Design and analysis of *in vitro* pharmacokinetic experiments

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PODE, INI Cambridge
7 July 2015

Outline

1. Motivating experiments
2. Transform-both-sides models
3. The Anova fitting method
4. Extending the basic model
5. Optimal design
6. Comparison with rich design
7. Conclusions and future research

In-vitro pharmacokinetic experiments

In checking for drug-drug interactions, we check for enzyme activity of human liver microsome (HLM) samples.

Samples are available from a number (e.g. 47) of subjects (or donors).

Donors vary and we can consider those used as a sample from the general population.

We also have covariate information on them, namely activities for six cytochrome P450 enzymes. Typically one or two of these will have a large effect on the activity depending on the substrate being studied.

Modelling enzyme activity

We are interested in the variance between subjects, as well as the Michaelis constant and the covariate effects.

The design problem is to choose donors and substrate concentrations for each run. Standard is two replicates of each combination of 9 contentrations with all 47 donors.

We need to model the activity as a function of substrate concentration and covariate levels, also estimating donor to donor variance and allowing for run to run variance. How should we build a realistic model and estimate its parameters?

Simple additive errors might not be reasonable.

Transform-both-sides Model

$$Y^{(\lambda)} = \left(\frac{Vx}{K + x} \right)^{(\lambda)} + \epsilon,$$

where

$$z^{(\lambda)} = \begin{cases} z^{\lambda}, & \lambda \neq 0; \\ \log \lambda, & \lambda = 0 \end{cases}$$

and $\epsilon \sim N(0, \sigma^2)$ (Ruppert, Cressie and Carroll, 1989).

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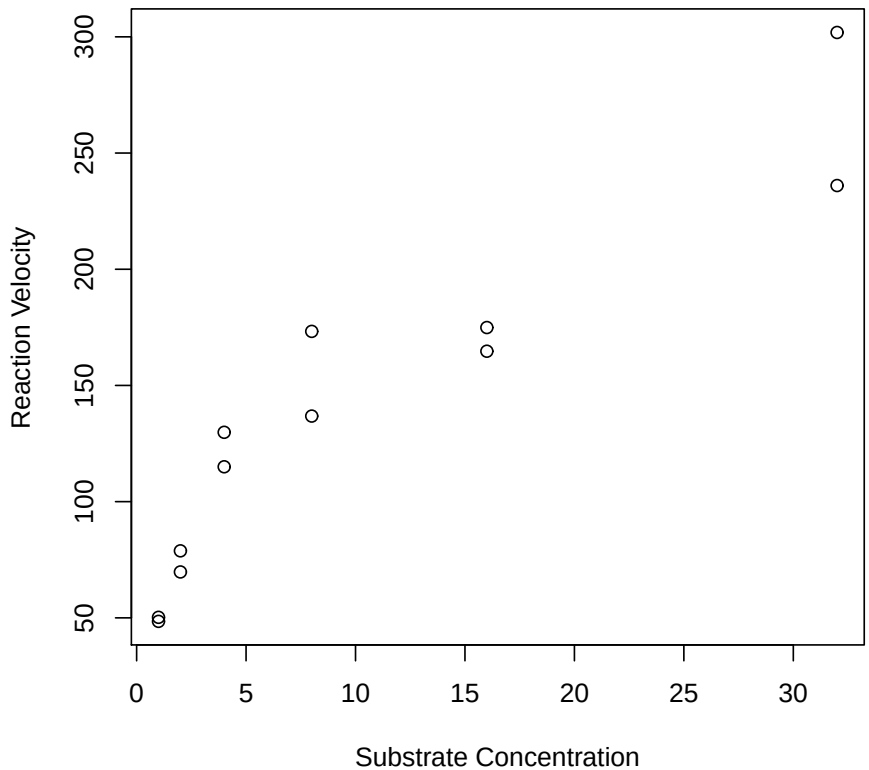
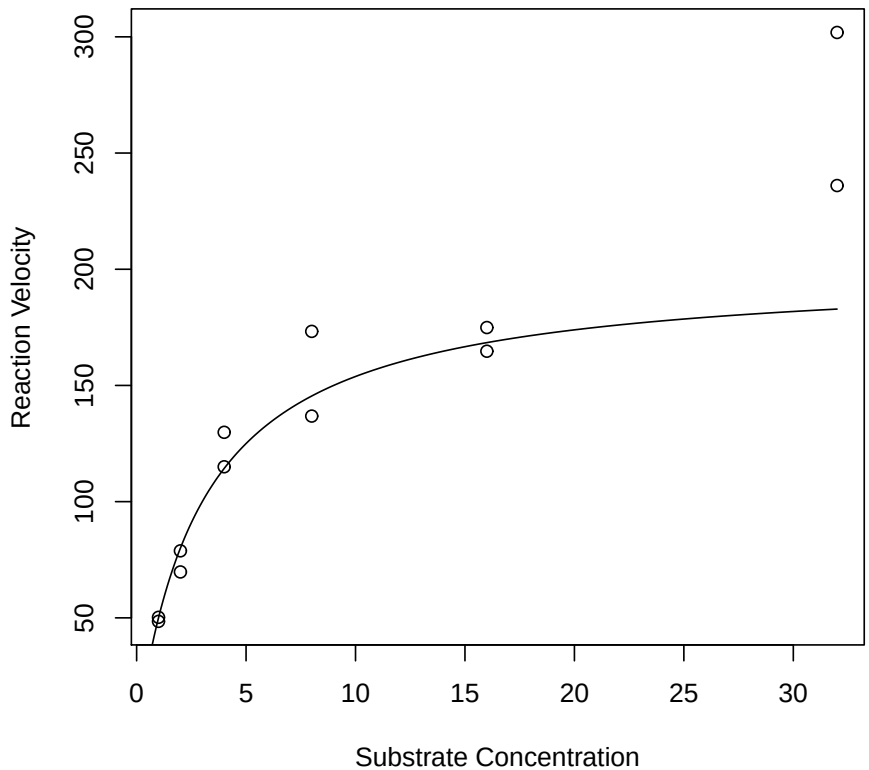
Data from experimental studies create problems and opportunities.

Typical Data



What is wrong with the basic model?



What is happening?

By eye, it is difficult to tell if:

- V is wrong (and σ^2 is large);
- σ^2 increases with $E(Y)$; or
- the systematic part of the model should be $\frac{V_1 x}{K_1+x} + \frac{V_2 x}{K_2+x}$.

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Unfortunately, likelihood methods don't do much better.

The likelihood is flat, with near optimal solutions at:

- $\lambda = 1$, $V_2 = 0$, large V_1 and large σ^2;
- $\lambda = -1$, $V_2 = 0$ and $V_1 = 200$;
- $\lambda = 1$, $V_1 = 200$ and $V_2 = 80$.

The Beauty of Replicated Designs

The parameters λ and σ^2 are unrelated to the systematic part of the model and tell us only about the relationship between the variance and the expectation. We can estimate them robustly without relying on assumptions about the systematic part of the model.

The levels of substrate concentration are treatments and we can estimate λ and σ^2 from the full treatment model:

$$Y_{i(r)}^{(\lambda)} = \mu + \tau_r + \epsilon_i,$$

where $Y_{i(r)}$ is the response from run i, which has treatment r applied. This is just estimation of a Box-Cox transformation in a one-way analysis of variance model.

We then estimate V and K by ML conditional on $\hat{\lambda}$.

We call this the anova method.

The real beauty of the anova method

Since the first stage of the estimation is just transformation in linear models, it extends trivially to:

- more complex nonlinear models, such as that for two binding sites;
- models with other treatment factors in addition to substrate concentration;
- block designs, row-column designs and any orthogonal block structure;
- split-plot and other multi-stratum structures.

Blocking factor effects can be considered as fixed or random.

Mixed Treatment Models

Reminder: checking for drug-drug interactions, we check for enzyme activity of human liver microsome samples.

Donors vary and we can consider those used as a sample from the general population. We also have covariate information on them.

Model: $Y_{ij}^{(\lambda)} = \left(\frac{V_i x_{ij}}{K+x_{ij}}\right)^{(\lambda)} + \epsilon_{ij}$, where $\ln V_i \sim N(\mu_v, \sigma_v^2)$,
$\mu_v = \beta_0 + \beta_1 z_{1i} + \cdots + \beta_6 z_{6i}$.

The anova method works again, although the second stage fitting involves a nonlinear mixed model.

Design optimality criterion

The design problem is to choose HLM and substrate concentration for each run.

Initially look for locally optimal designs, which are optimal at point prior estimates of the parameters.

To match the proposed analysis, we suggest a compound optimality criterion

$$-\log V_a(\hat{\lambda}) + \log \left| \mathbf{M}(\hat{\beta}, \hat{K}, \hat{\sigma}^2_V | \lambda = \lambda_0) \right|,$$

where V_a denotes the variance under the one-way anova model, $\mathbf{M}$ is the information matrix for the nonlinear model given λ and λ_0 is the prior estimate of λ.

Design search

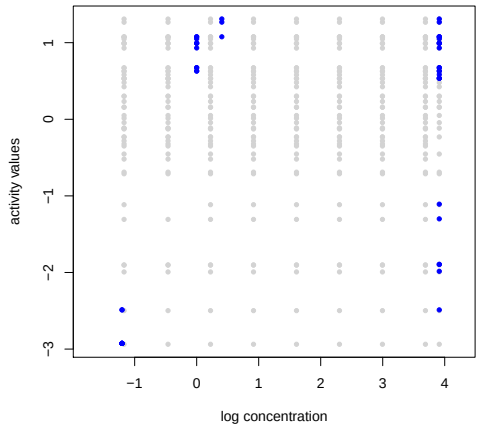
For large N, it is natural to find continuous optimal designs.

This fails, since $\mathbf{M}$ is not proportional to N.

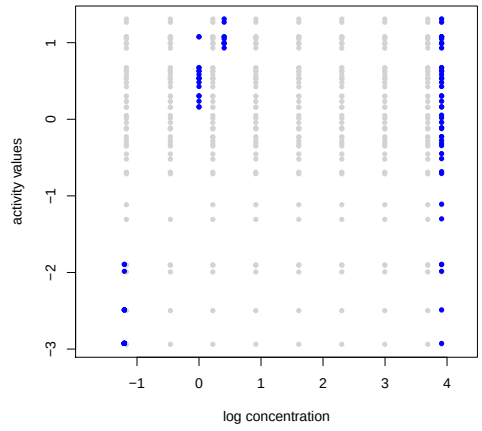
Instead, for specific N, use a modified Fedorov algorithm to search for optimal designs, i.e. start with a set of candidate support points, choose a random design and then exchange points to improve the design until convergence.

This imposes a major computational burden, e.g. the $N = 846$ of the commonly used design is out of reach.

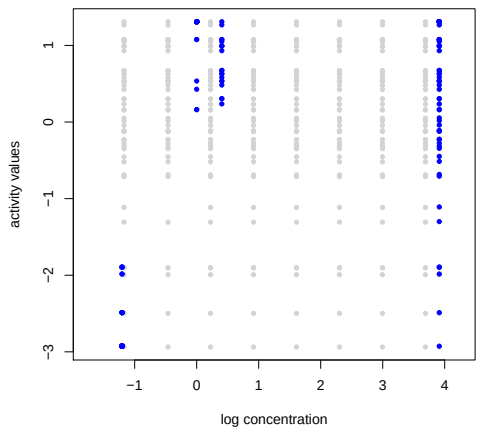
Some optimal design support points



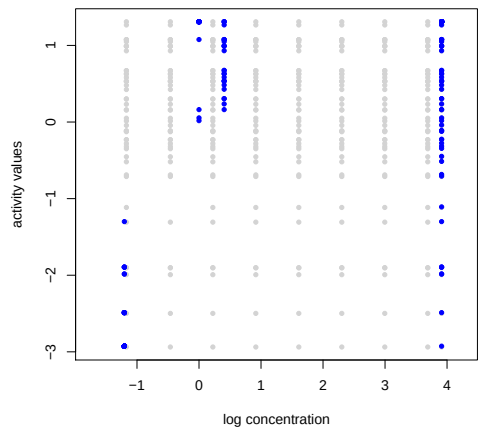
(a) $N = 50$



(b) $N = 100$

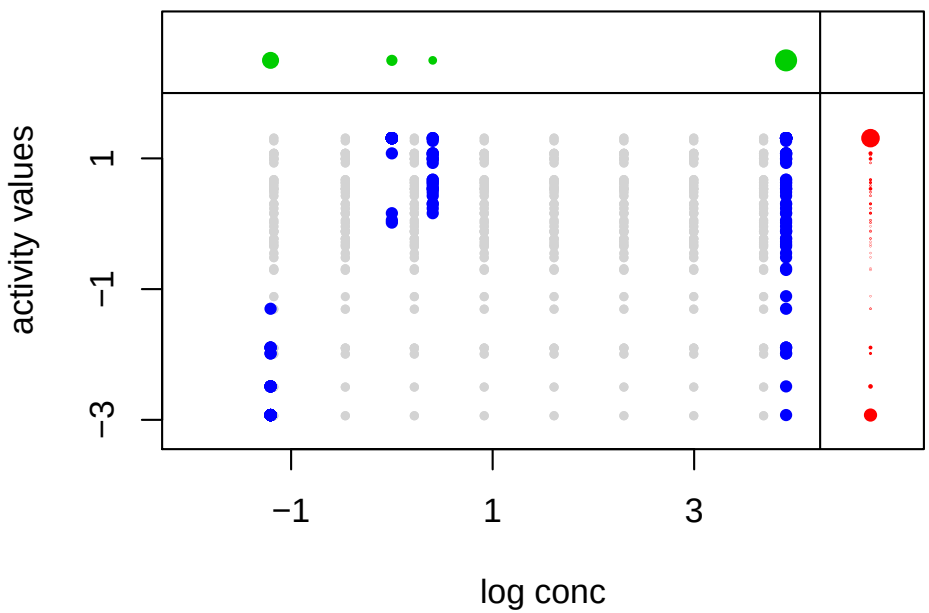


(c) $N = 150$



(d) $N = 200$

Optimal design for $N = 200$ with marginal replication

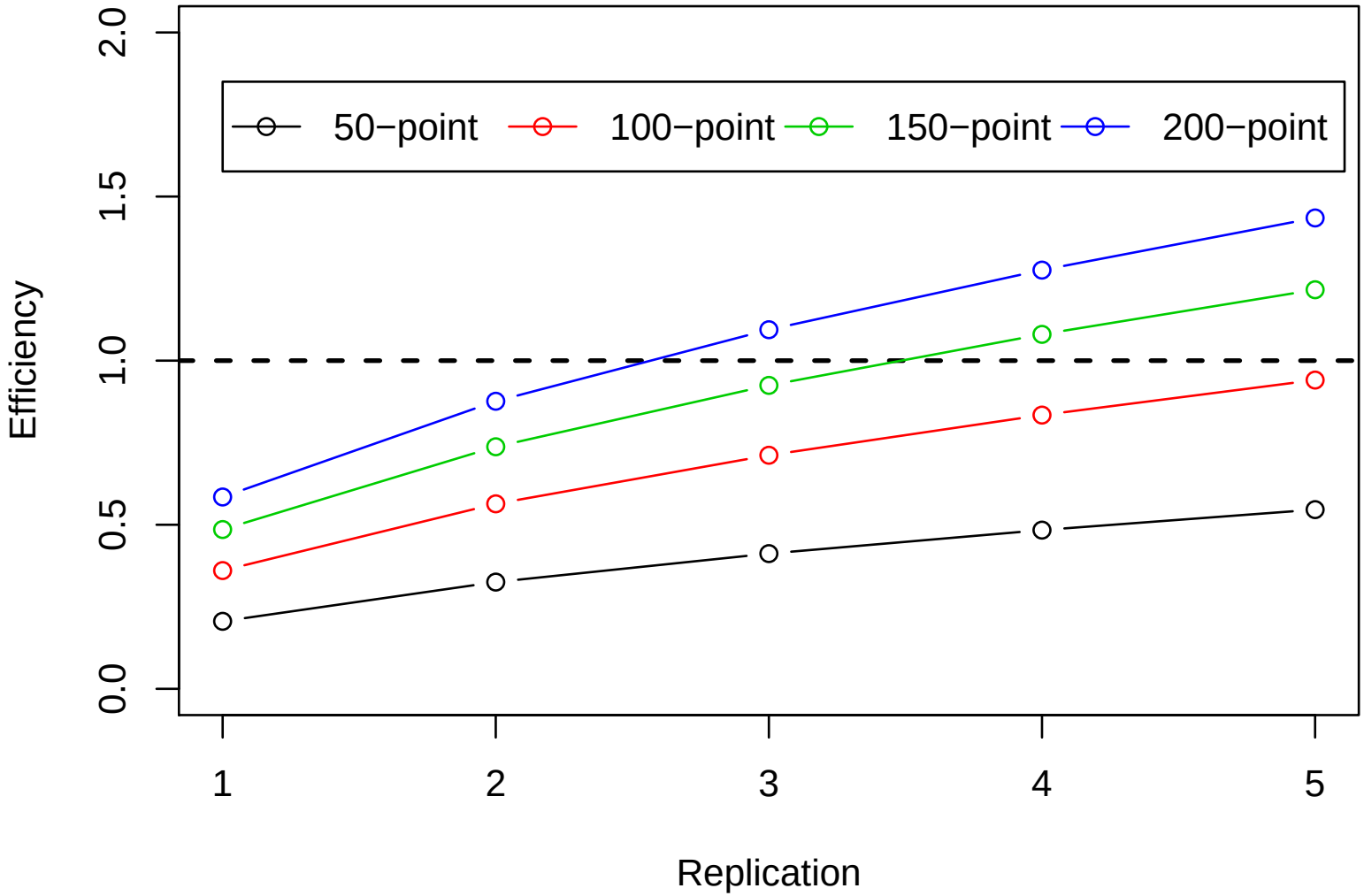


Comparison with a rich design

We considered an 846-point rich design consisting of two replicates of each point shown in grey in the plots.

The relative efficiencies of our optimal designs, and replicates of them, to the rich design are compared.

Relative efficiencies with respect to rich design



Conclusions

- The anova method is widely applicable, easy to compute and has good properties.
- Its use is limited to replicated data and therefore to designed experiments.
- It also focuses the data analyst's attention on the design structure.
- We can find designs which are better than standard designs, but are limited by computing time.
- Research on design is continuing, e.g. pseudo-Bayesian designs.
- Computation is the limiting factor in choosing big designs.