A COMPUTER PROGRAM FOR THE DETERMINATION OF KINETIC PARAMETERS FROM SIGMOIDAL STEADY-STATE KINETICS

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1. Introduction

Current computer programs for the determination of kinetic parameters [1] are either limited only to the treatment of hyperbolic v vs. s curves* [2, 3] or based on specified models [4, 5] which are not generally applicable to non-hyperbolic saturation curves.

In many cases, sigmoidal v vs. s curves are analysed on the basis of models developed for the treatment of allosteric enzymes [6, 7]. However, it should not be overlooked that also models of non-allosteric enzymes could yield sigmoidal saturation curves [5, 8, 9]. Generally, it should be noted that, if the parameter-finding process is model-oriented, the data-processing procedure and the interpretation of the results obtained are mixed, which might lead to erroneous results. In principle, therefore, it is useful to separate data-processing and interpretation.

Separation of the two operations is achieved, if the data-processing is based on a general function $v = f(s)$ with only the minimum number of parameters necessary for the description of the experimental data. We here describe a computer program, which exclusively serves the parameter fitting of sigmoidal and hyperbolic v vs. s characteristics excluding any parameter interpretation based on models.

2. Equations, iteration procedure and computer program

Sigmoidal v vs. s curves can be described, analogously to the classical Hill equation [10], with the parameters V_{max}, $K_{0.5}$ and n_H:

$$v = \frac{V_{max}}{1 + \left(\frac{K_{0.5}}{s}\right)^{n_H}} \tag{1}$$

With the following transformations, the fit-problem can be reduced to a linear least-squares fit:

$$\log \frac{v}{V_{max} - v} = n_H . \log s - n_H . \log K_{0.5} \tag{2}$$

$$\frac{s^{n_H}}{v} = \frac{1}{V_{max}} . s^{n_H} + \frac{K_{0.5}^{n_H}}{V_{max}} \tag{3}$$

V_{max} being known, n_H and $K_{0.5}$ can be computed according to equation (2); and n_H being known, V_{max} and $K_{0.5}$ are obtained from equation (3). However, only two parameters can be determined at a time, since one parameter is always part of the variables. All three parameters can be obtained by iterative applications of equations (2) and (3). The procedure is schematically represented in fig. 1.

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* The following symbols are used:
v = initial velocity,
s = substrate concentration,
V_{max} = maximal velocity,
$K_{0.5}$ = substrate concentration giving $v = V_{max}/2$,
n_H = "interaction coefficient",
α = limitation factor.

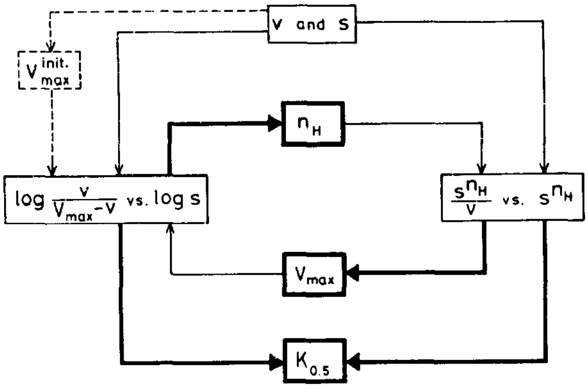


Fig. 1. Scheme of the iteration procedure.

From the experimental data v and s, an initial value of V_{max} (V_{max}^{init}) is produced and a least-squares fit of $\log(v/(V_{max}\text{-}v))$ vs. $\log s$ according to equation (2) is performed. The resulting value of n_H is used in equation (3), which on least-squares-fitting of $s^{n}\text{H}/v$ vs. $s^{n}\text{H}$ delivers a better value of V_{max}, which again is incorporated into equation (2). This procedure is repeated, until the changes of n_H and V_{max} in two consecutive loops pass a limit which is fixed in the program. $K_{0.5}$ is not used for iteration. For this estimation of the parameters, all the experimental data were used.

In order to analyse for asymmetry of the v vs. s curves as well as for linearity in equation (2), a second iteration procedure follows (see general remarks). Now, only the experimental data are used, which fulfil the condition $(1\text{-}\alpha).\ V_{max} < v < \alpha.\ V_{max}$. The limitation factor α can arbitrarily be chosen.

The results of both iteration procedures are collected in form of tables (see figs. 2 and 3) and graphically (fig. 4). For further evaluation of the results of fitting, two additional linear transformations from equation (1) are used: $1/v$ vs. $1/s^{n}\text{H}$ and v vs. $v/s^{n}\text{H}$(fig. 2).

The least-squares procedure implies that the experimental error of the data is exclusively connected with the determination of v and that s is accurately determined (see [1]). However, the error prolongation resulting from $s^{n}\text{H}$ is considered. In contrast to the program of Cleland [4] no weighting factor for v is used, however, an indirect weighting is implied by the quotient $s^{n}\text{H}/v$ in equation (3) [1, 11].

On routine application we have found that the iteration procedure is convergent with respect to n_H and V_{max}. Nevertheless, errors implied in the data set ($n_H < 0.5$; $V_{max} < v$) will result in specified comment statements, in order to avoid erroneous computations. The program written in FORTRAN IV is developed for an IBM 360/44 H-level computer and occupies 32 K bytes of system 360 memory. It is available at cost from the authors.

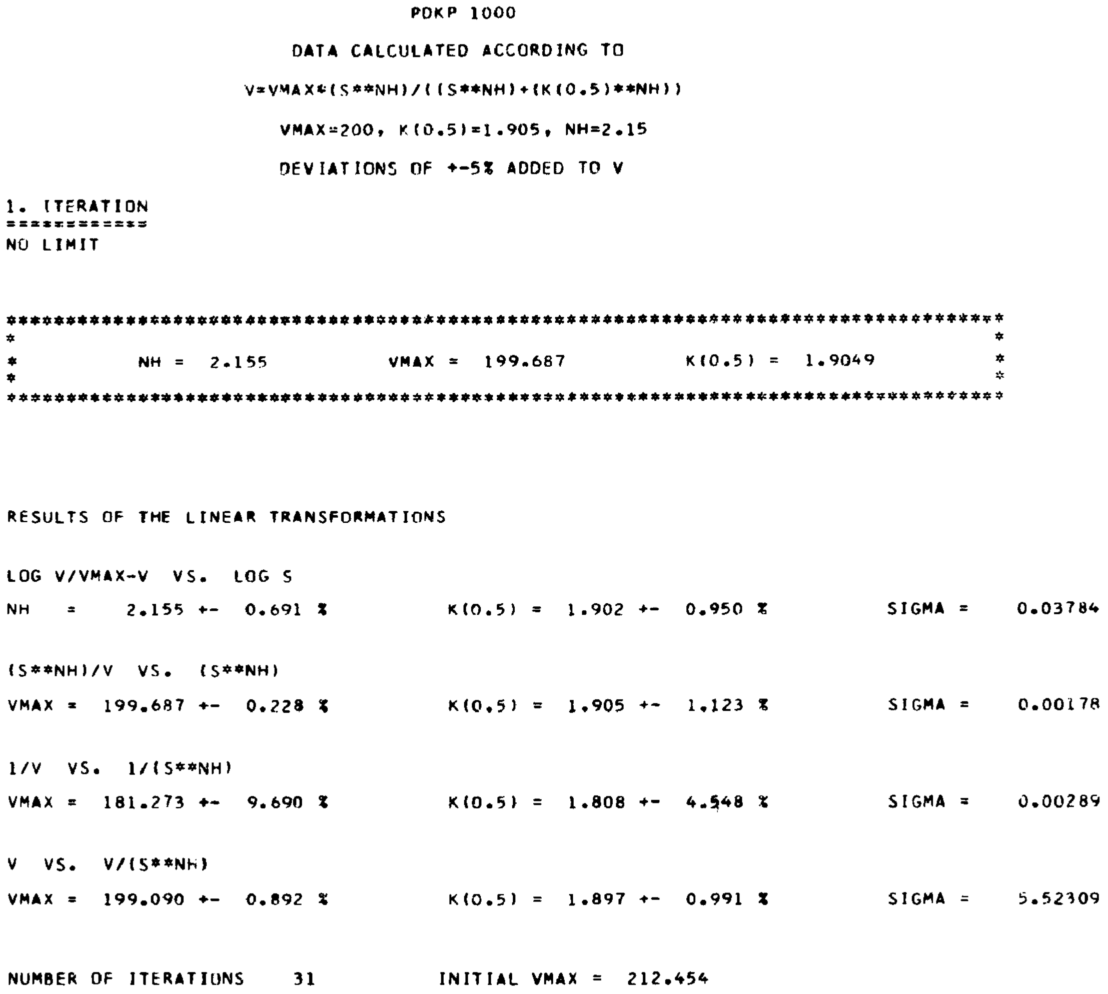
3. General remarks

Any interpretation of the results obtained necessitates a critical inspection of the computed and experimental data, looking for the following discrepancies in the curves of numerical values.

1) A large deviation of each individual experimental point from the respective computed point, as numerically given in the output (fig. 3, "error"),
2) a serial error along the curve with equal sign,
3) a larger error accumulated in the derived parameters,
4) discrepancies of V_{max} resulting from the three linear transformations (fig. 2),
5) discrepancies between the first and second (limited) iteration procedures.

Any error may result from the experimental data or from the inapplicability of the Hill equation. Whereas a discussion of errors in the experimental data set is out of the scope of this paper, the applicability of the equation system must always be questioned. As mentioned above, equation (1) displays a sigmoidal v vs. s curvature with the minimum number of three parameters. For this reason, equation (1) cannot be used for systems which can only be described with at least four parameters, such as e.g. substrate inhibition mechanism, systems which operate with a mixture of positive and negative cooperativity [12] or systems which exhibit asymmetry.

Equation (1) implies simplifications which are not fulfilled in all enzymic reactions, especially the assumption that the "interaction coefficient" n_H is



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                                    PDKP 1000

                             DATA CALCULATED ACCORDING TO

                        V=VMAX*(S**NH)/((S**NH)+(K(0.5)**NH))

                           VMAX=200, K(0.5)=1.905, NH=2.15

                           DEVIATIONS OF +-5% ADDED TO V

1. ITERATION
============
NO LIMIT


****************************************************************************************
*                                                                                      *
*           NH =  2.155           VMAX =  199.687           K(0.5) =  1.9049          *
*                                                                                      *
****************************************************************************************



RESULTS OF THE LINEAR TRANSFORMATIONS

LOG V/VMAX-V  VS.  LOG S

NH    =    2.155 +-  0.691 %           K(0.5) =  1.902 +-  0.950 %           SIGMA =    0.03784

(S**NH)/V  VS.  (S**NH)

VMAX =  199.687 +-  0.228 %           K(0.5) =  1.905 +-  1.123 %           SIGMA =    0.00178

1/V  VS.  1/(S**NH)

VMAX =  181.273 +-  9.690 %           K(0.5) =  1.808 +-  4.548 %           SIGMA =    0.00289

V  VS.  V/(S**NH)

VMAX =  199.090 +-  0.892 %           K(0.5) =  1.897 +-  0.991 %           SIGMA =    5.52309


NUMBER OF ITERATIONS    31          INITIAL VMAX =  212.454
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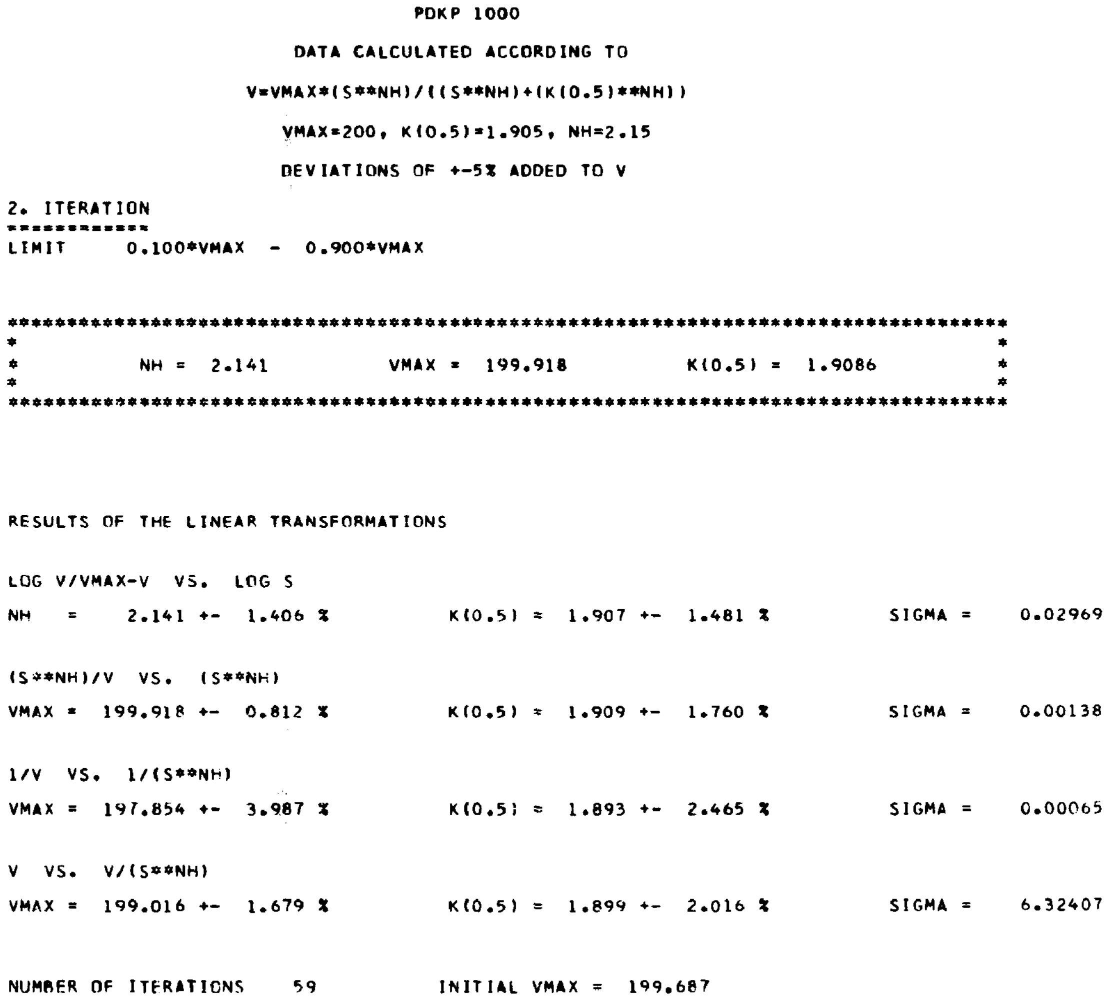
Fig. 2a



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                                  PDKP 1000

                        DATA CALCULATED ACCORDING TO

                   V=VMAX*(S**NH)/((S**NH)+(K(0.5)**NH))

                      VMAX=200, K(0.5)=1.905, NH=2.15

                      DEVIATIONS OF +-5% ADDED TO V

2. ITERATION
************
LIMIT     0.100*VMAX  -  0.900*VMAX


*************************************************************************************
*                                                                                   *
*          NH =  2.141          VMAX =  199.918          K(0.5) =  1.9086           *
*                                                                                   *
*************************************************************************************



RESULTS OF THE LINEAR TRANSFORMATIONS


LOG V/VMAX-V  VS.  LOG S

NH    =    2.141 +-  1.406 %           K(0.5) =  1.907 +-  1.481 %           SIGMA =    0.02969


(S**NH)/V  VS.  (S**NH)

VMAX =  199.918 +-  0.812 %           K(0.5) =  1.909 +-  1.760 %           SIGMA =    0.00138


1/V  VS.  1/(S**NH)

VMAX =  197.854 +-  3.987 %           K(0.5) =  1.893 +-  2.465 %           SIGMA =    0.00065


V  VS.  V/(S**NH)

VMAX =  199.016 +-  1.679 %           K(0.5) =  1.899 +-  2.016 %           SIGMA =    6.32407


NUMBER OF ITERATIONS    59           INITIAL VMAX =  199.687
```

Fig. 2b

Fig. 2. Computer output: results of the linear transformations, Sigma = standard deviation = $\sqrt{\Sigma(x_{\mathrm{i}}-x^{-})^2/n\text{-}2}$. a) First iteration, b) Second iteration.

```
                                  PDKP 1000

                        DATA CALCULATED ACCORDING TO

                    V=VMAX*(S**NH)/((S**NH)+(K(0.5)**NH))

                       VMAX=200, K(0.5)=1.905, NH=2.15

                       DEVIATIONS OF +-5% ADDED TO V

1. ITERATION
============
NO LIMIT


************************************************************************************
*                                                                                  *
*          NH =  2.155           VMAX =  199.687            K(0.5) =  1.9049       *
*                                                                                  *
************************************************************************************



             S             V           V(CALC)         ERROR

          0.10000        0.35335        0.34744        1.70194 %     +
          0.20000        1.52761        1.53862       -0.71560 %     +
          0.40000        6.87252        6.67697        2.92873 %     +
          0.50000       10.66490       10.58248        0.77887 %     +
          0.70000       19.76716       20.68761       -4.44927 %     +
          0.80000       28.13981       26.66595        5.52714 %     +
          0.90000       31.57753       33.09545       -4.58650 %     +
          1.00000       40.00000       39.84947        0.37774 %     +
          1.10000       46.02166       46.80733       -1.67852 %     +
          1.20000       56.71321       53.85885        5.29970 %     +
          1.40000       64.61767       67.87107       -4.79350 %     +
          1.50000       74.82569       74.68340        0.19052 %     +
          1.60000       83.05723       81.29217        2.17125 %     +
          1.70000       89.54476       87.65869        2.15161 %     +
          1.80000       92.00235       93.75567       -1.87009 %     +
          2.00000      105.19386      105.08058        0.10780 %     +
          2.20000      121.08892      115.21788        5.09559 %     +
          2.40000      121.81598      124.20262       -1.92157 %     +
          2.60000      134.85774      132.11667        2.07473 %     +
          2.80000      132.20238      139.06370       -4.93394 %     +
          3.00000      145.25167      145.15301        0.06797 %     +
          3.30000      156.07275      152.90793        2.06975 %     +
          3.60000      156.21403      159.28930       -1.93062 %     +
          4.00000      166.24229      166.11668        0.07561 %     +
          5.00000      177.66999      177.51097        0.08958 %     +
          5.50000      185.05156      181.24895        2.09800 %     +
          6.00000      184.34455      184.15680        0.10195 %     +
          6.50000      182.92140      186.45469       -1.89499 %     +
          7.00000      188.50716      188.29673        0.11175 %     +
          8.00000      191.24980      191.02180        0.11936 %     +
          9.00000      193.14020      192.89853        0.12528 %     +
```

Fig. 3a

```
                                  PDKP 1000

                        DATA CALCULATED ACCORDING TO

                    V=VMAX*(S**NH)/((S**NH)+(K(0.5)**NH))

                       VMAX=200, K(0.5)=1.905, NH=2.15

                       DEVIATIONS OF +-5% ADDED TO V

2. ITERATION
============
LIMIT     0.100*VMAX  -  0.900*VMAX


************************************************************************************
*                                                                                  *
*          NH =  2.141          VMAX =  199.918          K(0.5) =  1.9086          *
*                                                                                  *
************************************************************************************

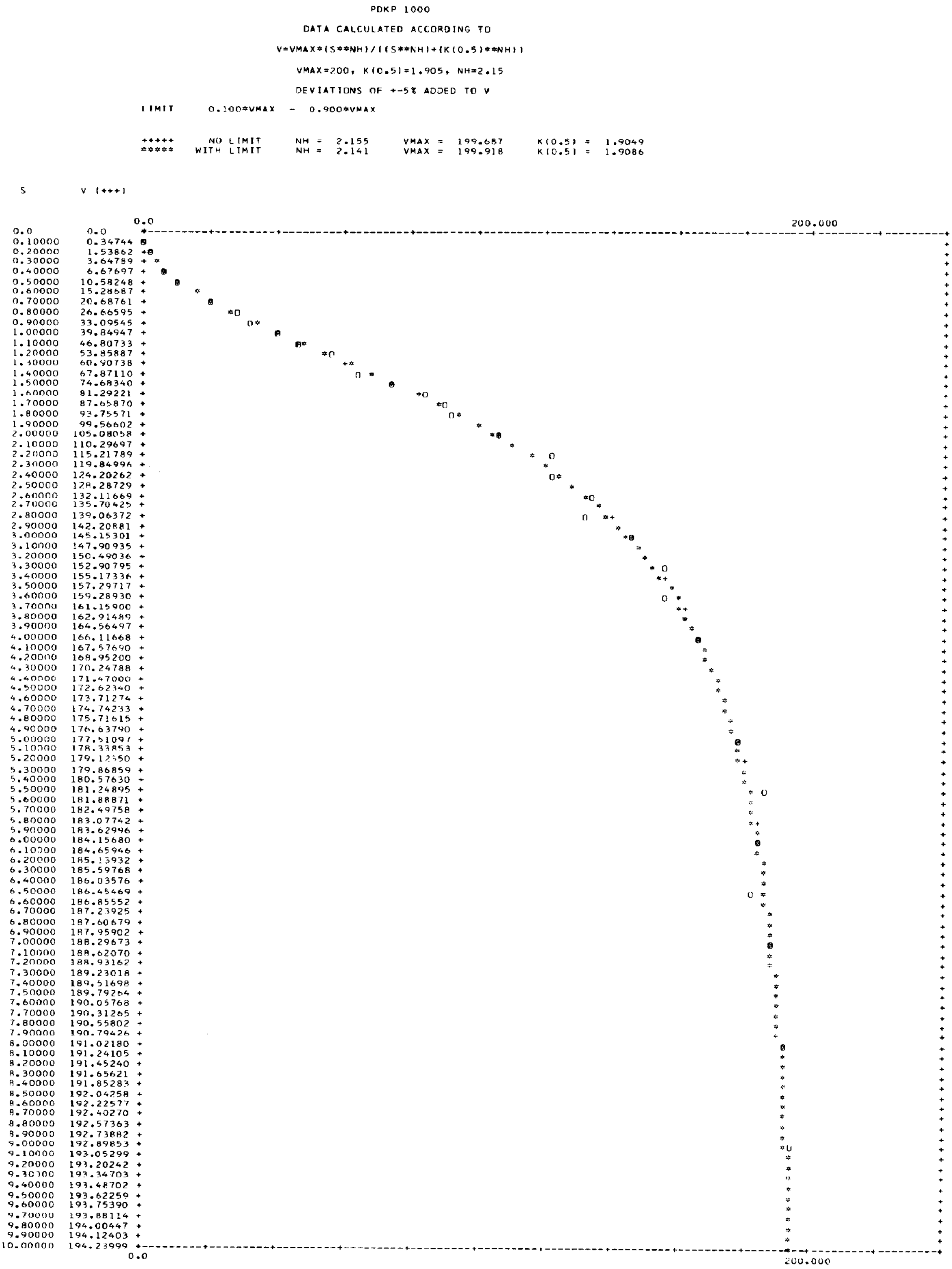


             S            V           V(CALC)          ERROR

          0.10000         0.35335        0.36165       -2.29578 %      -
          0.20000         1.52761        1.58515       -3.63020 %      -
          0.40000         6.87252        6.80668        0.96731 %      -
          0.50000        10.66490       10.75079       -0.79888 %      -
          0.70000        19.76716       20.90769       -5.45508 %      -
          0.80000        28.13981       26.89557        4.62621 %      +
          0.90000        31.57753       33.32329       -5.23885 %      +
          1.00000        40.00000       40.06506       -0.16239 %      +
          1.10000        46.02166       47.00167       -2.08506 %      +
          1.20000        56.71321       54.02477        4.97630 %      +
          1.40000        64.61767       67.96632       -4.92692 %      +
          1.50000        74.82569       74.73999        0.11467 %      +
          1.60000        83.05723       81.30991        2.14897 %      +
          1.70000        89.54476       87.63855        2.17508 %      +
          1.80000        92.00235       93.69955       -1.81132 %      +
          2.00000       105.19386      104.96050        0.22233 %      +
          2.20000       121.08892      115.04644        5.25221 %      +
          2.40000       121.81598      123.99272       -1.75554 %      +
          2.60000       134.85774      131.88019        2.25777 %      +
          2.80000       132.20238      138.81082       -4.76075 %      +
          3.00000       145.25167      144.89209        0.24817 %      +
          3.30000       156.07275      152.64688        2.24431 %      +
          3.60000       156.21403      159.03810       -1.77572 %      +
          4.00000       166.24229      165.88785        0.21366 %      +
          5.00000       177.66999      177.35378        0.17829 %      +
          5.50000       185.05156      181.12711        2.16669 %      -
          6.00000       184.34455      184.06757        0.15048 %      -
          6.50000       182.92140      186.39489       -1.86351 %      -
          7.00000       188.50716      188.26322        0.12958 %      -
          8.00000       191.24980      191.03232        0.11384 %      -
          9.00000       193.14020      192.94370        0.10184 %      -
```

Fig. 3b

Fig. 3. Computer output of tabulated input data (s, ν), calculated values of ν (ν (calc) and the relative error between ν and ν (calc), for a) first and b) second iteration. + indicates the values used in the iteration procedures.



```
                              PDKP 1000

                     DATA CALCULATED ACCORDING TO

               V=VMAX*(S**NH)/((S**NH)+(K(0.5)**NH))

                   VMAX=200, K(0.5)=1.905, NH=2.15

                     DEVIATIONS OF +-5% ADDED TO V

LIMIT      0.100*VMAX  -  0.900*VMAX


+++++      NO LIMIT     NH =  2.155     VMAX =  199.687     K(0.5) =  1.9049
*****    WITH LIMIT     NH =  2.141     VMAX =  199.918     K(0.5) =  1.9086


  S          V (+++)

   0.0          0.0
   0.10000      0.34744
   0.20000      1.53862
   0.30000      3.64789
   0.40000      6.67697
   0.50000     10.58248
   0.60000     15.28687
   0.70000     20.68761
   0.80000     26.66595
   0.90000     33.09545
   1.00000     39.84947
   1.10000     46.80733
   1.20000     53.85887
   1.30000     60.90738
   1.40000     67.87110
   1.50000     74.68340
   1.60000     81.29221
   1.70000     87.65870
   1.80000     93.75571
   1.90000     99.56602
   2.00000    105.08058
   2.10000    110.29697
   2.20000    115.21789
   2.30000    119.84996
   2.40000    124.20262
   2.50000    128.28729
   2.60000    132.11669
   2.70000    135.70425
   2.80000    139.06372
   2.90000    142.20881
   3.00000    145.15301
   3.10000    147.90935
   3.20000    150.49036
   3.30000    152.90795
   3.40000    155.17336
   3.50000    157.29717
   3.60000    159.28930
   3.70000    161.15900
   3.80000    162.91489
   3.90000    164.56497
   4.00000    166.11668
   4.10000    167.57690
   4.20000    168.95200
   4.30000    170.24788
   4.40000    171.47000
   4.50000    172.62340
   4.60000    173.71274
   4.70000    174.74233
   4.80000    175.71615
   4.90000    176.63790
   5.00000    177.51097
   5.10000    178.33853
   5.20000    179.12550
   5.30000    179.86859
   5.40000    180.57630
   5.50000    181.24895
   5.60000    181.88871
   5.70000    182.49758
   5.80000    183.07742
   5.90000    183.62996
   6.00000    184.15680
   6.10000    184.65946
   6.20000    185.13932
   6.30000    185.59768
   6.40000    186.03576
   6.50000    186.45469
   6.60000    186.85552
   6.70000    187.23925
   6.80000    187.60679
   6.90000    187.95902
   7.00000    188.29673
   7.10000    188.62070
   7.20000    188.93162
   7.30000    189.23018
   7.40000    189.51698
   7.50000    189.79264
   7.60000    190.05768
   7.70000    190.31265
   7.80000    190.55802
   7.90000    190.79426
   8.00000    191.02180
   8.10000    191.24105
   8.20000    191.45240
   8.30000    191.65621
   8.40000    191.85283
   8.50000    192.04258
   8.60000    192.22577
   8.70000    192.40270
   8.80000    192.57363
   8.90000    192.73882
   9.00000    192.89853
   9.10000    193.05299
   9.20000    193.20242
   9.30000    193.34703
   9.40000    193.48702
   9.50000    193.62259
   9.60000    193.75390
   9.70000    193.88114
   9.80000    194.00447
   9.90000    194.12403
  10.00000    194.23999
```

Fig. 4. Graphical output of ν vs. s. Experimental values: ○, fitted values: + first iteration, * second iteration.

independent of s. If n_H is a function of s, the Hill plot (equation 2) cannot be linear over the total range of log s [8, 13]. However, as pointed out by Frieden [14], Hill plots usually are linear in the optimum experimental range (0.1 to 0.9. V_{max}), which indeed is approached in the second iteration procedure used here. It should be pointed out that for theoretical reasons [7, 13] this range of the Hill plot served for the interpretation of the "interaction coefficient" n_H. Thus, on practical and theoretical grounds, the introduction of the limitation factor α is fully justified and serves finally as the test of the linearity of the Hill plot. For greater refinement [7], the magnitude of the factor α can be varied arbitrarily.

The three parameters obtained with the procedure described here immediately lead to the process of parameter interpretation by one of the known models in enzyme kinetics, as exemplified in the case of the steady-state kinetics of yeast pyruvate kinase [15]. Furthermore, the program can be used for parameter evaluation in any type of binding studies.

References

[1] W.R.Gardiner and J.H.Ottaway, FEBS Letters 2, Suppl. (1969) S34.
[2] K.R.Hanson, R.Ling and E.Havir, Biochem. Biophys. Res. Commun. 29 (1967) 194.
[3] W.W.Cleland, Nature 198 (1963) 463.
[4] W.W.Cleland, Adv. Enzym. 29 (1967) 1.
[5] J.Kowalik and J.F.Morrison, Mathem. Biosciences 2 (1968) 57.
[6] J.Monod, J.Wyman and J.-P.Changeux, J. Mol. Biol. 12 (1965) 88.
[7] D.E.Koshland Jr., G.Némethy and D.Filmer, Biochemistry 5 (1966) 365.
[8] C.C.Griffin and L.Brand, Arch. Biochem. Biophys. 126 (1968) 856.
[9] W.Ferdinand, Biochem. J. 98 (1966) 278.
[10] A.V.Hill, Biochem. J. 7 (1913) 471.
[11] G.N.Wilkinson, Biochem. J. 80 (1961) 324.
[12] A.Levitzki and D.E.Koshland Jr., Proc. Natl. Acad. Sci. U.S. 62 (1969) 1121.
[13] M.M.Rubin and J.-P.Changeux, J. Mol. Biol. 21 (1966) 265.
[14] C.Frieden, J. Biol. Chem. 242 (1967) 4045.
[15] H.-J.Wieker, K.-J.Johannes and B.Hess, Abstract No. 398, VI. Meeting of FEBS, Madrid, 1969.