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Notes

Elimination of linear parameters in non-linear regressions: a fast and effective method for the determination of binding parameters

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Abstract

A semi-linear least-squares regression methodology is proposed to extract binding parameters from experimental binding data. After legitimation of simplification and suitable mathematical transformation, the only non-linear parameter which remains to be searched for by a non-linear least-squares regression is the affinity constant. As a result, number of sites and affinity constants are easier to grasp than with a full non-linear regression. This methodology can be applied to saturation as well as to displacement binding studies. It has been tested with binding data of QNB and αBTX on rat hippocampus synaptosomes.

Keywords: Binding parameters; Nonlinear regression; Semilinear regression; Least-squares fitting procedure; Cholinergic receptor

The determination of 'binding parameters' (affinity constants, binding capacities and non specific binding) by a non-linear least-squares fitting procedure may be time-consuming. Firstly, it requires the identification of the model (one, two... or more classes of sites). Secondly, for each of these models, the speed of convergence towards the minimum minimorum of the cost function is iterative, and therefore, frequently slow, especially when many parameters are effective. The following method is considerably faster than those commonly used for the determination of binding parameters. It is based on the reduction of the number of non-linear parameters.

For saturation studies, the equations currently used to calculate the whole binding level of the ligand i (B_i: specific + non-specific levels) is, according to the terminology of Feldman (1972), Munson and Rodbard (1979, 1980) and Munson (1983):

$$\left(B_i^{\text{calc}}\right) = \sum_i \frac{K_{ij} R_j \left(L_i - B_i^{\text{calc}}\right)}{1 + K_{ij}\left(L_i - B_i^{\text{calc}}\right)} + N_i\left(L_i - B_i^{\text{calc}}\right) \quad (1)$$

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Table 1
Results from saturation curve of a radiolabelled ligand: comparison with the fully non-linear process results

	QNB (L_i from 1×10^{-11} to 1×10^{-3} M)			αBTX (L_i from 1×10^{-11} to 1×10^{-9} M)		
	Theoretical minimum U [a]					
	24.473			Semi-linear regression	16.51	
	U minimum minimorum				U minimum minimorum	
	One specific receptor		Two specific receptors	One specific receptor	Two specific receptors	
	50.49		568.80	30.82	236.66	
	Values of the parameters obtained (1st model, 2nd model excluded) [b]			Values of the parameters obtained (1st model, 2nd model excluded) [b]		
	Semi-linear programme	'Ligand' programme [c]	Our programme [d]	Semi-linear programme	Ligand programme	Our programme
K_{i1}	1.298×10^{9}	1.157×10^{9}	1.403×10^{9}	1.117×10^{9}	4.377×10^{8}	8.151×10^{8}
R_1	1.216×10^{-9}	1.338×10^{-9}	1.188×10^{-9}	3.404×10^{-11}	4.922×10^{-11}	3.862×10^{-11}
N_1	4.883×10^{-2}	3.463×10^{-3}	4.889×10^{-2}	1.7×10^{-3}	1.082×10^{-3}	1.496×10^{-3}

[a] Taking into account only the pure errors from repeated points (Draper and Smith, 1981).
[b] A sequential F-test (Draper and Smith, 1981) immediately excluded the second model.
[c] Ligand programme (Rovati et al., 1988).
[d] Our programme is derived from the Hooke and Jeeves (1961) minimization process and will be published elsewhere.

Table 2
Results from the displacement of a fixed labelled ligand concentration (L) by variable concentrations (l_i) of the same unlabelled one: comparison with the fully non-linear process results

	QNB ($L = 1\times10^{-10}$ M: l_i from 0 to 1×10^{-7} M)				α-BTX ($L = 5\times10^{-10}$ M: l_i from 0 to 1×10^{-7} M)		
	Theoretical minimum U				Theoretical minimum U		
	14.678				14.117		
	Semi-linear regression				Semi-linear regression		
	U minimum minimorum				U minimum minimorum		
	One specific receptor		Two specific receptors		One specific receptor		Two specific receptors
	21.73		236.374		86.022		5112.241
	Values of the parameters obtained				Values of the parameters obtained		
	(1st model, 2nd model excluded)				(1st model, 2nd model excluded)		
	Semi-linear	Ligand	Our programme		Semi-linear	Ligand	Our programme
K_{i1}	1.422×10^{9}	1.116×10^{9}	1.26×10^{9}		7.85×10^{8}	2.74×10^{8}	7.156×10^{8}
R_1	7.944×10^{-10}	9.997×10^{-10}	8.24×10^{-10}		2.931×10^{-11}	4.559×10^{-11}	3.012×10^{-11}
N_1	5.695×10^{-2}	1.723×10^{-2}	5.742×10^{-2}		1.493×10^{-3}	1.387×10^{-3}	1.473×10^{-3}
	($L = 1\times10^{-9}$ M: l_i from 0 to 1×10^{-7} M)						
	Theoretical minimum U						
	12.368						
	Semi-linear regression						
	U minimum minimorum						
	One specific receptor		Two specific receptors				
	13.383		900.478				
	Values of the parameters obtained						
	(1st model, 2nd model excluded)						
	Semi-linear	Ligand	Our programme				
K_{i1}	1.71×10^{9}	1.345×10^{9}	1.559×10^{9}				
R_1	9.69×10^{-10}	1.345×10^{-9}	1.025×10^{-9}				
N_1	4.562×10^{-2}	2.648×10^{-2}	4.16×10^{-2}				

where K_{ij} is the affinity constant of the class of sites j, L_i denotes the total concentration of the ligand i, R_j is the binding capacity of the class of sites j, and N_i represents the non-specific binding constant.

This identification of the model implies performing the computation of (B_i) successively with $j = 1,2...$ Moreover, this computation implies solving a $j+1$ degree equation in (B_i) to perform a strictly pure simulation (i.e., without mixing experimental and calculated (B_i) values: the best way to avoid, as much as possible, the propagation of errors (Sands, 1974)).

It is noteworthy that in Eq. 1, the parameters we are looking for, K_{ij}, R_j and N_i can be gathered in the linear terms $(K_{ij}R_j)$and N_i and in the non-linear one K_{ij}, provided that, in the right member of Eq. 1 (B_i^{calc}) are replaced by the known experimental values (B_i^{exp}). This reduced classification is frequently adopted for the treatment of mathematical models in physics and has been studied elsewhere (Lawton and Sylvestre, 1971; Barham and Drane, 1972; Harville, 1973); it should be noted that this methodology has never been applied in the field of binding studies. As a result, the model (Eq. 1) becomes a non-linear one only in the parameter(s) K_{ij}. An initial guess of the K_{ij} values allows us, with total certainty, to obtain immediately the parameters $(K_{ij}R_j)$ and N_i by a strictly linear least-squares proce-

Table 3
Results from the displacement of variable labelled ligand concentrations (L_i) by a fixed concentration (l) of the same unlabelled one: comparison with the fully non-linear process results

	QNB (L_i from 1×10^{-11} to 1×10^{-8} M: $l = 1\times10^{-9}$ M)			αBTX (L_i from 1×10^{-11} to 5×10^{-8} M: $l = 5\times10^{-10}$ M)		
	Theoretical minimum U			Theoretical minimum U		
	12.735			26.032		
	Semi-linear regression			Semi-linear regression		
	U minimum minimorum			U minimum minimorum		
	One specific receptor		Two specific receptors	One specific receptor		Two specific receptors
	34.541		258.994	94.879		9108.004
	Values of the parameters obtained			Values of the parameters obtained		
	(1st model, 2nd model excluded)			(1st model, 2nd model excluded)		
	Semi-linear	Ligand [a]	Our programme	Semi-linear	Ligand [a]	Our programme
K_{i1}	1.228×10^{9}		1.183×10^{9}	7.871×10^{8}		8.085×10^{8}
R_1	1.086×10^{-9}		1.117×10^{-9}	3.138×10^{-11}		3.113×10^{-11}
N_1	4.866×10^{-2}		4.777×10^{-2}	1.396×10^{-3}		1.399×10^{-3}
	(L_i from 1×10^{-11} to 1×10^{-8} M: $l = 1\times10^{-8}$ M)					
	Theoretical minimum U					
	14.96					
	Semi-linear regression					
	U minimum minimorum					
	One specific receptor		Two specific receptors			
	32.18		635.588			
	Values of the parameters obtained					
	(1st model, 2nd model excluded)					
	Semi-linear	Ligand [a]	Our programme			
K_{i1}	1.284×10^{9}		1.125×10^{9}			
R_1	1.26×10^{-9}		1.389×10^{-9}			
N_1	4.903×10^{-2}		3.423×10^{-2}			

[a] Ligand is not programmed for such calculations.

dure. Indeed, deriving simultaneously the U cost function vs the $(K_{ij}R_j)$ and N_i parameters:

$$\frac{\delta U}{\delta(K_{ij}R_j)} = 0 \quad \frac{\delta U}{\delta N_i} = 0$$

leads to the classical set of linear equations in $(K_{ij}R_j)$ and N_i parameters which are easily solved. Using the optimal parameters resulting from this derivation in the expression of U gives, in fact, a value which depends only on the K_{ij} parameter(s). The process is repeated with only different K_{ij} term(s) until the minimum minimorum is obtained. This can be achieved by the usual ways of minimization of the objective function (Walsh, 1979).

This 'semi-linear' method has been applied to original binding results of quinuclidinyl 4-[*phenyl*-^{3}H]benzilate (47 Ci/mmol; QNB) and *N*-[*propionyl*-^{3}H]propionylated α-bungarotoxin (54 Ci/mmol; αBTX) with cholinergic receptors from synaptosomes of rat hippocampus homogenized according to classical methodologies (Whittaker and Barker, 1972; Yamamura and Snyder, 1974; Marks and Collins, 1982; Schwartz et al., 1982; Gurwitz et al., 1984; Hulme and Birdsall, 1992).

This methodology can be extended to experiments of the displacement of a radiolabelled ligand by the same unlabelled one. This can readily be achieved by an original modification of Eq. 1 using an experimentally known α term:

$$\left(B_i^{\text{calc}}\right) = \sum_i \frac{K_{ij}R_j\left(L - B_i^{\text{calc}}\right)}{1 + \alpha_i K_{ij}\left(L - B_i^{\text{calc}}\right)} + N_i\left(L - B_i^{\text{calc}}\right) \quad (2)$$

or

$$\left(B_i^{\text{calc}}\right) = \sum_i \frac{K_{ij}R_j\left(L_i - B_i^{\text{calc}}\right)}{1 + \alpha_i K_{ij}\left(L_i - B_i^{\text{calc}}\right)} + N_i\left(L_i - B_i^{\text{calc}}\right) \quad (3)$$

where L is the fixed and L_i the variable concentration(s), in each i point, of the labelled ligand, according to the type of experiments.

In Eq. 2

$$\alpha_i = \frac{L + l_i}{L}$$

and in Eq. 3

$$\alpha_i = \frac{l + L_i}{l}$$

L representing the radiolabelled ligand and l corresponding to the same unlabelled ligand.

The introduction of the α_i parameters is very efficient, since Eq. 2 and 3 exhibit a very close analogy with Eq. 1 and so can be handled with the described semi-linear methodology by grouping α_i with the $(L_i - B_i^{\text{calc}})$ or $(L - B_i^{\text{calc}})$ terms.

It is noteworthy that the values resulting from the semi-linear regression are very close to those obtained by the true non-linear methodology and can, of course, be used as starting values for the fully non-linear regression.

It appears that with the proposed methodology the binding parameters as well as the number of receptors can be obtained faster than by using the usual strictly non-linear methodology.

This procedure may be very useful to any authors studying the field of binding.

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