Parameter Estimation in Enzyme-Kinetics with Consideration of Heteroscedasticity and Low Dose Data

Marcus Brunnert
Department of Statistics
University of Dortmund
D-44221 Dortmund
Germany

Frank Gilberg
Biometrics Department TD-BS1
Roche Diagnostics GmbH
Pharma Development Mannheim
D-68305 Mannheim
Germany

Abstract

In this paper we propose a simulation study in order to discuss four statistical models dealing with the problem of parameter estimation in enzyme-kinetics. The pseudo-maximum-likelihood estimators for the transform-both-sides-model and the weighted TBS-model are compared with least-square-estimators of the classical nonlinear regression model and the linearized Eadie-Hofstee-plot. Due to heteroscedasticity of enzyme-kinetic data in low dose experiments the proposed estimators are investigated.

Key words: Nonlinear regression model; Pseudo-maximum-likelihood estimation; Heteroscedastic error variance; Michaelis-Menten-kinetic; Low dose data; Simulation study.

1. Introduction

The description of complex enzyme-kinetic-reactions is strongly connected with the statistical analysis of conventional Michaelis-Menten-kinetic. The Michaelis-Menten-equation that refers to the relation between the velocity v of the reaction and the concentration [S] of substrate is as follows:

$$v = \frac{v_{max}[S]}{k_m + [S]}, \qquad (1)$$

where v_{max} characterises the maximum velocity of the reaction and k_m is the half-saturation constant. This constant defines the concentration of substrate achieving $v_{max}/2$ in (1).

Selecting an appropriate statistical model in order to estimate the parameters (v_{max} and k_m) of a Michaelis-Menten-kinetic is of great importance. In Frei et al. (1999) the statistical evaluation of enzyme-kinetic experiments investigating the potent liver carcinogen NTDMA (N-nitro-dimethylamine) to rats has been described. One conclusion of Frei et al. (1999) refers to similar estimators of the TBS and the weighted TBS-model after analysing low dose data. This may cause in biased parameter estimators in this special application. In this technical report a better validation of both statistical models will be connected with an investigation of parameter estimation of the Eadie-Hofstee-plot (Eadie, 1942) and the classical (unweighted) nonlinear regression.

On the one hand a validation has to consider the specific structure of data in enzyme-kinetic experiments. Because of toxic effects, many experiments in high dose levels are not practicable as described in Frei et al. (1999). In such an experiment observations of low dose substrate concentrations are only available. On the other hand the statistical analysis of former experiments indicate nonconstant error variances (heteroscedasticity). In these cases an important assumption of nonlinear statistical models referring to constant error variances is not fulfilled.

Hence simulations studies are an important adviser for future selection of a statistical model in a real data situation and can validate the selection of statistical models of former evaluations. Simulation studies are described in Currie (1982) or in Dowd and Riggs (1965). The aim of Currie (1982) was to find an appropriate experimental design and Dowd and Riggs (1964) compared linearization methods referring to statistical analysis of Michaelis-

Menten-kinetic. Zivin and Waud (1982) described the advantages of the Eadie-Hofstee-plot in comparison to other linearization methods.

In this technical report a new simulation study is described in order to discuss four statistical models. The four statistical models dealing with parameter estimation are explained in the following paragraph. In paragraph three the simulation study is described. Especially the specific simulated data structure is mentioned. All results of the study are summarized in paragraph four. In sequence of three assumed error variance structures the presented figures enable a comparison of the statistical models. Finally a conclusion of the whole simulation study will be drawn.

2. Statistical Models

Various methods were discussed in the literature (Ruppert et al., 1989; Zivin and Waud, 1982) in order to estimate the parameters v_{max} and k_m referring to the Michaelis-Menten-kinetic. In biology, chemistry or medicine the nonlinear function of the velocity of the enzyme-catalyzed reaction is often handled by standard methods of linearization. For example, the Eadie-Hofstee-plot (Eadie, 1942) belongs to these methods. Alternatively, the statistical models of nonlinear regression consider nonlinearity in a direct way. Moreover, the transformation and/or weighting of these nonlinear models yields a wider class of statistical models dealing with the estimation of v_{max} and k_m

2.1 The Eadie-Hofstee-plot

The reciprocal of both sides of equation (1) and the multiplication with v_{max} and k_m results in a linearization of the Michaelis-Menten-function. By rearranging this new equation we yield:

$$v = v_{max} - k_m \frac{v}{s}. \qquad (2)$$

Plotting v versus $\frac{v}{s}$ from (2) results in a line with intercept v_{max} and slope $-k_m$. Therefore the linear regression model is appropriate. It is defined as:

$$v_i = v_{max} - k_m \frac{v_i}{s_i} + e_i \text{, where} \qquad (3)$$

e_i, i=1, ... , n, are assumed to be identical and independent distributed (i.i.d.) with a constant variance.

The least-square-method leads to estimators of the intercept v_{max} and the slope $-k_m$ by solving the minimization problem

$$\min_{v_{max},\, k_m} \sum_{i=1}^{n} \left[v_i - (v_{max} - k_m \frac{v_i}{s_i}) \right]^2 \qquad (4)$$

(for details see for example Zivin and Waud, 1982).

A problem of this method concerns the quotient of dependent variable y_i and independent variable x_i. Biased estimators can be the result of this problem.

In this simulation study the *SAS* procedure *Proc Reg* has been used for computing the estimators.

2.2 The classical nonlinear regression model

The application of classical (unweighted) nonlinear regression model to Michaelis-Menten-kinetic results in the model

$$v_i = \frac{v_{max} s_i}{k_m + s_i} + e_i \quad , \qquad (5)$$

where the errors e_i, i= 1, ... , n, are assumed to be independent and identical distributed with constant variances.

From the model (5) we yield estimators for v_{max} and k_m with ordinary least-square-method. The minimization of

$$RQS = \sum_{i=1}^{n} \left[v_i - \left(\frac{v_{max} s_i}{k_m + s_i} \right) \right]^2 \qquad (6)$$

requires an application of a nonlinear optimization algorithms. In the presented simulation study the DuD-algorithm (<u>d</u>oesn't <u>u</u>se <u>d</u>erivatives-algorithm) has been applied. This method approximates the nonlinear function at each iteration step by an affine function that agrees with the approximated functions of previous iteration steps. Thereby a linear least-square-problem leads to estimators of the parameters (Ralston and Jennrich, 1978).

The estimation of parameters by the least-squares-method is optimal and yields ML-estimators if additionally the errors are normally distributed.

Violations of the assumptions especially heteroscedasticity may lead to biased estimators. Transformation and/or weighting of the classical nonlinear regression model are well known methods to handle this problem.

The *SAS* procedure *Proc Nlin* has been used for computing the estimators.

2.3 The TBS-model

The TBS-model (transform-both-sides-model) is a modified nonlinear regression model which deal with the problem of heteroscedasticity by transforming data. Here the transformation rule proposed by Box and Cox (1964) is used. This transformation is defined as follows:

$$h(z,\lambda) = z^{(\lambda)} = \begin{cases} (z^{\lambda} - 1)/\lambda & \text{if } \lambda \neq 0 \\ \log z & \text{if } \lambda = 0. \end{cases} \qquad (7)$$

The application of the Box-Cox-transformation to both sides of equation (5) is expressed by the following TBS-model:

$$v_i^{(\lambda)} = [\frac{v_{max} s_i}{k_m + s_i}]^{(\lambda)} + e_i, i = 1, \ldots, n. \qquad (8)$$

The Box-Cox-transformation of v_i causes a symmetric distribution of errors and a constant error variance. Besides this the transformation of the regression function preserves the relation between v_i and s_i in absence of the stochastic error term. The functional relationship holds in case of a deterministic model.

2.4 The weighted TBS-model

An alternative approach to handle heteroscedasticity is the definition of a flexible weighting function. By applying the weighted nonlinear regression model to the Michaelis-Menten-kinetic we yield:

$$v_i = \frac{v_{max} s_i}{k_m + s_i} + g_i(\theta, s_i) e_i, \qquad (9)$$

where g_i, i=1, ... , n, are functions depending on an additional parameter θ and the concentration of substrate s_i.

The weighting of the errors with consideration of the concentration of substrate counteracts heteroscedasticity. Former analyses of experiments of enzyme-kinetics indicate that especially the power function $g_i(\theta) = s_i^{\theta}$, i=1, ..., n, is appropriate for weighting the errors in order to consider the influence of the concentration of substrate on the variability of the observed velocities (Gilberg, 1996; Ruppert et al., 1989).
Combining approach (9) and transformation (7) we yield the weighted TBS-model:

$$v_i^{(\lambda)} = [\frac{v_{max} s_i}{k_m + s_i}]^{(\lambda)} + s_i^{\theta} e_i . \qquad (10)$$

Consequently, we have to estimate two additional parameters λ and θ which give an insight into the underlying error structure. In addition, if $\theta = 0$ the TBS-model is a special case of the weighted TBS-model and if $\theta = 0$ and $\lambda = 1$ the classical nonlinear model is a special case of the weighted TBS-model.

For simultaneously estimation of the parameters v_{max}, k_m, λ and θ using the weighted TBS-model (10) a pseudo-maximum-likelihood-method by Giltinan and Ruppert (1989) has been applied. The resulting pseudo model can be implemented in *SAS* using the procedure *Proc Nlin*. For details of implementation see Giltinan and Ruppert (1989) and Gilberg (1996).

3. Description of the simulation study

Simulation of data and computation of estimators has been performed using *SAS*, version 6.12 TS level 045 on an Intel 200 MHz workstation under *Windows NT*, version 4.0.

3.1 Error structures

An aim of this simulation study is to consider different error structures in order to compare the four statistical models. Hence we suppose the following error structures, i=1, ... , n:

- *Constant error variance*: $Var(e_i)=0.002$.

  Together with normally distributed errors this error variance is an important assumption of nonlinear regression. Therefore this error structure has the function of control if we compare different error structures.

- *Relative constant error variance*: $Var(e_i)=[0,2\ E(v_i)]^2$.

  An error variance that is connected with the expected velocities $E(v_i) = \frac{v_{max} s_i}{k_m + s_i}$.

  Former statistical analysis of enzyme kinetics indicates that the quantity of variability may depend on the substrate doses (Edler et al., 1997). This defined structure of variance corresponds to this experience.

- *Heteroscedastic error variance*: $Var(e_i)=[(0,05+0,1s_i)/(k_m+s_i)]^2$.

  An error variance that varies and has no relation to the expected velocities $E(v_i)$. In Currie (1982) this heteroscedastic condition has been used in a simulation study comparing different statistical models to the proposed.

3.2 Simulation of the data

An example of substrate doses that can be analysed is:

0.008, 0.016, 0.024, 0.048, 0.096, 0.192, 0.384, 0.768, 1.536.

These values indicate a normal dose level, if $v_{\max} = 1$ and $k_m = 0.1$ as defined in this example. A data set of a low or high dose level can be generated by multiplication of these normal values with a constant factor k. In this simulation study $k \in \{0.1,\ 0.2,\ 0.3,\ ...\ ,0.9\}$ are factors for generating low dose level data sets and $k \in \{2,\ 3,\ ...\ ,\ 10\}$ are factors for generating high dose level data sets.

For each data set the velocities v_i are calculated by:

$$v_j = \frac{v_{max} s_j}{k_m + s_j} + Var(e_j)\ \text{pseudo},\ j=1,..,9,$$

where $Var(e_j)$ depends on the assumed error structures (3.1) and pseudo is a standard normal distributed random number.

All together on each data set 500 Monte-Carlo simulations have been performed. With consideration of each error structure we yield 1500 simulated data sets consisting of 9 substrates and 9 velocities for k=0.1, 0.2, ... , 0.9, 1, 2, ... , 10.

The evaluation of all simulated data in low/high dose levels for each error structure results in 13.500 estimations. An additional evaluation of simulated data in normal dose levels (k=1) results in 1500 estimations. For each error structure and low or high dose levels the computations result in 4.500 estimations and for normal dose level data sets in 1.500 estimations.

The evaluation of all four discussed statistical models and error structures results in 114.000 estimations.

4. Results

The following statistics are considered in order to describe the results of the simulation study:

- *Arithmetic mean* of all simulated estimators for each error structure and statistical model.
- *Simulation error* $\sigma = \frac{s}{\sqrt{N}}$, where s is standard deviation of N simulated estimators.
- *Coefficient of variation* CV, calculated as quotient of standard deviation and arithmetic mean.

In this simulation study the simulation error of estimated k_m varies from 0.0001 to 0.0027 and the simulation error of estimated v_{max} varies from 0.0006 to 0.0154. Therefore the variation of mean estimators is small and the bias of estimators can be quantified by the examination of the arithmetic mean of simulated estimators and the coefficient of variation.

4.1 Figures

First we compare all estimators of the simulated data with constant error structure. The arithmetic mean of the estimators v_{max} and k_m are plotted in figure 1 and 2 and the coefficients of variation (CV) referring to the estimators of v_{max} are plotted in figure 3. The coefficients of variation of the estimators of k_m are not presented because they are similar to those of v_{max}.

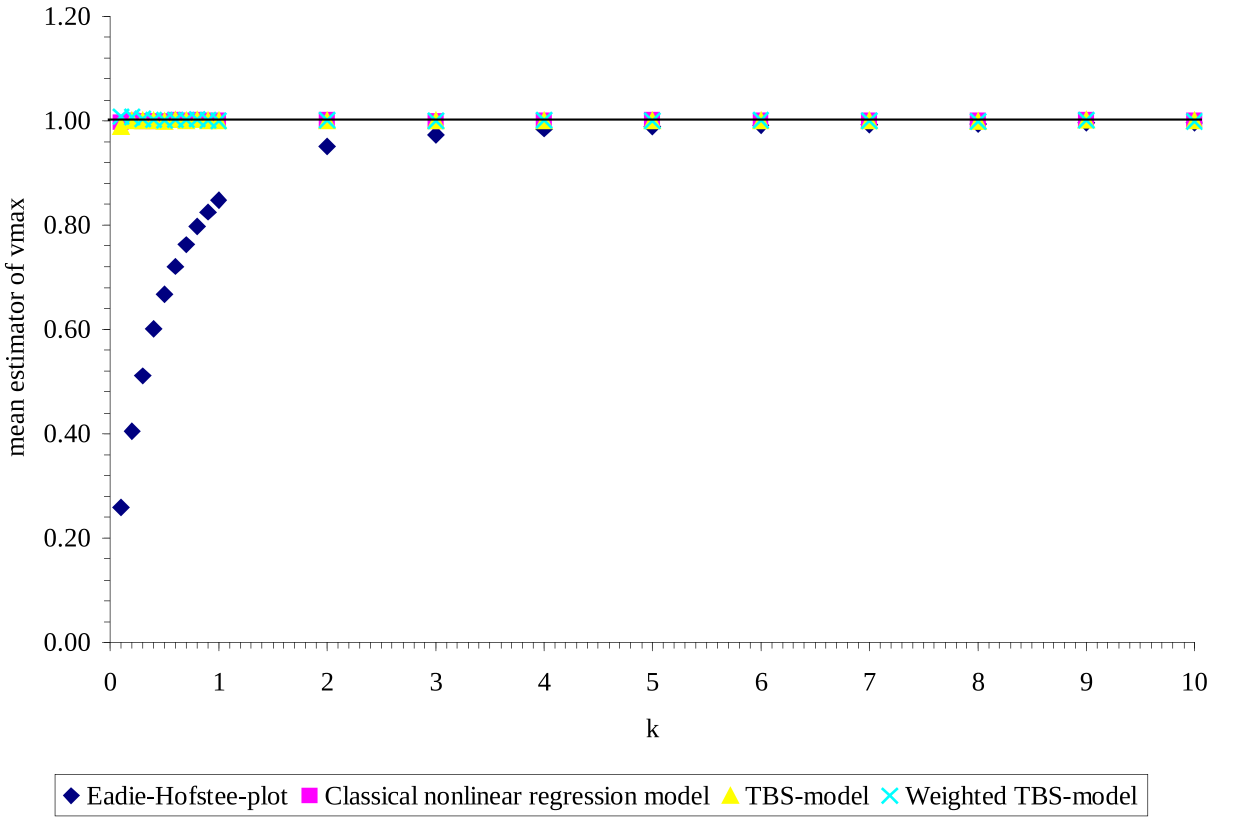

Figure 1: Mean estimator of $v_{max,}$ constant error structure.

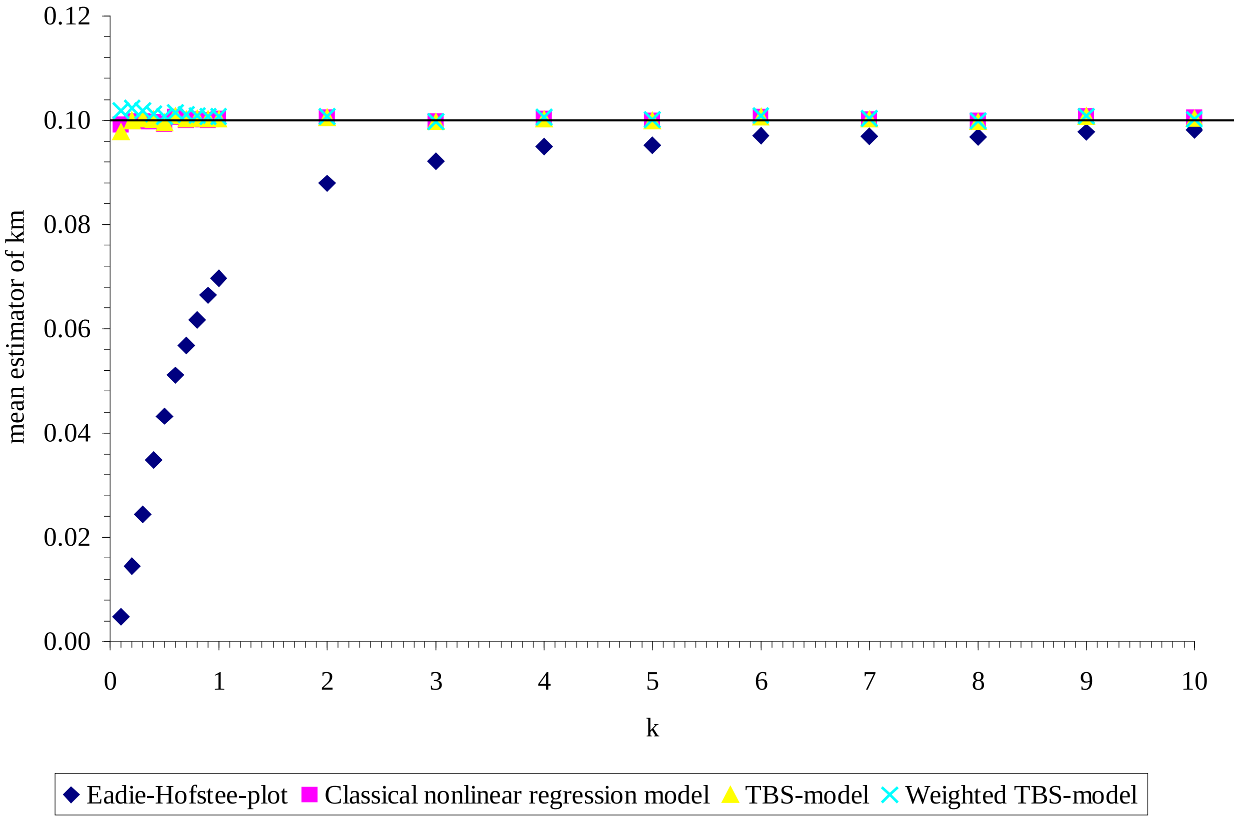

Figure 2: Mean estimator of k_m, constant error structure.

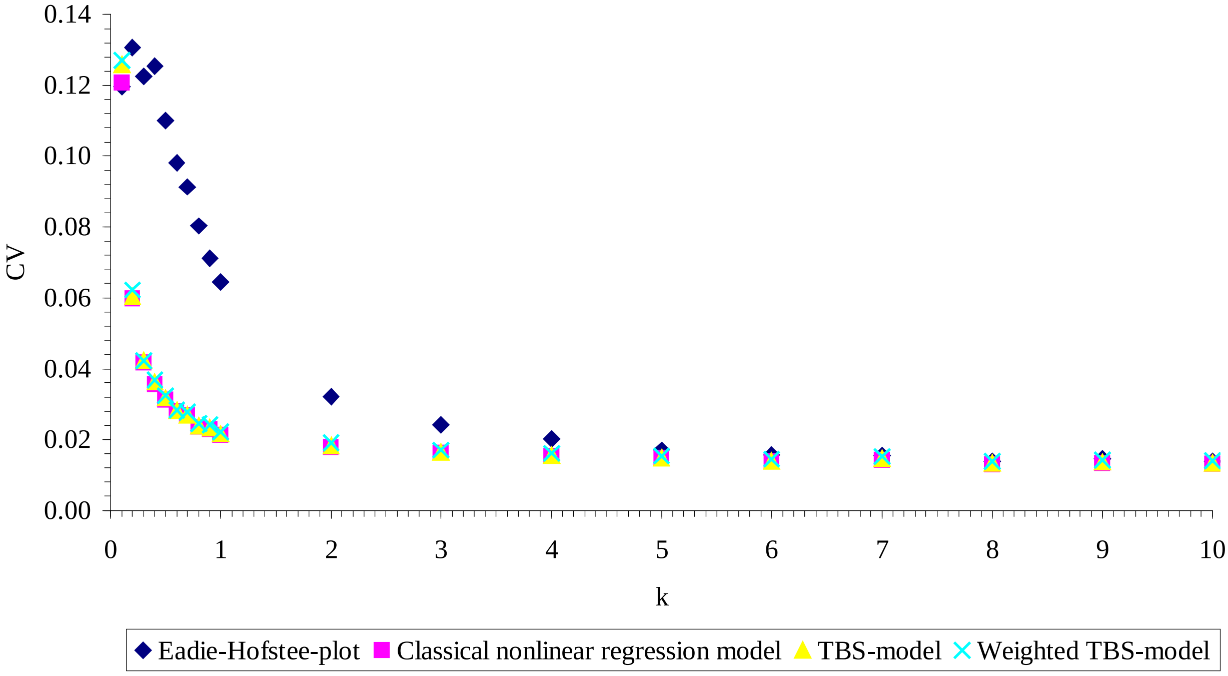

Figure 3: Coefficient of variation referring to estimators of v_{max}, constant error structure.

If we consider constant error structure figures 1 and 2 indicate similar mean estimators referring to the classical nonlinear regression, the TBS-model and the weighted TBS-model. Little overestimated k_m by the weighted TBS-model and little underestimated k_m by the TBS-model and nonlinear regression can be pointed out in figure 2. Taking into account the quantity of this overestimated/underestimated parameters these results can be neglected. It is remarkable that the mean estimators of the Eadie-Hoftsee-plot strongly differ from true parameters (horizontal reference lines). Therefore these estimators are comparatively useless. By increasing k the mean estimators of the Eadie-Hoftstee-plot are less biased. But they are comparatively more biased if we compare them to the other estimators. In the important low dose level ($0.1<k<1$) figure 1 and 2 show that Eadie-Hoftsee-plot should not be used in evaluations.

Two results can be stated by figure 3. First in low dose level (excepted k=0.1) the coefficient of variation referring to the Eadie-Hofstee-plot ranges from 0.06 to 0.14 and coefficient of variation referring to the other models ranges from 0.02 to 0.06. These two ranges stress that Eadie-Hofstee-plot should not be used in low dose level. Second in dose levels of $k>4$ all CV's are below 0.02. This result and the decrease of all CV's show estimators with decreasing standard deviation. In other words standard deviation amounts less than 2% of mean estimator. For this reason figure 3 confirms that the least-square-method yields optimal estimators in the case of normally distributed errors and constant error variance.

As the last result should have been expected in every simulation study the control of simulation study by constant error structure is fulfilled.

Next we compare all estimators of the simulated data with relative constant error structure. The arithmetic mean of the estimators v_{max} and k_m are plotted in figure 4 and 5 and the coefficients of variation (CV) referring to the estimators of v_{max} are plotted in figure 6. The coefficients of variation of the estimators of k_m are not presented because they are similar to those of v_{max}.

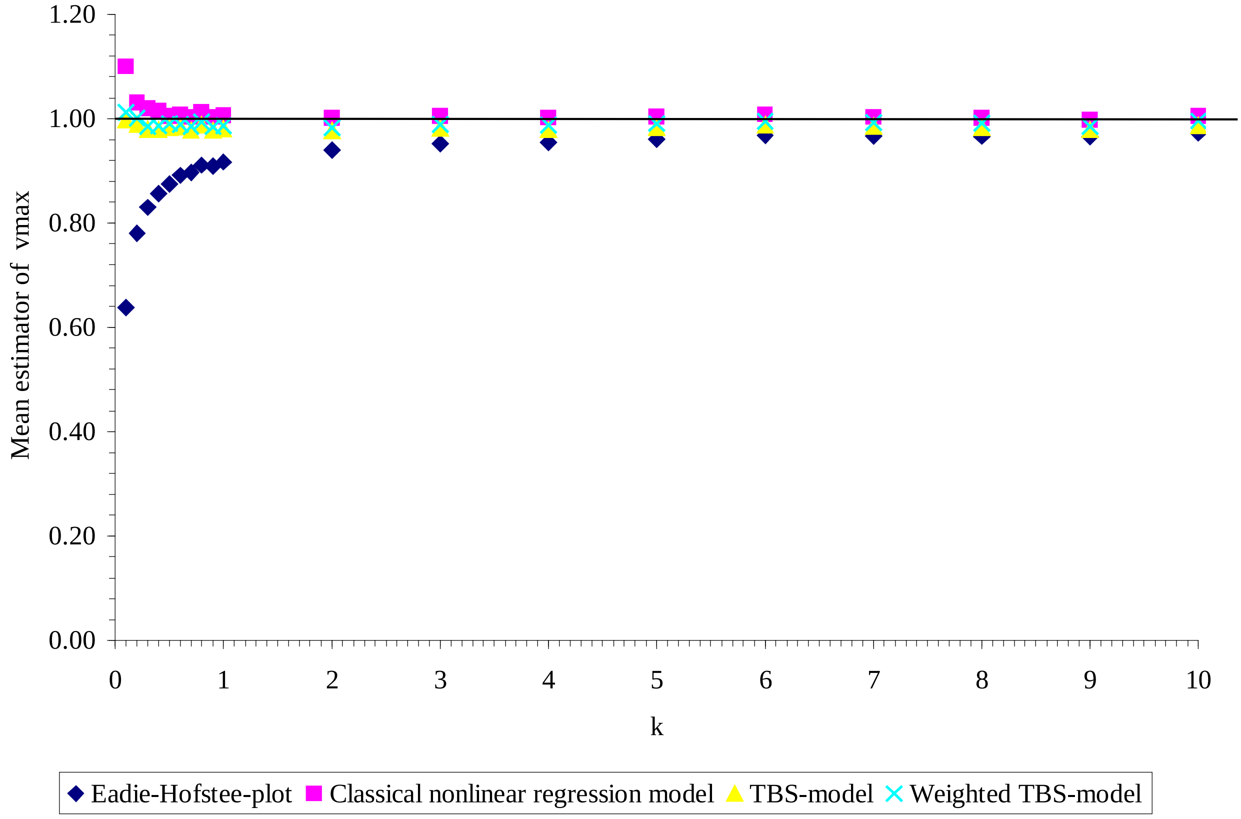

Figure 4: Mean estimator of $v_{max,}$ relative constant error structure.

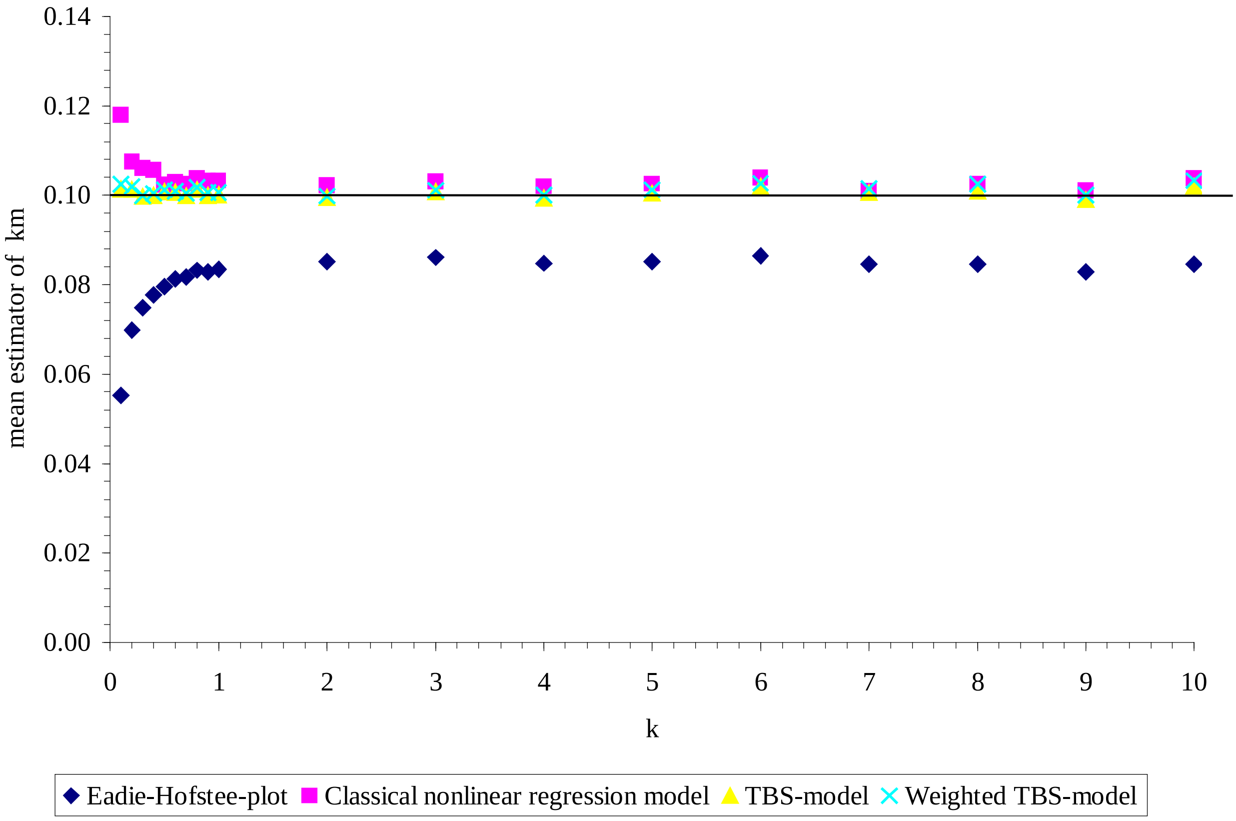

Figure 5: Mean estimator of k_m, relative constant error structure.

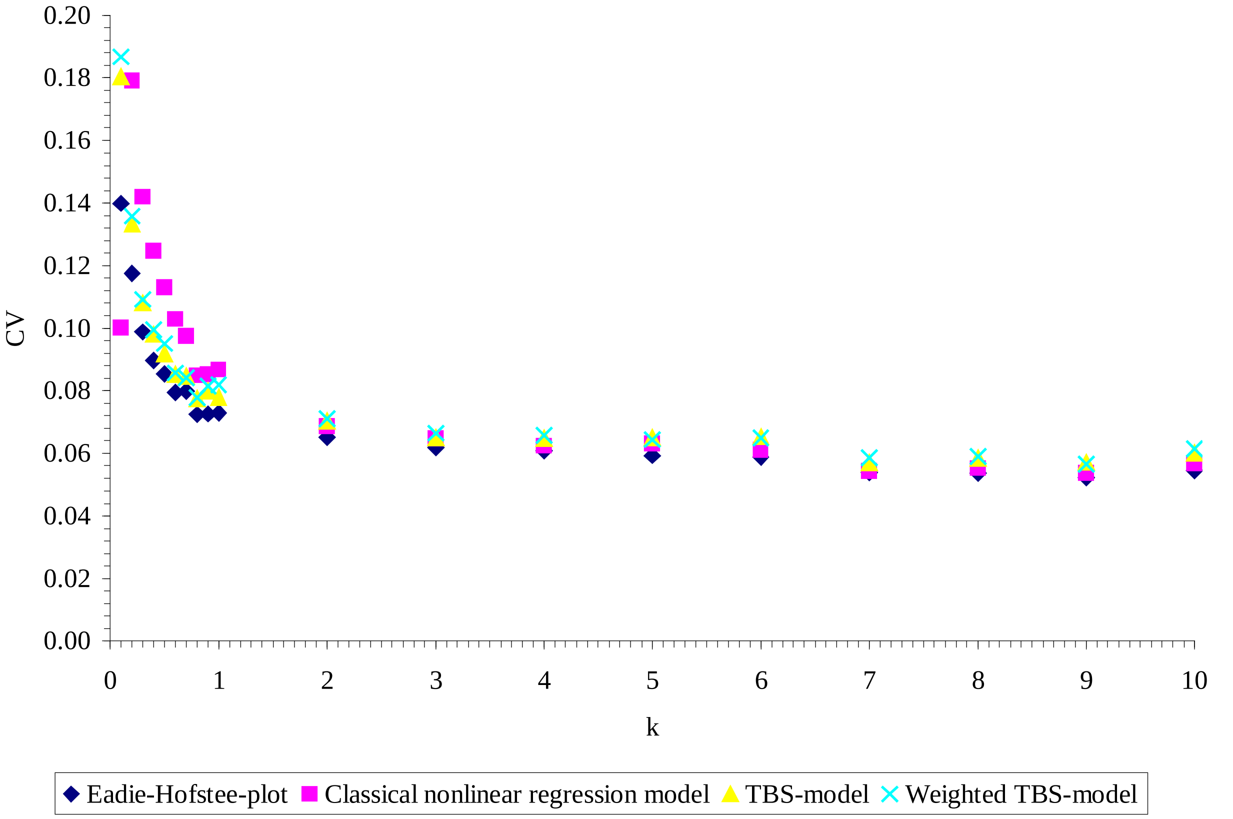

Figure 6: Coefficient of variation referring to estimators of v_{max}, relative constant error structure.

If we consider the relative constant error structure figures 4 and 5 indicate that the mean estimators of the TBS-model and the weighted TBS-model are near the true parameters. Underestimation can be pointed out by the Eadie-Hofstee-plot and overestimation can be pointed out by the classical nonlinear regression model. The mean estimators of Eadie-Hoftsee-plot and the classical nonlinear regression model show a strong increase/decrease in dose levels with k<0.4. The estimated v_{max} of the Eadie-Hofstee-plot amount about 0.09 in dose level $k \geq 0.6$. Therefore a relative constant error structure can be better aggregated by the Eadie-Hoftsee-plot in dose levels with $k \geq 0.6$ than a constant error structure.

Figure 6 stresses the last result. Because now, the coefficients of variation of Eadie-Hofstee-plot in low dose levels are lower than CV's of other statistical models (excepted k=0.1). Considering k between 0.2 and 1 the coefficients of variation can be ordered by their quantities. Thereby CV's of the Eadie-Hofstee-plot are lower than CV's of the TBS-model. Then CV's of the TBS-model are lower than CV's of the weighted TBS-model and furthermore CV's of the weighted TBS-model are lower than CV's of the classical nonlinear regression model. A ranking of statistical models referring to these coefficient of variation should be made with consideration of systematically underestimation by Eadie-Hofstee-plot and overestimation by classical nonlinear regression model and additional estimation of θ by the weighted TBS-model. An additional estimator decreases number of degrees of freedom referring to the standard deviation of the estimator and a lower number of degrees of freedom increases standard deviation. In high dose levels all CV's have a range of 0.03 which is lower than the range in low dose levels. This indicates better estimators in high dose levels.

Finally we compare all estimators of the simulated data with heteroscedastic error structure. The arithmetic mean of the estimators v_{max} and k_m are plotted in figure 7 and 8 and the coefficients of variation (CV) referring to the estimators of v_{max} are plotted in figure 9. The coefficients of variation of the estimators of k_m are not presented because they are similar to those of v_{max}.

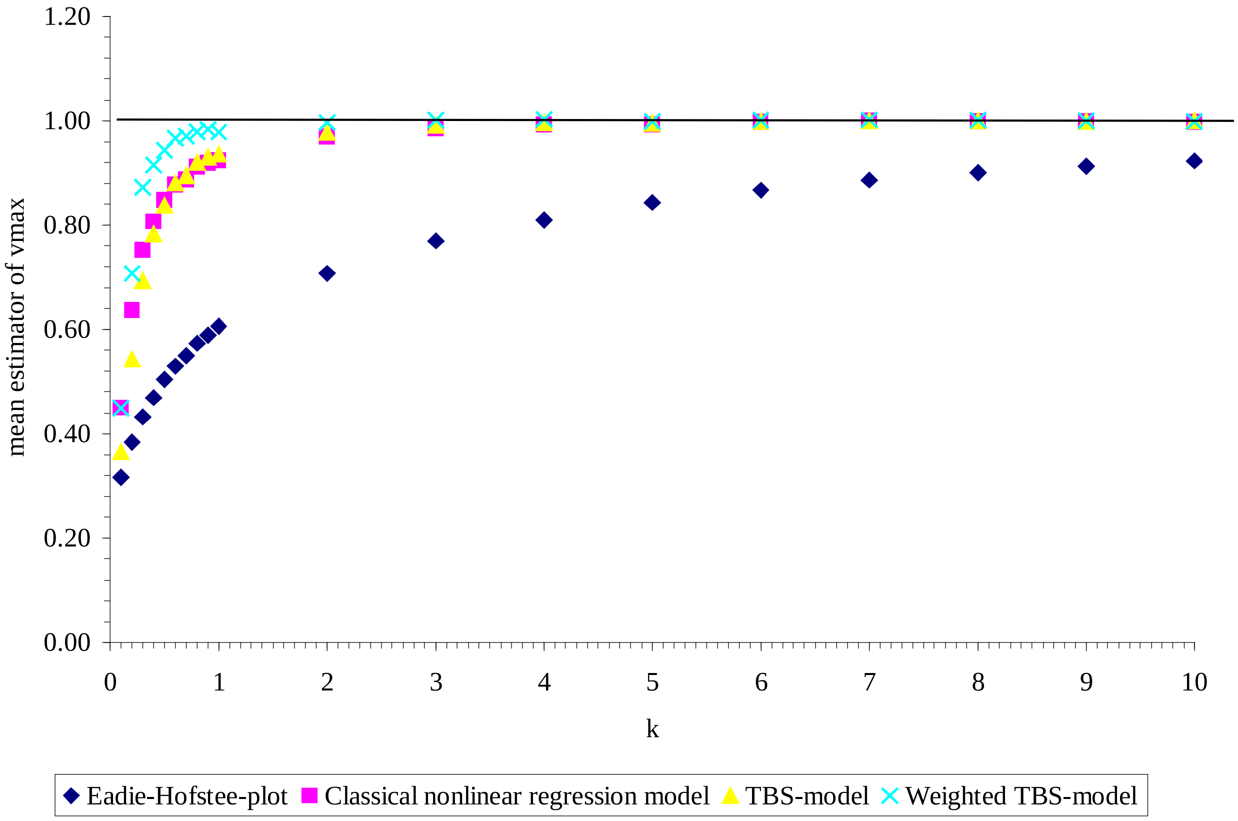

Figure 7: Mean estimator of $v_{max,}$ heteroscedastic error structure.

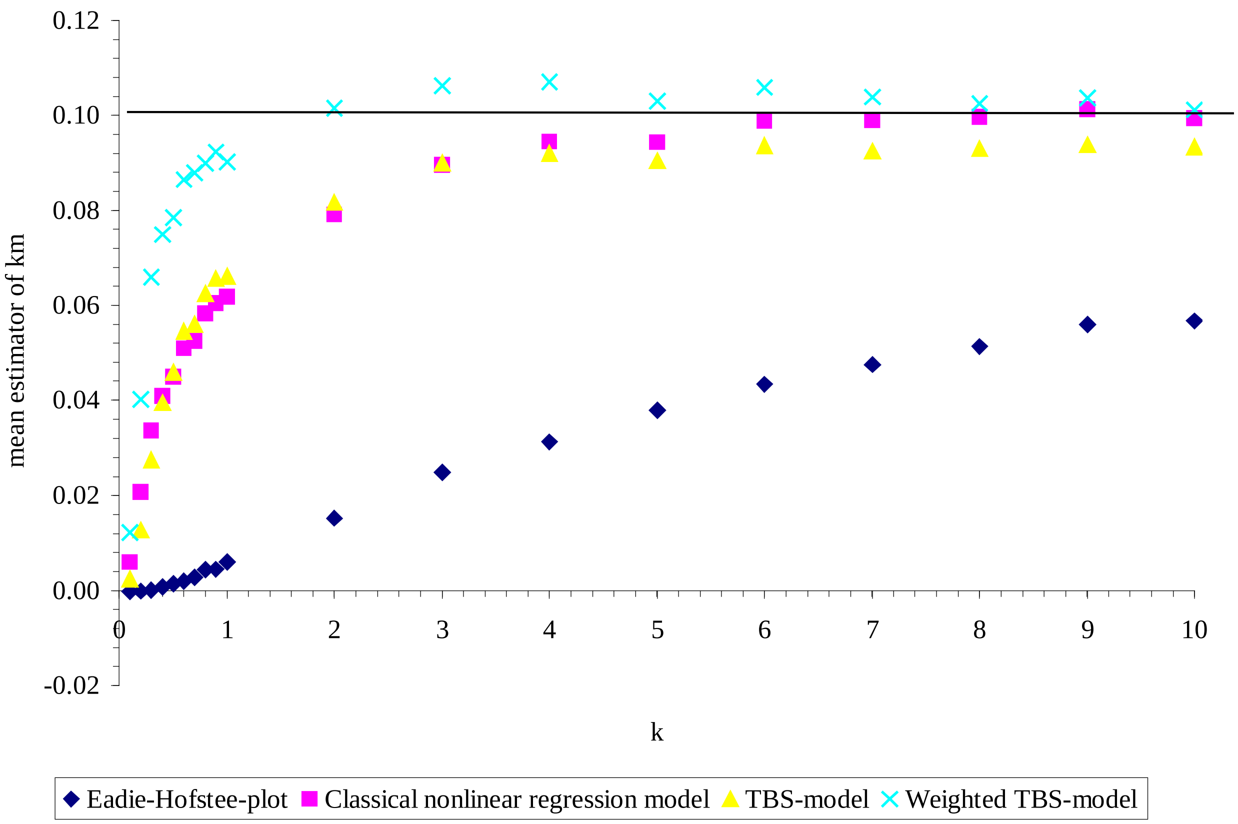

Figure 8: Mean estimator of k_m, heteroscedatic error structure.

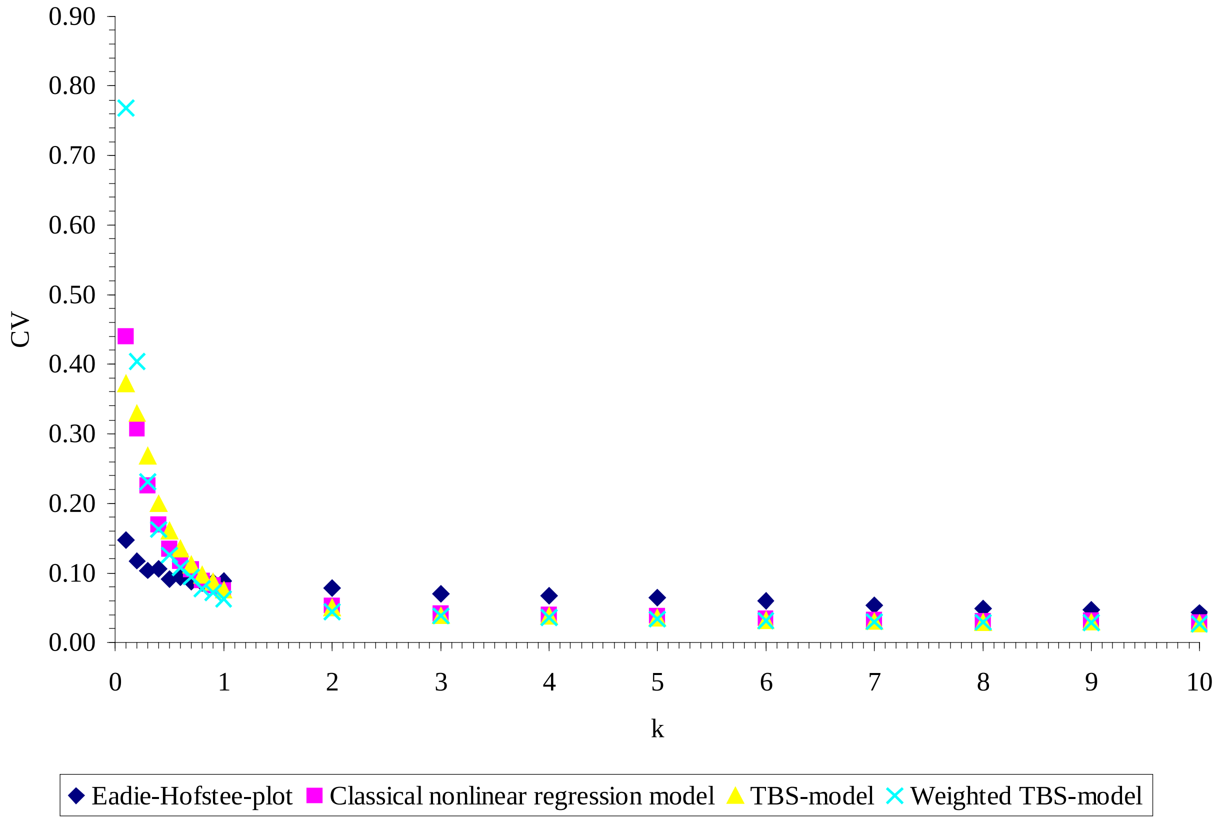

Figure 9: Coefficient of variation referring to estimators of v_{max}, heteroscedastic error structure.

If we consider the heteroscedastic error structure figure 7 indicates the best estimation of v_{max} with the weighted TBS-model. Estimators of the classical nonlinear regression model and the TBS-model are similar. Extremely biased estimators of v_{max} are achieved by the Eadie-Hofstee-plot. The same result can be seen in figure 8 referring to estimators of k_m. Moreover, overestimated parameters will be found in figure 8 if we consider the mean estimators of the weighted TBS-model in high dose levels (k>1). It is remarkable that the mean estimators of Eadie-Hofstee-plot are negative in dose levels with k=0.1 and k=0.2. The increase of the mean estimator of Eadie-Hofstee-plot is lower than in figure 7 and the mean estimators of k_m are not higher than 0.06. For these two reasons the Eadie-Hofstee-plot is not an appropriate statistical model referring to the heteroscedastic error structure.

Figure 9 makes clear that a low range of 0.06 to 0.16 of the coefficient of variation referring to the Eadie-Hofstee-plot indicate comparatively useful estimators in low dose levels with k<1. In contrast, the quantities of these estimators strongly deviate from true parameter (cf. figure 7 and 8). Therefore estimators referring to Eadie-Hofstee-plot are not appropriate. Heteroscedastic error structure will be indicated by this plot in low dose level if we consider the comparatively high range of CV (0.08 to 0.78) referring to the classical nonlinear regression model, the TBS-model and the weighted TBS-model. Useful estimators are indicated by values of CV between 0.04 to 0.08 in high dose levels. Furthermore CV's of the weighted TBS are minimal in comparison to all other models in dose levels with $k \geq 0.8$.

The assumed error variance structures can be recognized by the estimators of transforming parameter λ and the estimators of weighting parameter θ. For example, if we consider a constant error variance the mean estimators of θ of the TBS-model are between 0.7908 and 0.9873 (cf. table 5 in appendix). Therefore the constant error structure is well recognized. Flexibility in the TBS-model and in the weighted TBS-model will be found if we consider heteroscedastic error variance (cf. table 6 in appendix.).

5. Conclusions

From the point of expected results assuming constant error structure and of the low simulation error ($\sigma < 2$ %) we can justify the proposed simulation study. Furthermore the recognition of error structure secure the possibility of a careful discussion of the results.
Summarizing the TBS-model and the weighted TBS-model will be more appropriate than the Eadie-Hofstee-plot or the classical nonlinear regression model. Only in one case (estimator of k_m referring to relative constant error structure) the Eadie-Hofstee-plot will be more appropriate than the classical nonlinear regression model if we accept an underestimation of k_m to be a conservative method for example in toxicokinetics. In all other cases the Eadie-Hofstee-plot should not be applied.
The results of this simulation study lead to this conclusion because of several reasons.

- Especially the estimators of the TBS-model and the weighted TBS-model are acceptable in the important low dose level if we consider all three error structures.
- The coefficients of variation assuming relative constant and heteroscedastic error structure are comparatively low if we consider TBS and weighted TBS-model.

- In a real data situation the error structure is unknown and the TBS-model and the weighted TBS-model are able to recognize the underlying error structure by estimating the transformation parameter λ and weighting parameter θ.

This simulation study does indicate an influence of overparameterisation referring to the weighted TBS-model in the case of relative constant error structure. In future evaluations the choice of the weighted TBS-model with two additional parameters or of the TBS-model with only one additional parameter should be made in consideration of similar estimators of k_m or v_{max} but different standard deviations. In Frei et al. (1999) similar estimators of the TBS-model and the weighted TBS-model caused the choice of the TBS-model.
Nevertheless it has to be discussed if different nonlinear optimization algorithm than DuD-algorithm yields better TBS- or weighted TBS-estimators.

Acknowledgement

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Appendix

	Eadie-Hofstee-plot				*Classical nonlinear regression model*				*TBS-model*				*Weighted TBS-model*			
k	***mean of*** $\hat{v}_{max}$	***s.d.***	***mean of*** $\hat{k}_m$	***s.d.***	***mean of*** $\hat{v}_{max}$	***s.d.***	***mean of*** $\hat{k}_m$	***s.d.***	***mean of*** $\hat{v}_{max}$	***s.d.***	***mean of*** $\hat{k}_m$	***s.d.***	***mean of*** $\hat{v}_{max}$	***s.d.***	***mean of*** $\hat{k}_m$	***s.d.***
0.1	0.2581	0.0309	0.0048	0.0025	0.9970	0.1203	0.0991	0.0230	0.9889	0.1244	0.0977	0.0238	1.0074	0.1280	0.1018	0.0248
0.2	0.4049	0.0529	0.0145	0.0066	0.9996	0.0598	0.0998	0.0141	0.9994	0.0603	0.0999	0.0142	1.0069	0.0626	0.1023	0.0150
0.3	0.5109	0.0626	0.0244	0.0094	0.9990	0.0417	0.0998	0.0112	0.9992	0.0422	0.1002	0.0113	1.0034	0.0424	0.1018	0.0115
0.4	0.6008	0.0753	0.0349	0.0125	0.9989	0.0356	0.0997	0.0098	0.9998	0.0361	0.1001	0.0100	1.0015	0.0368	0.1012	0.0103
0.5	0.6673	0.0734	0.0432	0.013	0.9984	0.0312	0.0993	0.0095	0.9986	0.0316	0.0995	0.0097	1.0007	0.0324	0.1007	0.0102
0.6	0.7209	0.0707	0.0511	0.0138	1.0009	0.0281	0.1006	0.0092	1.0012	0.0282	0.1008	0.0093	1.0022	0.0284	0.1015	0.0096
0.7	0.7627	0.0697	0.0568	0.0139	0.9998	0.0269	0.1000	0.0088	1.0001	0.0269	0.1002	0.0089	1.0013	0.0278	0.1011	0.0093
0.8	0.7976	0.0641	0.0617	0.0141	1.0016	0.0236	0.1001	0.0081	1.0019	0.0238	0.1003	0.0082	1.0027	0.0247	0.1009	0.0086
0.9	0.8248	0.0587	0.0664	0.0134	0.9998	0.0229	0.1000	0.0081	0.9999	0.0232	0.1002	0.0082	1.0007	0.0242	0.1007	0.0086
1	0.8469	0.0546	0.0697	0.0129	0.9994	0.0212	0.1003	0.0079	0.9992	0.0215	0.1002	0.0082	0.9998	0.0222	0.1007	0.0085
2	0.9501	0.0305	0.0879	0.0107	1.0002	0.0178	0.1005	0.0070	1.0001	0.0182	0.1005	0.0074	1.0002	0.0192	0.1007	0.0079
3	0.9735	0.0234	0.0921	0.0094	0.9990	0.0162	0.0998	0.0069	0.9997	0.0164	0.0997	0.0071	0.9998	0.0171	0.0998	0.0073
4	0.9841	0.0198	0.0950	0.0090	1.0000	0.0154	0.1003	0.0069	0.9999	0.0155	0.1002	0.0069	1.0002	0.0161	0.1006	0.0072
5	0.9880	0.0168	0.0952	0.0082	1.0003	0.0148	0.0999	0.0071	1.0001	0.0148	0.0999	0.0073	1.0000	0.0153	0.1001	0.0075
6	0.9913	0.0155	0.0970	0.0084	1.0000	0.0138	0.1006	0.0072	0.9999	0.0139	0.1006	0.0075	1.0003	0.0144	0.1009	0.0076
7	0.9925	0.0154	0.0969	0.0083	0.9997	0.0142	0.1001	0.0076	0.9995	0.0146	0.1002	0.0079	0.9995	0.0152	0.1004	0.0081
8	0.9934	0.0137	0.0968	0.0084	0.9997	0.0129	0.0999	0.0075	0.999	0.0132	0.0997	0.0078	0.9991	0.0139	0.0999	0.0081
9	0.9951	0.0145	0.0977	0.0084	1.0009	0.0133	0.1007	0.0073	1.0006	0.0135	0.1007	0.0076	1.0006	0.0141	0.1008	0.0079
10	0.9955	0.0138	0.0981	0.0081	1.0000	0.0131	0.1005	0.0079	0.9995	0.0133	0.1003	0.0079	0.9989	0.0140	0.1002	0.0082

Table 1: Constant error structure - mean estimators of $v_{\max}$ *and* k_m *, standard deviation (s.d.) in dark columns.*

	Eadie-Hofstee-plot				***Classical nonlinear regression model***				***TBS-model***				***Weighted TBS-model***			
k	***mean of*** $\hat{v}_{max}$	***s.e.***	***mean of*** $\hat{k}_m$	***s.d.***	***mean of*** $\hat{v}_{max}$	***s.d.***	***mean of*** $\hat{k}_m$	***s.d.***	***mean of*** $\hat{v}_{max}$	***s.d.***	***mean of*** $\hat{k}_m$	***s.d.***	***mean of*** $\hat{v}_{max}$	***s.d.***	***mean of*** $\hat{k}_m$	***s.d.***
0.1	0.6379	0.0892	0.0552	0.0097	1.0998	0.1101	0.1179	0.0580	0.9963	0.1796	0.1014	0.0218	1.0117	0.1889	0.1024	0.0227
0.2	0.7799	0.0917	0.0699	0.0108	1.0306	0.1846	0.1074	0.0363	0.9886	0.1317	0.1013	0.0175	1.0005	0.1359	0.1020	0.0180
0.3	0.8304	0.0821	0.0749	0.0101	1.0192	0.1446	0.1060	0.0317	0.9791	0.1058	0.0997	0.0147	0.9857	0.1076	0.0999	0.0151
0.4	0.8565	0.0768	0.0777	0.0103	1.0151	0.1265	0.1056	0.0294	0.9781	0.0958	0.0999	0.0141	0.9867	0.0981	0.1004	0.0145
0.5	0.8746	0.0746	0.0795	0.0100	1.0044	0.1134	0.1023	0.0268	0.9825	0.0900	0.1008	0.0139	0.9903	0.0941	0.1013	0.0143
0.6	0.8910	0.0708	0.0813	0.0099	1.0071	0.1036	0.1029	0.0266	0.9832	0.0837	0.1007	0.0131	0.9886	0.0847	0.1009	0.0134
0.7	0.8966	0.0717	0.0817	0.0107	1.0030	0.0977	0.1026	0.0235	0.9768	0.0826	0.0998	0.0137	0.9854	0.0830	0.1004	0.0137
0.8	0.9111	0.0660	0.0832	0.0097	1.0121	0.0859	0.1037	0.0218	0.9872	0.0764	0.1012	0.0127	0.9934	0.0773	0.1017	0.0129
0.9	0.9087	0.0660	0.0829	0.0096	1.0029	0.0854	0.1031	0.0208	0.9768	0.0781	0.0999	0.0128	0.9847	0.0802	0.1006	0.0132
1	0.9166	0.0669	0.0835	0.0107	1.0059	0.0872	0.1033	0.0236	0.9804	0.0764	0.1001	0.0135	0.9866	0.0809	0.1006	0.0143
2	0.9394	0.0611	0.0852	0.0115	1.0008	0.0687	0.1022	0.0200	0.9761	0.0685	0.0994	0.0139	0.9819	0.0698	0.0998	0.0142
3	0.9520	0.0589	0.0861	0.0126	1.0043	0.0650	0.1030	0.0203	0.9815	0.0637	0.1008	0.0152	0.9882	0.0657	0.1011	0.0153
4	0.9549	0.0581	0.0848	0.0135	1.0014	0.0625	0.1019	0.0208	0.9785	0.0633	0.0993	0.0170	0.9878	0.0649	0.1001	0.0169
5	0.9607	0.0569	0.0852	0.015	1.0034	0.0633	0.1025	0.0224	0.9823	0.0638	0.1004	0.0188	0.9900	0.0638	0.1013	0.0189
6	0.9679	0.0569	0.0864	0.0153	1.0076	0.0614	0.1039	0.0205	0.9876	0.0644	0.1020	0.0188	0.9942	0.0646	0.1027	0.0193
7	0.9669	0.0521	0.0846	0.0151	1.0025	0.0544	0.1009	0.0192	0.9853	0.0561	0.1005	0.0181	0.9924	0.0581	0.1015	0.0185
8	0.9659	0.0518	0.0846	0.0163	1.0007	0.0552	0.1025	0.0228	0.9822	0.0574	0.1009	0.0206	0.9917	0.0584	0.1024	0.0209
9	0.9645	0.0505	0.0829	0.0179	0.9973	0.0535	0.1010	0.0236	0.9783	0.0558	0.0990	0.0217	0.9858	0.0557	0.1001	0.0218
10	0.9723	0.0530	0.0845	0.0199	1.0049	0.0570	0.1037	0.0258	0.9867	0.0591	0.1019	0.0247	0.9958	0.0613	0.1033	0.0254

Table 2: Relative constant error structure - mean estimators of v_{max} *and* k_m *, standard deviation (s.d.) in dark columns.*

	Eadie-Hofstee-plot				*Classical nonlinear regression model*				*TBS-model*				*Weighted TBS-model*			
k	***mean of*** $\hat{v}_{max}$	***s.d.***	***mean of*** $\hat{k}_{m}$	***s.d.***	***mean of*** $\hat{v}_{max}$	***s.d.***	***mean of*** $\hat{k}_{m}$	***s.d.***	***mean of*** $\hat{v}_{max}$	***s.d.***	***mean of*** $\hat{k}_{m}$	***s.d.***	***mean of*** $\hat{v}_{max}$	***s.d.***	***mean of*** $\hat{k}_{m}$	***s.d.***
0.1	0.3171	0.0467	0.0003	0.0003	0.4501	0.1981	0.0059	0.0256	0.3649	0.1358	0.0023	0.0139	0.4488	0.3449	0.0122	0.0517
0.2	0.3839	0.0451	0.0002	0.0006	0.6368	0.1959	0.0207	0.0360	0.5428	0.1785	0.0127	0.0256	0.7074	0.2859	0.0402	0.0611
0.3	0.4319	0.0448	0.0001	0.0012	0.7524	0.1694	0.0336	0.0377	0.6935	0.1854	0.0274	0.0346	0.8715	0.2012	0.0659	0.0559
0.4	0.4696	0.0497	0.0007	0.0018	0.8069	0.1365	0.0409	0.0364	0.7833	0.1559	0.0395	0.0395	0.9153	0.1489	0.0749	0.0525
0.5	0.5037	0.0459	0.0014	0.0025	0.8476	0.1137	0.0450	0.0312	0.8374	0.1344	0.0458	0.0349	0.9436	0.1189	0.0785	0.0441
0.6	0.5292	0.0496	0.0020	0.0031	0.8774	0.1024	0.0509	0.0329	0.8802	0.1183	0.0545	0.0378	0.9674	0.1038	0.0865	0.0459
0.7	0.5504	0.0479	0.0028	0.0037	0.8872	0.0933	0.0525	0.3070	0.8940	0.0995	0.0559	0.0329	0.9705	0.0914	0.0879	0.0410
0.8	0.5739	0.0494	0.0043	0.0047	0.9117	0.0813	0.0583	0.0304	0.9199	0.0889	0.0624	0.0336	0.9791	0.0758	0.0899	0.0396
0.9	0.5887	0.0509	0.0045	0.0045	0.9185	0.0751	0.0604	0.0307	0.9305	0.0807	0.0656	0.0343	0.9828	0.0706	0.0923	0.0403
1	0.6065	0.0535	0.0060	0.0058	0.9244	0.0694	0.0618	0.0281	0.9353	0.0703	0.0660	0.0294	0.9781	0.0614	0.0902	0.0353
2	0.7078	0.0551	0.0152	0.0116	0.9691	0.0510	0.0791	0.0294	0.9774	0.0488	0.0816	0.0299	0.9965	0.0446	0.1015	0.0331
3	0.7699	0.0535	0.0249	0.0161	0.9858	0.0406	0.0895	0.0301	0.9913	0.0379	0.0899	0.0298	1.0006	0.0384	0.1062	0.0329
4	0.8091	0.0547	0.0313	0.0200	0.9929	0.0399	0.0944	0.0311	0.9958	0.0374	0.0919	0.0302	1.0024	0.0362	0.1070	0.0324
5	0.8433	0.0542	0.0379	0.0238	0.9927	0.0377	0.0943	0.0299	0.9950	0.0350	0.0904	0.0286	0.9988	0.0335	0.1029	0.0293
6	0.8663	0.0516	0.0434	0.0244	0.9978	0.0346	0.0988	0.0301	0.9989	0.0317	0.0935	0.0290	1.0009	0.0318	0.1059	0.0320
7	0.8861	0.0476	0.0475	0.0247	0.9992	0.0323	0.0989	0.0298	0.9997	0.0298	0.0924	0.0280	1.0016	0.0301	0.1038	0.0303
8	0.9006	0.0439	0.0514	0.0253	0.9991	0.0308	0.0996	0.0285	1.0000	0.0286	0.0930	0.0272	1.0010	0.0288	0.1025	0.0283
9	0.9124	0.0429	0.0559	0.0281	0.9983	0.0311	0.1013	0.0312	0.9986	0.0288	0.0937	0.0289	0.9993	0.0285	0.1036	0.0303
10	0.9226	0.0394	0.0567	0.0259	0.9980	0.0293	0.0994	0.0307	0.9998	0.0271	0.0933	0.0292	0.9986	0.0266	0.1012	0.0289

Table 3: Heteroscedastic error structure - mean estimators of v_{max} *and* k_m*, standard deviation (s.d.) in dark columns.*

TBS-model	***Constant error structure***		***Relative constant error structure***		***Heteroscedastic error structure***	
k	***mean of*** $\hat{\lambda}$	***s.d.***	***mean of*** $\hat{\lambda}$	***s.d.***	***mean of*** $\hat{\lambda}$	***s.d.***
0.1	0.8764	0.1505	0.0051	0.1299	0.4827	0.1785
0.2	0.8989	0.1369	0.0147	0.1326	0.6196	0.2356
0.3	0.9152	0.1288	0.0132	0.1432	0.7515	0.2598
0.4	0.9293	0.1369	0.0070	0.1675	0.8471	0.2621
0.5	0.9366	0.1552	0.0166	0.1633	0.9111	0.2694
0.6	0.9565	0.1400	0.0171	0.1724	0.9571	0.2804
0.7	0.9608	0.1452	0.0097	0.1762	0.9927	0.2571
0.8	0.9759	0.1508	0.0103	0.1893	1.0280	0.2648
0.9	0.9725	0.1521	0.0208	0.2006	1.0953	0.2851
1	0.9873	0.1667	0.0253	0.1998	1.0939	0.2751
2	0.9697	0.2286	0.0084	0.2656	1.2557	0.2783
3	0.9680	0.2902	0.0355	0.3177	1.3576	0.3408
4	0.9625	0.3403	0.0518	0.3809	1.4615	0.3590
5	0.9376	0.3934	0.0746	0.4320	1.5759	0.3912
6	0.9014	0.4472	0.1110	0.4496	1.6255	0.3935
7	0.8799	0.5143	0.1180	0.4534	1.6995	0.3905
8	0.8877	0.5407	0.1326	0.5044	1.7679	0.3998
9	0.9064	0.5742	0.1316	0.5416	1.8469	0.5173
10	0.7908	0.5789	0.1605	0.5593	1.8589	0.5178

Table 5: Mean estimator of transforming parameter λ, standard deviation (s.d.) in dark columns.

Weighted TBS-model	***Constant error structure***				***Relative constant error structure***				***Heteroscedastic error structure***			
k	***mean of*** $\hat{\lambda}$	***s.d.***	***mean of*** $\hat{\theta}$	***s.d.***	***mean of*** $\hat{\lambda}$	***s.d.***	***mean of*** $\hat{\theta}$	***s.d.***	***mean of*** $\hat{\lambda}$	***s.d.***	***mean of*** $\hat{\theta}$	***s.d.***
0.1	0.5275	0.3140	-0.2748	0.2175	0.3943	0.7956	0.3327	0.6859	0.4582	0.2114	-0.0457	0.1509
0.2	0.4971	0.3234	-0.3176	0.2432	0.3034	0.7165	0.2193	0.5603	0.5395	0.2188	-0.1528	0.1701
0.3	0.5103	0.3517	-0.3054	0.2583	0.2167	0.6508	0.1414	0.4742	0.5506	0.2531	-0.2573	0.1663
0.4	0.5464	0.3964	-0.2748	0.2744	0.1839	0.5996	0.1141	0.4004	0.5729	0.2753	-0.2821	0.1451
0.5	0.5815	0.4224	-0.2479	0.2739	0.1714	0.6310	0.0962	0.4040	0.5509	0.2637	-0.3123	0.1423
0.6	0.6429	0.4785	-0.2113	0.2939	0.1671	0.5419	0.0891	0.3326	0.5166	0.2855	-0.3463	0.1346
0.7	0.6455	0.4653	-0.2023	0.2815	0.1607	0.5431	0.0852	0.3195	0.5288	0.2938	-0.3589	0.1474
0.8	0.7210	0.4790	-0.1591	0.2776	0.1639	0.5737	0.0819	0.3137	0.5053	0.2951	-0.3671	0.1418
0.9	0.7686	0.4917	-0.1242	0.2687	0.1934	0.6128	0.0895	0.3273	0.5231	0.3176	-0.3741	0.1431
1	0.7545	0.5437	-0.1335	0.2884	0.2259	0.5824	0.1019	0.3079	0.5179	0.3166	-0.3733	0.1444
2	0.8394	0.6957	-0.0582	0.2696	0.1885	0.5751	0.0714	0.2354	0.5154	0.3295	-0.3926	0.1417
3	0.8383	0.7812	-0.0485	0.2482	0.2529	0.6736	0.0762	0.2185	0.5599	0.3769	-0.3699	0.1407
4	0.7239	0.8403	-0.0742	0.2298	0.3282	0.6704	0.0905	0.1875	0.6217	0.4254	-0.3475	0.1387
5	0.7452	0.9636	-0.0509	0.2281	0.3102	0.7535	0.0717	0.1804	0.7256	0.4818	-0.3056	0.1446
6	0.7019	0.9891	-0.0509	0.2123	0.3607	0.7315	0.0679	0.1682	0.7435	0.5263	-0.2956	0.1453
7	0.6566	1.1341	-0.0478	0.2084	0.3499	0.7181	0.0621	0.1518	0.8087	0.5446	-0.2750	0.1473
8	0.6295	1.2189	-0.0519	0.2109	0.4194	0.7496	0.0771	0.1528	0.9121	0.5211	-0.2397	0.1302
9	0.7374	1.2751	-0.0333	0.1986	0.3952	0.7579	0.0681	0.1482	0.9236	0.6813	-0.2429	0.1448
10	0.5525	1.3819	-0.0416	0.2096	0.4802	0.8022	0.0820	0.1491	0.9015	0.7225	-0.2282	0.1347

Table 6: Mean estimator of transforming parameter λ and weighting parameter θ, standard deviation (s.d.) in dark columns.

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