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THE USE OF CHEBYSHEV POLYNOMIALS FOR THE REPRESENTATION OF ENZYME KINETIC PARAMETERS

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Chebyshev polynomials have been used for the on-line determination of the Michaelis–Menton constant, K_m, and the maximum velocity, V_{max}, for four enzymes (lactic-, malic-, isocitric-dehydrogenase and trypsin). Advantages of the use of Chebyshev polynomials over other polynomial equations are indicated.

1. Introduction

Most classical manual methods for the study of enzyme kinetics involve the spectrophotometric measurement of transmittance as a function of time. Determination of the kinetic parameters which attract the most interest requires repetition of this procedure while some other experimental variables are changed. For example, the determination of the Michaelis constant requires several measurements at different substrate concentrations followed by a laborious graphical or mathematical analysis. Typically this procedure requires two full days of effort by a skilled technician and a noticeable amount of enzyme. The objective of this project was to develop an automated method which eliminates most of the manual steps, eliminates sample variations by using only one sample and quickly yields results. A further objective was to use conventioal laboratory equipment in order to make the resulting method useful to a wide variety of laboratories.

2. Equipment

The PDP-9 computer was connected to an Hitachi Perkin–Elmer Model 139 spectrophotometer. It is an analytical instrument designed for spectroscopic studies of the radiant energy absorbed by samples at wavelengths from 170 to 800 nm.

The output of the photocell amplifier is linear with respect to light intensity and with proper calibration, linear with respect to the transmittance of the sample [1,4].

3. Theoretical considerations

In recent years the Gaussian method, polynomial equations up to the 10th order, Cleland's method [5], the non-linear least-square procedure and BMD–OIR (UCLA Biomed program of Dixon [7], which employed a modified Gauss–Newton method) have been extensively used in studies on enzyme systems to obtain K_m and V_{max}. When the same methods were employed in this work, the polynomial coefficients were far from ideal and the enzyme kinetic parameters were far from satisfactory. In this communication, the Chebyshev polynomials have been used. This new method gave good results when applied to enzyme systems.

The following derivations and assumptions employed in this work were proposed in terms of transmittance, T, and absorbance, A. Both terms were assumed to fit a polynomial over an interval of several data points as

$$T = a'_0 + a'_1 s^1 + a'_2 s^2 + a'_3 s^3 + \cdots + a_n s^n, \qquad (1)$$

where s is the substrate concentration and $a'_0, a'_1, a'_2,$

a_n' are the polynomial coefficients. Since substrate concentration is a function of time, t, time can be used instead of substrate concentration in eq. (1) to give

$$T = a_0 + a_1 t^1 + a_2 t^2 + \cdots + a_n t^n. \quad (2)$$

The coefficients of eqs. (1) and (2) are different. Differentiating eq. (1) with respect to time, t, gives

$$dT/dt = a_1 + 2a_2 t^1 + 3a_3 t^2 + \cdots + na_n t^{n-1}. \quad (3)$$

By definition, however, absorbance, A, is given by

$$A = -\log_{10} T \quad (4)$$

and $A = abc$, where a is the molar absorptivity, c is the concentration, and b is the cell length and is equal to 1 cm. Therefore, $A = ac$, and

$$c = A/a. \quad (5)$$

Differentiating eq. (5),

$$\frac{dc}{dt} = \frac{1}{a}\frac{dA}{dt}$$

$$= \frac{-0.4343}{Ta}(a_1 + 2a_2 t^1 + 3a_2 t^2 + \cdots + na_n t^{n-1}), \quad (6)$$

where dc/dt is the velocity or rate. In other words the rate equation is

$$\frac{dA}{dt} = \frac{-0.4343}{Ta}(a_1 + 2a_2 t^1 + 3a_2 t^2 + \cdots + na_n t^{n-1}), \quad (7)$$

where

$$\frac{dA}{dt} = \frac{-1}{a}\frac{ds}{dt} = \frac{1}{a}\frac{dp}{dt},$$

where p is the product concentration. When t is small the polynomial converges rapidly, and the values of the coefficients in the expression for dA/dt stay constant, regardless of the order of the polynomial.

The representation of dA as a series of polynomials in t largely overcomes the difficulties discussed above. Orthogonal polynomials $f_i(t)$ satisfy the condition $\Sigma_m f_i(t_m) f_j(t_m) = 0$, $(i \neq j)$ where m is the number of points to be fitted. This means that for the series

$$\frac{dA}{dt} = \sum_{i=0}^{n} c_i f_i(t),$$

where n is the order of fit, any coefficient c_j can be represented by

$$c_j = \sum t_m (dA/dt) f_j(t_m) / \sum_m \{f_j(t_m)\}^2.$$

Thus, if the series for dA/dt were developed for i terms, the addition of the $(i+1)$th term would not alter any of the coefficients c_i already obtained. The coefficients of each polynomial are specific for the m data points considered, and it is therefore an advantage for the polynomials to be converted to a Chebyshev series of polynomials because evaluation of this series requires a knowledge only of $(n+1)$ coefficients for a fit or order n (and not of the internal coefficients of all the f_i polynomials). If the polynomials are rearranged as a power series, again only $(n+1)$ coefficients are required but this method involving large coefficients and cancellation among the terms, can lead to a loss of accuracy in evaluation of the series.

In the treatment adopted A is defined by the series:

$$A = a_0 + a_1 E_1(x) + a_2 E_2(x) + \cdots + a_s E_s(x) + \cdots a_n E_n(x), \quad (8)$$

where $E_s(x) = \cos(s \cos^{-1} x)$ is the Chebyshev polynomial in x of degree s, and where x is a function of time, varying between the limits 1 and -1, defined as

$$x = \{A - (A_{max} + A_{min})\}/(A_{max} - A_{min}), \quad (9)$$

A_{max} and A_{min} being two kinetic absorbances respectively just above and below the extreme absorbances of the measured values. The first few Chebyshev polynomials are $E_0(x) = 1$; $E_1(x) = x$; $E_2(x) = 2x^2 - 1$; $E_3(x) = 4x^3 - 3x$; $E_4(x) = 8x^2 - 8x^2 + 1$. They are related by the recurrence relation

$$E_{s+1}(x) - 2xE_s(x) + E_{s-1}(x) = 0. \quad (10)$$

Any polynomial in the series can therefore be calculated from its two predecessors, a fact which makes the series especially useful for computer programming, and the series can be easily summed and differentiated. To sum the series the coefficients $b_n, b_{n-1}, \cdots b_s, \cdots b_0$ are formed from $b_s = 2xb_{s+1} - b_{s+2} + d_s$ (where $b_{n+1} = b_{n+2} = 0$), and then $A = b_0 - b_2 = a_0 + 2xa_1 + \cdots$. The programming of a computer to fit data to orthogonal polynomials and to reduce these polynomials to a Chebyshev series has been described [3,6]. The output of the program consists of the Chebyshev coefficients [2] and the residuals calculated from the ob-

Table 1
Summary of the K_m values of dehydrogenases at 25 ± 1.0°C

Enzyme source	Mean experimental values[a]		Literature values	
	V_{max}	K	K_m	pH
Lactic (bovine heart)	1.25×10^{-2}	1.40×10^{-4}	1.40×10^{-4}	7.5
Lactic (rabbit muscle)	1.25×10^{-2}	8.97×10^{-5}	9.0×10^{-5}	7.5
Lactic[b] (rabbit muscle	1.26×10^{-2}	4.13×10^{-5}	—	—
Malic (pig heart)	3.55×10^{-2}	4.6×10^{-4}	4.2×10^{-4}	7.4
Isocitric (pig heart)	1.23×10^{-3}	1.11×10^{-5}	1.0×10^{-5}	7.4
Trypsin[c] dl-BAPA	1.86×10^{-2}	9.69×10^{-5}	9.39×10^{5}	8–8.5
Trypsin TAME	3.65×10^{-2}	4.87×10^{-5}	5.0×10^{-5}	7.8–8.0

Units: K_m—molar, V_{max}—mole·min^{-1} · (mg enzyme)$^{-1}$.
a) pH = 7.5 b) Temperature = 36 ± 1.0°C. c) PH - 8.15, bovine panacreatic trypsin.

served values for all the orders of fit up to a chosen maximum. No precisely logical procedure can be laid down for determining an order for the final equation which is neither too low nor too high; clearly no more coefficients should be used than are required for an adequte fit, however that may be defined. The following critieria were applied in this study. (i) Sum of squares of the residuals: This sum rises rapidly at first as the order of fit is increased until an order is reached beyond which little improvement occurs. (ii) Magnitudes of the residuals: These should be comparable with the estimated errors of the data, and should be randomly scattered about zero. (iii) Smoothness of fit: Analysis of dA/dt or of the first differences obtained at regular intervals of t will show whether a smooth fit has been obtained.

4. Results

The work described in this paper deals with a study of experimental results for the following enzymes: lactic-, malic-, isocitric-dehydrogenase and trypsin, and it was found that the equation $A = \Sigma_{s=0}^{n} a_s E_s(x)$ for $n \geqslant 4$ gave very satisfactory results (table 1, fig. 1).

The method of evaluation of a Chebyshev polynomial is by taking the calculated value of t and x from eq. (9), and the coefficients

$$b_5 = a_5, \qquad b_4 = 2xb_5 + a_4,$$

$$b_3 = 2xb_4 - b_5 + a_3, \qquad b_2 = 2xb_3 - b_4 + a_2,$$

$$b_1 = 2xb_2 - b_3 + a_1, \qquad b_0 = 2xb_1 - b_2 + a_0.$$

For example, a first value is obtained from the equation:

$$A = b_0 - b_2 \cong a_0 + 2xa_1, \tag{11}$$

so that a close approximation to the correct values is obtained by using only two constants. The inverse cal-

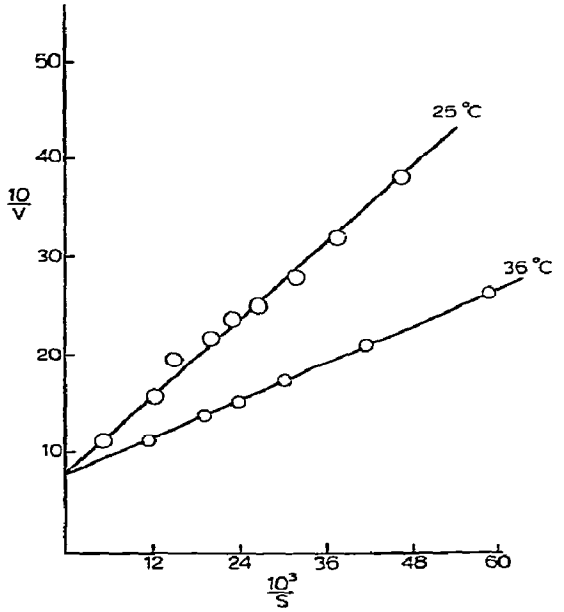

Fig. 1. Lactic dehydrogenase (rabbit muscle).

culation of A from t can be done only by iteration of the procedure just given, but the approximate values of A or the values of A from eq. (11) will allow the correct value of A to be obtained without difficulty. The needless use of excessive numbers of digits produced by the computer (8 for each constant in this work) increases the possibility of error in transcription, and no more digits should be quoted than are necessary. If the equation is written in full,

$$A = a_0 + a_1 x + a_2(2x^2 - 1) + a_3(4x^3 - 3x) + a_4(8x^4 - 8x^2 + 1) + \cdots, \quad (12)$$

it may be seen that none of the constants is ever multiplied by a factor greater than +1 so that the number of decimals required for all the constants is determined by the number required for A.

Fig. 1 presents a Lineweaver–Burk plot of typical results obtained for rabbit muscle lactic dehydrogenase at two different temperatures: 25 ± 0.1 and 36 ± 0.1°C. The mean $K_m = 8.97 \times 10^{-5}$ M for rabbit muscle lactic dehydrogenase at 25°C compares favorably with the literature value of 9.0×10^{-5} M although the calculated standard deviation is 5.5% or 5×10^{-6}. On the other hand, the calculated errors, associated with the individual velocity values are very small. For example, rabbit muscle lactic dehydrogenase has a mean V_{max} of 1.245×10^{-2} with a typical absolute error less than 1.0×10^{-4} or less than 0.8%. Table 1 summarizes the mean values for all of the enzymes, substrates and temperatures studied. The mean values obtained in this work for K_m and V_{max} for the enzymes, included in table 1, are in good agreement with the corresponding literature values.

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