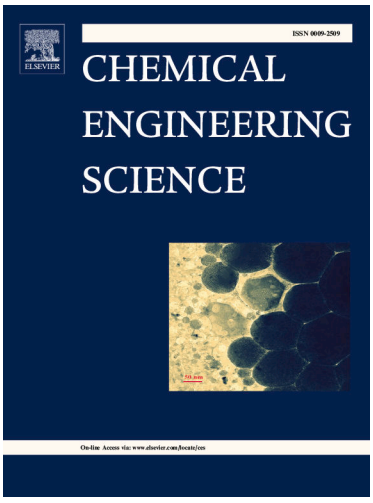
Journal Pre-proofs



Optimal design of infusion tests for the identification of physiological models of acquired von Willebrand syndrome

F. Galvanin, E. Galletta, A. Bertomoro, V. Daidone, A. Casonato

PII: S0009-2509(23)01216-2
DOI: https://doi.org/10.1016/j.ces.2023.119660
Reference: CES 119660

To appear in: *Chemical Engineering Science*

Received Date: 22 August 2022
Revised Date: 3 December 2023
Accepted Date: 18 December 2023

Please cite this article as: F. Galvanin, E. Galletta, A. Bertomoro, V. Daidone, A. Casonato, Optimal design of infusion tests for the identification of physiological models of acquired von Willebrand syndrome, *Chemical Engineering Science* (2023), doi: https://doi.org/10.1016/j.ces.2023.119660

Optimal design of infusion tests for the identification of physiological models of acquired von Willebrand syndrome

F. Galvanin[2], E. Galletta[1], A. Bertomoro[1], V. Daidone[1], A. Casonato[1]

[1]*Department of Medicine, University of Padua Medical School*

[2]*Department of Chemical Engineering, University College London,*

Abstract

Acquired von Willebrand syndrome (AVWS) is a bleeding disorder resembling inherited Von Willebrand disease (VWD) characterised by a qualitative and/or quantitative deficiency of von Willebrand factor (VWF) that occurs in patients with no personal or family history of bleeding as a result of underlying pathological conditions. To treat AVWS patients, desmopressin (DDAVP) and plasma-derived VWF concentrates are the primary therapies for spontaneous acute bleeding episodes and for preventing bleeding during invasive or surgical procedures. Pharmacokinetic (PK) models have been recently developed and applied to characterize VWD and AVWS, but these models cannot be calibrated from infusion tests data, and their calibration requires stressful 24-hours long tests to be carried out on subjects to achieve a satisfactory estimation of the individual haemostatic parameters. The objectives of this paper are: *i*) to present a new physiological model of VWD including exogenous infusion of plasma-derived VWF concentrates, suitable to describe AVWS; *ii*) to validate the newly proposed model from clinical data; *iii*) to quantify the information that can be obtained from clinical tests using different VWF concentrates by applying model-based design of experiments (MBDoE) techniques. Results show that the newly developed model calibrated from infusion data allows to estimate precisely the full set of haemostatic parameters for a subject affected by AVWS preserving the same level of information obtained from conventional tests. Most importantly, results show that the overall duration of infusion tests for the identification of key haemostatic parameters can significantly be reduced from 24 hours to 2.5 hours.

I. Introduction

Von Willebrand disease (VWD) is one of the most diffuse bleeding disorders in humans, caused by an alteration of von Willebrand factor (VWF), a key multimeric glycoprotein present in the bloodstream playing a crucial role in the haemostatic process (Lillicrap, 2007). VWF mediates platelet aggregation and thrombus growth and it binds, transports and protects coagulation factor VIII. VWD-induced alteration of VWF in the bloodstream causes symptoms ranging from sporadic nosebleeds and mild bleeding from small lesions in skin to acute thrombocytopenia or prolonged bleeding episodes (Sadler, 1998). Diagnosis of VWD is complex due to the heterogeneous nature of the disorder, characterised by a number of VWD types and subtypes (Groot et al., 2009). Acquired von Willebrand syndrome (AVWS) is a rare and very heterogenous bleeding disorder (Tiede et al., 2011) resembling inherited VWD that occurs in patients with no personal or family history of bleeding. AVWS is not caused by any genetic defects but may be the result of underlying pathological conditions, including lympho- and myeloproliferative disorders, solid tumours, immune diseases, cardiovascular disorders, hypothyroidism, diabetes, and infectious diseases, or the side effects of drugs (Galletta et al., 2021). Subjects affected by AVWS present severe bleeding symptoms requiring urgent and often multiple treatment (Tiede et al., 2011).

Kinetic models of different degree of complexity have been recently proposed for the characterisation of VWD (Gezsi et al., 2010; Casonato et al., 2011; Galvanin et al., 2014a; Ferrari et al., 2017; Taverna et al., 2019) and AVWS (Galletta et al., 2021) based on the estimation of subject-specific haemostatic parameters to elucidate the critical pathways involved in the disease characterization and paving the way to model-based approaches to VWD diagnosis (Galvanin et al., 2014b; Castaldello et al., 2017). However, the complexity of the proposed models requires the execution of time-consuming (24 h long) and cumbersome non-routine tests like the desmopressin response test (DDAVP) to be carried out on the subjects to precisely estimate the individual haemostatic parameters. During DDAVP test desmopressin is administered subcutaneously at a prescribed dose to patients (0.3 – 0.4 μg/kg body weight) (Casonato et al., 2006, Galletta et al., 2021), and blood samples are collected at regular fixed times (after 15, 30, 60, 120, 180, 240, 480 and 24 h from the injection). DDAVP induces an acute release of VWF stored in the Weibel Palade bodies of the endothelial cells, so the time course of VWF antigen (VWF:Ag) and VWF collagen binding (VWF:CB) can be quantitatively analysed after DDAVP by mean of dynamic models describing the kinetics of variation in VWF concentration (Casonato et al., 2006). VWF kinetics in plasma depend on three key factors: *i*) the amount of VWF released and the rate of release; *ii*) ADAMTS-13 proteolytic activity (i.e. the capability of the enzyme to reduce VWF into smaller multimeric forms); and *iii*) VWF clearance, i.e. elimination from blood stream. As illustrated by Budde at al. (2006) DDAVP administration is not effective to treat all the types of VWD and may be contraindicated in patients with certain co-morbidities including atherosclerosis, heart failure or other conditions requiring diuretic treatment, as well as in very young children or in patients older than 65–70 years. For these reasons, plasma-derived VWF/FVIII concentrates (Berntorp, 2009) are becoming the current standard for controlling acute bleeding episodes or as prophylaxis for invasive or surgical procedures. The available VWF concentrates differ in their purification and pathogen removal as well as in VWF multimer content and activity (Auerswald and Kreuz, 2008), aspects which affect therapeutic safety and efficacy. So far there have been no specific studies or kinetic models developed to characterise the exogenous VWF infusion of VWF concentrates, and the quantification of the intrinsic information that can be obtained from infusion tests when different VWF concentrates are used. A key challenge in the identification of physiological models of VWD is the estimability of subject-specific haemostatic parameters, i.e. their precise estimation from potentially limited amount of data, and the level of information acquired from clinical tests is strictly related to the protocol used for dynamic model calibration (Villaverde et al., 2022).

In this paper a new kinetic model including VWF exogenous infusion is developed, based on modifications of the post-DDAVP model proposed by Ferrari and coworkers (2017). The new model is calibrated from clinical data to characterise a subject affected by AVWS and its capability

on estimating subject-specific haemostatic parameters is compared against a standard desmopressin response test. Model-based design of experiments techniques (MBDoE) (Franceschini and Macchietto, 2008a; Chakrabarty et al., 2013) are then applied to the newly developed model to *i)* quantify the distribution of information in time, suggesting the optimal allocation of sampling points during the test (Galvanin and Bezzo, 2018); *ii)* to compare the information that can be obtained from the optimised infusion test when different VWF concentrates are used. Results show the potential of infusion tests in drastically decreasing the time and effort required for the disease characterization and diagnosis, by allowing a quick and precise identification of the full set of haemostatic parameters. The subject-specific calibrated model can then be used for personalised AVWS monitoring purposes and to improve the dosage of VWF concentrates in the treatment of severe forms of AVWS.

II. Available clinical dataset

The features of the available clinical dataset used in this study are illustrated in Table 1. AVWS patient and normal subjects were studied in accordance with the Helsinki Declaration, after obtaining their written informed consent, and our ethical board's approval of the study. Clinical data have been supplied by the Hospital of Padua. DDAVP (1-desamino-8-D-argine vasopressin; Emosint, Sclavo, Italy) was administered subcutaneously at a dose of 0.3 μg kg^{-1}. Blood samples were collected before and 15, 30, 60, 120, 180, 240, 480 min and 24 h after administering DDAVP for a pool of health subjects (O/non-O blood group) and for a subject affected by AVWS. The same subject was also treated with exogenous intravenous administration of Haemate P, a VWF concentrate commonly used in the treatment of VWD. After administering 2,000 U of Haemate P (Behring GMBH, Hattersheim am Main, Germany), blood samples were collected at 4, 15, 30, 60, 120, 180, 240, 360, 480 minutes and at 24 hours. Note that in clinical practice sampling points are concentrated at the beginning of the test when Haemate P is infusion, as intravenous administration produces faster dynamics in VWF as compared to subcutaneous DDAVP administration.

Table 1. *Illustration of the features of the available dataset.*

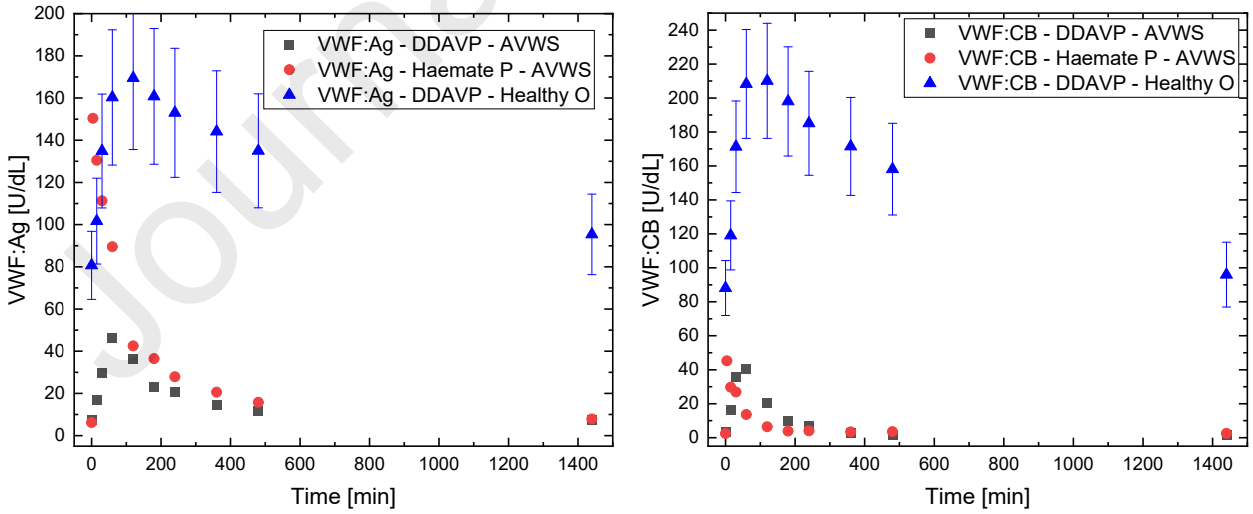
Subjects	Number of subjects (Origin)	Age years	Sex M/F	Body Weight kg	Blood group O/nonO	VWF:Ag U/dL	VWF:CB U/dL	DDAVP/Haemate P
AVWS	1 (Caucasian)	75	1/0	78	1/0	9.0	4.4	√ / √
Normal subjects (Control)	42 (Caucasian)	19-52	20/22	43-95	17/25	96.3±46.5	99.4±45.9	√ / x
Normal	-	-	-	-	-	*60-160*	*65-150*	-

range								

Figure 1a and Figure 1b show the collected data from clinical tests for O healthy subjects and the subject affected by AVWS in terms of VWF antigen (VWF:Ag) and VWF collagen binding (VWF:CB). As VWF exists across a multimeric range, VWF:Ag represents a measure of the overall VWF amount in plasma for the subjects, including high and low molecular weight species, while VWF:CB is a measure of the amount of high molecular weight species only. High molecular weight multimers are more active in the coagulation process, and their deficiency leads to prolonged bleeding in subjects even after small lesions or scars. There are several important aspects to observe from Figure 1:

- AVWS leads to significantly reduced VWF:Ag and VWF:CB levels before and after DDAVP administration; these levels are considerably low also at basal state, i.e. see values at $t = 0$ min when compared to healthy O subjects;
- The infusion of Haemate P in the AVWS patient prompted a sudden increase in low molecular weight species (see VWF:ag peak in the first observation after 4 minutes), but produced a limited release of high multimeric species (relatively low VWF:CB levels) during the test;
- For the AVWS patient very low VWF:CB levels are observed both after DDAVP and after Haemate P infusion;
- The infusion of Haemate P forces a very fast dynamic VWF response, i.e. by 240 minutes after the administration most of the VWF:CB was no longer detectable.

Based on these observations it is of primary importance to understand from this study: *i*) if infusion tests be exploited for the identification of subject-specific kinetics; *ii*) if infusion tests can be more or less informative than a standard DDAVP test, i.e. if this test can provide a more precise and accurate estimation of haemostatic parameters; *iii)* if the proposed sampling point allocation in time is optimal, and if the test duration can be shortened preserving the required level of information.



(a) (b)

Figure 1. *Examples of (a)VWF:Ag and (b) VWF:CB measurements after DDAVP for healthy O (blue triangles), AVWS subject (black squares) and after Haemate P administration for the AVWS subject (red*

circles).

III. Methodology

The study involves the quantitative characterization of the metabolic pathways involved in post-DDAVP and VWF infusion studies by developing and validating kinetic models that can be tailored to the specificity of each single subject. The following sections contain a description of the models used in the study (Sections III.1-III.2) and the techniques used for model validation and model-based design of clinical tests (Section III.3).

III.1. Post-DDAVP model

The Ferrari and coworkers (2017) dynamic model has been developed to represent the evolution in time of different multimeric species after DDAVP administration. This model structure is illustrated in Figure 2a.

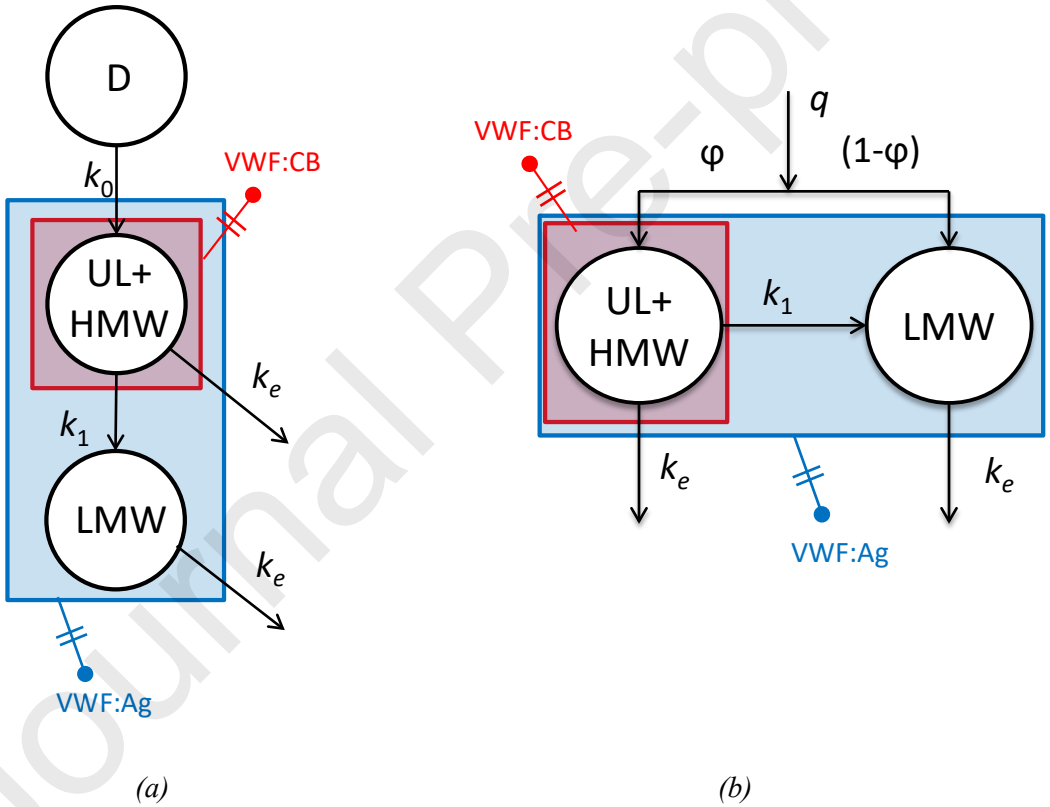


Figure 2. (a) *Compartmental structure of the post-DDAVP model of VWD proposed by Ferrari et al. (2017) representing the distribution of ultralarge + high (UL + HMW) and low molecular weight (LMW) multimers in the blood; (b) Compartmental structure of the model proposed in this study including exogenous VWF infusion, where q is the infusion rate [U/min] and φ is an effective partition constant defining the split between (UL + HMW) and LMW. For both models the accessible compartments through VWF:Ag and VWF:CB measurements are indicated by the blue and red box respectively.*

The model assumes that after DDAVP administration both high molecular weight (HMW) and ultralarge molecular weight (UL) VWF multimers are released from the endothelial cells. Then, HMW and UL multimers are cleaved to low molecular weight (LMW) multimers by the

metalloprotease ADAMTS-13 before being finally eliminated from the bloodstream. This model is described by a system of differential and algebraic equations (DAEs) described by equations (1-6). Differential equations are written as

$$\frac{dx^{UL+HMW}}{dt} = k_0 D\, e^{-k_0(t-t_{max})} - k_1\left(x^{UL+HMW} - x_b^{UL+HMW}\right) - k_e\left(x^{UL+HMW} - x_b^{UL+HMW}\right) \quad (1)$$

$$\frac{dx^{LMW}}{dt} = k_1\left(x^{UL+HMW} - x_b^{UL+HMW}\right) - k_e\left(x^{LMW} - x_b^{LMW}\right) \quad (2)$$

where x^{UL+HMW} and x^{LMW} are the amount of UL+HMW and LMW multimer units [U] contained in the plasma; the subscript b refers to the basal state (i.e. the state of the subject before the DDAVP test starts); t is the test execution time and t_{max} is the time at which the release profile peaks. In the kinetic model k_0 [min^{-1}] represents the kinetics of VWF release from endothelial cells; k_1 [min^{-1}] the proteolytic conversion of large and ultra-large VWF multimers into LMW multimers and k_e [min^{-1}] represents the clearance of VWF from the circulation, which is assumed to be the same for both the UL+HMW multimers and the LMW multimers (Casonato et al., 2002). The amount of VWF released, Q^{DDAVP} [U], can be calculated from

$$Q^{DDAVP} = \int_0^{\tau} k_0 D e^{-k_0(t-t_{max})} dt \quad (3)$$

where D [U/dL] is a release parameter and τ is the overall test duration [min]. It is important to notice that, for a given subject, parameter k_0 quantifies the rate of release, while D is related to the amount of VWF released from the endothelial cells after a standardised DDAVP dose of 0.3 μg/kg body weight. A limitation of this model is that it does not include the amount of DDAVP administered to the subject as explicit variable. The measured responses are the antigen concentration y^{AG} [U/dL] and collagen binding concentration y^{CB} [U/dL] which are defined, respectively, by the following algebraic equations:

$$y^{AG} = \frac{x^{UL+HMW} + x^{LMW}}{V_d} \quad (4)$$

$$y^{CB} = \frac{x^{UL+HMW}}{V_d} \quad (5)$$

It is assumed that VWF:CB measurements can quantify the amount of UL and HMW multimers in plasma, while VWF:Ag measurements quantify the overall amount of VWF multimers (i.e. UL + HMW + LMW). A correction was introduced in the definition of the collagen binding measurements in order to account for the different affinity of multimers to collagen observed in clinical tests using the following algebraic equation:

$$y^{CB\prime} = k y^{CB} \frac{y_b^{AG}}{y_b^{CB}} \quad (6)$$

where k is a correction factor to be estimated from data, and y_b^{AG} and y_b^{CB} are antigen and collagen binding concentration measurements [U/dL] determined at basal state. In (4) and (5) V_d = 40 mL/kg$_{bw}$ is the approximate distribution volume according to Menache and coworkers [15]. Initial conditions for differential state variables (i.e. at t = 0) can be calculated from basal antigen and collagen binding concentrations:

$$x(0) = \left[x_b^{UL+HMW} \quad x_b^{LMW} \right] = \left[y_b^{CB} V_d \quad y_b^{AG} V_d - y_b^{CB} V_d \right]. \tag{7}$$

The full set of model parameters to be estimated from available post-DDAVP VWF:Ag and VWF:CB measurements is $\boldsymbol{\theta}^{\mathbf{DDAVP}} = \left[k_0 \quad k_1 \quad k_e \quad D \quad k \quad y_b^{CB} \quad t_{max} \right]$. The DDAVP test needs to be carried out on each single subject (Ferrari et al., 2017) to achieve a statistically precise estimation of the individual kinetic parameters to accurately quantify the rate of VWF release, proteolysis and elimination from plasma.

III.2. Model including exogenous infusion of VWF concentrates

In the infusion models the infused VWF is distributed among the (UL + HMW) and LMW compartments, and the release from endothelial cell is assumed to be negligible, as illustrated in Figure 1b. The model is represented by the following differential equations

$$\frac{dx^{\mathrm{UL+HMW}}}{dt} = \varphi q - k_1\left(x^{\mathrm{UL+HMW}} - x_b^{\mathrm{UL+HMW}}\right) - k_e\left(x^{\mathrm{UL+HMW}} - x_b^{\mathrm{UL+HMW}}\right) \tag{8}$$

$$\frac{dx^{\mathrm{LMW}}}{dt} = (1-\varphi) q + k_1\left(x^{\mathrm{UL+HMW}} - x_b^{\mathrm{UL+HMW}}\right) - k_e\left(x^{\mathrm{LMW}} - x_b^{\mathrm{LMW}}\right) \tag{9}$$

where φ is the effective partition constant, representing the relative amount of high and low molecular weight multimers that are present in the injected dose, which is specific for each VWF concentrate and can be calculated from the specific collagen binding capacity

$$\varphi = \frac{VWF:CB^{IV}}{VWF:Ag^{IV}} \tag{10}$$

where $VWF:CB^{IV}$ and $VWF:Ag^{IV}$ are collagen binding and antigen VWF measurements carried out on the infused VWF concentrate. Intravenous administration is modeled through the infusion rate q [U/min]:

$$q = \begin{cases} D^{IV} & t \le t_{inj} \\ 0 & t_{inj} < t \le \tau \end{cases} . \tag{11}$$

In (11) D^{IV} is the discrete injection rate [U/min] and t_{inj} is the injection time [min]. The model is subject to the following additional constraint on infusion dose:

$$Q^{IV} = \int_0^{\tau} q\,dt \tag{12}$$

where Q^{IV} is the actual injected dose of VWF concentrate [U]. The full model is constituted by the system of differential and algebraic equations (8-10) including (4-6) and (11) to be solved with the initial conditions provided by (7). For this model the full set of model parameters to be estimated from VWF:CB and VWF:Ag data is

$$\boldsymbol{\theta}^{\mathbf{IV}} = \left[\, k_1 \quad k_e \quad D^{IV} \quad k \quad y_b^{CB} \quad t_{inj} \right]. \tag{13}$$

The parameter sets in both models (i.e. $\boldsymbol{\theta}^{\mathbf{DDAVP}}$ and $\boldsymbol{\theta}^{\mathbf{IV}}$) are determined for each subject by iteratively solving a nonlinear optimization problem based on maximum likelihood parameter estimation (Bard, 1977) following the procedure described in Taverna et al. (2019) and carried out using the commercial software gPROMS® ModelBuilder (Siemens Process Systems Enterprise, 2023). VWF:Ag and VWF:CB measurements are assumed to be normally distributed with a standard deviation of 2 U/dL as evaluated from repeated measurements on the AVWS subject, as reported in Galletta et al. (2021). Parameter estimation results are assessed in terms of estimated values and a posteriori statistics including *t*-values and confidence intervals. For a statistically precise estimation the *t*-value for each model parameter is calculated from

$$t_i = \frac{\hat{\theta}_i}{\sigma_{\theta_i}} \qquad\qquad i = 1 \ldots N_\theta \tag{14}$$

where $\hat{\theta}_i$ represents the estimated value from maximum likelihood parameter estimation and σ_{θ_i} the corresponding standard deviation. Each *t*-value calculated from (14) is compared against a tabulated reference *t*-value related to ($N - N_\theta$) degrees of freedom and 95% confidence level, where N is the total number of test samples and N_θ the total number of model parameters. A *t*-value higher than the reference *t*-value indicates a precise parameter estimation. Model adequacy is evaluated using a lack-of-fit (LOF) χ^2 test, by comparing the calculated chi-square

$$\chi^2 = \sum_{i=1}^{N} \frac{r_i^2}{\sigma_i^2} \tag{15}$$

with a tabulated reference chi-square at a 95% confidence level for ($N - N_\theta$) degrees of freedom (χ^2_{ref}) (Snedecor and Cochran, 1989). In (15) r_i and σ_i^2 are, respectively, the residual (difference between measured value and model prediction) for the *i*-th observation and the corresponding variance of measurement error. If $\chi^2 < \chi^2_{ref}$ the model is adequate to represent the test data.

III.3 Model-based design of clinical tests

Information content analysis has been executed on both the post-DDAVP model of VWD and on the proposed modified PK model of VWD with the following goals: *i*) study the distribution of information during clinical tests and evaluate the impact of information distribution on the overall test duration required to precisely estimate the set of PK parameters; *ii*) suggest the optimal allocation of sampling points for VWF:Ag and VWF:CB measurements; *iii*) quantify and rank the relative information that can be obtained using different VWF concentrates. The metric that is used to evaluate the overall information content of a clinical test is the trace of dynamic Fisher Information Matrix (FIM), which is defined by

$$I_d(\hat{\boldsymbol{\theta}}, t) = tr\left[\mathbf{H}_\theta(\hat{\boldsymbol{\theta}}, t)\right]. \qquad . \tag{16}$$

In Equation (16) $\mathbf{H}_\theta$ is the dynamic FIM calculated at the estimated value of model parameters, which are calculated from

$$\mathbf{H}_\theta(\hat{\boldsymbol{\theta}}, t) = \left[\mathbf{V}_\theta(\hat{\boldsymbol{\theta}}, t)\right]^{-1} \cong \sum_{j=1}^{N_m} \left[\frac{1}{\sigma_j^2}\left(\frac{\partial \hat{y}_j(\hat{\boldsymbol{\theta}}, t)}{\partial \theta_k} \frac{\partial \hat{y}_j(\hat{\boldsymbol{\theta}}, t)}{\partial \theta_l}\right)\right]_{k,l = 1 \ldots N_\theta} . \tag{17}$$

In Equation (17) the FIM, which is the inverse of the variance-covariance matrix of model parameters $\mathbf{V}_\theta$, is expressed as the product of the sensitivity of the *j*-th output variable with respect to each of the N_θ parameter in the conditions investigated in the *i*-th test, divided by the corresponding variance of measurement error (σ_j^2) for the *j*-th measured response (VWF:Ag or VWF:CB). Information from (17) can be decomposed to analyse the contribution to the information related to the estimation of the *i*-th model parameter (h_{ii}):

$$I_d(\hat{\boldsymbol{\theta}},t) = tr\left[\mathbf{H}_\theta(\hat{\boldsymbol{\theta}},t)\right] = \sum_{i=1}^{N_\theta} h_{ii}(\hat{\boldsymbol{\theta}},t). \tag{18}$$

A maximum in I_d defines the most informative time points to take samples during the clinical test. When this maximum is located at the end of the test information acquisition is favored by long test durations. If an information peak is located at the very beginning of the test, samples can be concentrated in the first few hours of test execution and the test duration can significantly be reduced. Optimal sampling allocation can be obtained by solving the following optimal model-based design of experiments (MBDoE) problem (Fedorov and Leonov, 2014):

$$\mathbf{t}^{sp} = \arg\min \psi\left[\mathbf{V}_\theta\right] = \arg\min \psi\left[\left(\sum_{i=1}^{N_{sp}} \mathbf{H}_\theta(\theta,t_i)\right)^{-1}\right] \tag{19}$$

where $\mathbf{t}^{sp} = \begin{bmatrix} t_1 & t_2 & \dots & t_{N_{sp}} \end{bmatrix}$ is the optimal vector of sampling times and ψ [.] is a metric function of the variance-covariance matrix of model parameters, identifying the chosen experimental design criterion. Popular choices for ψ are the determinant (D-optimality), the trace (A-optimality), the largest eigenvalue (E-optimality) of $\mathbf{V}_\theta$ (Pukelsheim, 1999). The optimization in (19) is carried out considering practical constraints on sampling points allocation in time, including, for fixed number of samples N_{sp}: *i*) minimum time between consecutive measurements; *ii*) test duration. This set of constraints $\mathbf{C} = [C_{1i}\ C_2]$ are formulated as

$$C_{1i} = \Delta t_i = t_i - t_{i-1} \geq MTBM \qquad i = 1 \dots N_{sp} \tag{20}$$

$$C_2 = \sum_{i=1}^{N_{sp}} \Delta t_i \leq \tau^{MAX} \tag{21}$$

where MTBM is the minimum time between consecutive measurements (here set to 15 minutes to propose a practical, clinically feasible test) and τ^{MAX} is the maximum allowed duration for the test (here fixed to 24 hours, which is the maximum duration of a standard DDAVP test). The optimization (19) subject to (20) and (21) and the model equations (8-10) including (4-6) and (11) has been carried out in gPROMS ModelBuilder (Siemens Process Systems Enterprise, 2023) using a sequential quadratic programming (SQP) optimization solver with multiple shooting to solve the resulting NLP problem.

IV. Results and discussion

IV.1 Model calibration: DDAVP Vs Haemate P infusion

Data available for the AVWS subject (see Table 1) have been used to calibrate the DDAVP model and the newly developed model including VWF infusion. Results after model identification are illustrated in Figure 3a (post-DDAVP test) and Figure 3b (Haemate P administration test). Parameter estimation results are reported in Table 2. As shown in Figure 3a and 3b, both the models are adequately fitting the available clinical data for the AVWS subject, providing very limited

deviations from the measured VWF:Ag and VWF:CB concentrations, as underlined by the corresponding low χ^2 values reported in Table 2. A more detailed analysis of residuals reveal that the post-DDAVP model has some minor limitations on representing the initial VWF:Ag peak observed in the clinical test, while the infusion model tends to underestimate the observed VWF:Ag decay realised after 300-500 minutes from VWF infusion. When analysing the results from maximum likelihood parameter estimation, it can be observed from Table 2 that the available clinical data allows a precise estimation of the key haemostatic parameters for the description of proteolysis (parameter k_1) and elimination (parameter k_e) pathways, as confirmed by the low 95% confidence intervals. However, the description of release parameters D and t_{max} (post-DDAVP model) and infusion parameters D_{IV} and t_{inj} (Haemate P infusion model) is more problematic, due to the strong correlation between forced by the corresponding model equations and the limited amount of data points available to capture the initial transient behaviour after drug administration (see Section IV.2 for further details). Interestingly, the Haemate P infusion test allows for a more precise estimation of the elimination parameter k_e compared to a standard DDAVP test, while there are no significant differences when comparing the precision in the estimate for proteolytic parameter k_1.

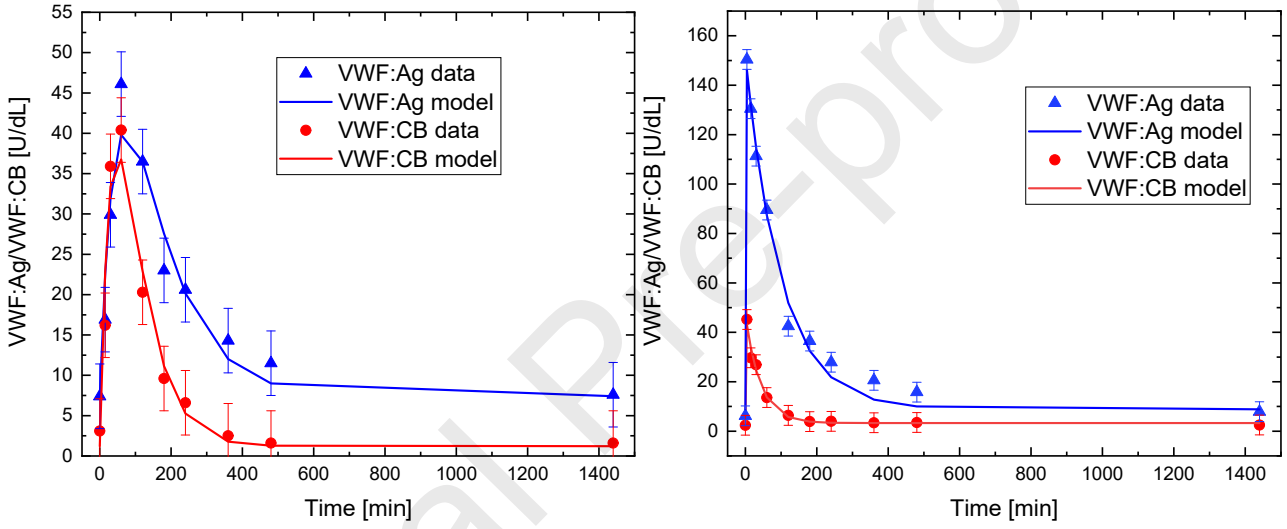
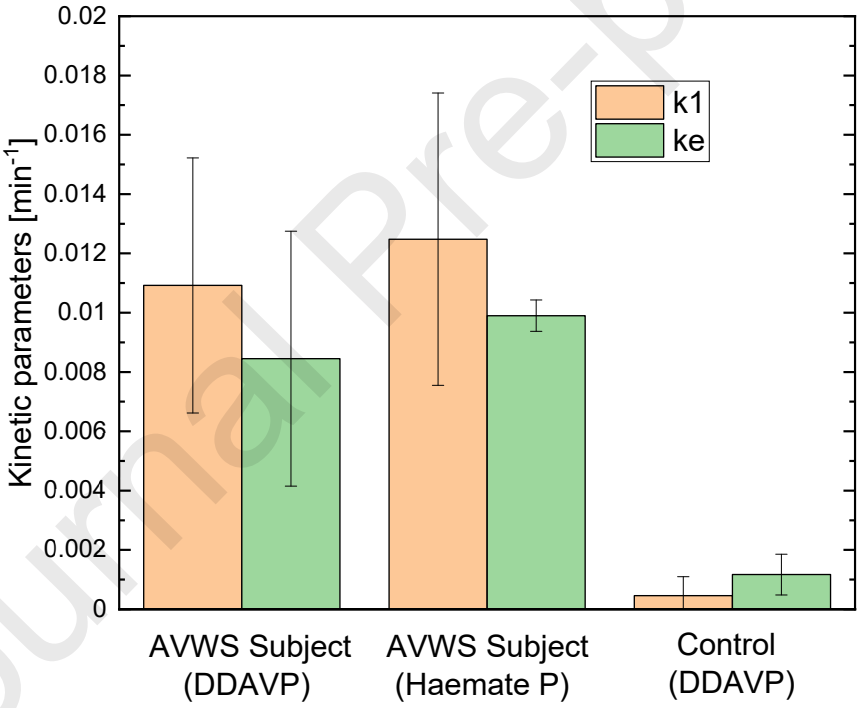


Figure 3. *Model identification results from AVWS data using (a) the post-DDAVP model proposed by Ferrari et al. (2017) and (b) the model proposed in this study including exogenous VWF infusion. VWF:Ag and VWF:CB measurements are indicated, respectively, by blue triangles and red circles; error bars indicate the standard deviation in the data.*

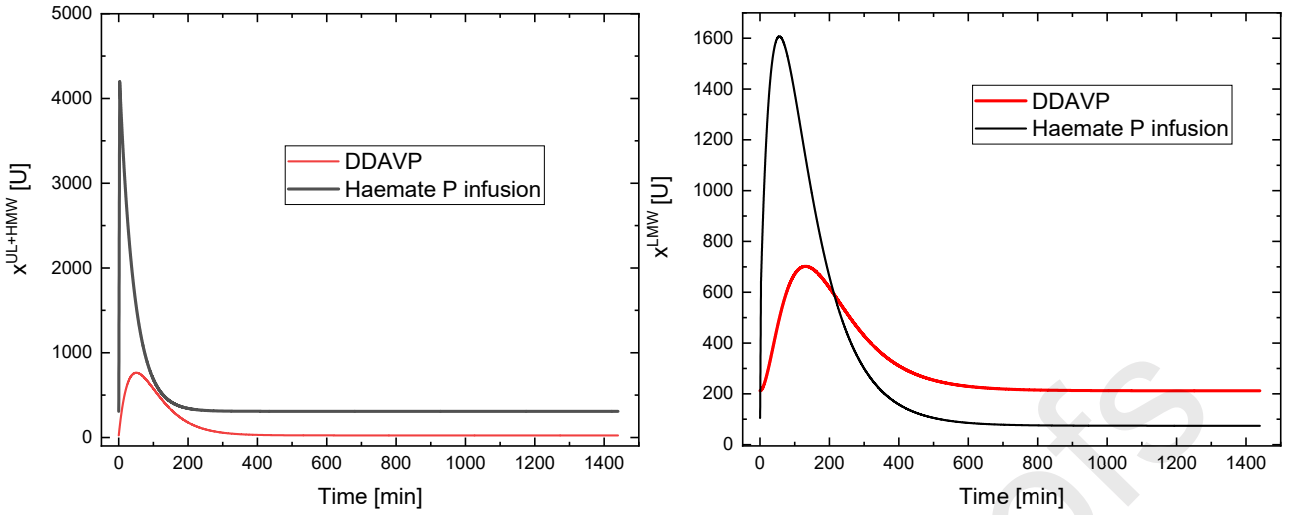
Table 2. *Estimated values of model parameters and a-posteriori statistics, including 95% confidence intervals, t-test and χ^2 lack of fit test results for the post-DDAVP model and Haemate P infusion model. Asterisk * indicates parameters failing the t-test.*

Post-DDAVP Model				**Haemate P Infusion Model**			
Model Parameters	**Estimated value**	**Confidence Interval**	**t-value (Ref: 1.75)**	**Model Parameters**	**Estimated value**	**95% Confidence Interval**	**t-value (Ref: 1.73)**

k_1	0.01092	0.0044	2.13	k_1	0.01248	0.0049	2.53
k_e	0.00845	0.0042	2.49	k_e	0.00990	0.0005	18.60
k_0	0.02051	0.0095	1.98	D_{IV}	1658.254	8913.9784	**0.186***
D	1304.1110	762.5731	**1.71***	t_{inj}	2.7228	14.4830	**0.188***
t_{max}	219.6	10980.0105	**0.02***	-	-	-	-
χ^2	23.3	χ^2_{ref}	26.3	χ^2	25.5	χ^2_{ref}	28.9



(a)



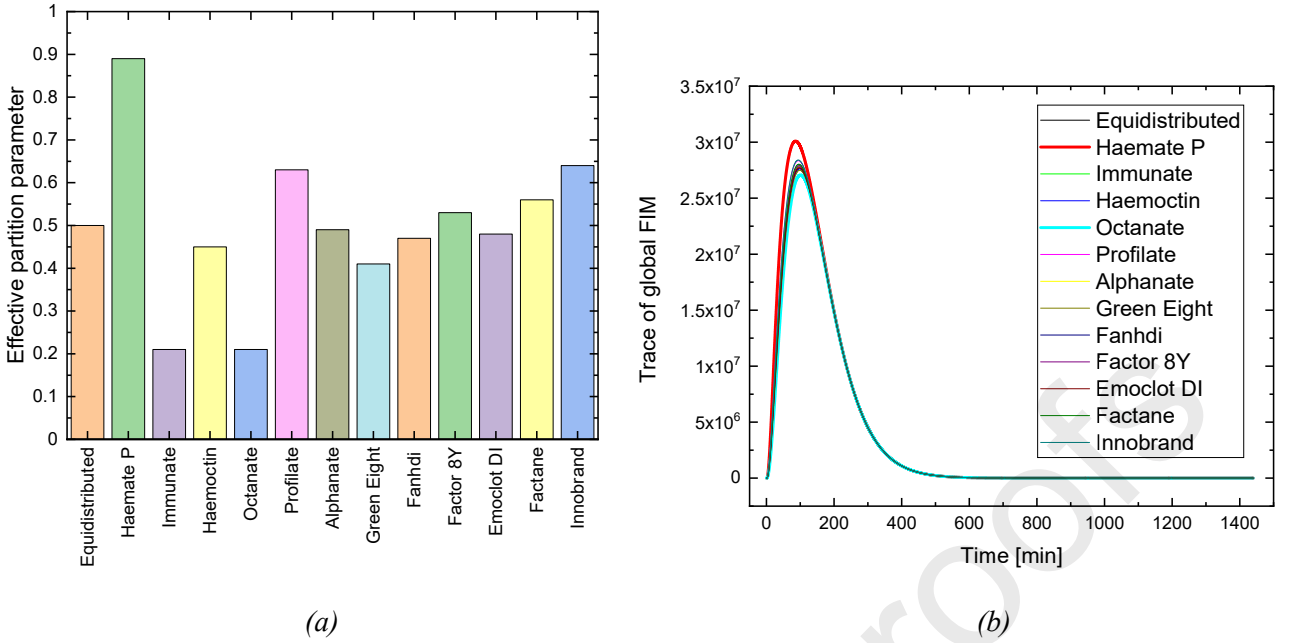
(b) *(c)*

Figure 4. *Parameter estimation results for key haemostatic parameters related to proteolysis (k1) and elimination (ke) of VWF from plasma for the AVWS subject after DDAVP and Haemate P infusion, and comparison with healthy (O + non-O) control subjects. Bars on columns indicate 95% confidence intervals.*

By analysing the estimates for key parameters k_1 and k_e (Figure 4a) it is apparent that the estimated parameter values obtained from the two different tests (DDAVP ad Haemate P infusion) are very similar, and undistinguishable considering the uncertainty in parameter estimates. As illustrated in the figure, the AVWS subject shows accelerated proteolysis and elimination pathways compared to healthy control subjects, resulting in a lack of high molecular weight species in the blood stream and, consequently, a reduced haemostatic activity. The precise estimation of k_e and k_1 (minimum variance in the estimation of these parameters) is crucial for the model-based diagnosis of the subject and to achieve a clear distinction between AVWS subjects and subjects affected by other types of VWD. The time profiles of (UL + HMW) (Figure 4b) and LMW (Figure 4c) multimeric concentrations (Figure 4b) and (Figure 4c) show the very different dynamics realised during DDAVP and Haemate P infusion tests and how both the models are capable to represent the distribution of multimeric species in time. The fast infusion of HMW multimers in Haemate P test produces a peak in $x^{UL\ +\ HMW}$ at the very first minutes after infusion (Figure 4b) that quickly disappears as the high molecular weight species are converted to LMW (Figure 4c).

IV.2 Information analysis for selected VWF infusion concentrates

Information content analysis has been carried out on the new model of VWD including exogenous infusion, for different VWF infusion concentrates, as these are characterised by different values of the effective partition parameter φ as given in equation (10). The range of variability of the effective partition parameter is around 0.2 – 0.9 as illustrated in Figure 5 based on the comparison between functional activities reported in Auerswald and Kreuz (2008). In this study, 13 potential scenarios have been simulated to analyse the potential impact of the choice of concentrates on the expected information of the clinical test, including a scenario (Scenario 1) in which HMW and LMW multimers are equally distributed in the administered dose and additional scenarios (Scenarios 2-13) to consider the relative functional activities of different VWF concentrates.



(a) *(b)*

Figure 5. *(a) Values of the effective partition parameter used in simulated scenarios for different VWF concentrates; (b) dynamic profile of the trace of global FIM for each concentrate. Thick lines indicate maximum and minimum information profiles for selected scenarios.*

Results in terms of global FIM analysis are reported in Figure 5b. From the point of view of the overall information that can be obtained on the haemostatic parameters we can observe that there is no specific impact on information dynamics for different VWF concentrates, as all of them exhibit a maximum on information within the first two hours. When Haemate P is used as concentrate the most informative sampling point in time for the estimation of haemostatic parameters can be found at around t = 88 minutes. If concentrates characterised by a lower amount of HMW species are used (for example Octanate) the maximum information peak is reduced (i.e. the test is less informative) and moved towards slightly longer experimental times (t = 102 min). This means that the use of concentrates characterised by a higher amount of HMW species is beneficial for the precise estimation of kinetic parameters, and that VWF:Ag and VWF:CB measurements should be concentrated around the optimal sampling point identified by the information peak, so within the first two hours after drug infusion. Results are particularly interesting when the overall Fisher information is decomposed into its parameter-specific components as illustrated in Figure 6a-d. Haemate P represents the most suitable VWF concentrate to precisely estimate the proteolytic parameter k_1, provided that sampling points are taken at the very beginning of the test (t < 100 min), as the corresponding information has a peak at around t = 45 min.

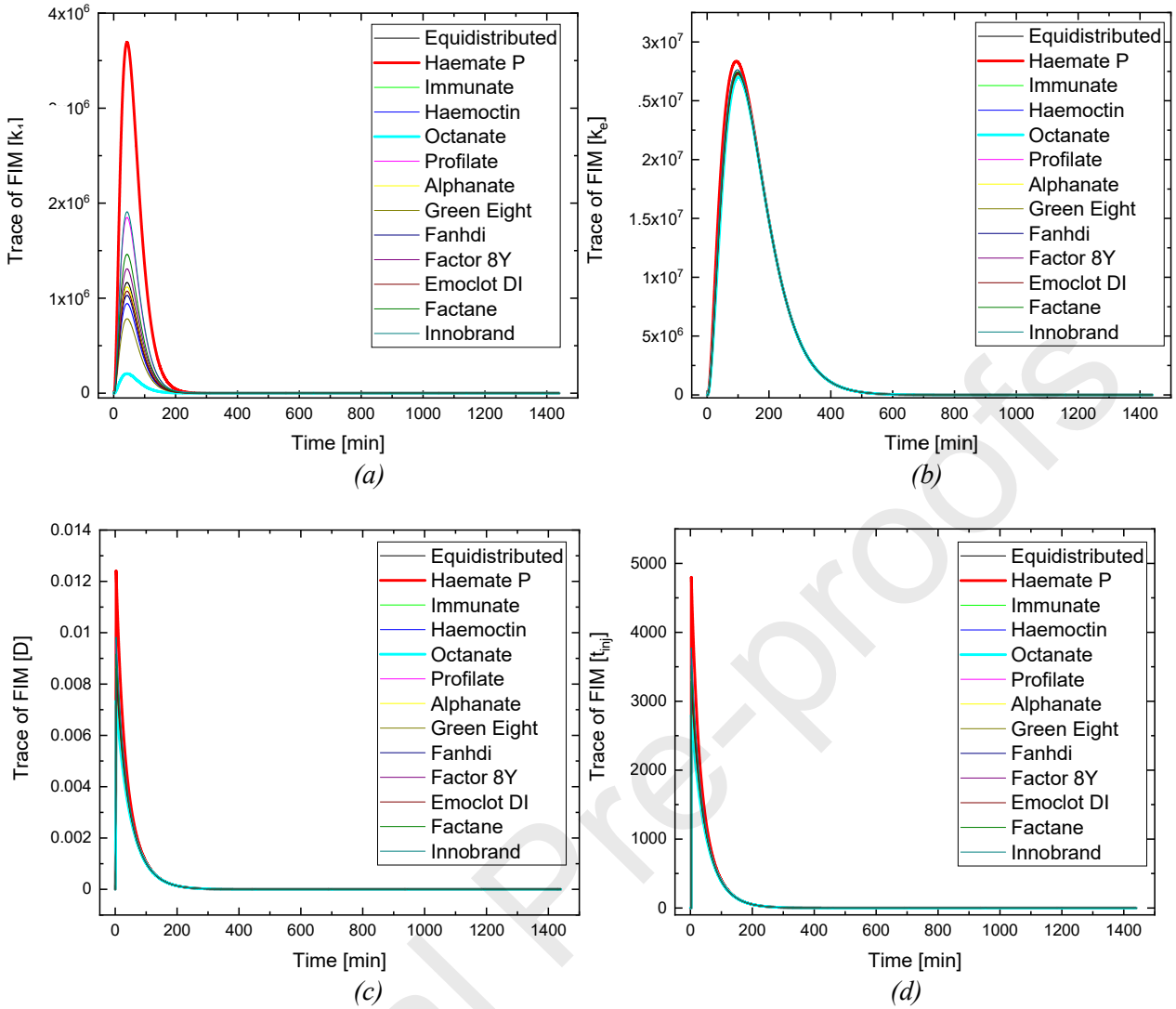


Figure 6. *Trace of FIM for representative subjects from each category of subjects) dynamic profile of the trace of global FIM for each simulated scenario. Thick lines indicate maximum and minimum information profiles for selected scenarios.*

The use of VWF concentrates characterised by a low effective partition parameter (for example Octanate) is detrimental to the information that can be obtained from the infusion test. Interestingly, the use of different concentrates has little to no effect on the estimation of the elimination parameter k_e (Figure 6b), but still the sampling points should be concentrated in the first few hours of the test, as the information is the highest approximately 2 hours after infusion. Results for the infusion parameters D_{IV} and t_{inj} show that extremely fast information dynamics are required for a precise estimation of these parameters. This explains why, with the current sampling limitations (earliest sample can potentially be taken only after 10 minutes from infusion), only an uncertain estimation of infusion parameters can be obtained (see Table 2). The estimation is further complicated by the extremely high correlation between D_{IV} and t_{inj}, as illustrated in Table 3.

Table 3. *Correlation matrix obtained after parameter estimation from Haemate P infusion test data. High values of the correlation coefficients are indicated in boldface.*

Parameter	k_1	k_e	D_{IV}	t_{inj}

k_1	1.00000			
k_e	-0.114767	1.00000		
D_{IV}	**-0.808363**	0.164162	1.00000	
t_{inj}	**0.808625**	-0.161232	**-0.999993***	1.0000

Results clearly illustrate that a moderate correlation is also present between parameters k_1 and D_{IV} and t_{inj}, showing that during the test a very accurate control of the Haemate P infusion rate q, defined by equation (10), is needed to avoid bias in the estimation of kinetic parameters.

IV.3 Optimal design of Haemate P infusion test

A D-optimal MBDoE has been carried out by solving the optimal experimental design optimization problem given by equation (18) to determine the optimal allocation of sampling points $\mathbf{t}^{sp}$ in time. In the everyday clinical procedures, there is a non-negligible uncertainty in the definition of each sampling time, as it is impractical to sample with a resolution in time lower than 10 minutes. Therefore, a conservative constraint on the minimum time between measurements of 15 minutes has been assumed in the MBDoE optimisation. Optimal experimental design results are illustrated in Table 4 in terms of experimental design variables and in Figure 7 in terms of simulated VWF:Ag and VWF:CB profiles and optimal allocation of samples. As expected, given the faster information dynamics realized in infusion experiments (see Figure 6b) the sampling points are concentrated at the very beginning of the test. Albeit not shown for the sake of conciseness, this result does not change significantly if different experimental design criteria are used (i.e. A- or E- optimal).

Table 4. *Allocation of sampling points in time and duration for DDAVP, Haemate P infusion and D-optimal designed Haemate P infusion tests.*

Clinical test protocol	**Sampling point allocation** **($\mathbf{t^{sp}}$)** [min]	**Test duration** [h]
DDAVP	[0 15 30 60 120 180 240 360 480 1440]	24
Haemate P Infusion	[0 4 15 30 60 120 180 240 360 480 1440]	24
D-optimal MBDoE	[0 15 30 45 60 75 90 105 120 135 150]	2.5

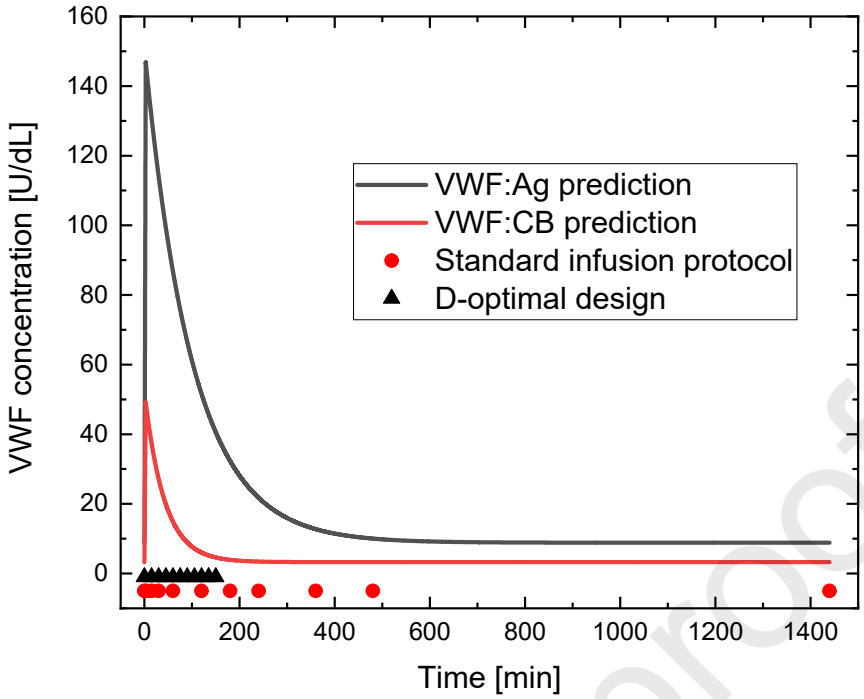


Figure 7. *Predicted profiles of VWF:Ag and VWF:CB and allocation of sampling points as obtained from the D-optimal designed test (black triangles) as compared to the original allocation of sampling points in standard Haemate P infusion tests (red circles).*

Table 5. *Estimated values of model parameters and a-posteriori statistics, including 95% confidence intervals, t-test and χ^2 lack of fit test results obtained from the D-optimally designed Haemate P infusion test. Asterisk * indicates parameters failing the t-test.*

Haemate P Infusion Model after MBDoE			
Model Parameters	**Estimated value**	**95% Confidence Interval**	**t-value (Ref: 1.73)**
k_1	0.0124	0.0049	3.15
k_e	0.0098	0.0005	19.60
D_{IV}	1658.2542	8913.9784	**0.13***
t_{inj}	2.7228	14.4830	**0.12***
χ^2	23.5	χ^2_{ref}	28.9

Results in terms of parameter estimation are reported in Table 5 and show that this proposed design is more efficient to precisely estimate the kinetic parameters k_1 and k_e when compared to the original sampling used in Haemate P infusion tests (Table 2), as demonstrated by the reduced confidence intervals, while preserving a similar value in parameter estimates. Most importantly, this optimal sampling schedule would allow to adopt a considerably shorter test than the currently adopted infusion protocol (150 min ~ 2.5 h against 24 h of the currently proposed infusion test), maintaining the same level of information for the determination of key metabolic parameters. Still, as underlined by the parameter estimation results obtained from the standard Haemate P infusion test, the individual precise characterization of infusion parameters D_{IV} and t_{inj} is particularly challenging. It is interesting to confirm that, if we assume that a precise injection time can be guarantee during the infusion, i.e. by fixing the infusion time at t_{inj} = 3 minutes, a precise estimation of key parameters k_1, k_e and D_{IV} can be achieved, as shown in Table 5.

Table 6. *Estimated values of model parameters and a-posteriori statistics, including 95% confidence intervals, t-test and χ^2 lack of fit test results obtained from the D-optimally designed Haemate P infusion test assuming a fixed injection time (t_{inj} = 3 min).*

Haemate P Infusion Model after MBDoE (fixed t_{inj})			
Model Parameters	**Estimated value**	**95% Confidence Interval**	**t-value (Ref: 1.73)**
k_1	0.0140	0.0078	1.80
k_e	0.0099	0.0020	4.99
D_{IV}	1658.2500	159.9083	10.37
χ^2	23.7	χ^2_{ref}	28.9

Results show that a small variation in the injection time from 2.7 seconds (as reported in Table 5 after parameter estimation) to fixed 3 seconds does not affect the estimated values of model parameters, but it slightly affects the precision of the estimates for parameters k_1 and k_e (see the increase in confidence intervals values from Table 5 to Table 6). The goodness of fit test is still passed and does not change significantly when t_{inj} is fixed (χ^2 = 23.7 against χ^2 = 23.5 when t_{inj} is estimated).

V. Conclusions

Kinetic models for VWD have been proposed recently to quantify the mechanisms of VWF release (k_0), proteolysis (k_1) and elimination (k_e) in the blood stream, and to characterise the distribution of high and low molecular weight multimers for different types of VWD. These models are affected by

several limitations: *i*) their calibration requires a 24 hours-long DDAVP test to achieve a statistically satisfactory estimation of the PK metabolic parameters for each subject; *ii*) they have been developed based on a fixed DDAVP administration dose, and their formulation does not explicitly include the exogenous infusion of VWF concentrates, which are the main form of treatment for severe forms of VWD. In this paper, a new VWD model including exogenous VWF infusion has been proposed. The model has been calibrated based on clinical data from a subject affected by acquired Von Willebrand syndrome (AVWS), a form of VWD whose treatment requires exogenous VWF administration. Results show that the new model allows to maintain the same level of information on key proteolysis (k_1) and elimination (k_e) parameters than the one obtained from a DDAVP-calibrated model. A dynamic Fisher information matrix (FIM) analysis has been carried on the new model to evaluate the estimability of model parameters and the dynamics of information during the identification test considering different VWF concentrates. Results show that VWF concentrates characterised by a larger partition coefficient (high VWF:CB/VWF:Ag ratio) are found more adequate to improve the estimation of proteolysis parameter k_1, while shorter test durations can be used for the estimation of the elimination parameter k_e, a parameter usually characterised by slower information dynamics. This is a particularly relevant result, as the current test protocol including VWF infusion requires 24 hours. Further relevant results show that information dynamics for infusion parameters D^{IV} and t_{inj} are extremely fast and these parameters are highly correlated. These aspects make their precise estimation a particularly challenging task. A D-optimal MBDoE has then been used to redesign the Haemate P infusion test by optimally allocating the sampling points to maximise the expected information acquired from clinical test. Using MBDoE, the test duration has been successfully reduced from 24 hours to 2.5 hours proposing a new clinically applicable sampling protocol where the injection time can be controlled to precisely estimate the full set of kinetic model parameters. The possibility to reduce test duration associated to a conventional infusion test is a remarkable achievement because it allows patients to undergo a less stressful clinical procedure and it facilitates clinical management in terms of both economical and organizational aspects. The precise estimation of subject-specific haemostatic parameters allows to obtain a subject-specific calibrated model that can be used for personalised AVWS monitoring and to improve the dosage of VWF concentrates, a key aspect to address when treating severe forms of AVWS. Future work will be carried out to extend the validity of the infusion model and further investigating the effect of infusion parameters uncertainty on model identification by *i*) adopting model reparametrisation techniques (Quaglio et al., 2019) and MBDoE anticorrelation criteria (Franceschini and Macchietto, 2008b) to decrease the degree of correlation between parameters; *ii*) designing specific tests for the practical estimation of infusion parameters only, considering clinically realizable injection settings and the uncertainty affecting the infusion process; *iii*) extending the model applicability to the estimation of PK parameters for new subjects affected by different types of VWD.

References

1. Lillicrap, D. (2007). Von Willebrand disease-phenotype versus genotype: Deficiency versus disease. *Thrombosis Research*, 87, 57-64.

2. Sadler, J. E. (2003). Von Willebrand disease type 1: a diagnosis in search of a disease. *Blood*, 101, 2089-2093.

3. Groot, E., Fijnheer, R., Sebastian, S. (2009). The active conformation of von Willebrand factor in patients with thrombotic thrombocytopenic purpura in remission. *J. Thromb. Haemost.,* 7, 962-969.

4. Galletta, E., Galvanin, F., Bertomoro, A., Daidone, V., Casonato, A. (2021). Acquired von Willebrand syndrome in patients with monoclonal gammopathy of undetermined significance investigated using a mechanistic approach. *Blood Transfus.* doi: 10.2450/2021.0121-21.
5. Casonato, A., Daidone, V., Padrini, R. (2011). Assessment of von Willebrand factor propeptide improves the diagnosis of von Willebrand disease. Semin. Thromb. Hemost., 37, 456-463.
6. Galvanin, F., Barolo, M., Padrini, R., Casonato, A., Bezzo, F. (2014). A model-based approach to the automatic diagnosis of von Willebrand disease. *AIChE Journal*(60), 1718-1727.
7. Gezsi, A., Budde, U., Deak, I., Nagy, E., Mohl, A., Schlammadinger, A., Boda, Z., Masszi, T., Sadler, J. E., Bodo, I. (2010). Accelerated clearance alone explains ultra-large multimers on von Willebrand disease Vicenza. *J. Thromb. Haemost.*, 8, 1273–1280.
8. Ferrari, M., Galvanin, F., Barolo, M., Daidone, V., Padrini, R., Bezzo, F., Casonato, A. (2018). A Mechanistic Model to Quantify von Willebrand Factor Release, Survival and Proteolysis in Patients with von Willebrand Disease. *Thromb. Haemost.*, 118(02), 309-319.
9. Galvanin, F., Bezzo, F. (2018). Advanced Techniques for the Optimal Design of Experiments in Pharmacokinetics. *Computer Aided Chemical Engineering*, 42, 65-83.
10. Taverna, B., Casonato, A., Bezzo, F., Galvanin, F. (2019). A framework for the optimal design of a minimum set of clinical trials to characterize von Willebrand disease. *Comp. Meth. Prog. Biomed.*, 179, 104989.
11. Galvanin, F., Monte, A., Casonato, A., Padrini, R., Barolo, M., Bezzo, F. (2014). Towards model-based diagnosis of von Willebrand disease. *Computer Aided Chemical Engineering*, 33, 583-588.
12. Castaldello, C., Galvanin, F., Casonato, A., Padrini, R., Barolo, M., Bezzo, F. (2018). A model-based protocol for the diagnosis of von Willebrand disease. *Canadian Journal of Chemical Engineering*, 96, 628-638
13. Casonato, A., Pontara E., Sartorello F. (2006). Identifying type Vicenza von Willebrand disease. *J. Lab Clinical Medicine*, 147, 96-102.
14. Budde, U, Metzner, H. J., Muller, H. G. (2006). Comparative analysis and classification of von Willebrand factor/factor VIII concentrates: impact on treatment of patients with von Willebrand disease. *Semin. Thromb. Hemost.*, 32, 626–35.
15. Berntorp E. (2009). Haemate P/Humate-P: a systematic review. *Thromb Res.*, 124, S11-S14.
16. Auerswald, G., Kreuz, W. (2008). Haemate P/Humate-P for the treatment of von Willebrand disease: considerations for use and clinical experience. *Haemophilia*, 14, 39–46.
17. Casonato, A., Pontara, E., Sartorello, F., Cattini, M. G., Sartori, M. T., Padrini, R., Girolami, A. (2002). Reduced von Willebrand factor survival in type Vicenza von Willebrand disease. Blood, 99, 180-184.
18. Menache, D., Aronson, D. L., Darr, F. et al (1996). Pharmacokinetics of von Willebrand factor and factor VIIIC in patients with severe von Willebrand disease (type 3 VWD): estimation of the rate of factor VIIIC synthesis. *Br. J. Haematol.*, 94, 740-745.
19. Snedecor, G. W., Cochran, W. G. (1989), *Statistical Methods (Eighth Edition)*, Iowa State University Press.
20. Bard, Y. (1974). *Nonlinear parameter estimation,* Academic Press.
21. Pukelsheim, F. (1995). *Optimal design of experiments*, Chapman and Hall.
22. Fedorov, V., Leonov, S. (2014). *Optimal design for nonlinear response models.* Boca Raton: Taylor & Francis Group.

23. Quaglio, M., Waldron, C., Pankajakshan, A., Cao, E., Gavriilidis, A., Fraga, E. S., Galvanin, F. (2019). An online reparametrisation approach for robust parameter estimation in automated model identification platforms. *Computers & Chemical Engineering*, 124, 270-284

24. Siemens Process Systems Enterprise (2023), gPROMS, https://www.siemens.com/global/en/products/automation/industry-software/gproms-digital-process-design-and-operations.html.

25. Tiede, A., Rand, J. H., Budde, U., Ganser, A., Federici, A. B. (2011). How I treat the acquired von Willebrand syndrome. *Blood*, 117, 6777–6785.
26. Chakrabarty, A., Buzzard, G.T. and Rundell, A.E. (2013), Model-based design of experiments for cellular processes. *Syst. Biol. Med.*, 5, 181-203.
27. Franceschini, G., Macchietto, S. (2008a), Model-based design of experiments for parameter precision: State of the art. *Chem. Eng. Sci.*, 63, 4846–4872.
28. Villaverde, A. J., Pathirana, D., Fröhlich, F., Hasenauer, J., Banga, J. R. (2022). A protocol for dynamic model calibration. *Briefings in Bioinformatics*, 23 (1), 1-19.
29. Franceschini, G., Macchietto, S. (2008b), Novel anticorrelation criteria for model-based experiment design: Theory and formulations. AIChE J., 54, 1009-1024.

HIGHLIGHTS:

- A new mathematical model is proposed to describe acquired von Willebrand syndrome.
- The model includes exogenous infusion and is calibrated from clinical data.
- Sampling points allocation is optimised using model-based design of experiments.
- Infusion preserves the haemostatic information obtained from conventional tests.
- Using exogenous infusion test duration can be reduced from 24 hours to 2.5 hours.

30.