Efficient and cost-effective experimental determination of kinetic constants and data: the success of a Bayesian systematic approach to drug transport, receptor binding, continuous culture and cell transport kinetics

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Abstract Details about the parameters of kinetic systems are crucial for progress in both medical and industrial research, including drug development, clinical diagnosis and biotechnology applications. Such details must be collected by a series of kinetic experiments and investigations. The correct design of the experiment is essential to collecting data suitable for analysis, modelling and deriving the correct information. We have developed a systematic and iterative Bayesian method and sets of rules for the design of enzyme kinetic experiments. Our method selects the optimum design to collect data suitable for accurate modelling and analysis and minimises the error in the parameters estimated. The rules select features of the design such as the substrate range and the number of measurements. We show here that this method can be directly applied to the study of other important kinetic systems, including drug transport, receptor binding, microbial culture and cell transport kinetics. It is possible to reduce the errors in the estimated parameters and, most importantly, increase the efficiency and cost-effectiveness by reducing the necessary amount of experiments and data points measured.

Key words: Tyrosine transport; Dopamine receptor; 10,11-Dihydroxy-*N*-*n*-propylnorapomorphine; Kinetics; Microbial culture

1. Introduction

There are many research fields and applications where information about kinetic systems and their parameters is essential for progress, including medical and pharmaceutical research, clinical diagnosis and biotechnology research. Details of the kinetics provide crucial information about how the system or components of the system will behave or respond in a given situation. For example, they are vital to the prediction of the toxic or metabolic effects of drugs in the body. Enzyme kinetics, drug transport kinetics, cell transport kinetics, microbial culture kinetics and receptor binding kinetics are a few examples of key kinetic systems about which information is fundamental to making advancements in research and their applications.

The correct design of an experiment is critical to collecting kinetic data suitable for analysis, modelling and deriving the correct information. Indeed this is the fundamental starting point in efficient research as the incorrect design can lead to poor and/or insufficient measurements, which can, in turn, lead to misleading fits and parameter estimates. An experimental design must ensure that the data provides the necessary information required to fit and discriminate between models and obtain good parameter estimates. The kinetics of a system are typically determined using a steady-state approximation and by measuring the initial rates at different substrate concentrations to fit to the model. There are many features of a design to be considered in its optimisation, including the substrate range and the number of data points required.

The importance of experimental design and its role in successful kinetic analysis is becoming increasingly recognised in pharmaceutical research and commercial research [1–3]. Classical approaches to design [4] are based on knowledge of experimental statistics and not the biological details of the system under study. Such methodologies are obtained analytically or by simple computation from the likelihood. The main disadvantages of these approaches are that they can provide highly variable results owing to their dependence on the initial parameter values chosen and their unsuitability for the study of more complex kinetic systems. In contrast, a Bayesian approach to experimental design involves the use of prior knowledge about the kinetic system. A Bayesian design is based around prior distributions of the model parameter estimates and their variance, rather than on chosen single-point values, and each design can be tailored to the type of kinetics in question. Chaloner and Verdinelli [5] originally suggested Bayesian statistics could be applied to the design of experiments and not simply in their analysis. They suggested it would be possible to use a decision-theoretic approach [6] to specify a Utility function reflecting the purpose of the experiment (i.e. to estimate the parameters as precisely as possible). The best design is the one optimising the Utility value (U). Little has been reported on the use of this proposal and Bayesian methodology is not yet well established in experimental practice but as prior information is already available in existing data, it is logical that it should be used to aid

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the design of the increasingly complex experiments required to advance medical and biotechnology research.

In a previous publication [7], we reviewed the design methodologies available to help maximise the accuracy of enzyme kinetic experiments. Information about enzyme kinetics is important in a number of fields, including areas of drug development where it is essential for understanding the pharmacokinetics and interactions within the formulation, as well as for stability testing and predictions of metabolic clearance of the drug. We suggested that a Bayesian approach would be both valid and helpful in evaluating kinetic parameters to a high degree of accuracy. Initial findings, using a number of kinetic data sets of varying degrees of complexity as examples, showed that through careful design major gains could be made that are quantifiable in terms of the productivity and accuracy of each experiment.

More recently we developed a set of rules and procedures for Bayesian experimental design for enzyme kinetics, starting with a very rough prior K_M estimate [8]. It was shown that a number of features of the design, including the substrate range and individual concentrations measured at, greatly affect the success of and error in the subsequent analysis of the data. A systematic series of Bayesian studies enabled us to identify trends and sets of design rules for both simple and complex kinetics. We now show that these rules are applicable to a wide variety of biological steady-state processes.

2. Materials and methods

The initial Bayesian studies involved the development of Utility functions from the models used to fit data. The Utility functions were written using a Mathematical program (Mathematica Version 4.1, Wolfram Research) as the procedure involves complex algebra.

The prior distributions for the unknown parameters and the variance vector were first written as a variance–covariance matrix. The utility function was then developed from expressions for the variance of the parameters in the kinetic model, incorporating the prior distribution. All of this involved differentiation of the model expressions and transposing, multiplying and inverting large matrices. Inferences about the parameters were the main aims and thus equal weighting was put on each of them in the utility function. The weighting was determined according to the relative magnitudes of the prior parameter values. This decided how much weight must be put on minimising each of the parameter variances.

The general form of a utility function is:

$$U = 1/[c_1\{V(P_1)\} + c_2\{V(P_2)\} + \ldots + c_p\{V(P_p)\}]$$

where U is the utility value for a model equation with p parameters; $\{V(P_i)\}$ is the variance expression derived from the variance–covariance matrix for the model parameter P_i; and c_i is the weight for each parameter expression (where $i = 1, 2, \ldots p$), chosen subjectively to reflect the relative importance of estimating that parameter.

Once the correctly weighted algebraic Utility function was embedded in a workbook, the Utility value for each design could be calculated. This was achieved by inserting the chosen set of individual substrate concentrations measured in the theoretical experiment and the prior parameter values. The key features of the optimum design were identified by observing trends and changes in the U value with design choices. That design maximising the Utility value could be selected as the optimum.

For confirmation and to convert the results terms more specific to general understanding, kinetic data for each model was computationally simulated and fitted for each designed substrate value set. Data simulation was achieved using a program written into a Mathematica workbook. This generates experimental data for different designs so each set can be fitted and the results compared [8]. All data was fitted using a Windows package called Simfit (version 5.40, release 4.005). This reports the best-fit model and the parameters with standard errors. The errors for each parameter were expressed as percentage standard errors, which are the errors expressed as a percentage of the parameter value fitted.

Computational simulation is a simple way to generate data for a large number of different designs so the results can be fitted to the models and the results easily compared, i.e. the errors in the fitted model parameters. Information about the actual reduction in error when using the optimum design is more useful.

In the simulation program the total number of substrate concentrations to be measured and the values of each of those concentrations was entered along with the prior model parameter values. The kinetic model was entered and the expected velocity value calculated for each substrate concentration. At this stage, the calculated sets of values were of course the exact ones specified to fit the model, i.e. without any experimental error. The program was then used to generate a list of random error values from a normal distribution. Adding one error term to each expected velocity provided a set of simulated data to later plot and fit to the kinetic model.

For example, this is the theory behind the simulation of data for a Michaelis–Menten enzyme:

If the number of substrate values being used is termed i and $i = 1,\ldots n$, then the model equation can be written:

$$v_i = \frac{V_{max} S_i}{S_i + K_M} + \varepsilon_i$$

The set of substrate values $(S_1,\ldots S_n)$ in the design and the prior values of V_{max} and K_M are input into the program. The set of expected velocity values, with no error terms added, is calculated.

The error term ε has a normal distribution: $\varepsilon_i \sim N(0,1)$ with a mean of zero and standard deviation of 1.

A list of n number error values $(\varepsilon_1,\ldots \varepsilon_n)$ is then generated from a normal distribution. Each error value must be multiplied by the standard deviation known for the prior V_{max} to correct from the standard deviation of 1. These error values are added to the equation to give a simulated set of data $v_1,\ldots v_n$. The end result is a set of v and s values to fit and analyse as if real and estimate V_{max} and K_M.

Our approach produces sets of step-by-step rules for designing the optimum kinetic experiments to obtain data suitable for modelling and accurate analysis. Table 1 summarises the rules for the design of a simple (two-parameter model) kinetic experiment and Table 2 for the design of a complex kinetic (four-parameter model) experiment. The key starting points to the design method are: the need for a very rough prior estimate of the K_M parameter (or the ratio of K_M values if more than one in the model); and, knowledge of the number of parameters in the kinetic model equation that the data is to be fitted to. The rules vary according to the number of parameters in the model being studied. The following features of the experimental design can be chosen: the substrate range; the total number of substrate concentrations to take measurements at; the distribution of data points across the range; and, the choice of individual important points in that range. Importantly, each choice is based on the K_M or ratio value.

The importance of basing the design on the K_M becomes more apparent when looking at the other choices to be made about the data points used within the chosen substrate range. Firstly, a trend emerges relating to the percentage of total data points measured that are below and above the K_M. The Utility value rises to a peak at an optimum choice of 60% points below (or on) the K_M, and falls off either side of this but more markedly when reduced to less than 60% [8]. That is the optimum data point distribution is when 60% of the data points fall below or on the K_M substrate concentration value but above half the K_M (the starting point of the range). This has been demonstrated for a 15-data point design of five points measured in triplicate [8].

For complex functions, again there are definite optimum lower and upper range points and the detailed study reveals that these are related to the ratio of the two K_M values (K_{m1}/K_{m2}) [8]. The range must extend from 10 times below the ratio K_{m1}/K_{m2} up to 100 times the ratio. The importance of basing the design on the K_{m1}/K_{m2} ratio is reiterated when looking at the other choices to be made about the data points used within the chosen substrate range. There is an optimum data point distribution around the K_M ratio. The Utility value rises to a peak at an optimum choice of 50% points measured below/on the K_M ratio and falls off either side, most markedly from 30% or below. This has been demonstrated for a 25-data point

Table 1
Design rules for Michaelis–Menten type (two-parameter) kinetics

Experiment design decision	Bayesian rule
(1) Start with a prior estimate of K_M.	Use prior information for an estimate or a rough guess.
(2) Choose the substrate range for measurements.	The range should extend from half the K_M concentration up to 100 times it.
(3) Choose the total number of data points to measure.	Measure at five different substrate concentrations in triplicate (the first concentration is the lower point of the range and the fifth is the upper point).
(4) Choose the middle (third) concentration to measure at.	This measurement should be made at the K_M concentration.
(5) Choose the second concentration to measure at.	This concentration should be the value which is 1/4 the distance towards the K_M concentration from the first point (the lower range point).
(6) Choose the fourth concentration to measure at.	This measurement should be made at the concentration which is 20% below the upper point.
(7) Perform the experiment to obtain data. Fit the two-parameter model to the kinetic data (plotting the mean of each of the data points measured in triplicate) to obtain parameter estimates and errors.	N.B. Take an iterative approach if the prior estimate of K_M that the design is based on is known to be poor. That is, carry out the experiment at five concentrations only (i.e. no triplicates) and fit to obtain a better estimate of K_M. Redesign based on new K_M estimate and perform a second five-point experiment. Redesign again and for the third experiment perform the experiment for the five points in triplicate to obtain final low variance parameter estimates.

design with each point measured at a different substrate concentration [8].

With all the examples tested, the error decreases with an increasing number of replicates up to three; increasing the numbers of replicates beyond three produces no improvement in the standard error of parameter estimation [8].

As the number of measurements required is based on the number of model parameters to be fitted rather than the actual equation, it is possible to design and also differentiate through fitting between kinetic models with the same number of parameters. The rules also suggest an iterative experiment if prior information about K_M is very poor (that is a series of three small experiments to obtain more information about the parameter).

It is clear that this method of Bayesian design and the rules are potentially applicable to other kinetic systems, particularly where it is important to have rapid but accurate and cost-effective methods in clinical or industrial research. We show here how four kinetic experiments for different systems can be markedly improved by designing using the rules. Definite improvements can be made by designed choices of features such as the concentration range, percentage distribution of points and the individual selection of points.

3. Results

3.1. Cell transport kinetics: using a Bayesian experimental design to improve the accuracy and efficiency of model parameter estimation

For the purpose of this example, we used kinetic data for the transport of the radiolabelled amino acid 3-[^{123}I]iodo-L-α-methyltyrosine ([^{123}I]IMT) in human GOS3 glioma cells [9]. The use of [^{123}I]IMT is a promising tool for the diagnosis and monitoring of brain tumours (both in the non-invasive grading of gliomas and the delineation of tumour extent) using single-photon emission tomography. Critically, it has been established that there is a specific transport of [^{123}I]IMT

Table 2
Design rules for complex equation kinetics (four parameters)

Experiment design decision	Bayesian rule
(1) Start with a prior estimate of the ratio of the two K_M values (if more than one).	Use prior information for an estimate or a rough guess.
(2) Choose the substrate range for measurements.	The range should extend from 10 times below the K_M ratio concentration up to 100 times above it.
(3) Choose the total number of data points to measure.	Measure at 25 different substrate concentrations (no replicates) (the first concentration is the lower point of the range and the 25th is the upper point).
(4) Choose the middle (13th) concentration to measure at.	This measurement should be made at the K_M ratio concentration.
(5) Choose the second concentration to measure at.	This concentration should be the value which is 1/4 the distance towards the K_M ratio concentration from the first point (the lower range point).
(6) Choose the 12th concentration (point below the K_M ratio) to measure at.	This measurement should be made at the concentration which is 10% below the K_M ratio value.
(7) Choose the 14th concentration (point above the K_M concentration)	This measurement should be made at the concentration which is five times the K_M ratio concentration.
(8) Choose the 24th concentration (point below the upper range point).	This measurement should be made at the concentration which is 20% below the upper range point.
(9) Choose the intermediate points – points 3–11 and 15–23.	These should be as evenly spaced as possible between the pre-decided points.
(10) Perform the experiment to obtain data. Fit the four-parameter model to the kinetic data to obtain parameter estimates and errors.	N.B. Take an iterative approach if the prior estimate of K_M that the design is based on is known, or thought, to be poor. That is, carry out the experiment at 10 concentrations only (still selecting the individual points according to their position within the 10 points) and fit to obtain a better estimate of K_M. Redesign based on the new K_M estimate and perform a second 10-point experiment. Redesign again and for the third experiment perform the experiment for the 25 points to obtain final low variance parameter estimates.

Table 3
A comparison of percentage standard errors in the fitted kinetic model parameters when fitting data obtained with the classical experimental design to those obtained with data from our Bayesian design

Calculated Utility values for each experimental design and standard errors of model kinetic parameters fitted to data	Classical design	Bayesian design
Cell transport:		
Utility value	5.505e-02	3.618e-01
% Standard error in V_{max}	4.35	1.07
% Standard error in K_M	8.86	4.11
Continuous culture:		
Utility value	2.891e-04	1.186e-03
% Standard error in $(q_p{}^E)_{max}$	3.86	0.61
% Standard error in K_s*	10.34	2.27
Drug transport:		
Utility value	1.799e-2	2.798e-2
% Standard error in V_M	3.81	2.21
% Standard error in n_H	15.12	14.93
% Standard error in K_M	7.36	4.42
Receptor binding:		
Utility value	1.24e-03	2.022e-03
% Standard error in B_{max1}	16.74	8.01
% Standard error in B_{max2}	12.32	5.72
% Standard error in K_{d1}	25.49	20.60
% Standard error in K_{d2}	38.99	14.56

The Utility values for each design are also compared. Four different kinetic systems are compared: cell transport [3], continuous culture [7], drug transport [8] and receptor binding [9].

across the blood–brain barrier [10,11] and it is metabolically stable and not incorporated into proteins [12]. However, in order to optimise its medical use, more work is needed to obtain precise kinetic details of its uptake in human glioma cells. Such details aid in the identification of the cell systems that mediate the transport and any differences between cell lines and types. This knowledge is essential to aid further in vitro studies and help identify suitable model cells.

The cell transport kinetics of [^{123}I]IMT are consistent with Michaelis–Menten type kinetics:

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

where v is the transport velocity of [^{123}I]IMT; [S] is the concentration of [^{123}I]IMT; V_{max} is the maximum transport velocity; and K_M is the apparent Michaelis–Menten constant. The latter two parameters are the unknown kinetic parameters to be estimated. The uptake of [^{123}I]IMT (in terms of its initial transport rate) was measured over a range of [^{123}I]IMT concentrations from 2.5 to 50 μM. Eight experimental points were performed in triplicate and each experiment was carried out at least twice with similar results. After fitting the data a K_M of 20.1 ± 1.5 μM and a maximum transport velocity (V_{max}) of 34.8 ± 1.9 nmol/mg protein/10 min were calculated.

It is possible to improve this experimental design using an iterative and systematic Bayesian approach. That is, following our rules for designing the optimum experiment for a system with two-parameter equation kinetics [8], it is possible to make marked gains in terms of the efficiency and the accuracy of the parameters estimated.

In the first instance we can compare the Bayesian Utility values calculated for the two experimental designs: that used by Riemann et al. and our Bayesian design. The authors' design is that as described above. The Bayesian design is based on the prior estimate of K_M and the concentration range and individual measurements made within that are chosen according to the rules. With a prior estimate of K_M of 20.1 μM, the concentration range specified by the rules is from 10 to 2000 μM with measurements taken at five different concentrations in triplicate. The two designs are very different with the concentration range of the original one running from eight times below the K_M value to two and a half times above it, a sharp contrast to that of the Bayesian design which runs from two times below the K_M to one hundred times above it. The calculated Utility values are compared in Table 3. Observe that the Utility for the Bayesian design is over six times higher than that for the original design. Recall that these Utility functions have been designed to reflect the purpose of the experiment, i.e. to minimise the parameter variance, theoretically achieved when the Utility value is maximised. This comparison confirms the Bayesian design offers a marked improvement and the use of this design will result in more effective parameter estimation.

In order to verify these Utility function conclusions, a simulation program was used to simulate experimental data for the kinetic model for the two different designs using the prior specified parameter values. The mean of each data point was calculated before fitting to the Michaelis–Menten equation to obtain parameter estimates. Table 3 also shows a comparison of the percentage standard errors in the fitted kinetic model parameters when fitting the data obtained with the original experimental design used in the authors' work to those obtained with data from our Bayesian design. The results show that when designing by the Bayesian rules the standard errors in the parameter estimates are considerably less than if obtained via the authors' route.

This demonstrates that following the Bayesian experimental design rules, which use prior information about the K_M value, leads to better parameter estimates with lower errors. Indeed the careful choice of the design and points at which measurements are made can improve the experiment, and reduce the numbers of data points required. This is assuming the prior information on K_M is good. However, prior information on the K_M value is often poor or unavailable and in this case it is possible to use our proposed iterative and systematic Bayesian approach [8]. This a three-step approach still using the Baye-

Table 4
The results of designing the cell transport [3] experiment using the iterative three-step Bayesian rules

Results	Experiment design step 1 (based on prior $K_M = 1$)	Experiment design step 2 (based on prior $K_M = 13.2$)	Experiment design step 3 (based on prior $K_M = 21.5$)
Concentrations of [^{123}I]IMT (μM) at which uptake transport velocity measurements made	0.5, 0.625, 1, 90, 100	6.6, 8.3, 13.2, 1192, 1324	10.75, 13.4, 21.5, 1935, 2150, in triplicate
V_{max} fitted (pmol/mg protein/10 min)	34.11	36.21	33.54
K_M fitted (μM)	*13.24*	*21.51*	19.71
Standard error in V_{max}	1.45	0.91	0.39
Standard error in K_M	4.01	2.22	0.72
% Standard error in V_{max}	–	–	1.16
% Standard error in K_M	–	–	3.70

The initial prior K_M information is very poor and a guess of 1 μM, which is 20 times out from the actual prior estimated value of 20.1 μM. In both steps 1 and 2, new K_M estimates are obtained for redesigning the set of concentrations for the measurements to be made at in the next experimental step. Step three of the experiment provides the final parameter estimates with minimised standard errors.

sian rules but collecting new estimates of the K_M at each step around which to redesign. Table 4 shows the results of using the iterative three-step Bayesian rules. The initial K_M value is a guess of 1 μM, which is 20 times out from the actual prior estimated value of 20.1 μM. In both steps 1 and 2, the experiment is designed according to the Bayesian rules and using the current K_M estimate but measurements are only made at five single concentrations. The aim of these two steps is to obtain new and better K_M estimates for redesigning the set of concentrations for the measurements to be made at in the next experimental step. Step 3 of the experiment provides the final parameter estimates with minimised standard errors. The results show that it is possible to get good final estimates with very low variances at the end of the three steps. It should be noted that this is also a shorter route than that used by Riemann et al. and the parameters have been more successfully estimated with lower variances. This was possible with only a very rough guess of the K_M value and after measuring a total of 25 points over three experiments. The original design used at least 48 points measured over two experiments and the errors were considerably higher for each parameter. The reduction in the number of data points is a key feature of our approach. The step-by-step Bayesian design means that the set of points measured at each stage in the experiment is specifically designed to the most recent data gained about the K_M value, rather than simply repeating points in triplicates and repeat experiments as in the original design. In conclusion, the iterative approach making use of prior information markedly increases the efficiency and cost-effectiveness of the experiment.

3.2. *Bayesian experimental design is a generic approach: examples of its applicability to the study of continuous culture, drug transport and receptor binding kinetics*

In addition to the study of enzyme and cell transport kinetics, the specified Bayesian rules and method of design are applicable to the study of a number of other important kinetic systems. Continuous culture, drug transport and receptor binding kinetics are three further examples. Table 3 shows the improvements made when redesigning three such experiments for these kinetic systems. These examples are discussed below.

The analysis of the kinetics of energy spilling-associated product formation in substrate-sufficient continuous culture can be very important in the development of the optimised bioprocess for maximising valuable product formation. For instance, quantified information about the model parameters may be helpful in designing the optimal medium and reactor operating conditions. In our chosen example, Liu et al. looked at the effect of residual methanol concentration on the specific production rate of acetic acid in a nitrogen-limiting continuous culture of a *Bacillus* strain [13]. The authors show that the kinetics are consistent with Michaelis–Menten type kinetics. Table 3 compares the Bayesian Utility values calculated for the two experimental designs: that used by Liu et al. and the Bayesian design. The rate of product formation was determined over a range of residual substrate concentrations. The authors measured five concentrations in triplicate over a range from 10 to 90 mM. In contrast, the Bayesian design, based on the prior estimate of K_s* (K_M equivalent) of 23.6 mM and chosen according to the rules, is five concentrations measured in triplicate over a concentration range of 12 to 2360 mM. Observe that the Utility for this design is over four times higher than for the authors'. The table also compares the percentage standard errors of the parameters fitted to simulated data for the two different designs. The results confirm that when designing by the Bayesian rules, the standard errors in the parameter estimates are markedly less than if obtained via the authors' route.

In general, information about the kinetics of drug transport is essential to understand the mode of action of a drug, its efficacy and toxicity. There is a great demand for accurate and efficient methods to determine such details. The widespread use of antibiotics and drugs has also led to the emergence of defence mechanisms that, are at present, the major drawback to the drug-based treatment of infectious diseases and cancers. Garnier-Suillerot et al. looked at the uptake of a number of drugs into multi-drug resistant cells [14]. Kinetic information about this process has implications for drug action and is essential in the search for an inhibitor. In this example, we compared experimental designs for the study of the kinetics of the P-glycoprotein mediated efflux of the anthracycline derivative 'WP401'. Garnier-Suillerot et al. have described the kinetics with a three-parameter model, including a value for the Michaelis constant. The rate of drug efflux by the pump was measured over a range of intracellular free drug concentrations. The authors' design used a concentration range from 0.1 to 2.1 μM measuring at 13 concentrations. The Bayesian design, based on the prior K_M estimate of 0.115 μM and selected according to the rules for a three-parameter experiment, is seven concentrations measured in triplicate over a concentration range of 0.0575 to 11.5 μM. Table 3 shows that the calculated Utility value for the Bayesian design is over one and a half times higher than that for the authors'.

Comparing the percentage standard errors in the parameters fitted to data simulated for both designs verifies that the estimates are more accurate with fewer data points when using the Bayesian design.

The analysis of equilibrium data for receptor–ligand binding helps to identify the mechanism and specificity of the interaction of a receptor with a ligand. Such knowledge is also essential in the search for inhibitors for medical or research applications. In our final example of a kinetic system, we used an experiment looking at the binding of the ligand 10,11-[^{3}H]dihydroxy-*N*-*n*-propylnorapomorphine ([^{3}H]NPA) to the D2-dopamine receptor[1]. The kinetics were consistent with second-order Michaelis–Menten type kinetics. This means they were described by a four-parameter equation. The total binding was measured for a range of [^{3}H]NPA concentrations. The original design used in this study was to measure binding at 12 [^{3}H]NPA concentrations in triplicate over a concentration range of 0.006 to 15.6 pM. The Bayesian design, suggested by the rules for a four-parameter equation and based on the prior K_{d1}/K_{d2} ratio of 0.06 (the ratio of the two equilibrium constants), was 25 concentrations measured once over the range of 0.0058 to 5.770 pM. The individual concentrations measured within that range were chosen according to the rules. Table 3 shows that the utility calculated for the Bayesian design is nearly two times higher. The percentage standard errors for the parameters estimated by fitting the data obtained by simulating the Bayesian design are also considerably lower than for the original. Again a higher degree of accuracy is obtained using the Bayesian method of design.

4. Discussion

We have shown that our Bayesian rules for the systematic design of kinetic experiments can be successfully applied to a number of different systems. The rapid evaluation of kinetics proves critical in many processes and research and the effective utilisation of this method has the potential to increase the efficiency of these experiments. The optimum data is collected following the rules and the iterative approach provides a more cost-effective route to accurate parameter estimates, as fewer data points are needed.

This method is timely to aid the design of the increasingly complex kinetic experiments in rapidly advancing research fields and the rules logically use existing data and information. The concept is easily transferred to any area of study requiring the analysis of steady-state kinetics.

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