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COMMUNICATIONS

The use of empirical equations to describe dynamic dialysis "escape curves" in drug-macromolecule binding measurements

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The introduction of the dynamic dialysis technique to study the binding of small molecules to macromolecules (Meyer & Guttman, 1968; 1970a, b), has prompted a number of workers to utilize this approach in view of the apparent advantages inherent in this method (Dearden & Tomlinson, 1970; Goto, Ohki & others, 1971; Asghar & Roth, 1971; Brown & Crooks, 1973; Crooks & Brown, 1973; 1974a, b; Tukamoto, Ozeki & others, 1974). It became evident during studies utilizing this technique for the determination of the binding parameters of several steroidal anti-inflammatory agents and collagen (Kanfer, 1975), that methods available for obtaining the value of the slope of the curvilinear dynamic dialysis plot at various values of time in the presence of macromolecules, could lead to anomalies in the subsequent Scatchard plot. These values for the slope are necessary to obtain the instantaneous rate of escape at a value of Dt (total drug concentration) (Meyer & Guttman, 1968; 1970a).

Meyer & Guttman (1968), in their original publication suggested that the slopes of the escape curve, in the presence of protein, at various values of time, could be estimated graphically. However, in view of the convenience and accuracy of fitting the data from plots of Dt versus time with the aid of a digital computer, the latter approach was employed by them. This involved the use of a six parameter tri-exponential equation ($Dt = \sum_{i=1}^{3} A_i e^{-b_i t}$) (Guttman, personal communication).

From their studies of the binding of polymethylene bisquaternary ions to chondroitin *in vitro*, Asghar & Roth (1971) pointed out that the graphic evaluation of the instantaneous rates yielded only approximate values.

Dearden & Tomlinson (1970) used the dynamic dialysis technique to study the binding of *p*-substituted acetanilides to bovine serum albumin (BSA). A modified single exponential equation, which included a constant term ($Dt = Ae^{-Bt} + C$), was employed to fit their dialysis data in the presence of protein (Dearden, personal communication).

Table 1. *Summary of the sum of squares of the deviations (SSQ. of DEV.) from the fitting of kinetic dialysis data to various empirical equations.*

Function	Equation no.	Fig. 1	SSQ. of DEV.
$Dt = Ae^{-bt} + C$	1	A	$5{\cdot}11 \times 10^{-5}$
$Dt = Ae^{-at} + Be^{-bt}$	2	B	$3{\cdot}36 \times 10^{-6}$
$Dt = Ae^{-at} + Be^{-bt} + Ce^{-ct}$	3	C	$1{\cdot}42 \times 10^{-6}$
$Dt = Ae^{-at} - Be^{-bt} + Ce^{-ct}$	4	D	$3{\cdot}36 \times 10^{-6}$
$Dt = Dt^0 + at + bt^2 + ct^3 + dt^4$	5	E	$1{\cdot}04 \times 10^{-5}$

Dt^0 is the initial concentration of drug in the protein solution and a, b, c and d are constants.

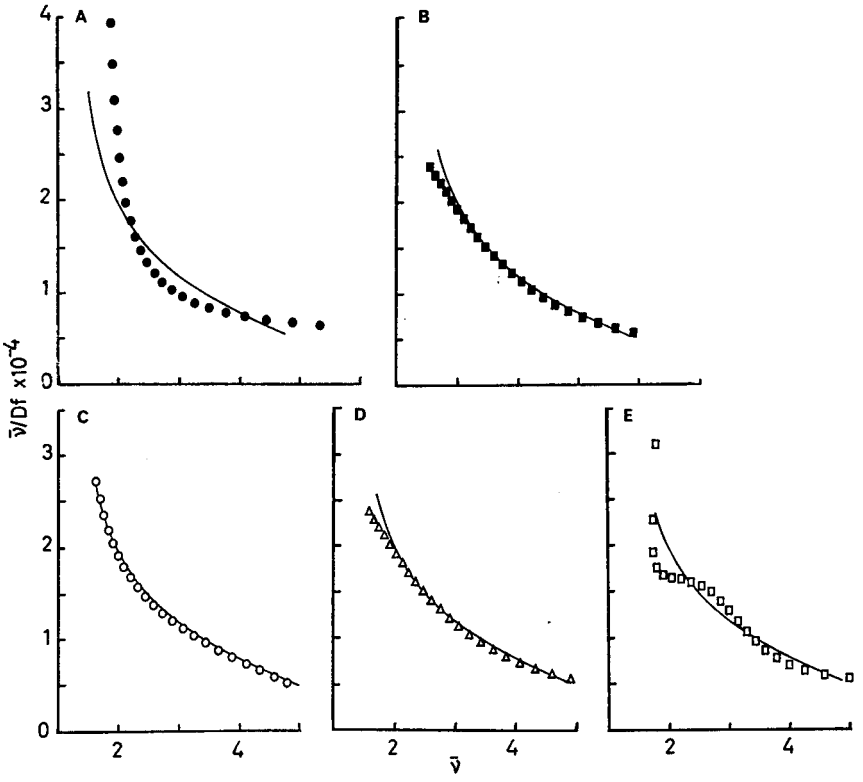

FIG. 1. Scatchard plots of the binding data obtained from fitting theoretical values of Dt and time ● eqn 1; ■ eqn 2; ○ eqn 3; △ eqn 4; □ eqn 5 (see Table 1). The solid curve was generated from the binding parameters, $n_1 = 1$; $n_2 = 6$; $k_1 = 1{\cdot}74 \times 10^5$; $k_2 = 1{\cdot}97 \times 10^3$.

Brown & Crooks (1973) and Crooks & Brown (1973) fitted their plots of Dt versus time in the presence of protein, to a fourth order polynomial equation which included the value of the initial concentration of drug in the protein solution.

In view of the fact that different empirical equations which have no clear physical significance, have been utilized by a number of workers to describe the kinetic behaviour observed experimentally, a study was undertaken to test the fitting of the curvilinear plots of Dt against time in the presence of protein, to the various equations claimed to conform closely to experimental data.

The equations listed in Table 1 were used.

All curve fitting was done with the aid of Fortran IV computer programs and an ICL 1901A computer.

In order to test the above equations, a standard set of theoretical binding data was generated from equations derived by Kruger-Thiemer (1966) and modified by Meyer & Guttman (1970b). The latter sets of equations describe the rates of loss of unbound and total concentrations of small molecules capable of being bound, from a protein compartment. Using these equations, a series of values for Dt and time were obtained. These calculations were made using a BASIC computer program written for a Hewlett-Packard 2100A computer. The values of n_i (number of binding sites) k_i (association constants) used were the same as those reported by Meyer & Guttman (1968) for the binding of phenol red to BSA ($n_1 = 1$; $n_2 = 6$; $k_1 = 1{\cdot}74 \times 10^5$; $k_2 = 1{\cdot}97 \times 10^3$).

The results obtained from the independent application of equations 1 to 5 to the theoretical binding data are illustrated in the form of Scatchard plots in Figure 1 A to E. The solid curves represent the theoretical Scatchard plot based on the published values for n_1, n_2, k_1 and k_2 (Meyer & Guttman, 1968; 1970a), while the symbols in each graph represent the binding data ($\bar{\nu}$ and $\bar{\nu}$/Df) obtained from the application of the particular function used to fit the curvilinear plot of Dt versus time in the presence of protein and subsequent computation of the slopes of these curves for various values of time.

Inspection of these Scatchard plots indicates that the use of the tri-exponential equation (eqn 3) used by Meyer & Guttman (1968; 1970a, b), resulted in the best representation of the theoretical binding curve for phenol red-BSA (Fig 1, eqn 3). In spite of the fact that this is an empirical equation, the excellent reproduction of the data indicates the applicability of this equation to fit the binding curves obtained from the dynamic dialysis system. Fig. 1 suggests that the use of either the bi-exponential (eqn 2) or the tri-exponential (eqn 4) also results in a reasonable approximation of the data. However, in view of the fact that the intercept on the abscissa gives Σn_i whereas the ordinate intercept gives $\Sigma n_i k_i$, subsequent application of these data (values of $\bar{\nu}$ and Df) to a hyperbolic regression program for estimates of n_i and k_i leads to erroneous values for these binding parameters. This is due to the deviations at the ends of the Scatchard curves. Fig. 1 also indicates that erroneous results may be obtained when the data from the curvilinear plot of Dt versus time is fitted to eqn. 1 used by Dearden (personal communication). When equation 5 (Brown & Crooks, 1973; Crooks & Brown, 1973) was used, a relatively poor representation of the data was obtained (Fig. 1).

Table 1 lists the "goodness of fit" with respect to the application of the various empirical equations, on the basis of the respective sum of the squares of the deviations. It is evident from this table that the "best fit" was obtained when the tri-exponential eqn 3 was used.

In view of the anomalies observed with the application of the various empirical equations to the curvilinear plots obtained from dynamic dialysis experiments, it is apparent that eqn 3 is the function of choice regarding treatment of dynamic dialysis data.

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