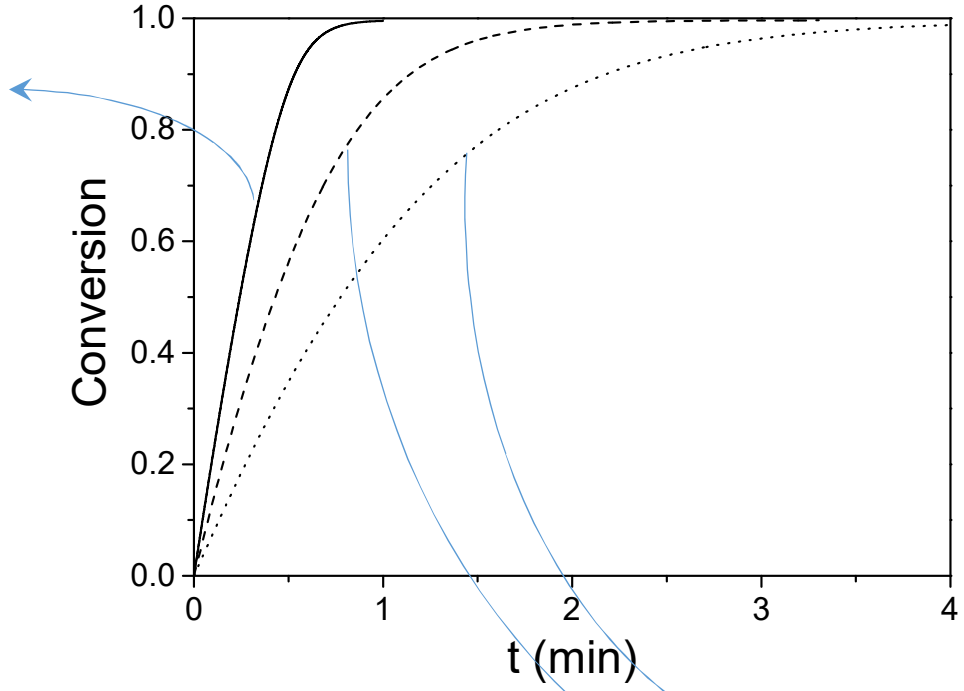
Catálisis enzimática heterogénea



Determinación parámetros cinéticos

Enzima soluble

$$\frac{dS}{dt} = -v$$



Enzima inmovilizada

$$\frac{\partial S}{\partial t} = D_e\left(\frac{\partial^2 S}{\partial r^2} + \frac{2}{r}\frac{\partial S}{\partial r}\right) - v$$

Catálisis enzimática heterogénea

Problema 2. Determinación del coeficiente de difusión efectiva (D_{ef})

$$\frac{\partial S}{\partial t} = D_e\left(\frac{\partial^2 S}{\partial r^2} + \frac{2}{r}\frac{\partial S}{\partial r}\right) - v$$

Catálisis enzimática heterogénea

Problema 2. Determinación del coeficiente de difusión efectiva (D_{ef})

Determination of Effective Diffusion Coefficients — an Important Parameter for the Efficiency of Immobilized Biocatalysts

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West Germany

Introduction

The limitation of mass transport by diffusion plays an important role in heterogeneous catalysis especially when the insoluble catalyst has a porous structure. Therefore, knowledge of diffusion processes influencing the reaction rate is of particular interest when the preparation of immobilized enzymes (or cells) must be optimized with respect to their catalytic activity. It is often assumed that it is difficult to investigate the diffusion behaviour of substrate molecules near the surface of a carrier or within its matrix. In this paper an experimental procedure is described that allows the determination of the effective diffusion coefficient D_e for low molecular weight substrates using a ball-shaped carrier for enzyme immobilization.

only approximately with the square root of enzyme concentration.

(3) Further, the influence of the substrate concentration gradient on the reaction rate v is diminished if the diameter d of the beads containing the catalyst is reduced. v is inversely proportional to d.

(4) Measuring v as a function of substrate concentration c leads to an enhanced K_M value, as v increases more slowly with c.

The relation between the efficiency η and these different parameters has been treated in detail in this journal by Roig *et al.*[1]

The expression for the diffusion of molecules in a ball-shaped matrix with radius r is

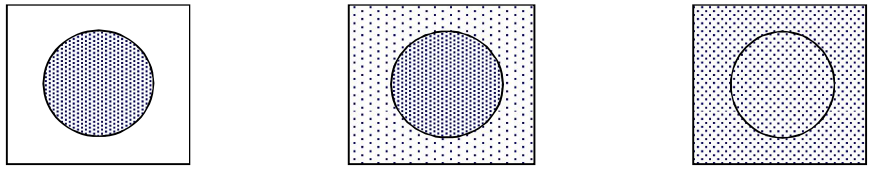
$$\frac{c_t - c_\infty}{c_0 - c_\infty} = \frac{6}{\pi^2} \sum_{n=1}^{\infty} n^{-2} \exp\left(-\frac{n^2 \cdot \pi^2 \cdot D_e \cdot t}{r^2}\right)$$

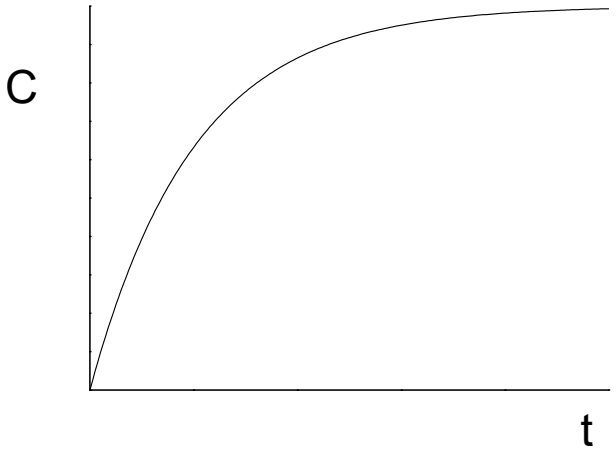
For sufficiently large values of t, this expression can be approximated by the first term of the series:

$$\frac{c_t - c_\infty}{c_0 - c_\infty} \simeq \frac{6}{\pi^2} \exp\left(-\frac{\pi^2 \cdot D_e \cdot t}{r^2}\right)$$

Catálisis enzimática heterogénea

Problema 2. Determinación del coeficiente de difusión efectiva (D_{ef})

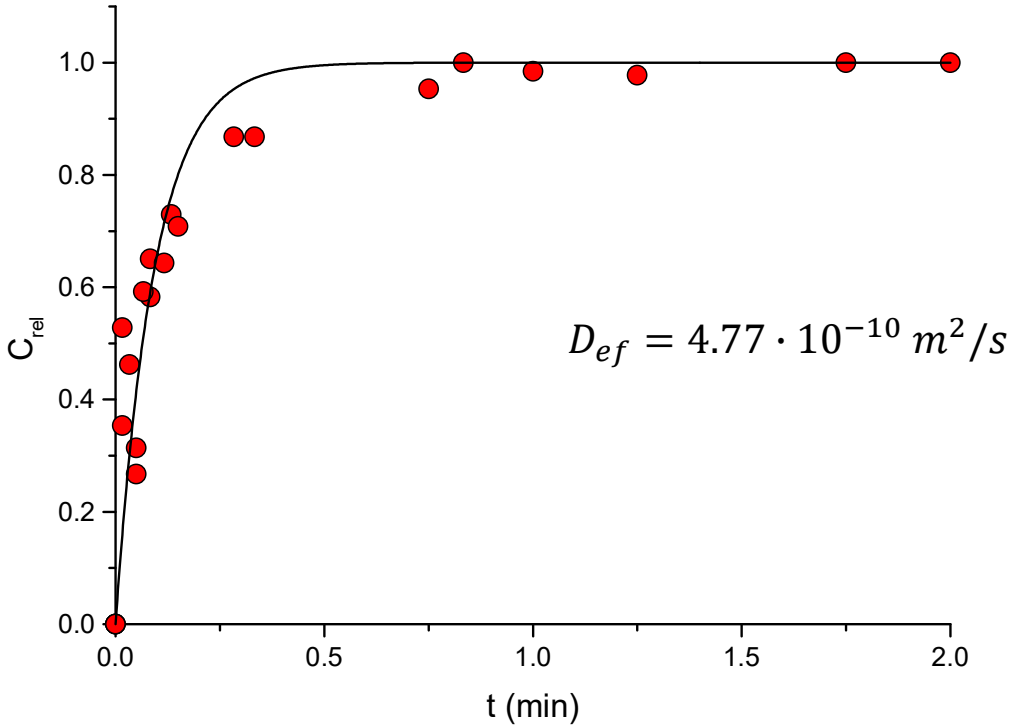




$$c_t = c_\infty - c_\infty e^{-\frac{\pi^2 D_e}{R^2} t}$$

Estimación de parámetros

Problema 2. Determinación del coeficiente de difusión efectiva (D_{ef})



Estimación del coeficiente de difusión efectiva

Objetivo

Estimar el coeficiente de difusión efectiva en catalizadores de enzima inmovilizada utilizando la ecuación de difusión sin aproximación analítica

Actividades

1. **Deducir la ecuación de difusión para partículas esféricas en medio líquido.**
2. **Implementar algoritmo de estimación del coeficiente de difusión.**
3. **Validar método con datos simulados.**

Catálisis enzimática heterogénea

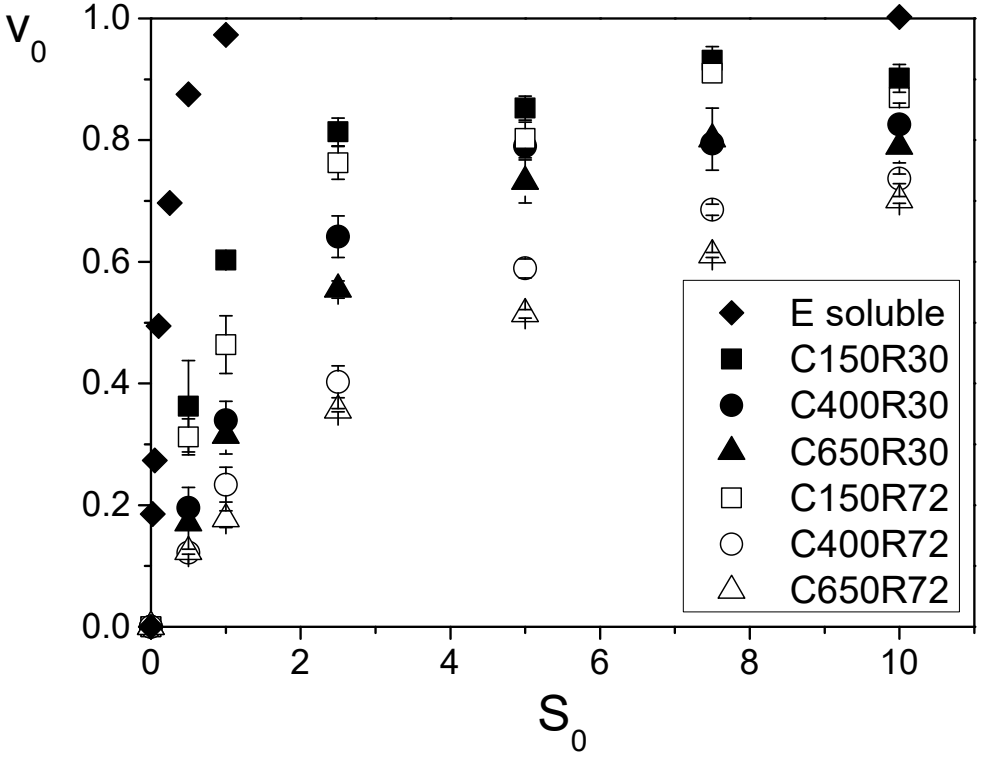
Problema 3. Determinación de constantes cinéticas intrínsecas.

$$\frac{\partial S}{\partial t} = D_e\left(\frac{\partial^2 S}{\partial r^2} + \frac{2}{r}\frac{\partial S}{\partial r}\right) - v$$

$$\frac{\partial S}{\partial t} = D_e\left(\frac{\partial^2 S}{\partial r^2} + \frac{2}{r}\frac{\partial S}{\partial r}\right) - \frac{V_{\max} S}{K_m + S}$$

Catálisis enzimática heterogénea

Problema 3. Determinación de constantes cinéticas intrínsecas.



Catalizador	K_m (mM)
E Soluble	0.23
C150R30	0.73
C400R30	1.70
C650R30	2.09
C150R72	1.04
C400R72	3.52
C650R72	4.55

Estimación de constantes cinéticas

Problema 3. Determinación de constantes cinéticas intrínsecas (V_{max}, K_m, K_s)

Modelo para inhibición acompetitiva por sustrato

$$\frac{\partial S}{\partial t} = D_e\left(\frac{\partial^2 S}{\partial r^2} + \frac{2}{r}\frac{\partial S}{\partial r}\right) - \frac{V_{\max} S}{K_m + S + \frac{S^2}{K_S}}$$

Determinación numérica de parámetros cinéticos de catalizadores usando un modelo de inhibición por sustrato

Modelo de inhibición por sustrato

$$\begin{cases} \dfrac{dS}{dt} = -\dfrac{VS}{K + S + \dfrac{S^2}{K_S}}, & t \in (0, T), \\ S(0) = S_0 \end{cases}$$

Problemática: Determinar los parámetros cinéticos V, K y Ks, a partir de mediciones de la concentración de sustrato S en un intervalo de tiempo [0,T].

Determinación numérica de parámetros cinéticos de catalizadores usando un modelo de inhibición por sustrato

Metodología

1. Plantear el problema usando el siguiente enfoque:

$$p = (V, K, K_s)$$

$$\min_{p \in P_{ad}} J(S, p) \text{ s.a } S \text{ resuelve el siguiente sistema:}$$

$$\begin{cases} \dfrac{dS}{dt} = -\dfrac{VS}{K + S + \dfrac{S^2}{K_S}}, & t \in (0, T), \\ S(0) = S_0 \end{cases}$$

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Metodología

2. Aplicar el método del gradiente para el resolver el problema del inciso 1:

$$p^{k+1} = \Pi_{ad}\left[p^k - \alpha_k J'(p^k)\right]$$

Para calcular $J'(p^k)$ usar el *Método adjunto*, el cual consiste en expresar $J'(p^k)$ en términos de la solución S de la ecuación diferencial del inciso 1 y una función λ que resuelve un sistema denominado *Sistema Adjunto*.

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Algoritmo

1. Parámetros iniciales p^0 .
2. Dado el vector de parámetros p^k, resolver el sistemas directo y adjunto.
3. Obtener la derivada del funcional, i.e., $J'(p^k)$.
4. Evaluar la proyección:

$$\Pi_{ad}\left[p^k - \alpha_k J'(p^k)\right]$$

5. Parar cuando $J(p^k) < TOL_1$ o $\|p^{k+1} - p^k\| < TOL_2$.

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Objetivo

Implementar algoritmo computacional usando la Metodología descrita para la determinación de los parámetros cinéticos V, K y K_s .

Actividades

1. **Implementar algoritmo computacional usando la metodología descrita.**
2. **Estudiar la sensibilidad del sistema al error de estimación de los parámetros.**
3. **Comparar resultados con mínimos cuadrados.**