In vitro model for predicting bioavailability of subcutaneously injected monoclonal antibodies

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ABSTRACT

Monoclonal antibodies (mAbs), which are now more frequently administered by subcutaneous (SC) injection rather than intravenously, have become a tremendously successful drug format across a wide range of therapeutic areas. Preclinical evaluations of mAbs to be administered by SC injection are typically performed in species such as mice, rats, minipigs, and cynomolgus monkeys to obtain critical information regarding formulation performance and prediction of PK/PD outcomes needed to select clinical doses for first-in-human studies. Despite extensive efforts, no preclinical model has been identified to date that accurately predicts clinical outcomes for these SC injections. We have addressed this deficiency with a novel in vitro instrument, termed Scissor, to model events occurring at the SC injection site and now further validated this approach using a set of eight mAbs for which clinical PK/PD outcomes have been obtained. Diffusion of these mAbs from the Scissor system injection cartridge into a large volume physiological buffer, used to emulate mAb movement from the SC injection site into the systemic circulation, provided distinct profiles when monitored over a 6 h period. Curve-fitting analysis of these profiles using the Hill equation identified parameters that were used, along with physicochemical properties for each mAb, in a partial least squares analysis to define a relationship between molecule and formulation properties with clinical PK outcomes. The results demonstrate that parameters of protein charge at neutral pH and isoelectric point (pI) along with combined formulation properties such as viscosity and mAb concentration can dictate the movement of the mAb from the injection cartridge to infinite sink compartment. Examination of profile characteristics of this movement provided a strong predictive correlation for these eight mAbs. Together, this approach demonstrates the feasibility of this in vitro modelling strategy as a tool to identify drug and formulation properties that can define the performance of SC injected medicines and provide the potential for predicting clinical outcomes that could be useful for formulation selection and a first-in-human clinical dosing strategy.

1. Introduction

The subcutaneous (SC) route is increasingly being used for the delivery of biopharmaceuticals such as therapeutic monoclonal antibodies (mAbs), as this route provides a more rapid, convenient, and cost-effective alternative to intravenous infusions. The most widely used preclinical species in the development and evaluation of mAb formulations are mice, rats and non-human primates [1], with the more recent adoption of minipigs [2]. Although progress has been made relating preclinical models to human outcomes of pharmacokinetic (PK)

parameters and pharmacodynamics (PD), especially in terms of clearance and volume of distribution [3], predicting human mAb absorption from the SC injection site using preclinical data remains a challenge [4]. For example, the percent bioavailability (%BA or F) of a recombinant human monoclonal anti-VEGF antibody administered SC has been reported as 100% in mice, 98% in cynomolgus monkeys and 69% in rats [5] and rituximab %BA in rats varied between 18 and 44% based upon dose and SC injection location [6]. More troubling is the fact that %BA outcomes in man often do not correlate with preclinical data: adalimumab %BA in monkeys is 96%, but 64% in man; omalizumab %BA in

mice is 90%, 64–104% in monkeys, but 62% in man; golimumab %BA in monkeys is 77%, but 53% in man; and ustekinumab %BA in monkeys is 97%, but 24–95% in man [7,8].

The poor correlation between outcomes in preclinical species and humans in terms of mAb absorption following SC injection has been attributed not only to species-specific physiological and anatomical features of the SC tissue, but also to the relatively larger injection volumes frequently used in animal models when compared to overall body size [9]. To take into account species differences, allometric scaling has been used for predicting PK/PD of mAbs based on pre-clinical data [10]. This method has been reported as successful for predicting mAb clearance, but appears unsuitable for predicting mAb absorption rate and %BA following SC injection [11]. Physiologically-based pharmacokinetic modelling (PBPK) has been used with moderate success to predict the maximum plasma concentration (C_{max}) and time to reach the peak plasma concentration (t_{max}) of subcutaneously administered therapeutic proteins, including mAbs [12], but this method failed to predict %BA. Correlating binding affinity of a mAb to the neonatal IgG receptor FcRn has shown promise in predicting %BA as mAbs having a higher binding to the FcRn receptor showed higher %BA [13]. It has also been reported that formulation properties have an influence on the bioavailability of subcutaneously administered mAbs; for example, increasing the formulation osmolarity of rituximab in a rat model led to an increased %BA [14].

Quality-by-design (QbD) is a paradigm that builds product and process parameters into a drug development program at the beginning to ensure therapeutic efficacy and safety of the active pharmaceutical ingredient (API) and its formulation rather than relying entirely on testing of the end product. The QbD concept is based on the identification of critical quality attributes (CQAs) that define product attributes critical for the efficacy and safety of the final product [15]. For mAbs, CQAs include parameters related to physical properties of the molecule, such as size, charge, oxidation level, and Fc chain glycosylation [16]. There are also CQAs related to formulation composition, adventitious agents, and product specific attributes such as visible and sub-visible particulates and sterility for liquid formulations [16].

For drugs delivered *via* the oral route, *in vitro* dissolution testing is used to identify CQA parameters that define a robust dosage formulation where a desired cumulative release drug profile can be consistently achieved [17]. For SC injected drugs, including protein therapeutics such as mAbs, however, have not had a method to assess CQA parameters related to absorption of a biopharmaceutical from an injected formulation. The lack of an assessment tool plays strongly into the rationale for variable and unpredictable clinical %BA outcomes for SC injected mAbs, as noted above. The SC injection site simulator (Scissor) mimics a number of the chemical, physical and physiological properties of the human SC injection site and enables monitoring events related to the stability of injected formulation components and their migration from the injection site [18]. In some ways, the Scissor system provides an *in vitro* method that parallels the examination of oral dosage form performance to define CQA parameters. Importantly, Scissor can provide a tool to examine injection site events as the environment in which the injected drug is exposed transitions from being in a drug product formulation to conditions consistent with the homeostatic state of the body. In the current study, we used the Scissor system to obtain data describing the movement of eight mAbs from the artificial injection site into the infinite sink reservoir of this instrument to model their absorption from the SC injection site into the systemic circulation.

The Hill equation can be used to describe the binding of a ligand to a macromolecule, which can be modified by the previous binding of that ligand to other sites on the macromolecules or by the presence of other ligands [19,20]. We considered the possibility that an individual mAb could be a ligand whose interactions with ECM components of the SC injection site could be affected by other agents in or properties of the formulation environment. Our studies now demonstrate that analysing Scissor-generated dissolution data using the Hill equation as well as the

molecule and formulation properties for that specific mAb provided a strong correlation to human %BA outcomes for this set of mAbs. Thus, this approach may be useful for screening potential mAbs formulations *in vitro* early in development to possibly predict %BA outcomes in man and may be useful in the identification of formulation CQAs for this class of therapeutic agents.

2. Experimental

2.1. Materials

Sodium chloride (NaCl), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), potassium chloride (KCl), calcium chloride ($CaCl_2$), sodium bicarbonate ($NaHCO_3$), sodium phosphate (NaH_2PO_4) and sodium diphosphate (Na_2HPO_4) as well as sodium azide (NaN_3), phosphate buffered saline (PBS) tablets and hyaluronic acid (HA) from *Streptococcus equii* were purchased from Sigma (Gillingham, UK). Monoclonal antibody (mAb) formulations were donated by Genentech, Inc. (South San Francisco, CA, United States). Information provided for each anonymized antibody is listed under "results" without details for the specific methods used to determine the listed values for each mAb.

2.2. Scissor

The subcutaneous injection site simulator "Scissor" was obtained from Sirius Analytical Ltd. (now PION, Forest Row, UK). Using a 1 mL disposable syringe (Thermo Fisher, Cramlington, UK) and a 16 mm 25G needle (Becton Dickinson, Oxford, UK), 0.5 mL of each mAb formulation was injected into the Scissor cartridge (Sirius Analytical Ltd., UK, now PION) filled with 6.25 mg/mL hyaluronic acid (HA) in PBS solution that had been allowed to equilibrate at 34 °C in the surrounding "infinite sink" chamber conditions. The chamber was filled with physiological, carbonate based buffer solution, 1 L of which contains 6.4 g NaCl, 0.09 g $MgCl_2 \cdot 6H_2O$, 0.4 g KCl, 0.2 g $CaCl_2$ and 2.1 g $NaHCO_3$ dissolved in Milli-Q water, maintained at 34 °C and pH 7.4 by bubbling CO_2(g) through the solution. The buffer also contained 0.02% NaN_3 to prevent bacterial growth during the experiments. Aliquots of varying volumes, depending on assay limits of quantitation were taken from the buffer chamber at 2, 5, 10, 20, 30, 40, 50, 60, 90, 120, 180, 240, 300 and 360 min after injection. All mAb formulations were analysed in triplicate.

2.3. Analysis of mAb movement from Scissor cartridge

Aliquots taken from the infinite sink chamber were filtered with a low protein-binding 0.22 μm Millex GV disposable syringe filter (Merck Millipore, Feltham, UK) to remove particulates prior to analysis by high performance liquid chromatography using an Agilent 1260 Infinity HPLC system. The injection volume was set to 100 μL, flow rate to 0.5 mL/min, oven temperature to 25 °C, and detection wavelength to 214 nm. A Yarra SEC-3000 size exclusion chromatography column (300 mm × 7.8 mm) with compatible pre-column filter (all from Phenomenex, Macclesfield, UK) was used for the separation. The mobile phase was 0.2 M potassium phosphate buffer (pH 6.2) with 0.25 M potassium chloride. Standard samples, prepared by diluting each mAb formulation to cover the range of concentrations expected from samples captured from the infinite sink compartment, were used to determine the amount of injected mAb moving into the infinite sink compartment over time.

2.4. Parametrisation of Scissor data

KinetDS software was used to identify the mathematical model that best described Scissor profile data [21]. Based on this software screening, the Hill equation (Eq. (1)) was identified as the best model for fitting the data. SigmaPlot 12.5 (Systat Software Inc.) was used for

plotting and final parametrisation of data using the Hill equation

$$\%Released = \frac{a*x^b}{c^b + x^b} \tag{1}$$

where a represents the maximum fraction of protein moving from the injection cartridge to the infinite sink compartment, b is a shape factor for the curve, c is the time at which half of a has been achieved, and x is time.

2.5. Partial least squares analysis

Minitab 17 (Minitab Ltd., Coventry, UK) was used to analyse the dataset. First, principal component analysis (PCA) was conducted to identify possible correlations between system input variables to find out the number of components that adequately described relationships between input parameters of the dataset; PCA is a technique that enables analysis of relationships between a group of variables that may be collinear in nature, and which reduces the original input variables to a number of components that describe the underlying data in fewer dimensions [22]. The number of components that adequately described the input data was identified from the inflection point of the curve where it levelled off ("the elbow") within the scree plot [23] while also ensuring that the model described more than 80% of the variation within the dataset [24]. Partial least squares (PLS) analysis, which is a form of PCA analysis that allows relating the input parameters to output parameters was then conducted to see how the different formulation and molecule properties as well as the Hill equation parameters related to outcomes for %BA and mean absorption rate constant (ka) in humans.

2.6. Human in vivo data

Human SC %BA and absorption rate values for the eight mAbs in this study were obtained from a population PK analysis of all available clinical data at the time of this manuscript's preparation. For a given antibody, the clinical serum PK data across all patients was pooled and simultaneously fit to its population PK/PD model in order to estimate its PK parameters, including but not limited to %BA and absorption rate from the SC space.

3. Results

3.1. Material attributes of mAb molecules tested and their formulations

Commonly-assessed physicochemical properties of the mAbs examined in these studies and their formulations are summarised in Table 1. All mAbs have molecular weight (MW) values of ~145 kDa. Isoelectric point (pI) values for these mAbs varied between 6.1 and 9.1, resulting in theoretical charges between −5.3 and +16.2 at the
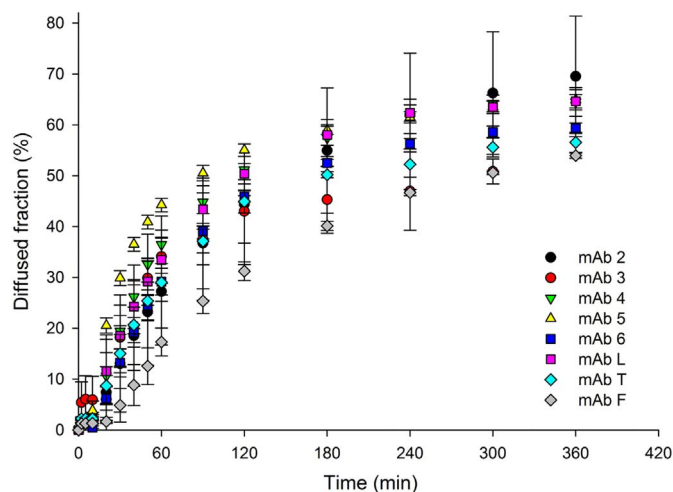

Fig. 1. Data points obtained from the Scissor experiments at 6.25 mg/mL HA concentration for the different mAbs. Data represents average ± S.D., n = 3.

physiological pH of 7.4. Protein concentration in the formulations for these mAbs ranged from 100 to 180 mg/mL. Formulation buffer concentrations varied between 20 and 200 mmol/L, but formulation pH was within a narrow range of 5.5 to 6.0. Formulation viscosities ranged between 5 and 80 cP. Additionally, the buffer species in all the formulations was histidine apart from mAbs 5 and T where arginine was used.

3.2. Profiles of mAb movement obtained using the Scissor system

In vitro concentration-time profiles of mAbs in the infinite sink for the eight mAbs tested using the Scissor system were determined over a 6 h period (Fig. 1). Data points, obtained by HPLC analysis, represent an average from three repeats with the error bars displaying the standard deviation between the experiments. Experimental work was carried out in a random order to ensure that differences observed reflected variations of the mAb formulation being examined rather than a systematic experimental error. In contrast to a previous publication using this type of in vitro model [18], the present studies included 0.02% (~3 mM) sodium azide to limit bacterial growth during the 6-hr time course used for infinite sink sampling. Importantly, four of the eight antibodies evaluated in this present study were also examined in the previous study in the absence of sodium azide with extremely similar outcomes in both cases. Of the mAbs investigated, mAb 5 left the injection cartridge the most rapidly, whereas the initial movement of mAb F was the slowest and the least overall after 6 h. Overall, mAb 2 showed the greatest extent of movement from the injection cartridge to the infinite sink compartment.

Table 1
A summary of mAb attributes and formulation parameters.

Molecule			Formulation parameters			
mAb designation	pI[a]	Theoretical charge at pH 7.4[b]	Protein concentration (mg/mL)	Buffer concentration (mmol/L)	pH	Viscosity (cP)[c]
mAb 2	7.6	1.0	125	30	6.0	80
mAb 3	8.5	4.9	100	30	5.5	3
mAb 4	9.0	8.8	150	20	5.8	7 (RT)
mAb 5	7.2	−3.3	150	200	5.5	10 (RT)
mAb 6	9.4	16.2	180	20	6.0	9 (RT)
mAb L	6.1	−5.3	125	20	5.7	7
mAb T	9.1	13.0	150	200	5.5	5
mAb F	8.7	9.0	150	30	5.5	12

[a] Determined by isoelectric focusing.
[b] Calculated based on pKa values of amino acids in the sequence.
[c] Measured at 25 °C except where otherwise denoted, RT = Room temperature.

Individual data sets and fitted curves allowed for a more refined assessment of profile variability for each mAb as it moved from the injection cartridge to the infinite sink compartment (Supplementary Fig. 1). Concentration-time profiles showed greater variability for mAbs 2 and 3 compared to the other mAbs (Supplementary Fig. 1). In the case of mAb 2, the greater variability between repeats may have been due to its higher formulation viscosity, making precise consistency between injections more challenging. Additionally, the pI of mAb 2 is close to physiological pH, where small changes in pH may influence interactions between this particular mAb and HA within the injection cartridge in a more dramatic fashion. Since the amount of mAb 3 detected in the infinite sink compartment at 300 and 360 min showed a decline compared to that observed at 240 min, data analysis was performed only up to the 240-min time point (Supplementary Fig. 1B). This decrease in mAb 3 concentration may be indicative of protein instability in the infinite sink buffer (and thus, possibly under physiological conditions as well) for longer periods of time. Indeed, some of the variability within results obtained for several of the mAb may have been due to protein instability. The inability to accurately measure mAb3 in the infinite sink after 240 min did not preclude the ability to define release characteristics for this antibody from the Scissor cartridge as there was ample data available from the earlier time points for curve fitting [25].

3.3. Parametrisation of Scissor data

Comparison of curve-fitting outcomes using the Hill equation for concentration-time averages demonstrated distinct profiles for these eight mAbs (Fig. 2). These plots demonstrate clear differences in the initial rate as well as total extent of mAb movement from the Scissor cartridge to the infinite sink compartment. These differences are reflected within the Hill curve parameters *a*, *b*, and *c*; values of *a* range from 54.49 to 82.93%, *b* from 1.32 to 1.75 and *c* from 35.60 to 116.80 min (Table 2). The standard estimate of error (SEE) for each fitted curve was also calculated, with the low values obtained demonstrating that the Hill equation fits the Scissor data well (Table 2).

3.4. Using Scissor outcomes to predict human PK parameters

Partial least squares (PLS) analysis was examined to develop a model to describe the relationship between a mAb and its formulation properties with regard to PK outcomes and identify formulation properties and physicochemical parameters of the mAbs that may potentially act as CQAs. In PLS analysis, a principal component analysis (PCA) is first conducted to determine the number of components that describe the variation within the dataset. PCA reduces the individual
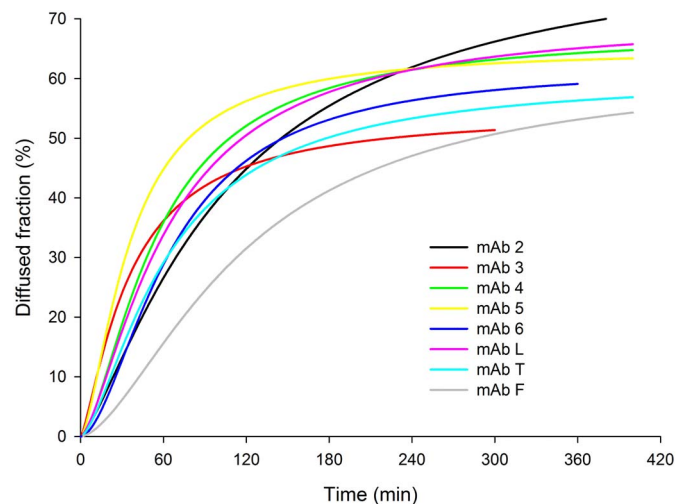
Table 2
The Hill equation analysis derived from Scissor concentration-time profiles.

	a ± SD (%)	*b* ± SD	*c* ± SD (min)	SEE
mAb 2	82.93 ± 12.04	1.32 ± 0.26	105.90 ± 30.35	7.71
mAb 3	54.49 ± 4.57	1.32 ± 0.23	35.87 ± 6.15	5.11
mAb 4	67.79 ± 2.45	1.55 ± 0.15	55.44 ± 4.01	3.87
mAb 5	64.94 ± 0.89	1.53 ± 0.07	35.60 ± 1.08	1.95
mAb 6	62.08 ± 0.90	1.75 ± 0.07	64.98 ± 1.73	1.48
mAb L	70.13 ± 2.31	1.47 ± 0.11	62.95 ± 4.20	3.01
mAb T	60.32 ± 1.66	1.51 ± 0.10	62.74 ± 3.44	2.27
mAb F	61.70 ± 2.52	1.62 ± 0.10	116.80 ± 8.14	1.88

Hill equation parameters *a*, *b* and *c* for the different mAbs and standard error of estimate (SEE) for each of the fits are presented. Values represent mean ± standard deviation (SD) (n = 3).

input parameters into latent parameters that describe the relationships that exist within the input parameters in fewer components [26]. We observed that, in addition to using the PCA results to identify the number of components to be used in the PLS-based prediction of %BA and ka values for these mAbs, the PCA analysis also furthered our understanding of Hill equation parameters; PCA for the dataset was conducted using molecule and formulation properties (Table 1) and Hill analysis parameters (Table 2) as input parameters.

A loading plot for this PCA described relationships between different input parameters in terms of the two first components of the data set (Fig. 3). Adjacent parameters within the loading plot are frequently considered as closely related [22]. A loading plot for the first two components demonstrated that the Hill equation parameter *a* was related to formulation viscosity, parameter *b* was related to mAb charge and isoelectric point as well as the formulation concentration, and parameter *c* was related to formulation pH; formulation buffer concentration was independent from the other input variables (Fig. 3).

A scree plot, which shows the fraction of total variance in a data set, was used to identify the number of components used in subsequent PLS analysis [22]. The number of components that described the variation within this dataset of mAbs obtained from the Scissor system was established at 4 for the PCA analysis (Fig. 4); the components selected should explain at least 80% of the variation within the data. In this case, a transition was observed between 3 and 4 components, with 77% or 88% of the variation explained using 3 or 4 components, respectively (Table 3). The transition from 3 to 4 components correlated with an inflection in the scree plot (Fig. 4; arrow).

Variance and correlation confidence for %BA and ka predicted values were derived using the PLS model and compared to human PK parameters.

The importance of different input parameters considered in these
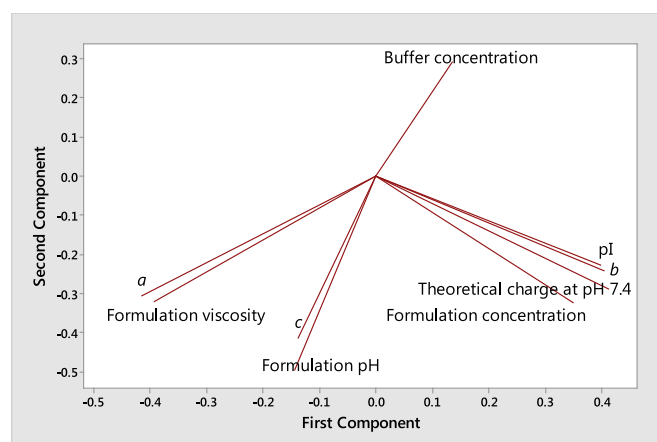

Fig. 2. Hill curves fitted to averaged data points shown in Fig. 1 for the different mAbs.

Fig. 3. Loading plot of the two first components describing the relationships between input parameters for the set of monoclonal antibodies tested in the Scissor system.
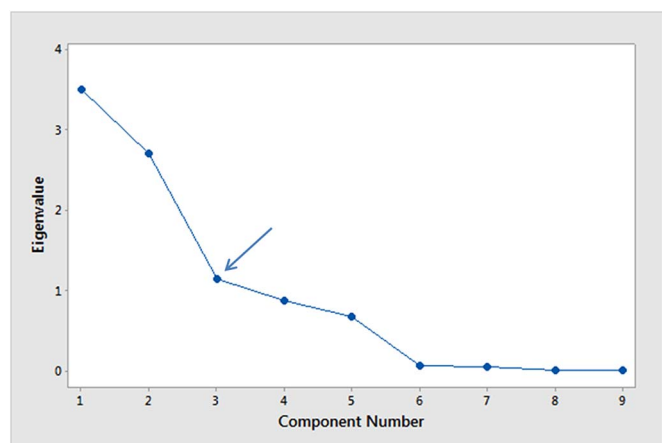
Fig. 4. Scree plot of PCA analysis with respect to input parameter number. Curve inflection (elbow) is highlighted with an arrow.

Table 3
Model selection for PLS analysis of human %BA and ka outcomes.

Number of components within the model	X-Variance	R^2 for %BA	R^2 for ka
1	0.33	0.16	0.76
2	0.53	0.73	0.80
3	0.78	0.85	0.81
4	0.88	0.88	0.88

studies within the model predicting %BA and ka can be assessed by considering the standard coefficients for each of the parameters independently in the context of these two PK outputs (Fig. 5). In the case of predicting %BA, formulation pH dominated the predictive outcomes, with values obtained for the Hill equation *b* parameter and formulation mAb concentration having diminished, but still positive, correlations (Fig. 5A). It should be noted that, individually, factors such as the Hill equation *a* parameter, formulation viscosity, formulation buffer concentration, pI, theoretical mAb charge at pH 7.4, and the Hill equation *c* parameter did not correlate with %BA outcomes. Formulation viscosity, both *a* and *c* parameters derived from the Hill equation fitting, as well as formulation buffer concentration demonstrated contributions towards the ka outcomes (Fig. 5B).
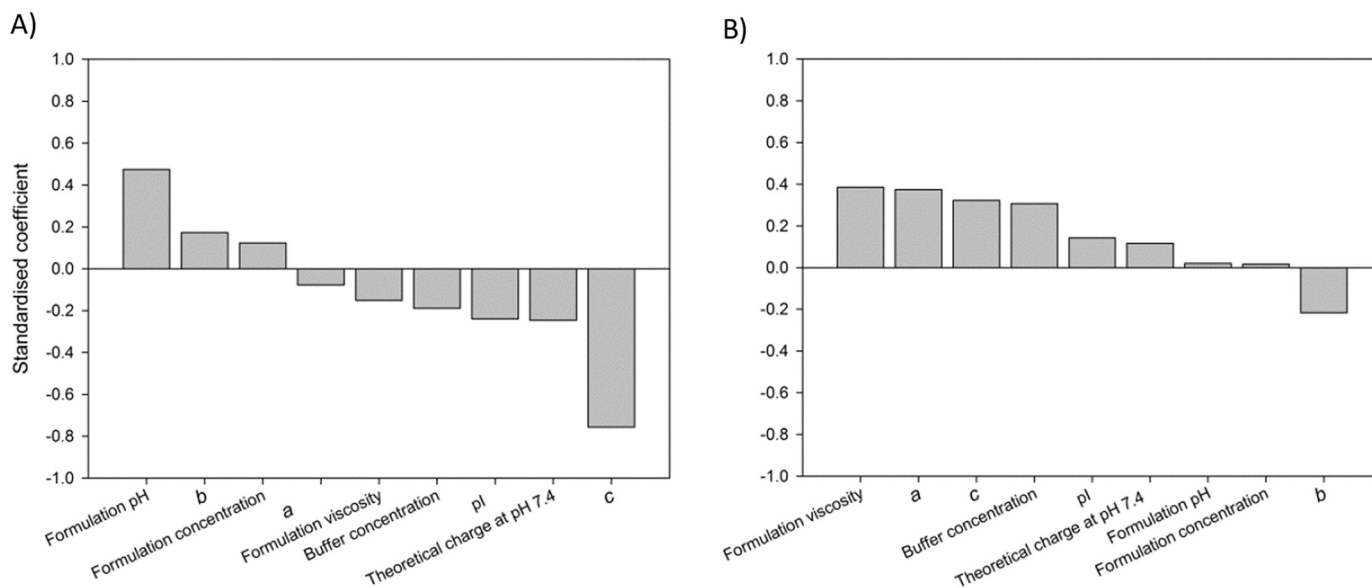
This assessment of parameters individually correlating with %BA and ka outcomes must be considered in light of the loading plot analysis performed for these elements (Fig. 3). While formulation pH dominated the predictive outcomes for %BA, there was an opposite correlation with the *c* parameter derived from the Hill equation fitting. The *b* parameter derived from the Hill equation fitting and formulation mAb concentration also showed a positive correlation with %BA outcomes. Parameters correlating with ka outcomes were much more diverse compared to that observed for %BA outcomes. Here, elements from essentially all sectors of elements identified in the loading plot analysis were represented. From this summary of data, we hypothesize that human ka outcomes for this set of mAbs are responsive to a wide range of the parameters identified and evaluated here and that a more composite perspective where multiple parameters are considered is required to obtain a predictive understanding of outcomes obtained from the Scissor system data.

The previous analysis points to multiple parameters impinging upon the movement of these eight mAbs from the injection cartridge to the infinite sink compartment in the Scissor system. We tested this hypothesis by using the PLS model to obtain predicted values for %BA and ka. Comparison of these predicted outputs correlated favourably with data obtained experimentally in humans for these same eight mAbs (Fig. 6). In this format, R^2 values were determined to be 0.87 and 0.88 for correlations of %BA and ka, respectively (Table 3).

3.5. Comparison of %BA obtained from pre-clinical models, scissor, and human

In order to better assess the potential predictive value of data obtained from the Scissor instrument, we compared %BA outcomes for the eight mAbs used in these studies in the context of any available %BA outcomes in preferred pre-clinical models of minipig and cynomolgus monkey (Fig. 7). Error bars have been omitted from the figure as different notations of error were used. Here, we could make comparisons for data in minipig for four of these antibodies and in cynomolgus monkey for four antibodies. In the case of mAb T and mAb F, preclinical information on %BA for both minipig and cynomolgus monkey was available. For mAbs 2 and 4 there is preclinical data in monkey and for mAbs 6 and L preclinical data in minipig was available. No preclinical information for mAb 4 and mAb 5 was available. The data demonstrates that the model developed in the current study for these eight mAbs
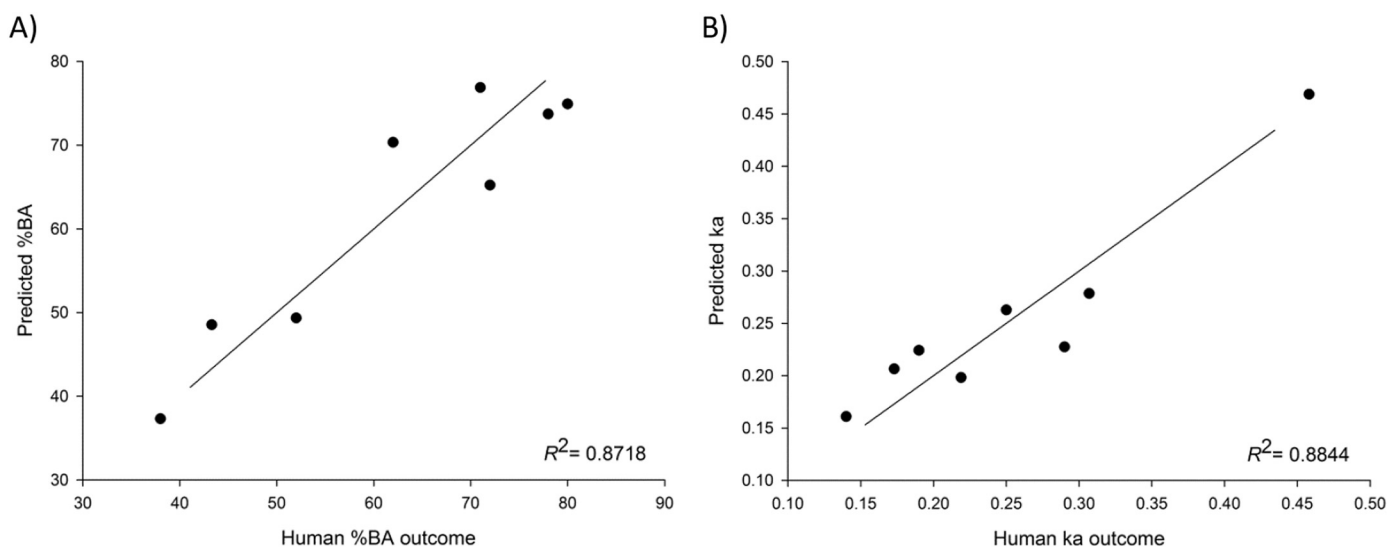

Fig. 5. Standardised coefficients calculated for the different input parameters within the model to predict A) human %BA and B) human ka outcomes for the antibodies evaluated by the Scissor system.

Fig. 6. Linear correlation assessment for human *in vivo* data and PLS-based modelling of Scissor system data outputs for A) %BA based on 4 components (R^2 = 0.87) and for B) ka based on 4 components (R^2 = 0.88).
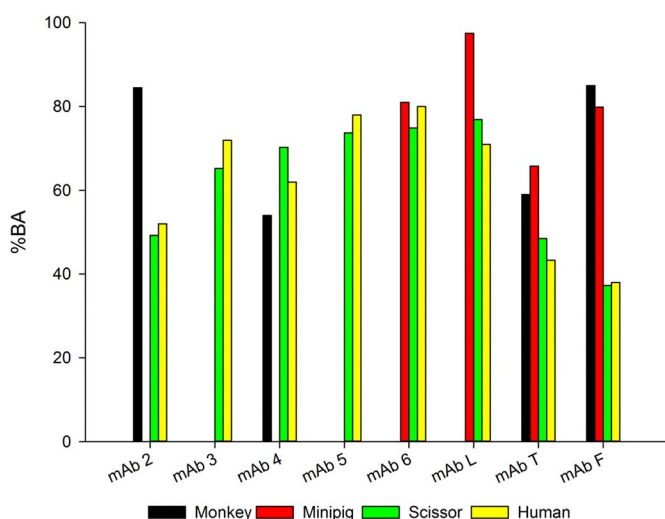

Fig. 7. %BA outcomes for the different mAbs in human, monkey and minipig as well as the %BA predicted using the model developed during the current study. The data for Scissor represents the value as predicted with the PLS model. The minipig data is adapted from [2]. For cynomolgus monkey the median value of reported ranges has been plotted. For human, minipig and cynomolgus monkey the %BA has been plotted. Error bars have been omitted due to inconsistencies in how variability was reported (SEE *vs.* RSE *vs.* %CV) for these values.

provides a better estimate of human %BA outcome than either of the preclinical species. This comparison also supported previously stated observations that %BA outcomes in monkeys are often higher than that observed in man and that neither monkey or minipig outcomes are predictive for human clinical outcomes [2,7,8]. While these preclinical models tend to overestimate %BA outcomes compared to that observed in humans, analysis described herein based upon Scissor system data provides a reasonable prediction of human %BA for this set of eight mAbs.

4. Discussion

Accurate prediction of PK/PD outcomes is critical for selecting clinical doses for first-in-human studies. Despite the value of such information, there is currently no validated *in vivo* preclinical or *in vitro* model to provide this data in the case of biopharmaceuticals, including

mAbs. Clinical outcomes for measured %BA of SC injected mAbs are frequently much lower than 100% and often in the range of 40–60% [27], despite pre-clinical model outcomes often demonstrating much higher %BA outcomes [28]. While clearance mechanism differences between humans and these non-human models may account for some of these discrepancies [29], a systematic examination of potential differences in the minipig model has failed to identify critical factors that might provide a predictive model [2]. While still highly valuable to assess a plethora of safety and toxicological concerns, the anatomical and physiological differences of the SC space for these various pre-clinical animal models and humans may provide an explanation for this lack of predictability for %BA and ka outcomes [30]. The recently-developed Scissor system potentially represents a model that may be useful to investigate events associated with the SC administration of a biopharmaceutical as it was established using parameters intended to emulate dynamic events occurring at the SC injection site that might occur after introduction of a non-physiological formulation [18]. Four mAbs were previously examined in a prototype version of the Scissor system, with a clear correlation of mAb movement from the artificial injection site being established with clinical %BA outcomes being identified [18]. We now extend those studies with the addition of four additional human mAbs to a make a total set of eight human mAbs where clinical %BA outcomes were available using commercially-available Scissor system.

The absolute concentration of HA in the human SC site is unknown [30]. In the prototype Scissor system, 10% HA content was used to establish a SC injection site environment that correlated with clinical %BA outcomes for the first four mAbs examined [18]. In the commercial Scissor system used in the current study, we found that 6.25% HA was sufficient for correlation with clinical %BA outcomes for the set of eight mAbs examined. This alteration in HA concentration accounted for a change in the injection site cartridge configuration from a dialysis cassette to a cuvette where the volume and path length for release into the infinite sink were different: in the prototype system, the cartridge volume was 3 mL and in the commercial version cartridge volume is now 5 mL. Thus, the total amount of HA within the cartridge remained constant between the two systems (Suppl. Fig. 3). The thin dialysis cassette used in the prototype system limited examination of differences of initial rates and profile of mAb movement from the simulated injection site to the infinite sink compartment; the current injection cartridge configuration provides greater diffusional distances prior to detection within the external buffer, emulating the infinite sink of systemic circulation in the case of the cassette *versus* the cartridge.

Thus, the commercial Scissor system appears to provide a better design to compared to the prototype format for examining diffusional properties of injected formulation components.

While the goal of the Scissor instrument is to mimic certain physical, chemical and physiological properties of SC tissue, we cannot definitively state that all of the essential elements of the SC injection site that define the fate of these mAbs in patients have been incorporated. What we can say is that the criteria used to evaluate the fate of this set of eight mAbs in the current system was sufficient to demonstrate different diffusional behaviours that correlated well with %BA and ka outcomes in man. Using the commercial Scissor system, we could identify a number of formulation component parameters that appeared to control the movement of these eight mAbs from the injection cassette into the infinite sink buffer reservoir. While all eight of the mAbs examined in these studies had very similar molecular weights, they had distinct surface charge properties and their formulations varied in the buffer used, pH, viscosity, and ionic strength. Thus, it is not surprising that we identified a combination of factors that correlated with the movement of these mAbs from the injection site cartridge into the infinite sink compartment and that could be envisioned to affect their fate following their SC injection in patients. It is unclear if additional parameters beyond those identified in the present studies, such as other physico-chemical properties of the mAbs or aspects of the formulations, could be used to further refine the predictive outcomes demonstrated presently. The information acquired through the present parameterization, however, appears to be potentially useful for a QbD approach for formulation design and optimisation.

Parametrisation of data obtained from the Scissor system was initiated by curve fitting with the Hill equation to describe binding interactions similar to those observed in a variety of biochemical and pharmacological processes [31,32]. The Hill equation parameter a describes the maximum amount of drug fraction moving from the Scissor cartridge to the infinite sink compartment. Using PCA, we could show this parameter was affected by formulation viscosity, further supporting the benefit of the commercial cartridge dimensions compared to the prototype dialysis cassette format as a refinement of the system that improved the ability of the Scissor system to characterize diffusional properties of injected materials. We failed to observe a direct correlation between a and %BA for this series of mAbs in humans (data not shown, $R^2 = 0.0173$). Thus, additional physical properties of the mAb and formulation components must affect the fate of these mAbs following SC injection, possibly through processes that involve both specific and/or non-specific electrostatic interactions between positively-charged domains of the mAb and the most common negatively-charged material present in the SC space, HA [33].

As b is a coefficient of the Hill equation that should describe the drug movement from the Scissor cartridge to the infinite sink compartment, we hypothesised that this parameter may help describe initial interactions between the mAb and injection compartment components that could involve initial solubility and/or binding events. PCA loading plot analysis demonstrated a close relationship between the pI of the mAb, its charge at physiological pH, concentration of mAb in the formulation and the value of b. This correlation is interesting as historically the value of b obtained from the Hill equation was considered as an indicator of binding reaction stoichiometry; the value is now more widely accepted as an indicator of the mass balance of binding events [34]. Therefore, non-specific electrostatic interactions between HA and any positively charged molecules within the Scissor injection site cartridge can potentially influence the rate and extent of drug movement from the injection cuvette. Thus, profiles of mAb release from the injection site compartment for the formulations tested in the current studies should have been sensitive to both the charge magnitude and mAb concentration available for these interactions.

We also hypothesised that parameter c, which describes the time at which half of a has been achieved, may be related to ka outcomes in humans as both parameters describe time dependence of the drug

absorption process. Interestingly, no direct correlation between ka and parameter c was observed (data not shown, $R^2 = 0.3888$). Based upon the PCA loading plot that demonstrates a relationship between c and formulation pH, the value of c appears to reflect how interactions between a mAb and SC tissue components might be altered as the environment of the injection site transitions from that of the formulation environment to conditions that better reflect the homeostatic state of the body [18]. Given that no direct correlation between any of the Hill parameters and human PK properties could be identified, a PLS analysis of the dataset with the aim of predicting %BA and ka based on mAb molecule and formulation properties as well as the Hill parameters was conducted. Importantly, PLS analysis is best suited for analysing data where the input parameters outnumber the observations and where the data is collinear [35].

Using the approach of PLS analysis that included Hill coefficients, protein physicochemical properties, and formulation characteristics for these mAbs, models for both %BA and ka were simultaneously developed, *i.e.* the same model was used for predicting both %BA and ka, not separate models. Here, correlations for a set of parameters to predict both %BA and ka were achieved with R^2 values of 0.8718 and 0.8844, respectively. These outcomes support the importance of the input parameters used within a model; the larger the magnitude of the standardised coefficient, the more important the parameter within the model. Using this approach, the Hill equation parameter c was identified as the most important parameter within the model for %BA with a coefficient of approximately -0.8. Formulation pH was the next most critical with a coefficient of approximately $+0.5$. Together, this suggests electrostatic interactions taking place between formulation components and the mAb may play a dominant role in determining %BA. For predicting ka, multiple parameters, including formulation viscosity, all the Hill parameters and buffer concentration have similar standardised coefficients of approximately $+0.3$ to $+0.4$. These findings indicate that the rate at which these mAbs are absorbed from the SC injection site is influenced by multiple factors.

Finally, it is also noteworthy that when the PLS prediction of the human PK properties for this set of mAbs was performed with either only the Hill parameters or only the molecule and formulation properties, poor correlations were obtained (Supplementary Figs. 2 and 3). Therefore, our data indicates that the knowledge of both the molecule and formulation properties as well as the diffusional properties of the mAbs in the Scissor model are required for meaningful predictions of human PK properties.

5. Conclusions

Previous studies with the prototype Scissor system identified a trend toward correlation with human %BA outcomes. We have now used the commercial system to refine parameters associated with the mAb and its formulation to better understand this correlation. The Hill equation was successfully used for parameterising these Scissor profiles, with parameters a, b, and c being attributable to molecule and formulation properties: parameter a being related to formulation viscosity, parameter b being related to pI, physiological mAb charge at neutral pH, and formulation concentration, while parameter c related to formulation pH. A PLS model was developed using molecule properties, formulation properties, and the three Hill parameters that provided strong predictive power for human %BA and ka outcomes. This approach, and the resultant model, provides a novel tool for the antibody engineer or formulation scientist to assess how an individual factor (*e.g.*, molecule charge at neutral pH, formulation pH, *etc.*) might directly impact PK outcomes of the molecule. The unavoidable shortcoming of this conclusion is the inability to validate specific hypotheses. For example, what happens when formulation pH is tuned by 0.5 units? As human PK data is required to adequately assess the outcome, this can be prohibitively expensive. Continued use of the Scissor system and this approach with additional mAbs will likely provide an opportunity for

further model validation and/or refinement and possibly for direct testing of such hypotheses.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jconrel.2018.01.015.

References

[1] J. Wang, S. Iyer, P.J. Fielder, J.D. Davis, R. Deng, Projecting human pharmacokinetics of monoclonal antibodies from nonclinical data: comparative evaluation of prediction approaches in early drug development, Biopharm. Drug Dispos. 37 (2016) 51–65.

[2] Y. Zheng, D.B. Tesar, L. Benincosa, H. Birnbock, C.A. Boswell, D. Bumbaca, K.J. Cowan, D.M. Danilenko, A.L. Daugherty, P.J. Fielder, H.P. Grimm, A. Joshi, N. Justies, G. Kolaitis, N. Lewin-Koh, J. Li, S. McVay, J. O'Mahony, M. Otteneder, M. Pantze, W.S. Putnam, Z.J. Qiu, J. Ruppel, T. Singer, O. Stauch, F.P. Theil, J. Visich, J. Yang, Y. Ying, L.A. Khawli, W.F. Richter, Minipig as a potential translatable model for monoclonal antibody pharmacokinetics after intravenous and subcutaneous administration, MAbs 4 (2012) 243–255.

[3] J. Ling, H. Zhou, Q. Jiao, H.M. Davis, Interspecies scaling of therapeutic monoclonal antibodies: initial look, J. Clin. Pharmacol. 49 (2009) 1382–1402.

[4] A.V. Kamath, Translational pharmacokinetics and pharmacodynamics of monoclonal antibodies, Drug Discov. Today Technol. 21-22 (2016) 75–83.

[5] Y.S. Lin, C. Nguyen, J.L. Mendoza, E. Escandon, D. Fei, Y.G. Meng, N.B. Modi, Preclinical pharmacokinetics, interspecies scaling, and tissue distribution of a humanized monoclonal antibody against vascular endothelial growth factor, J. Pharmacol. Exp. Ther. 288 (1999) 371–378.

[6] L. Kagan, D.E. Mager, Mechanisms of subcutaneous absorption of rituximab in rats, Drug Metab. Dispos. 41 (2013) 248–255.

[7] T.A. McDonald, M.L. Zepeda, M.J. Tomlinson, W.H. Bee, I.A. Ivens, Subcutaneous administration of biotherapeutics: current experience in animal models, Curr. Opin. Mol. Ther. 12 (2010) 461–470.

[8] W.F. Richter, S.G. Bhansali, M.E. Morris, Mechanistic determinants of biotherapeutics absorption following SC administration, AAPS J. 14 (2012) 559–570.

[9] A.M. Fathallah, S.V. Balu-Iyer, Anatomical, physiological, and experimental factors affecting the bioavailability of sc-administered large biotherapeutics, J. Pharm. Sci. 104 (2015) 301–306.

[10] J.Q. Dong, D.H. Salinger, C.J. Endres, J.P. Gibbs, C.P. Hsu, B.J. Stouch, E. Hurh, M.A. Gibbs, Quantitative prediction of human pharmacokinetics for monoclonal antibodies: retrospective analysis of monkey as a single species for first-in-human prediction, Clin. Pharmacokinet. 50 (2011) 131–142.

[11] R. Deng, S. Iyer, F.P. Theil, D.L. Mortensen, P.J. Fielder, S. Prabhu, Projecting human pharmacokinetics of therapeutic antibodies from nonclinical data: what have we learned? MAbs 3 (2011) 61–66.

[12] K.L. Gill, I. Gardner, L. Li, M. Jamei, A bottom-up whole-body physiologically based pharmacokinetic model to mechanistically predict tissue distribution and the rate of subcutaneous absorption of therapeutic proteins, AAPS J. 18 (2016) 156–170.

[13] R. Deng, Y.G. Meng, K. Hoyte, J. Lutman, Y. Lu, S. Iyer, L.E. DeForge, F.P. Theil, P.J. Fielder, S. Prabhu, Subcutaneous bioavailability of therapeutic antibodies as a function of FcRn binding affinity in mice, MAbs 4 (2012) 101–109.

[14] A.M. Fathallah, M.R. Turner, D.E. Mager, S.V. Balu-Iyer, Effects of hypertonic buffer composition on lymph node uptake and bioavailability of rituximab, after subcutaneous administration, Biopharm. Drug Dispos. 36 (2015) 115–125.

[15] S.M. Short, R.P. Cogdill, F. D'Amico, J.K. Drennen, C.A. Anderson 3rd, A new definition of pharmaceutical quality: assembly of a risk simulation platform to investigate the impact of manufacturing/product variability on clinical performance, J. Pharm. Sci. 99 (2010) 5046–5059.

[16] N. Alt, T.Y. Zhang, P. Motchnik, R. Taticek, V. Quarmby, T. Schlothauer, H. Beck, T. Emrich, R.J. Harris, Determination of critical quality attributes for monoclonal antibodies using quality by design principles, Biologicals 44 (2016) 291–305.

[17] M. Li, S. Sander, J. Duan, S. Rosencrance, S.P. Miksinski, L. Yu, P. Seo, B. Rege, Scientific and regulatory considerations in solid oral modified release drug product development, AAPS J. 18 (2016) 1406–1417.

[18] H.M. Kinnunen, V. Sharma, L.R. Contreras-Rojas, Y. Yu, C. Alleman, A. Sreedhara, S. Fischer, L. Khawli, S.T. Yohe, D. Bumbaca, T.W. Patapoff, A.L. Daugherty, R.J. Mrsny, A novel in vitro method to model the fate of subcutaneously administered biopharmaceuticals and associated formulation components, J. Control. Rel. 214 (2015) 94–102.

[19] A.V. Hill, The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curves, J. Physiol. 40 (1910) iv–vii.

[20] J.N. Weiss, The hill equation revisited: uses and misuses, FASEB J. 11 (1997) 835–841.

[21] A. Mendyk, R. Jachowicz, K. Fijorek, P. Dorożyński, P. Kulinowski, S. Polak, KinetDS: An Open Source Software for Dissolution Test Data Analysis, Dissolution Technol. 19 (2012) 6–11.

[22] R. Bro, K. Kjeldahl, A.K. Smilde, H.A. Kiers, Cross-validation of component models: a critical look at current methods, Anal. Bioanal. Chem. 390 (2008) 1241–1251.

[23] C. Hanson, M. Sieverts, E. Vargis, Effect of principal component analysis centering and scaling on classification of mycobacteria from Raman spectra, Appl. Spectrosc. 71 (2017) 1249–1255.

[24] E.E. Johnson, H.G. Mueller, T.A. Ricketts, Statistically derived factors of varied importance to audiologists when making a hearing aid brand preference decision, J. Am. Acad. Audiol. 20 (2009) 40–48.

[25] W. Cao, E.M. De La Cruz, Quantitative full time course analysis of nonlinear enzyme cycling kinetics, Sci. Rep. 3 (2013) 2658.

[26] P.S. Gromski, H. Muhamadali, D.I. Ellis, Y. Xu, E. Correa, M.L. Turner, R. Goodacre, A tutorial review: metabolomics and partial least squares-discriminant analysis–a marriage of convenience or a shotgun wedding, Anal. Chim. Acta 879 (2015) 10–23.

[27] R.J. Keizer, A.D. Huitema, J.H. Schellens, J.H. Beijnen, Clinical pharmacokinetics of therapeutic monoclonal antibodies, Clin. Pharmacokinet. 49 (2010) 493–507.

[28] I.P. Nnane, Z. Xu, H. Zhou, H.M. Davis, Non-clinical pharmacokinetics, prediction of human pharmacokinetics and first-in-human dose selection for CNTO 5825, an anti-Interleukin-13 monoclonal antibody, Basic Clin. Pharmacol. Toxicol. 117 (2015) 219–225.

[29] M.A. Tabrizi, C.M. Tseng, L.K. Roskos, Elimination mechanisms of therapeutic monoclonal antibodies, Drug Discov. Today 11 (2006) 81–88.

[30] H.M. Kinnunen, R.J. Mrsny, Improving the outcomes of biopharmaceutical delivery via the subcutaneous route by understanding the chemical, physical and physiological properties of the subcutaneous injection site, J. Control. Rel. 182 (2014) 22–32.

[31] N.D. Eddington, P. Marroum, R. Uppoor, A. Hussain, L. Augsburger, Development and internal validation of an in vitro-in vivo correlation for a hydrophilic metoprolol tartrate extended release tablet formulation, Pharm. Res. 15 (1998) 466–473.

[32] B.I. Kurganov, A.V. Lobanov, A. Borisov, A.N. Reshetilov, Criterion for Hill equation validity for description of biosensor calibration curves, Anal. Chim. Acta 427 (2001) 11–19.

[33] J.R. Fraser, T.C. Laurent, U.B. Laurent, Hyaluronan: its nature, distribution, functions and turnover, J. Intern. Med. 242 (1997) 27–33.

[34] S. Goutelle, M. Maurin, F. Rougier, X. Barbaut, L. Bourguignon, M. Ducher, P. Maire, The Hill equation: a review of its capabilities in pharmacological modelling, Fundam. Clin. Pharmacol. 22 (2008) 633–648.

[35] B. Hemmateenejad, M. Akhond, F. Samari, A comparative study between PCR and PLS in simultaneous spectrophotometric determination of diphenylamine, aniline, and phenol: effect of wavelength selection, Spectrochim. Acta A Mol. Biomol. Spectrosc. 67 (2007) 958–965.