

Adaptive optimal design for the concentration tiers in time-kill curve experiments

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Background and Objectives

Time-kill curves (TKC) experiments are performed to elucidate the pharmacodynamics of antibiotics. In order to quantify the antibacterial effect, TKCs are analysed by PK/PD modelling. Precise estimation of PD parameters is crucial, e.g. for reliable simulations. Conventionally, drug concentrations to be evaluated in TKC studies are usually based on multiples of the minimal inhibitory concentration (MIC) in two-fold increments.

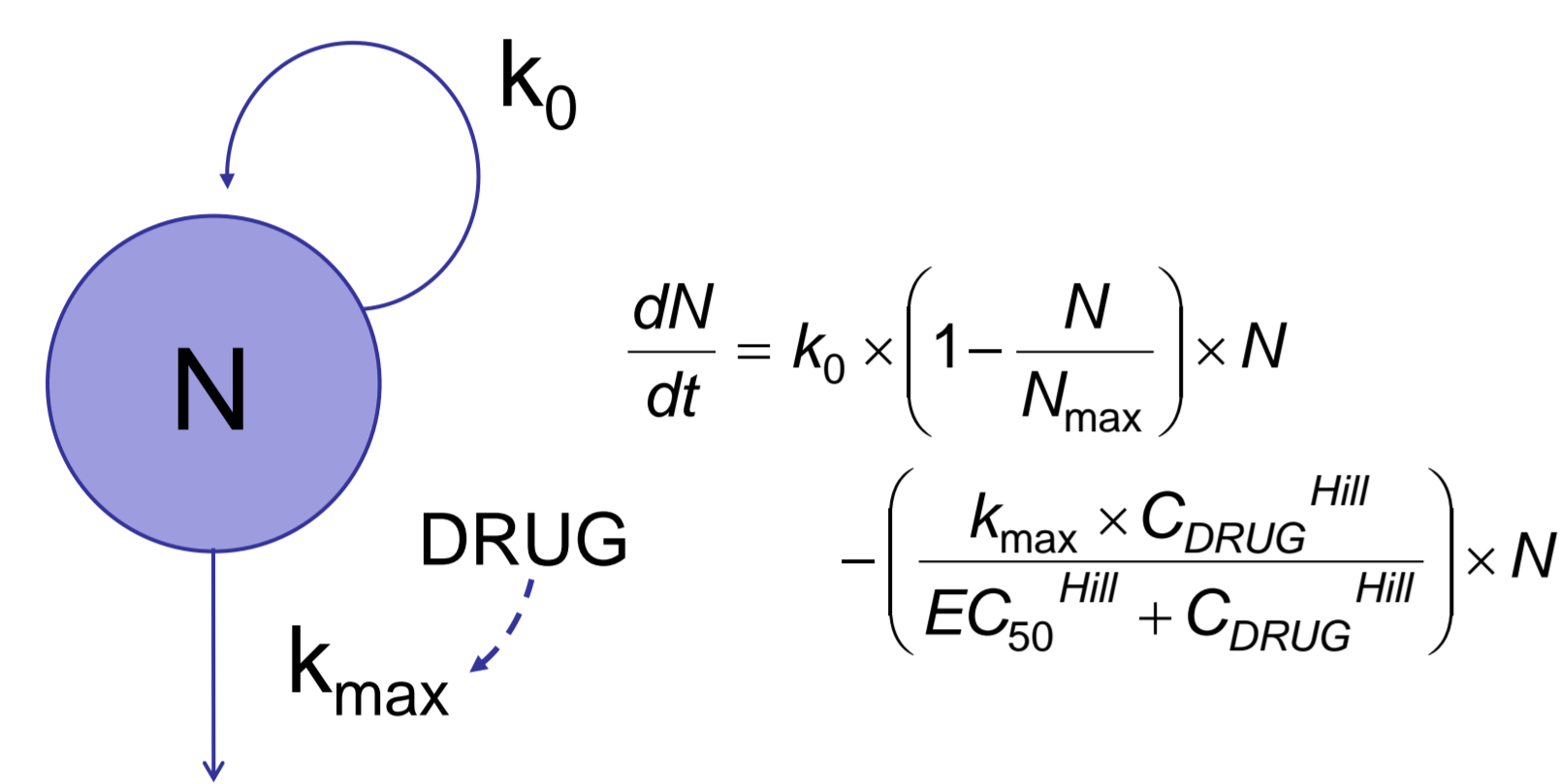
The objective of the present study was to assess and compare the performance of

- such a conventional design for the concentration tiers (CD)
 - a design with D-optimal concentration tiers (OD)
 - an adaptive D-optimal design approach (aOD)
- by a simulation-based study using a common TKC model [1].

Methods

Model and design

Modelling and simulations were performed in 'R' [2]. 500 parameter sets of the TKC model for the change of the bacterial concentration N over time (Fig. 1) were randomly sampled from a uniform distribution (Tab. 1).



Tab. 1: Parameter distribution.

Description	Parameter range
Inoculum size [log ₁₀ CFU/mL]	$4 \leq N_{t=0} \leq 6$
Replication rate [h ⁻¹]	$1.3 \leq k_0 \leq 1.8$
Maximum growth [log ₁₀ CFU/mL]	$8 \leq N_{max} \leq 10$
Maximum kill rate [h ⁻¹]	$1.8 \leq k_{max} \leq 2.1$
Conc. stimulating half-maximum kill rate [mg/L]	$0.5 \leq EC_{50} \leq 2$
Hill factor [-]	i) $1 \leq Hill \leq 4$ ii) $4 < Hill \leq 10$

Fig. 1: PK/PD model [1] for TKC modelling and simulation.

A 'virtual MIC' (minimal inhibitory concentration that kept concentration of bacteria below 10⁷ CFU/mL at 24 h) was determined with the sampled parameter set to build up the designs for the drug concentrations:

- **CD**: 9 antibiotic concentration tiers of 0-16x MIC (n = 90 data points).
- **OD**: 0x and 16x MIC was fixed whilst the other 7 concentration tiers were D-optimal (n = 90 data points).
- **aOD**:
 - *First stage*: a *reduced CD* (0, 0.5, 1, 2 and 16x MIC) was used for simulation of TKCs and parameters were estimated.
 - *Second stage*: Based upon the estimates of the *reduced CD*, two D-optimal concentration tiers were added to the reduced CD to obtain the aOD (n = 70 data points after second stage).

Sampling time points for colony count were set to 0, 2, 4, 8 and 24 h in all designs.

Computation of optimal designs

Optimal designs for the concentration tiers were determined by minimising the inverse of the determinant of the Fisher information matrix (FIM) given the different designs with the Nelder-Mead algorithm ('R', stats, 3.0.2): $OBJ = |\det(FIM)|^{-1}$

Design evaluation

Based on 500 simulations for each design assuming an additive error model (n=2 replicates per concentration tier, $\sigma = 0.3 \log \text{CFU/mL}$), distributions of

- **relative bias** (RB; 2.5th-97.5th percentile) for **accuracy** and
 - **relative standard error** (RSE; 95th percentile) for **precision**
- of the re-estimated versus the underlying (true) parameters were used to compare the performance of the designs.

Results

- **All designs** allowed for **accurate** (RB $\pm 20\%$) and **precise** (RSE $\leq 20\%$) estimation of the **bacteria-related parameters** of the TKC model (Fig. 2 and 3, $N_{t=0}$, k_0 and N_{max}).

- For **flat concentration-effect relationships** (Hill factor: $1 \leq Hill \leq 4$), the **drug-related parameters** were **accurately** and **precisely** estimated for **both ODs and CD** with all RBs between -13.8% and +18.3% and RSE $\leq 12.3\%$ (Fig. 2, k_{max} , EC_{50} and Hill).

- For **steep concentration-effect relationships** ($4 < Hill \leq 10$), the **ODs were superior** to the **CD** with respect to **drug-related parameters** of the TKC model (e.g. for Hill: **CD**: RE [-35.1%; 74.3%], RSE $\leq 133\%$ vs. **ODs**: RE [-12.9%; 16.7%], RSE $\leq 10.8\%$) (Fig. 3, k_{max} , EC_{50} and Hill).

- Similar to ODs, **aODs** performed comparably to the CD for $1 \leq Hill \leq 4$ (Hill: aODs: RB between -14.2% and +16.4% with RSE $\leq 12.7\%$) and were also **superior for $4 < Hill \leq 10$** (Hill: aODs: RB between -22.0% and 31.2% with RSE $\leq 17.8\%$) with respect to **drug-related parameters** by requiring only 7 instead of 9 concentration tiers (Fig. 3, k_{max} , EC_{50} and Hill).

- An example for a TKC comparing CD with aOD is given in Fig. 4 illustrating the additional concentration tiers in the aOD and their impact on RSEs (Tab. 2).

Results (cont.)

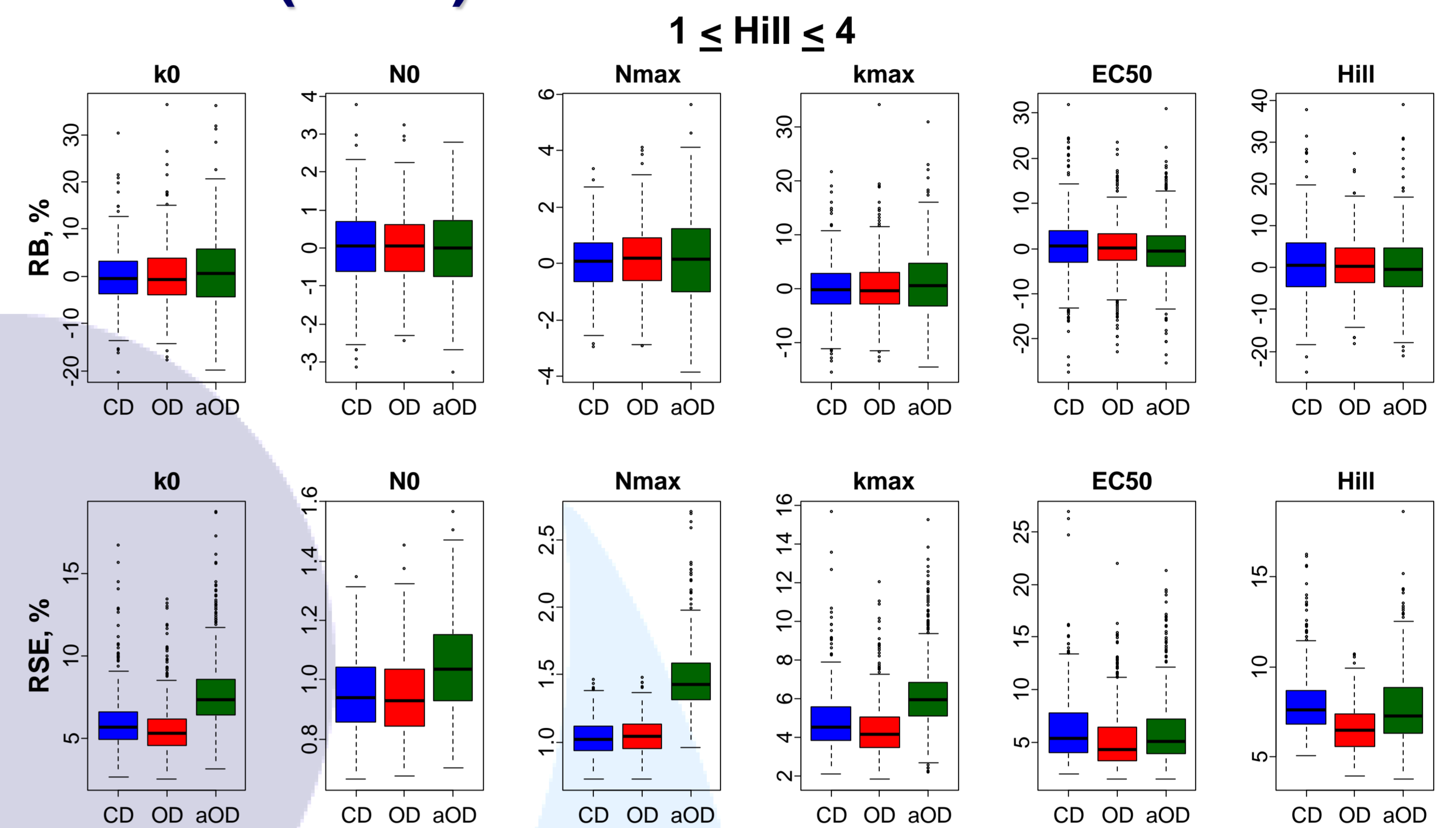


Fig. 2: Impact of the design of concentration tiers (CD, OD, aOD) on accuracy expressed as RB and precision expressed as RSE for 500 parameter sets with $1 \leq Hill \leq 4$ in a simulation-based analysis; whiskers represent 1.5x inter-quartile range; note different y-scales.

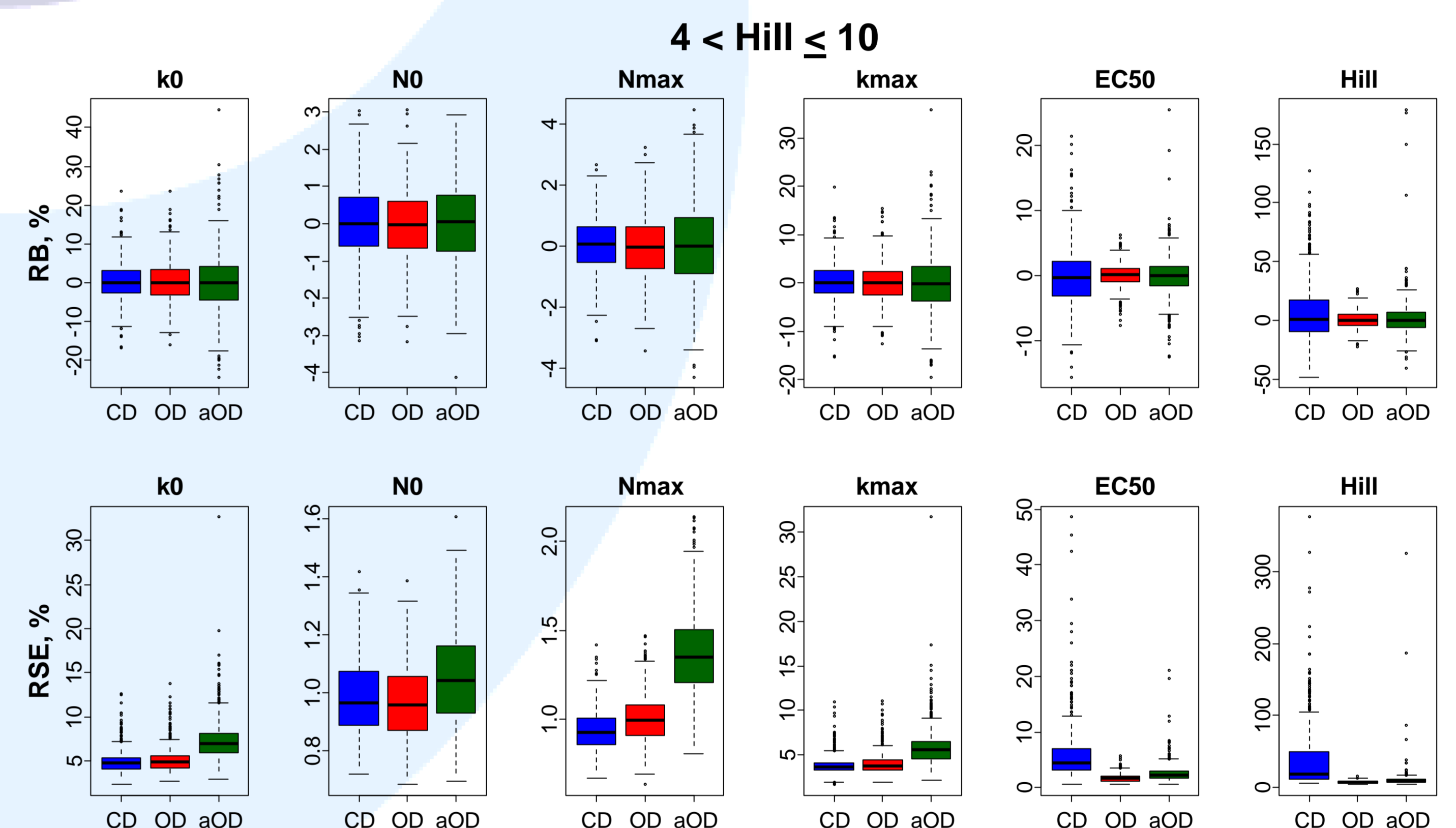
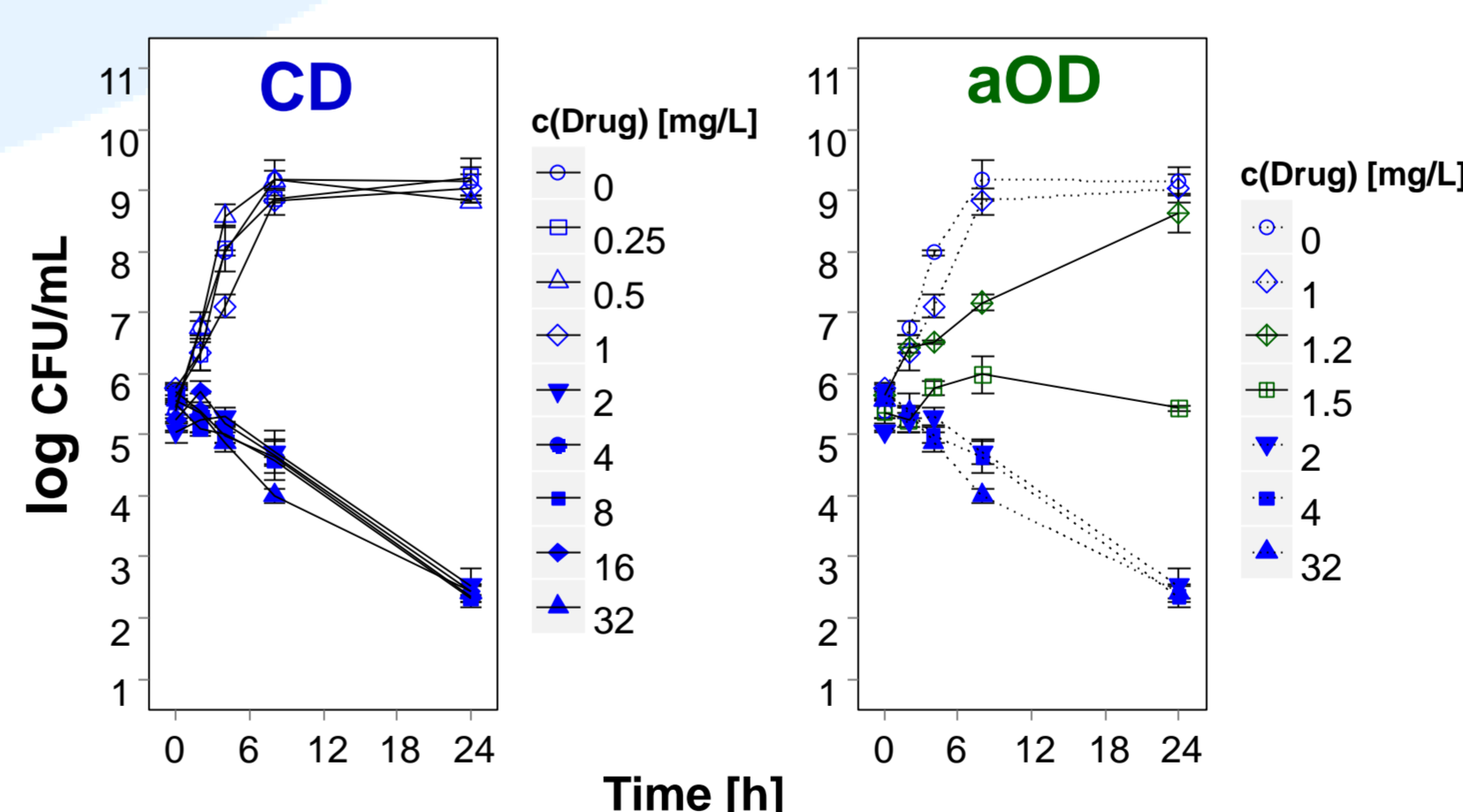


Fig. 3: See Fig. 2, but $4 < Hill \leq 10$.



Tab. 2: Parameter set for example in Fig. 4 with RSEs of the different designs

Parameter	Value	RSE CD	RSE aOD
$N_{t=0}$ [log ₁₀ CFU/mL]	5.5	0.9 %	1.0 %
k_0 [h ⁻¹]	1.5	5.2 %	8.0 %
N_{max} [log ₁₀ CFU/mL]	9	0.9 %	1.2 %
k_{max} [h ⁻¹]	1.8	4.2 %	6.5 %
EC_{50} [mg/L]	1.2	3.7 %	2.5 %
Hill [-]	7	19.0 %	8.2 %

Fig. 4: Example of simulated TKCs with parameter set from Tab. 2 visualizing **CD** and **aOD**; for aOD, reduced CD (*first stage*) is indicated by dotted lines, concentration tiers of subsequent OD (*second stage*) by solid green lines; error bars: range.

Discussion and Conclusions

For **antibiotics** with a considerably **steep concentration-effect relationship**, individual **adaption of the concentration tiers** in TKC studies by **optimal design techniques** might be **beneficial** for accuracy and precision of PD parameter estimates. Further research is necessary to confirm this *in silico* approach in experimental settings.

References

- [1] W. Treyaprasert, S. Schmidt, K.H. Rand, et al., *Int. J. Antimicrob. Agents*, 29: 263–70 (2007).
- [2] R Core Team. R: A language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.



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