EXPERIMENTS FOR ENZYME KINETIC MODELS

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Enzyme Kinetics

A catalyst is a substance that takes part in a chemical reaction, but is not itself consumed.
In a typical enzyme kinetics reaction, enzymes bind substrates and turn them into products. The binding step is reversible with the catalytic step irreversible:

$$S + E \longleftrightarrow ES \rightarrow E + P,$$

S, E and P denote substrate, enzyme and product, respectively. ES is a complex.
Examples: the degradation of alcohol in the liver; the curdling of milk in the stomach.
It is clear that E is not consumed in the reaction.
Interest is in the velocity of the reaction. This comes from 'heroic' assumptions about the kinetics.

Michaelis-Menten Model

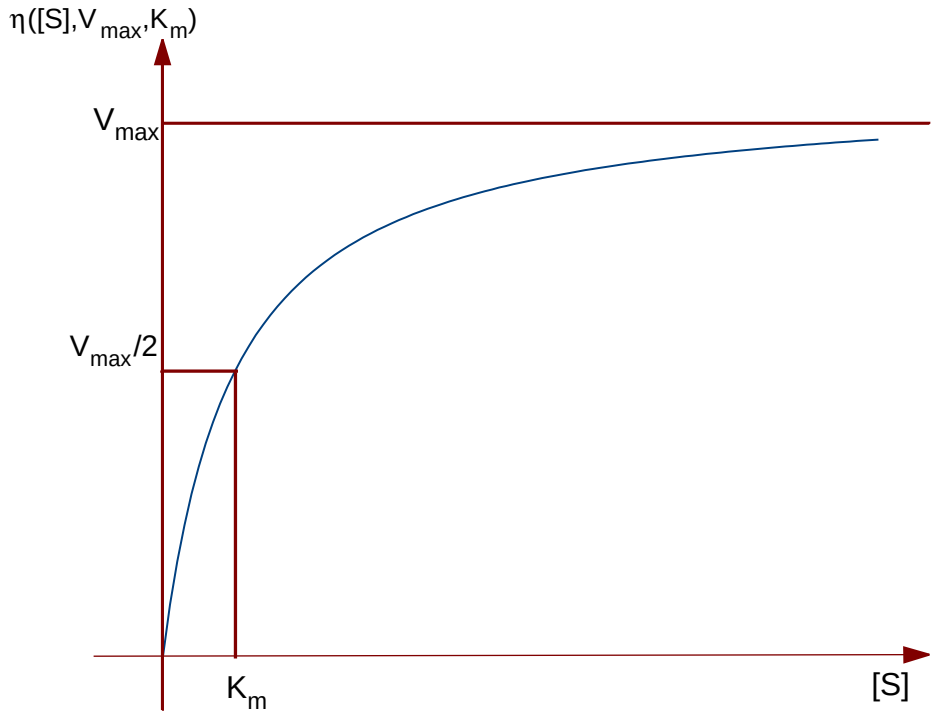
The reaction rate is represented by the Michaelis-Menten model

$$v = \frac{V_{max}[S]}{K_m + [S]},$$

where v = 'vee' not 'nu', $[S]$ is the concentration of the substrate and V_{max} and K_m are the model parameters:

- V_{max} denotes the maximum velocity of the enzyme and
- K_m is Michaelis-Menten constant, it is the value of $[S]$ at which half of the maximum velocity V_{max} is reached.

Michaelis-Menten Model



M-Menten Model. The response function: $\eta\left([S]; V_{max}, K_m\right)$ for $V^o_{max} = 1, K^o_m = 1.$

At what values of $[S]$ should measurements be taken to get 'good', i.e. accurate and inexpensive, estimates of the parameters V_{max} and K_m?

OPTIMUM EXPERIMENTAL DESIGN

Requires:

- **A model.** Many models are linear

$$y = \beta^T f(x) + \epsilon$$

  $f(x)$ is a $p \times 1$ vector of powers and products of x. The errors ϵ are independent, with variance σ^2

- In matrix form $E(Y) = F\beta$
- Some models are nonlinear in the parameters

$$y = 1 - e^{-\theta x} + \epsilon$$

Also requires:

- **A design region** $\mathcal{X}$. For MM-model [0,$[S]_{max}$]

OPTIMUM EXPERIMENTAL DESIGN

An experimental design is a set of n points in $\mathcal{X}$

- **Design Measure**

$$\xi = \left\{ \begin{array}{cccc} x_1 & x_2 & \dots & x_n \\ w_1 & w_2 & \dots & w_n \end{array} \right\}$$

- The design has
  - Support points $x_1 \dots x_n$
  - design weights $w_1 \dots w_n$
- **Exact design**

$$w_i = r_i/N, \quad \sum r_i = N$$

  r_i # replicates at x_i
- **Continuous/ Approximate Design** ξ is a measure over the design points. Removes dependence of design on N.

THE INFORMATION MATRIX

- The **Information Matrix** is often written

$$M(\xi) = F^T F/n$$

- **D-optimum** designs ξ^*

$$\max |F^T F| \qquad \text{so that} \qquad \max \det M(\xi) = \det M(\xi^*)$$

They minimise the volume of the confidence region

- In our comparisons of designs we make use of the D-efficiency, defined as

$$D_{\text{eff}}(\xi) = [\det\{M(\xi, \vartheta)\}/\det\{M(\xi^\star, \vartheta)\}]^{1/p}\,. \qquad (1)$$

- A design with an efficiency of 50% requires twice as many trials as the optimum design to give the same information about the value of ϑ.

GENERAL EQUIVALENCE THEOREM

The **standardised variance** of prediction at x is

$$d(x,\xi) = f^T(x)M^{-1}(\xi)f(x)$$

The (General) Equivalence Theorem (Kiefer and Wolfowitz, 1960) states that the following three conditions are equivalent:

- **Definition**

$$\xi^* \quad \text{maximises} \quad \det M(\xi)$$

- **Minimax Equivalence** ξ^* minimises

$$\max_{\mathcal{X}} d(x,\xi)$$

-

$$\max_{\mathcal{X}} d(x,\xi^*) = p$$

  So we can check any purported optimum design
- **Maximum** is at points of support of ξ^*

NONLINEAR MODELS

- The nonlinear model is

$$y_j = \eta(x_j, \vartheta) + \varepsilon_j$$

- Taylor series expansion of the model, at a prior ϑ^o, yields

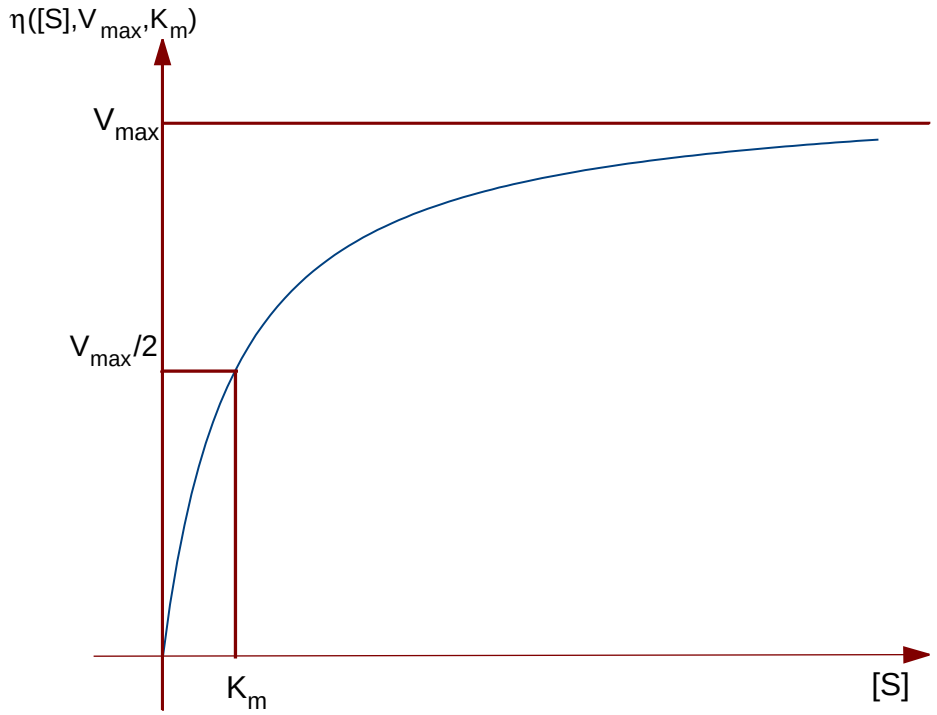
$$\eta(x, \vartheta) = \eta(x, \vartheta^o) + f^{\mathrm{T}}(x, \vartheta^o)(\vartheta - \vartheta^o) + \ldots$$

where

$$f^{\mathrm{T}}(x, \vartheta^o) = \left(\frac{\partial \eta(x, \vartheta)}{\partial \vartheta_1}, \frac{\partial \eta(x, \vartheta)}{\partial \vartheta_2}, \ldots, \frac{\partial \eta(x, \vartheta)}{\partial \vartheta_p} \right) \Big|_{\vartheta = \vartheta^o}.$$

- In the linearised model the design matrix is $F(\vartheta^o)$. The optimum design depends on ϑ^o
- Designs are only **locally** optimum

Back to Michaelis-Menten Model



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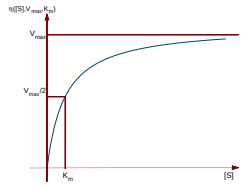
M-M Model: a design

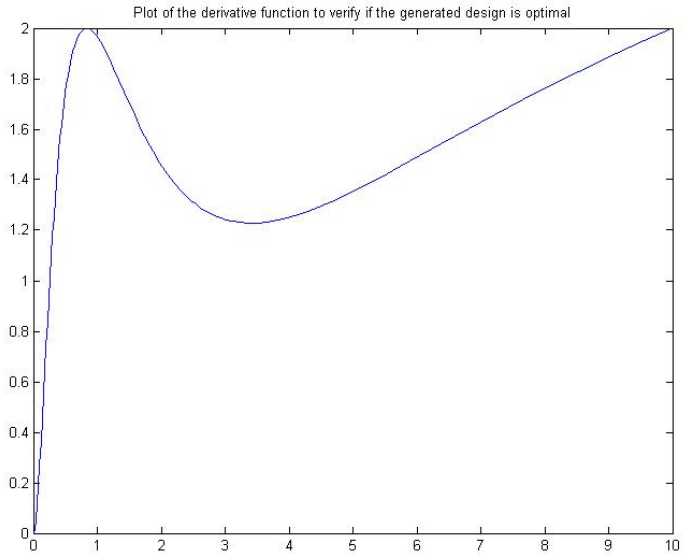


- The model is

$$y_i = \frac{V_{max}[S]}{K_m + [S]} + \varepsilon_i.$$

- D-optimum designs for NL models with p parameters often have p support points.
- Then we MUST have $w_i = 1/p$.
- So we guess the D-optimum design is of this form.
- Then $|F^T F| = |F|^2$.
- Guess one support point is $[S]_{max}$.
- straightforward mathematics maximizing $|F|$ yields algebraic expression for $[S]_s$

M-M Model: the Equivalence Theorem



- Plot of $d(x, \xi_s)$ is for design with weights 0.5 on $[S]_s$ and $[S]_{max}$ when $[S]_{max} = 10$.
- The two maxima of 2 (= p) are at the design points.
- This design is (locally) D-optimum.
- This is the procedure to be followed in some more complicated cases: guess, numerically optimize, verify.

Enzyme Inhibitors

- Enzyme inhibitors are molecules that bind to enzymes and decrease their activity. Since blocking an enzyme's activity can kill a pathogen (e.g. penicillin) or correct a metabolic imbalance, many drugs are enzyme inhibitors
- The interactions of drugs in 'cocktails' may also cause inhibition

Competitive Inhibition

$$\begin{array}{ccccc} & & EI & & \\ & & \updownarrow K_{ic} & & \\ S & + & E & \longleftrightarrow ES \longrightarrow & E + P, \\ & & + & & \\ & & I & & \end{array}$$

A competitive inhibitor is a substance that combines with an enzyme such that it prevents the substrate binding. This could be because enzyme and inhibitor bind to exactly the same site, or partly share a site; the inhibitor could mask or distort the substrate binding site. In any case, inhibitor and substrate binding are mutually exclusive.

Competitive Inhibition

The velocity equation is:

$$v = \frac{V[S]}{K_m\left(1 + \frac{[I]}{K_{ic}}\right) + [S]}, \tag{2}$$

where K_{ic} is the dissociation constant. For a fixed $[I]$ the limit of this function, when $[S] \to \infty$, is V. Although the same maximum velocity is obtained irrespective of the concentration of the inhibitor, higher values of $[I]$ make the reaction slower for a fixed value of $[S]$.
V occurs linearly in the model, so optimum designs will not depend on its value.

D-optimum Designs for Competitive Inhibition

- We, Bogacka *et al.* (2011) (really Maciej), obtain analytical solutions to the design optimization problem!
- For these and other inhibition models the number of support points is equal to p and, of course, $w_i = 1/p$.
- The design support points are often on the border of the design region

D-optimum Designs for Competitive Inhibition

Table: D-optimum designs for competitive inhibition

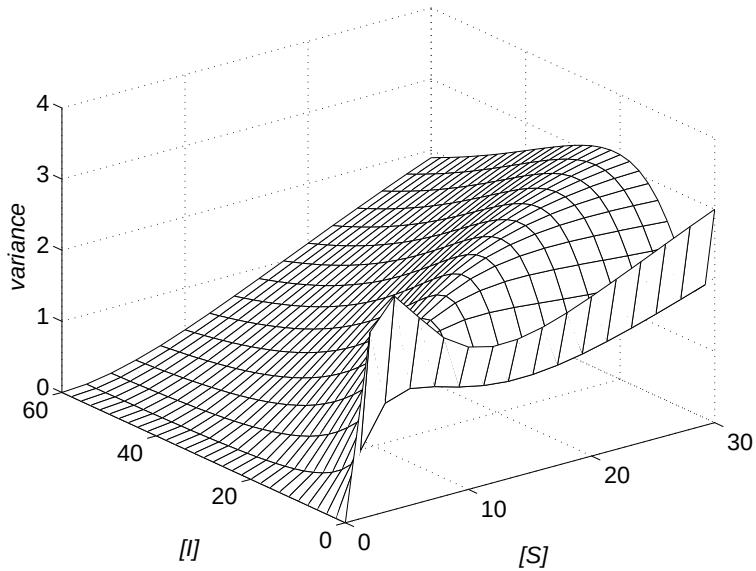
Inhibition MM model	Optimum design $\xi^\star$		
Competitive	$([S]_{\max}, [I]_{\min})$	$(s_2, [I]_{\min})$	(s_3, i_3)
	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$

$$s_2 = \max\left\{[S]_{\min},\ \frac{[S]_{\max}K_m(K_{ic}+[I]_{\min})}{2K_mK_{ic}+2K_m[I]_{\min}+[S]_{\max}K_{ic}}\right\}$$

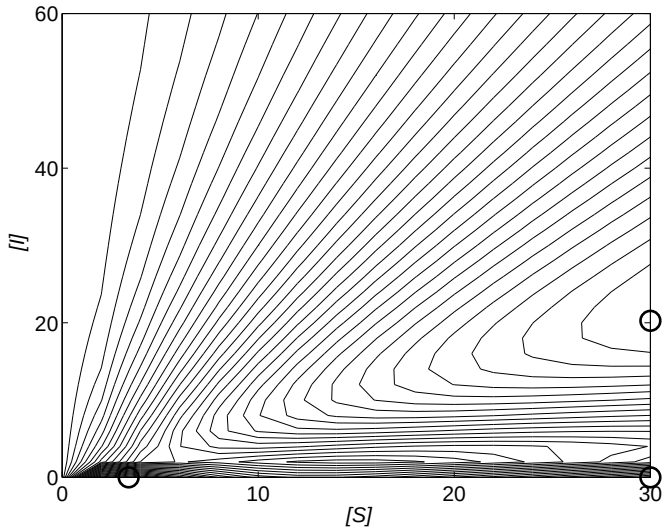
$$s_3 = \max\left\{[S]_{\min},\ \min\left\{\frac{K_m(K_{ic}+[I]_{\max})}{K_{ic}},\ [S]_{\max}\right\}\right\}$$

$$i_3 = \min\left\{\frac{2K_m[I]_{\min}+[S]_{\max}K_{ic}+K_mK_{ic}}{K_m},\ [I]_{\max}\right\}$$

The D-optimum Design



(a)



(b)

Figure: Dextrometorphan-Sertraline. Properties of the D-optimum design: (a) Plot of the variance of the response prediction, (b) Contour plot of the variance of the response prediction and support points of the D-optimum design

Typical Design

- A typical experiment using 15 different substrate (Sertraline) concentrations and 8 different inhibitor (Dextrometorphan) concentrations forming a grid of $n = 120$ points was performed and the velocity measured. We call this the “rich” design, since it contains observations at many points in $\mathcal{X}$.
- Many of the points of the design grid contribute hardly at all to the efficiency of estimation of the parameters and so are inefficient.

Design Efficiencies

- In our comparisons of designs we make use of the D-efficiency, defined as

$$D_{\text{eff}}(\xi) = [\det\{M(\xi, \vartheta)\} / \det\{M(\xi^{\star}, \vartheta)\}]^{1/p} . \qquad (3)$$

- The “rich” design has a paltry D-efficiency of 18.21%
- We run at 21 = 3 $\times 7$ trial D-optimum design for comparison

Design Efficiencies

The estimates of the parameters (and their standard errors) from the two experiments of very different sizes are:

	Rich data set, $n = 120$		D-optimum ($n = 21$)	
Parameter	Estimate	Standard error	Estimate	Standard error
V	7.29753	0.113163	7.15829	0.108512
K_m	4.38594	0.231031	4.15332	0.232656
K_{ic}	2.58218	0.143980	2.08923	0.127189

The match of the estimates from the observations for both designs is very good. Equally importantly, the standard errors of the estimates obtained from the optimum designs with 21 observations are similar (even slightly better for V and K_{ic}) to the standard errors obtained from the rich design with 120 observations, in line with the efficiencies

Robustness of Designs

- These optimum designs are locally optimum - depend on ϑ^0
- Bogacka *et al.* (2011) consider the sensitivity of the designs to these assumed values.
- Greatly simplified, compared with numerical investigations, since we can use our analytical expressions for the optimum designs.
- The analytical expressions provide insight into the structure of the optimum designs and so of the properties of the efficiencies.
- In particular, many points are on the boundaries of $\mathcal{X}$, so do not depend on ϑ^0.

Some Problems in Model Building

We now consider two problems that arise in model building - not mathematical statistics!

- One three-parameter model arises when two parameters in a four-parameter model are equal. How to design efficient experiments for testing this equality?
- We could have one of two competing three parameter models
  - D-optimality for a combined model
  - T-optimality - but intended for testing the truth of one model

D_1-optimum Designs and Model Building

An example in which two parameters may be equal.
Linear Mixed Inhibition. In this four-parameter model the deterministic velocity equation is:

$$v = \frac{V[S]}{K_m\left(1 + \frac{[I]}{K_c}\right) + [S]\left(1 + \frac{[I]}{K_u}\right)}, \tag{4}$$

Non-competitive Inhibition. When $K_u = K_c$ the model has a specific interpretation and becomes

$$v = \frac{V[S]}{(K_m + [S])\left(1 + \frac{[I]}{K_c}\right)}. \tag{5}$$

Require efficient designs for testing the equality of K_c and K_u

D_1-optimum Designs and Model Building

- Rewrite the larger model. Let $\theta_1 = 1/K_c$ and $\theta_2 = 1/K_u$, when (4) becomes

$$v = \frac{V[S]}{K_m\left(1+\theta_1[I]\right) + [S]\left(1+\theta_2[I]\right)}. \tag{6}$$

- Now make a reparameterization and write

$$\theta_1 = \theta + \delta \quad \text{and} \quad \theta_2 = \theta - \delta,$$

- Then (6) becomes

$$v = \frac{V[S]}{\left(K_m + [S]\right)\left(1+\theta[I]\right) + \delta[I]\left(K_m - [S]\right)}, \tag{7}$$

which reduces to (5) when $\delta = 0$.

D_1-optimum Designs and Model Building

In Table 3 we compare three designs for testing whether δ in (7) is zero.

- The first design is the analytically known D-optimum design of Patan.
- For the second design we keep the support points of the D-optimum design but perform a three-dimensional numerical search to find the D_S-optimum weights. The results are surprising. To five decimal places (we haven't explored further) the weights are identically 1/9, 2/9, 2/9 and 4/9.
- Patan's analytical results do not extend to D_S-optimality, so we found it numerically using the equivalence theorem to check our results.

D_1-optimum Designs and Model Building

Table: Enzyme Kinetics: D-optimum design and D_S-optimum designs for parameter equality

Criterion	i	1	2	3	4	Efficiency %
	$[S]$	100	5.8226	100	5.8226	
	$[I]$	0	0	1.35	1.35	
D	w	1/4	1/4	1/4	1/4	72.12
	$[S]$	100	5.8226	100	5.8226	
	$[I]$	0	0	1.35	1.35	
D_S weights	w	1/9	2/9	2/9	4/9	89.04
	$[S]$	100	4.1877	100	4.1877	
	$[I]$	0	0	1.9093	1.9093	
D_S	w	0.0858	0.2071	0.2071	0.5000	100.

D-optimum Designs and Model Building

We have assumed competitive inhibition

$$v = \frac{V[S]}{K_m\left(1 + \frac{[I]}{K_{ic}}\right) + [S]}.$$

Often it is not known whether the model for non-competitive inhibition is preferable in which

$$v = \frac{V[S]}{(K_m + [S])\left(1 + \frac{[I]}{K_{in}}\right)}.$$

Both models contain 3 parameters. The D-optimum designs have three support points, so are not informative about departures from either model.

A Combined Model

$$v = \frac{V[S]}{K_m\left(1 + \frac{[I]}{K_{ic}}\right) + [S]} \quad \text{or} \quad v = \frac{V[S]}{(K_m + [S])\left(1 + \frac{[I]}{K_{in}}\right)}.$$

Combine the models in a four parameter family and then test the parameter of combination.
Models are of the form a/b and a/c. The combined model is

$$\frac{a}{\lambda b + (1-\lambda)c} \qquad (0 \leq \lambda \leq 1).$$

Interest is, in particular, in whether $\lambda = 0$ or 1

Subsets of Parameters

The combined model is:

$$v = \frac{V[S]}{K_m\left\{1 + \frac{[I]}{K_\lambda}\right\} + [S]\left\{1 + \frac{(1-\lambda)[I]}{K_\lambda}\right\}}.$$

When interest is in estimation of a subset of parameters a D_S-optimum design is required. Here interest is in λ and $s = 1$

We compare D- and D_S-optimum designs with those that are T-optimum

Compound T-optimum Designs and Model Building

- The combined model provides a method of finding optimum designs for estimating the parameter of combination
- The found designs have good efficiencies for parameter estimation whether the competitive or non-competitive models hold.
- However, if only one model is of interest, T-optimum designs maximize the non-centrality parameter of the F-test for departures from that model.
- We now calibrate the Ds-optimum designs of the preceding section against the T-optimum designs for model discrimination.

T-optimum Designs

- With linear models the procedure is that of forming a combined linear model and then testing for the necessity of the extra terms.
- When the models differ by a single term the T-optimum design coincides with the Ds-optimum design for that term.
- For nonlinear models the situation is more complicated (because of linearisations).
- In our example an additional complication is that the competitive and non-competitive models are separate.
- We obtain the two T-optimum designs corresponding to departures from the component models.
- Since either model may be true, compound T-optimum designs must be investigated.

T-optimum Designs 2: Theory

- Atkinson and Fedorov (1975) describe designs for discrimination between two rival regression models $\eta_1(x, \theta_1)$ and $\eta_2(x, \theta_2)$ on the assumption that it is known which one is true.
- The models may be linear or nonlinear in the parameters which are estimated by least squares.
- With the first model true, the observations

$$y_i = \eta_t(x) + \epsilon_i = \eta_1(x, \theta_1) + \epsilon_i. \tag{8}$$

T-optimum Designs 3: Theory

- The lack of fit sum of squares for model 2 is made as large as possible by maximizing

$$\Delta_1(\xi) = \sum_{i=1}^{n} w_i \{\eta_t(x_i) - \eta_2(x_i, \hat{\theta}_{t2})\}^2, \tag{9}$$

where

$$\sum_{i=1}^{n} w_i \{\eta_t(x_i) - \eta_2(x_i, \hat{\theta}_{t2})\}^2 = \inf_{\theta_2 \in \Theta_2} \sum_{i=1}^{n} w_i \{\eta_t(x_i) - \eta_2(x_i, \theta_2)\}^2. \tag{10}$$

- Let ξ_T^* be the design maximizing (9).

T-optimum Designs 4: Efficiency

- We have two models which are present on an equal footing, neither being a special case of the other.
- We could equally well be interested in maximizing the non-centrality parameter $\Delta_2(\xi)$ in which indexes 1 and 2 in (9) are interchanged.
- To find designs that are informative about departures from either model we find a **compound T-optimum design** which is a function of both non-centrality parameters
- The T-efficiency of any design ξ relative to the T-optimum design ξ^*_{T1} when model 1 is assumed true can be written

$$\text{Eff}_{T1}(\xi) = \Delta_1(\xi)/\Delta_1(\xi^*_{T1}). \tag{11}$$

Compound T-optimum Designs 1

- A special case of compound DT-optimum designs for model discrimination and parameter estimation (Atkinson, 2008) when there are two models.
- Maximize a weighted product of the efficiencies

$$\{\text{Eff}_{\text{T1}}\}^{1-\kappa}\{\text{Eff}_{\text{T2}}\}^{\kappa} = \{\Delta_1(\xi)/\Delta_1(\xi^*_{\text{T1}})\}^{1-\kappa}\{\Delta_2(\xi)/\Delta_2(\xi^*_{\text{T2}})\}^{\kappa} \quad (0 \leq \kappa \leq 1). \tag{12}$$

- When $\kappa = 0$ we obtain T-optimality when model 1 is assumed true.
- We investigate the properties of designs as κ varies.

Compound T-optimum Designs 2

- To clarify the structure of the design criterion, take logs in (12), when the right-hand side becomes

$$\{(1-\kappa)\log\Delta_1(\xi)+\kappa\log\Delta_1(\xi)\}-\{(1-\kappa)\log\Delta_1(\xi^*_{\mathsf{T}1})+\kappa\log\Delta_2(\xi^*_{\mathsf{T}2})\}. \quad (13)$$

- The terms involving $\xi^*_{\mathsf{T}1}$ and $\xi^*_{\mathsf{T}2}$ are constants when a maximum is found over ξ, so that the criterion to be maximized is

$$\Phi_1^{(CT)}(\xi)=(1-\kappa)\log\Delta_1(\xi)+\kappa\log\Delta_2(\xi). \quad (14)$$

Numerical 1

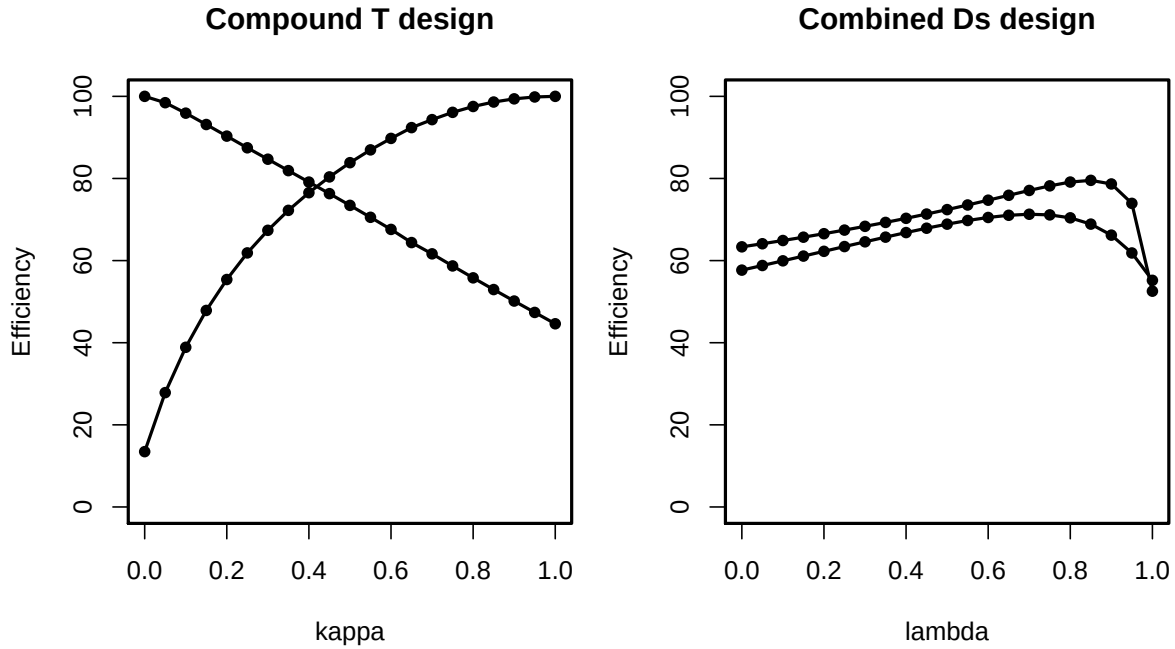


Figure: T-efficiencies (%) for the individual inhibition models. The two curves are efficiencies relative to the designs for the non-competitive inhibition model ($\kappa = 0$) and the competitive inhibition model ($\kappa = 1$). Left-hand panel: compound T-optimum designs as κ varies. Right-hand panel: Ds-optimum designs as λ varies. The Ds-design for $\lambda = 0.8$ has good properties

Numerical 2

Table: Some T-optimum, compound T-optimum ($\kappa = 0.5$) and Ds-optimum ($\lambda = 0.8$) designs and their T-efficiencies.

Design	$[S]$	$[I]$	w	Efficiencies (%) for $\kappa = 0$	Efficiencies (%) for $\kappa = 1$
$\kappa = 0$	30.000	0.000	0.067	100	13.47
	1.828	0.000	0.046		
	30.000	10.154	0.337		
	4.107	4.153	0.550		
$\kappa = 0.5$	30.000	0.000	0.056	73.45	83.84
	3.269	0.000	0.216		
	30.000	13.281	0.234		
	4.815	6.934	0.494		
$\lambda = 0.8$	30.000	0.000	0.082	70.41	79.12
	2.484	0.000	0.204		
	30.000	14.492	0.266		
	4.666	7.103	0.448		
$\kappa = 1$	30.000	0.000	0.059	44.61	100
	3.072	0.000	0.250		
	30.000	22.613	0.250		
	5.453	11.614	0.441		

Numerical 3

- The compound T-optimum designs for $\kappa = 0.5$ and the Ds-optimum design for $\lambda = 0.8$ are close in support points and design weights. As the values of the efficiencies show, the differences are only 3-4%.
- There is therefore little effect, for this example, in whether the optimum design for model choice maximizes the compound T criterion or that for Ds-optimality in the combined model.
- Important since T-optimum designs can be difficult to compute (although not here).

Discussion

- A problem with the T-optimum designs is that they may be tricky to compute - need NLLS for the “false” model.
- However, the Table shows that the Ds-optimum design has slightly lower D- and T-efficiencies than the T-optimum design, so the T-optimum design is recommended for scientific use.
- The designs found in this section are locally optimum. Are T- and Ds-designs close for all plausible parameter values?

SUMMARY

Parameter Estimation

- We have presented analytical expressions for 3 (and 4) point locally D-optimum designs
- It was easy to explore the robustness of these designs

Model Building

- D_1-optimum designs for parameter equality
- The combined model provided Ds-optimum designs for model building
- Compound T-optimum designs had slightly better properties (for one set of parameter values)

LITERATURE

Parameter Estimation

- D_1-optimum designs for K_{ic} are introduced in Youdim *et al.* (2010)

Model Building

- Bogacka *et al.* (2009) describe the D_1-optimum designs for parameter equality
- The combined model, Ds-optimum designs for model building and the compound T-optimum designs are in Atkinson (2012)

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