Robust Experimental Design for Choosing Between Models of Enzyme Inhibition

Anthony C. Atkinson and Barbara Bogacka

Abstract Models for enzyme inhibition form a family of extensions of the Michaelis-Menten model to two explanatory variables. We present four-point locally Ds-optimum designs for discriminating between competitive and non-competitive models of inhibition and explore the sensitivity of the designs to the values of the two nonlinear parameters in the model. We evaluate combinations of pairs of locally optimum designs. A robust design is found with six support points that has high minimum and average efficiencies over all considered parameter values.

1 Introduction

Enzymes are organic catalysts. In a typical enzyme kinetics reaction enzymes bind substrates and turn them into products. In the absence of inhibition the reaction rate is represented by the standard Michaelis-Menten model $v = V[S]/(K_m + [S])$, where V denotes the maximum velocity of the reaction, $[S]$ is the concentration of the substrate and K_m is the Michaelis-Menten constant—the value of $[S]$ at which half of the maximum velocity V is reached (Michaelis and Menten 1913).

Enzyme inhibitors are molecules that decrease the activity of enzymes. In order to model such behaviour, the Michaelis-Menten model is extended to include the effect of inhibitor concentration $[I]$. Two important mechanisms are competitive and non-competitive inhibition; see, for example, Segel (1993). Our paper presents a method of constructing robust experimental designs for discriminating between the mechanisms.

The two models, which have a similar structure, are introduced in Sect. 2. They may be combined in a single four-parameter model with parameter of combination λ

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(Atkinson 2011). The locally Ds-optimum designs of Atkinson (2012) yield efficient estimates of λ and provide a method of discriminating between the models. However, these locally optimum designs depend on the values of two of the parameters in the combined model. In Sect. 3 we find the minimum and average efficiencies of these designs over a set Θ of parameter values. The combination of pairs of locally optimum designs in Sect. 4 yields our robust design with an increased number of support points that has greatly improved minimum efficiency over Θ.

2 Models for Enzyme Inhibition and the Design Criterion

The velocity equation for *Competitive Inhibition* is

$$v = V[S]/\big\{K_m\big(1+[I]/K_{ic}\big)+[S]\big\}. \tag{1}$$

For *Non-competitive Inhibition* the model is

$$v = V[S]/\big\{\big(K_m+[S]\big)\big(1+[I]/K_{in}\big)\big\}, \tag{2}$$

where K_{ic} and K_{in} are the inhibition constants.

The nonlinear models (1) and (2) have some structure in common. Atkinson (2011) suggests combining the two models into the single four-parameter model

$$v = V[S]/\big[K_m\big\{1+[I]/K_\lambda\big\}+[S]\big\{1+(1-\lambda)[I]/K_\lambda\big\}\big]. \tag{3}$$

When $\lambda = 1$ the model is that for competitive inhibition and $K_\lambda = K_{ic}$, whereas, for $\lambda = 0$, $K_\lambda = K_{in}$ and we obtain non-competitive inhibition.

An experimental design involves the choice of substrate and inhibitor concentrations $x_i = ([S]_i, [I]_i)^{\mathrm{T}}$ at which measurements are to be taken. Interest is in precise estimation of λ, with the other three parameters being treated as nuisance parameters. We use Ds-optimality and investigate the robustness of designs to the values of the nuisance parameters. The linearized model in partitioned form is

$$y_i = \psi^{\mathrm{T}} f(x_i) + \varepsilon_i = \psi_1^{\mathrm{T}} f_1(x_i) + \psi_2^{\mathrm{T}} f_2(x_i) + \varepsilon_i, \tag{4}$$

where $\psi^{\mathrm{T}} = (\psi_1^{\mathrm{T}}\ \ \psi_2^{\mathrm{T}})$ is a p-dimensional vector of all parameters and ψ_1 is $s \times 1$. We assume $\varepsilon_i \sim \mathscr{N}(0, \sigma^2)$. For the design measure ξ putting weight w_i at the design point x_i in the design region $\mathscr{X}$, the information matrix for ψ for a design with n support points can be written in the partitioned form, with blocks given by

$$M_{jk}(\xi) = \sum_{i=1}^{n} w_i f_j(x_i) f_k^{\mathrm{T}}(x_i), \quad j,k = 1,2. \tag{5}$$

The covariance matrix for the estimator of ψ_1 is then

$$A^{-1}(\xi) = \big\{M_{11}(\xi) - M_{12}(\xi) M_{22}^{-1}(\xi) M_{12}^{\mathrm{T}}(\xi)\big\}^{-1}. \tag{6}$$

The Ds-optimum design for ψ_1 in the linear model (4) maximizes the determinant $|A(\xi)|$.

We linearize the model by Taylor series expansion. The information matrix is now a function of the vector of partial derivatives

$$f\left(x_i, \psi^0\right) = \left.\frac{\partial v(x_i, \psi)}{\partial \psi}\right|_{\psi^0} \tag{7}$$

of the response function with respect to the parameters ψ, often called the parameter sensitivities, where ψ^0 is a prior point estimate of the parameters. Optimum designs for this linearized model are called *locally-optimum* and depend, often strongly, on the value of ψ^0.

In our example the model is nonlinear, $\psi_1 = \lambda$ and $s = 1$, so that the locally Ds-optimum design maximizes $A(\xi, \psi^0)$. Throughout we will be interested in approximate designs in which the weights w_i are not constrained to be ratios of integers.

3 Design Sensitivity

Bogacka et al. (2011) find analytical expressions for locally D-optimum designs for several enzyme inhibition models including (1) and (2). However, Ds-optimum designs have to be found numerically. We base our numerical results on those for the system Dextrometorphan-Sertraline used by Bogacka et al. (2011) in which the rectangular design region is $\mathscr{X} = [0, [S]_{\max}] \times [0, [I]_{\max}]$, with $[S]_{\max} = 30$ and $[I]_{\max} = 40$. Bogacka et al. (2011) took parameter values $K_m^0 = 4.36$ and $K_{ic}^0 = 2.58$ with the value of V arbitrary. Our value of λ^0 was 0.8, since Atkinson (2012) demonstrates that this provides efficient locally optimum designs whether $\lambda = 0$ or 1. In (3) the inhibition coefficient is written as a general value K_λ. Atkinson (2011) argues that it is necessary to choose parameter values which are appropriate for modelling the same physical phenomenon, whichever component model is used. This is achieved by taking K_λ in (3) equal to $(2 - \lambda)K_{ic}$, so that $K_\lambda^0 = 1.2K_{ic}^0$. Since V occurs linearly in (3), we take the arbitrary value $V^0 = 10$. The parameter sensitivities required in the calculations are given by Atkinson (2011).

We calculated the optimum designs by numerical optimization using an unconstrained Quasi-Newton method with parameter transformation to satisfy the constraints on the design points and weights required for experimental designs (Atkinson et al. 2007, Sect. 9.5). For the design region used in this paper, and for all parameter values considered, the Ds-optimum designs for λ have the form

$$\xi^* = \left\{ \begin{array}{cccc} ([S]_{\max}, [I]_{\min}) & (s_2, [I]_{\min}) & ([S]_{\max}, i_3) & (s_4, i_4) \\ w_1 & w_2 & w_3 & w_4 \end{array} \right\}, \tag{8}$$

so that they can be found by a seven-dimensional numerical search, provided this structure holds. That this structure holds and that the optimum design had been

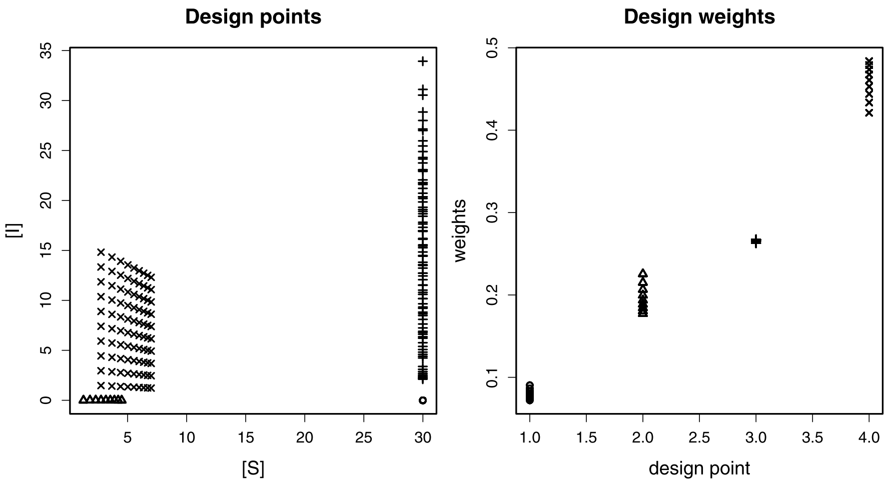

Fig. 1 90 locally Ds-optimum designs for elements of Θ. *Left-hand panel*, clustering of support points in $\mathscr{X}$; symbols $\circ$, $\triangle$, $+$ and $\times$ respectively denote x_1, x_2, x_3 and x_4. *Right-hand panel*, design weights

found for each case was checked by using the equivalence theorem (Atkinson et al. 2007, Sect. 10.3) over a grid of 81×81 points in $\mathscr{X}$.

To investigate the dependence of designs on the values of the two parameters in (3) let $\theta^{\mathrm{T}} = (K_{ic}, K_m)$. We take a grid of values $\theta_j \in \Theta$ defining the set Θ at all pairs such that

$$K_{ic} = (0.5, 1.0, \ldots, 5) \quad \text{and} \quad K_m = (2, 3, \ldots, 10). \tag{9}$$

There are therefore ten values of K_{ic}^0 and nine of K_m^0. The scatter of design points in $\mathscr{X}$ for the 90 locally optimum designs is shown in the left-hand panel of Fig. 1.

There is an appreciable structure in these designs which follows in part from (8). All designs have the same first support point $([S]_{\max}, [I]_{\min}) = (30, 0)$. There are 9 values of s_2 and of s_4, the variations in both of which therefore depend only on the value of K_m^0. The 90 values of i_3 range from 2.12 to 33.94 whereas those for i_4 have a maximum of 14.83.

The design weights in the right-hand panel are less dependent on the prior values θ_j^0. The minimum value is 0.048, so that, in this example, the Ds-optimum designs are not singular. However, Youdim et al. (2010) show that the Ds-optimum design for K_{ic} in (1) has only two points of support. Such singular designs are useful in calculating the efficiencies of practically useful designs.

To assess the designs requires the efficiency of ξ_{S}^i, the optimum design found for prior θ_i^0, evaluated at parameter $\theta_j \in \Theta$. Since $s = 1$, we define the efficiency as

$$\mathrm{Eff}_{\mathrm{s}}(i, j) = A\big(\xi_{\mathrm{S}}^i, \theta_j\big) / A\big(\xi_{\mathrm{S}}^j, \theta_j\big). \tag{10}$$

To assess each design we look at the minimum and average value of $\mathrm{Eff}_{\mathrm{s}}(i, j)$.

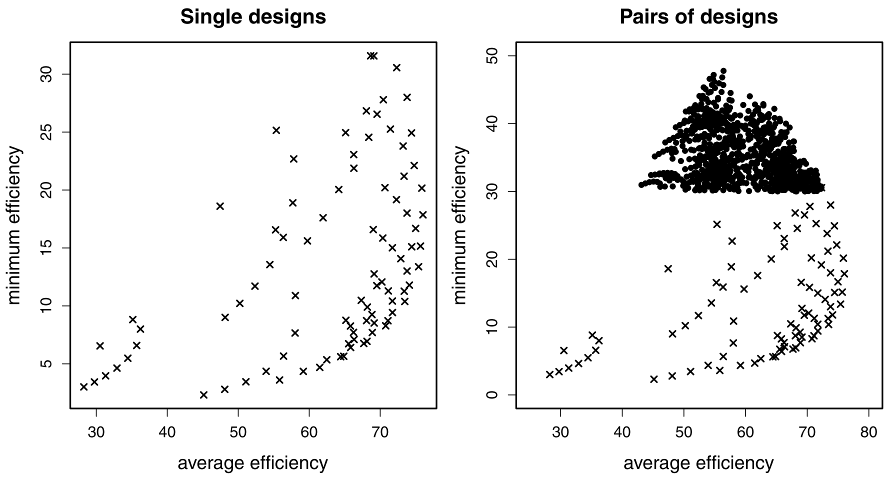

Fig. 2 *Left-hand panel*: Minimum and average efficiencies (×) over Θ for the 90 locally Ds-optimum designs. *Right-hand panel*: the same with the addition of efficiencies for selected pairs of locally optimum designs (•)

Table 1 Average and minimum efficiencies of six of the locally Ds-optimum designs shown in Fig. 2. K_{ic}^{min} and K_{m}^{min} are the parameter values at which each design has its minimum efficiency

Design	K_{ic}^{0}	K_{m}^{0}	Efficiency %		K_{ic}^{min}	K_{m}^{min}
			Average	Minimum		
1	2.5	5.0	76.03	17.86	0.5	10.0
2	2.5	6.0	75.87	20.16	0.5	10.0
3	1.5	4.0	68.65	31.58	0.5	10.0
4	1.5	5.0	69.11	31.58	5.0	2.0
5	0.5	10.0	28.25	3.01	5.0	2.0
6	5.0	2.0	45.14	2.32	0.5	10.0

These efficiencies are plotted in the left-hand panel of Fig. 2 with properties of six selected designs displayed in Table 1. Again there is some structure in the plot reflecting the grid of parameter values. Desirable designs will have both a high average efficiency and a high minimum efficiency. It is clear from the figure that there is a trade off, amongst the locally-optimum designs in the top right-hand corner of the figure, between average and minimum efficiency over Θ.

Some numerical details are in Table 1. The first two designs, for priors in the centre of the parameter range, are those with the highest average efficiency, 76.03 and 75.87 %. The second two designs, for smaller values of K_{ic}^{0}, have lower average efficiencies, 68.65 and 69.11 %, but higher minimum efficiencies; 31.58 % for both designs, rather than 17.86 and 20.16 %. The last two designs in the table, for parameter prior values on the boundary of Θ, have the lowest minimum efficiencies of those in Fig. 2.

Table 2 Six-point robust Ds-optimum design; a combination of locally optimum designs for parameter values (3.5, 4.0) and (0.5, 4.0). Left-hand panel, numerical; right-hand panel, notational from (8)

$[S]$	$[I]$	w	$[S]$	$[I]$
30.000	0.000	0.083	$[S]_{\max}$	$[I]_{\min}$
2.304	0.000	0.207	s_2	$[I]_{\min}$
30.000	20.195	0.133	$[S]_{\max}$	i_{31}
30.000	2.885	0.133	$[S]_{\max}$	i_{32}
4.414	9.738	0.222	s_4	i_{41}
4.414	1.391	0.222	s_4	i_{42}

The designs considered can be extended by including Bayesian-optimum designs over suitable prior distributions. For example, for a uniform distribution over the nine-point prior $[1, 2.5, 4] \times [3, 6, 9]$ which almost spans Θ, the design has four support points with the structure of (8); the average efficiency for this design is 75.12 %, similar to those of Designs 1 and 2 in Table 1, although the minimum is higher at 24.07. To find designs with higher minimum efficiencies we generate designs with more support points.

4 Robust Designs

The last two columns of Table 1 given the parameter values for which the minimum efficiency occurs for each locally optimum design. For four of the designs, these are (0.5, 10) and for the other two (5.0 and 2.0). Both are extreme points of Θ, yielding designs 5 and 6 in the table for which the minimum efficiency is smallest. The designs with high efficiencies are locally optimum for more central values of the prior values of the parameters. This suggests that a combination of locally optimum designs for central and extreme points in Θ will have a relatively high minimum efficiency.

The right hand-panel of Fig. 2 repeats the plot of minimum and average efficiencies of the 90 locally optimum designs and adds the efficiencies for all those pairs of locally optimum designs for which the minimum efficiency is greater than 30 %. As the plot shows, there are numerous designs with a minimum efficiency higher than the maximum value of 31.58 in Table 1. The design with the highest minimum efficiency, 47.78 %, has an average efficiency of 56.43. The numerical results for the design are given in the left-hand panel of Table 2 with notational expressions in the right-hand panel.

Because of the structure of the locally optimum designs shown in (8), the equally weighted combination of two designs only has six points of support. Points 1 and 2 have full weight whereas points 3 and 4 in (8) are divided between two points, although the values of s_4 are the same for the two parts of the two divided support points. The design is the combination of those for prior parameter values (3.5, 4.0) and (0.5, 4.0), not as extreme as those giving the minimum efficiencies in Table 1. However the three values of efficiency below 48 % for this design all occur at extremes of Θ: (5.0, 10.0), (5.0, 2.0) and (0.5, 10.0).

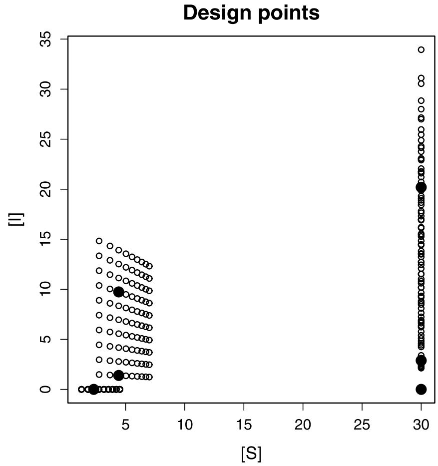

Fig. 3 The six support points of the robust design, •, and the points, ∘, of the 90 locally Ds-optimum designs for elements of Θ

5 Discussion

The combination of two locally optimum designs has led to a design with increased minimum efficiency. If a design with higher average efficiency but lower minimum efficiency is required, another design from the boundary in Fig. 2 could be used.

It is informative to look at the robust design points in the context of the locally optimum designs (8). Figure 3 repeats the left-hand panel of Fig. 1 with the addition of the points of the robust design. We see that the value of s_2 lies in the centre of the range of values for the locally optimum designs, the two values of i_3 almost span the range of locally optimum values and that there is a medium and extreme value of i_4, but not of s_4.

Intuitively some of the properties of the robust design are clear, such as an increase in the number of support points relative to the locally-optimum design. However it is not immediate from Fig. 1 which points should be divided. Dror and Steinberg (2006) find robust designs through the clustering of the support points of locally optimum designs, a procedure echoed in Fig. 3. D-optimality is used by Woods et al. (2006) to find robust designs for generalized linear models over link functions and parameters.

We have found designs which provide a compromise between the value of the average and minimum efficiencies. Calculation of the maximin design that maximizes the minimum efficiency is complicated by the non-convexity of the objective function. Recent results are given by Biedermann et al. (2011) for additive models. Dette et al. (2007) provide an equivalence theorem for maximin designs and apply it to a one parameter problem. King and Wong (2000) provide an algorithm for the construction of maximin designs.

Finally, we note that if only one model is of interest, T-optimum designs (Atkinson and Fedorov 1975) maximize the non-centrality parameter of the F-test for departures from that model. See Wiens (2009) for recent developments. However, since either model may be true, compound T-optimum designs are required (Atkinson 2008, Sect. 4) which maximize a function of the non-centrality parameters for departures from each model. Atkinson (2012) finds, for the parameter values of Bogacka et al. (2011), that the individual T-efficiencies for the T-optimum design are 3–4 % higher than those for the Ds-optimum design. In some cases T-optimum designs can be difficult to compute (but see Dette and Titoff 2008) so that Ds-optimum designs may be a useful surrogate.

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