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Reaction Mechanisms and Kinetic Constants used in Mechanistic Models of Coagulation and Fibrinolysis

A.M. Susree, B.M. Anand *

Department of Chemical Engineering, Indian Institute of Technology Hyderabad,
Kandi, Sangareddy 502285, Telangana, INDIA.

Abstract. Computational modeling has gained a strong foothold as a tool for understanding and simulating biological phenomena like blood coagulation. However, exceptional complexity of these phenomena (that include hundreds of individual biochemical processes and a few biophysical processes) complicates development of reliable models and interpretation of the results. It is thus of great importance that a model of hemostasis designed for a particular application should be reproducible, apart from making robust predictions. Such a model would further reflect useful pharmacological implications not only with respect to identifying drug targets but also in understanding drug effects on their respective targets. We show, using a sample model, that model predictions vary significantly with the use of different values available in the literature for the same kinetic constant. We thus highlight the importance of having consensus of kinetic constants used in the formulation of mechanistic models for coagulation, and document values for each kinetic constant that can be used in such models, and for varying reaction conditions (synthetic, *in vitro, in vivo*).

Key words:: computer simulation, consensus, hemostasis, kinetics, mathematical model

Mathematics Subject Classification: 80A30, 92C45

1. Introduction

Computational modeling has become a viable tool for understanding the kinetic interactions between individual components of a biological system at several levels (sub-cellular, cellular, and tissue-level), and for documenting the spatio-temporal behavior of such systems. Such models hold the key to revealing the quantitative behavior and design principles of biological systems. Further, they enable the phenomenon being studied to be simulated computationally, its behavior controlled predictively, and designed appropriately (Panteleev et al. [2007]). Conventional experimental methods may restrict the number of samplings, however, there are no such restrictions in case of mathematical models. Computational modeling thus fits the need for a non-invasive, large-scale and multivariate approach for simulation of natural phenomena.

Blood clot formation is one biological phenomenon that has been extensively modeled using mechanistic models incorporating the kinetics of individual reactions: see Anand et al. [2003], Mann et al. [2006] for

*Corresponding author. E-mail: anandm@iith.ac.in

exhaustive reviews. It is a dynamic process, governed by a network of several tens of interacting proteolytic enzymes, which has its own slow- and fast-proceeding portions. Orfeo and Mann [2005] remark in their review that mechanistic models and computer simulations provide the expediency of studying many situations *in silico* with minimal cost, and multiple hypotheses can be considered prior to engaging a human subject. In the pharmacological perspective, this can further aid not only in the selection of appropriate therapeutic targets but also in developing a patient-specific understanding of disease-risk and drug dosing (Diamond [2013], Brummel-Ziedins [2013]). The need to embed mathematical models in studying the pharmacokinetics and pharmacodynamics of various anti-thrombotic/anti-platelet drugs has been emphasized in the review by Panteleev et al. [2007]; pharmacological effects of drugs like rivaroxaban, argatroban, warfarin, etc have been studied using such models (Adams et al. [2003], Luan et al. [2007],Burghaus et al. [2011], Gulati et al. [2014], Zhou et al. [2015]). However, mechanistic models have too many parameters to be measured for each patient and hence, their predictive utility is a matter of debate (Hemker et al. [2012], Mann [2012]).

A mechanistic model for clot formation *in vivo* needs to cover the aspects of biochemistry, physiology and rheology that are involved, should match with experimental data for the same conditions, and also rule out that there is no other reaction/parameter set that yields the same results so as to validate the mechanism that is embedded within it (Hemker and Ataullakhanov [2005]). Predictions of such mechanistic models require reaction rate constants that are obtained from biochemical studies. These biochemical studies report rate constants along with the order of the reactions and it is essential that the model use these in the conditions they are measured in and/or intended for so as to enable an objective comparison: this care is lacking as on date. It is well known that changes in the reaction rate constants result in changes in the model predictions (Leipold et al. [1995]). The order and constants reported for a particular reaction can also vary from study to study and this is also a source of uncertainty for model predictions. Limitations of computational modeling owing to parameter variations and possible approaches to overcome these are briefly discussed by Danforth et al. [2009]. Inaccurate knowledge of the available kinetic rate constants hinders the formulation of a robust mathematical model. Hence, it is important to have consensus in kinetic constants so that a given model's predictions can be confirmed to hold within experimental error of each constant. We illustrate the differences arising in a model's predictions using our model in Susree and Anand [2016] for tissue factor-initiated *in vitro* blood coagulation in plasma containing platelets by using kinetic constants for the same reaction from different sources. Since most of the available models do not discuss platelet-dependent reactions, it is one of the strong features of the model in Susree and Anand [2016]. We note that the reaction mechanisms in this model and those we compare with are obtained for the view of coagulation espoused by Furie and Furie [2008] : this view represents the consensus view but does not include details like sub-populations of activated platelets, circulating tissue factor, etc. In this review, we aim to highlight the significance of better agreement in the kinetic parameters used in mechanistic models of blood coagulation, and identify different sets of constants for different conditions (*in vivo, in vitro*, in synthetic plasma).

We begin by giving a brief overview of mechanistic models in Section 2. The changes in model predictions, due to use of constants from different sources, are documented in Section 3. We then review the literature on enzyme kinetic studies for each reaction in the coagulation cascade in Section 4. We document a list of possible constants in Section 5, and conclude with remarks on how to use the list.

2. Literature Review

2.1. *Overview of Hemostasis*

Hemostasis is a physiological mechanism responsible for preventing blood loss due to injury and hence maintaining the structural as well as functional integrity of the human vasculature. The three main sub-processes of hemostasis are vasoconstriction, platelet plug formation, and blood coagulation. All these sub-processes are also involved in thrombosis: the formation of undesirable aggregates or clots in the vessels that might disrupt blood circulation with fatal consequences. An elaborate description of the

process of blood coagulation as it occurs *in vivo* is presented in Furie and Furie [2008], Butenas and Mann [2002], and we sketch the same in Figure 1.

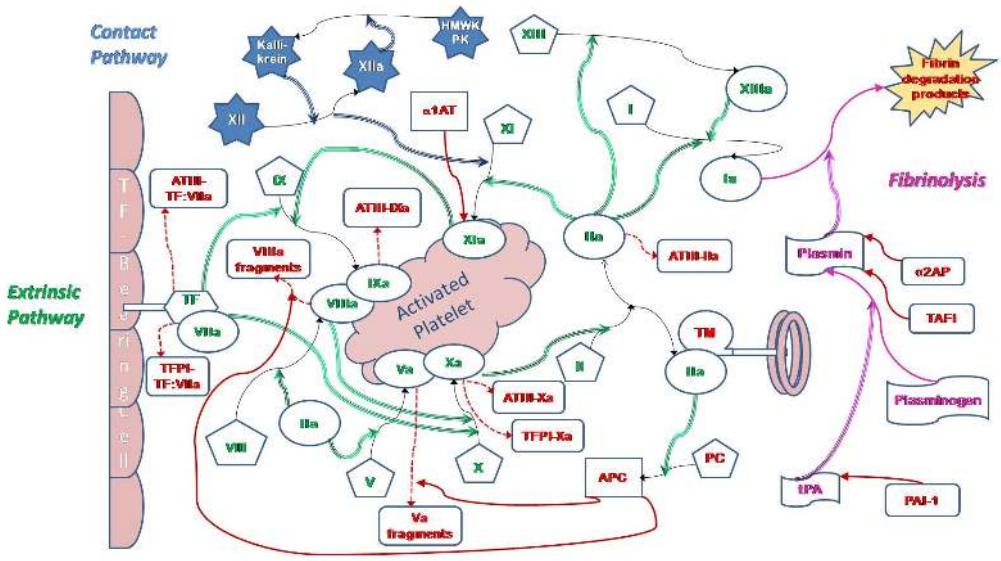

FIGURE 1. Schematic Diagram of Coagulation Pathway *in vivo*

2.2. *Types of Mechanistic Models of Blood Coagulation*

Owing to the rapid growth and awareness of mathematical modeling of biological phenomena in the last decade, many models have been devised to describe the clotting system as a whole or in modules. Several mechanistic models (at continuum, micro-, and nano-scales) have detailed how flow-mediated transport, platelet deposition, and coagulation factors interact and affect clot formation. There is a whole literature of multiscale models (Leiderman and Fogelson [2014], Xu et al. [2012], Tomaiuolo et al. [2014]) which, among others, discuss these sub-processes.

We limit our review to single scale models of blood coagulation, the categorization of which is done as: *homogeneous, pseudo-homogeneous*, and *heterogeneous* (Diamond [2013]). Models wherein concentrations are defined with respect to volume of plasma alone (single phase) are called homogeneous; models wherein species concentrations and kinetics are defined in different phases, i.e., liquid-phase and surface-phase, corresponding to plasma and platelets, respectively, are heterogeneous models. In pseudo-homogeneous models, the particle composition of blood is overlooked, and the heterogeneous system is treated as homogeneous by defining platelet concentration based on plasma (or blood) volume. Here, we discuss only certain key models developed under each category. For a more detailed review, see Anand et al. [2003].

Homogeneous Models

A simulation model for thrombin generation in plasma was developed in Willems et al. [1991] wherein they validated the model with experimental data using a nominal fit of the kinetic parameters (obtained from purified systems). They concluded that, in order to strengthen the model, such parameters need to be directly identified from plasma activation curves. Jones and Mann [1994] used a collection of empirical, estimated, and deduced kinetic rate constants in their model for the tissue factor pathway of coagulation. This model provides a satisfactory approximation of empirical data involving activation reactions for

various coagulation factors (IX, X, V, VIII, and thrombin), and the reactions for the assembly of the coagulation enzyme complexes (VIIIa:IXa - intrinsic tenase and Va:Xa - prothrombinase) on phospholipid membranes. One of the widely reviewed homogeneous models is that developed by Hockin et al. [2002] (which we refer to as the Hockin-Mann model) wherein fully activated platelets are assumed to be present from t = 0 with no limitation of platelet phospholipid surface. The model (which consists of 34 differential equations and 42 rate constants) describes production of thrombin via extrinsic pathway in synthetic plasma upon addition of exogenous TF. No distinction is made between soluble species and platelet-bound species. Lo et al. [2005] have solved this model stochastically using a kinetic Monte Carlo simulation to describe clotting in small volumes. Yet another mathematical model that has successfully predicted the action of serine protease inhibitors on the tissue factor (extrinsic) pathway, is the one developed by Leipold et al. [1995].

Spatiotemporal dynamics of thrombin production and fibrin polymerization have been studied in experiments (Ataullakhanov et al. [2002b]), and backed up theoretically (Ataullakhanov et al. [2002a]). The dependence of thrombus formation on surface concentration density and overall distribution of tissue factor was studied using a set of partial differential equations in Balandina et al. [2011]. In their review article, Anand et al. [2003] proposed a series of coupled convection-reaction-diffusion partial differential equations to describe the flow, generation, and depletion of hemostatic system components in flowing blood and combined them with shear-thinning viscoelastic fluid models for blood and clot. Their work was the first to combine both the biochemistry and rheology in an exact mathematical framework whereby the spatio-temporal changes during clot formation, growth, and lysis in flowing blood could be tracked. Anand et al. [2008] have listed a set of reaction-diffusion equations and certain criteria to simulate thrombus formation, growth, and thrombolysis in a quiescent pool of plasma exposed to a thrombogenic surface. The model, like the Hockin-Mann model (Hockin et al. [2002]), provides for an excess of platelet binding sites and does not include platelets as a separate entity. However, unlike the other models, this model includes equations for species involved in fibrinolysis as well. Also, the model can be studied along with flow thus identifying the location and size of clots formed in different flow profiles. The numerical solution for blood flow in an artery was obtained using the model in Anand et al. [2008] along with Newtonian fluid models in Bodnár and Sequeira [2008], and for the shear-thinning viscoelastic fluid models in Bodnár et al. [2014]. *In vivo*, the various reactions of the multi-stage coagulation cascade occur at different sites, and this redefines the way each constituent pathway of the process relates to the other and the process as a whole in a cascade model. LaCroix and Anand [2011] extended the model in Anand et al. [2008] and investigated the effect of the intrinsic pathway on clot formation, growth and dissolution *in vitro*.

Heterogeneous Models

Regulation of coagulation at different spatial locations and temporal stages by the different feedback pathways like, for example, fXa generation by intrinsic tenase which is later amplified by fIX activation in the fXI-mediated feedback, was modeled using reaction-diffusion equations by Panteleev et al. [2006]. The model highlighted the concept of spatial heterogeneity in coagulation control and used kinetic constants and initial concentrations from experimental reports without any fitting. The work in Panteleev et al. [2006] was extended to understand the effect of flow on coagulation regulation: Shibeko et al. [2010] modified the model to include convection terms to account for flow. They showed that removal of factor Xa by flow largely controls the initiation of clotting under flow (at high shear rates). A heterogeneous model consisting of 73 ODEs was developed in Bungay et al. [2003], which we shall refer to as the Bungay-Gentry model. This model differentiates clearly between the fluid phase zymogens/enzymes and lipid-bound factors and complexes. The role of lipid surface area and non-specific binding of clotting factors during extrinsic coagulation in quiescent conditions was highlighted in this model. The kinetic model developed by Kuharsky and Fogelson [2001] (the Kuharsky-Fogelson model) for the extrinsic pathway of thrombin production describes binding of coagulation factors to specific binding sites on activated platelets (unlike in Bungay-Gentry model). The Kuharsky-Fogelson model was developed for coagulation of normal plasma with wall bound TF exposed in a wound patch on healthy endothelium layer with

thrombomodulin binding sites (for production of activated protein C (APC)). This model was extended to perform a combined analysis on the vulnerability of a reaction cascade to pharmacological inhibition as well as the robustness of such mechanistic models in revealing such points of fragility (Luan et al. [2007]); this analysis could be helpful in designing suitable drugs. The Kuharsky-Fogelson model was later extended to include thrombin mediated feedback activation of XIa (Fogelson et al. [2012]). More recently, a convection-diffusion-reaction model was developed to investigate the roles of surface bound complexes, prothrombinase and extrinsic tenase, in production of thrombin and factor Xa, respectively (Haynes et al. [2011, 2012]). The production of Xa was reported to be transport limited when only extrinsic tenase was present; competition between prothrombinase and extrinsic tenase for lipid binding sites caused Xa production to enter an intermediate regime between reaction and transport limitation.

Pseudo-homogeneous Models

The Hockin-Mann model (Hockin et al. [2002]) was extended by Chatterjee et al. [2010] to understand clotting at picomolar concentrations of (as well as in absence of) added TF, and also included the intrinsic pathway. This Platelet-Plasma model assumes a 'platelet activation status' which accounts for the dynamics of platelet activation without explicitly including platelets as a distinct reacting variable. The model discusses the feedback activation of resting platelets by thrombin.
The model of Susree and Anand [2016] describes clot formation and growth *in vitro* in a quiescent pool of plasma containing platelets. We included platelet concentration as a distinct variable, and assumed platelet activation to be due to both thrombin as well as other activated platelets. Clotting factor activation and enzymatic reactions on platelet membranes are modeled to proceed via the extrinsic pathway. Inhibition of the active clotting factors is considered to be by TFPI and ATIII only since the absence of endothelial cells in the system rules out inhibition by APC. Also, we neglected the contact pathway (known to be activated upon contact of blood with any negatively-charged surface) by assuming it to be suppressed by action of corn-trypsin inhibitor.

3. Problem Formulation

Sample Model (Salient Features)

The sample model used for discussion in this paper is that in Susree and Anand [2016], and this model (as also the models in Hockin et al. [2002] and Kuharsky and Fogelson [2001]) consists of a system of ordinary differential equations. An extension of this formulation is found in Anand et al. [2008] where partial differential equations are given to account for both reaction and diffusion. In Susree and Anand [2016], each of the model equations is formulated in the following manner:

$$\frac{d[Y_i]}{dt} = G_i$$

where $[Y_i]$:the concentration of the constituent Y_i,
$\frac{d[Y_i]}{dt}$: the time derivative of $[Y_i]$, and
G_i the net rate of production of Y_i as a result of enzymatic reactions (equal to the rate of production minus the rate of depletion).
A sample equation for G_i is of the form below:

$$\begin{aligned} G_{Xa^m} = \quad & \frac{k_{10}[VIIIa^m : IXa^m][X^m]}{K_{10M} + [X^m]} - k^+_{PRO}[Va^m][Xa^m] \\ & + k^-_{PRO}[Va^m : Xa^m] + k^+_{10}N_{10}[AP][Xa] - k^-_{10}[Xa^m] \end{aligned}$$

Here $Xa, IXa, VIIIa$ refer to active forms of coagulation factors X, IX, and $VIII$. Superscript 'm' refers to 'membrane-bound', the membrane being found on the surface of activated platelets (AP).

$VIIIa^m : IXa^m$, $Va^m : Xa^m$ refer to membrane bound procoagulant complexes, intrinsic tenase and prothrombinase, respectively. N_{10} refers to number of binding sites specific to factor X/Xa on the surface of activated platelets. The entire set of reactions and mechanisms used in Susree and Anand [2016] are included in Table 1. Details of the reaction rate constants and respective references can be found in Susree and Anand [2016].

This implies that the model is a set of coupled non-linear ODEs that need to be solved subject to the appropriate initial conditions. The equations in Susree and Anand [2016] were then solved using the 'ode15s' algorithm (meant for stiff ODEs) in MATLAB (version R2012a); in general, any numerical method designed to solve non-linear coupled ODEs can be used, and a description of such numerical methods can be found in Conte and Boor [1980]. For a detailed description of the assumptions, and salient features of the sample model we refer the readers to Susree and Anand [2016]. Detailed analysis of mechanistic models includes sensitivity analysis of the equations, and we refer the readers to Danforth et al. [2009], Luan et al. [2007], Naidu and Anand [2014] for instances of such sensitivity analysis. Further, for a detailed view on numerical solution and analysis of mechanistic models for coagulation, refer to Luan et al. [2010]. It is apparent that the model solution depends critically on the parameters present in the equations for G_i as given above. We now discuss the impact of choosing different values from the literature for a given kinetic constant.

Table 1:**Reactions and Reaction Mechanisms from Susree and Anand [2016] (F: First order, M: Michaelis-Menten, S: Second order)**

(a) For reactions involving platelets

Reaction	Mechanism
$PL + AP \xrightarrow{kpp} 2AP$	**S**
$PL + IIa \xrightarrow{kp2} AP$	**S**

(b) For enzymatic reactions in plasma

Reaction	Mechanism
$TF + VII \underset{k_{T7}^-}{\overset{k_{T7}^+}{\rightleftharpoons}} TF : VII$	**S**
$TF + VIIa \underset{k_{T7a}^-}{\overset{k_{T7a}^+}{\rightleftharpoons}} TF : VIIa$	**S**
$TF : VIIa + VII \xrightarrow{k_{TF7}} TF : VIIa + VIIa$	**S**
$VII + Xa \xrightarrow{k_{10,7}} VIIa + Xa$	**S**
$VII + IIa \xrightarrow{k_{2,7}} VIIa + IIa$	**S**
$TF : VIIa + ATIII \xrightarrow{h_7^{AT}} TF : VIIa_i + ATIII$	**S**
$TF : VIIa + Xa : TFPI \xrightarrow{h_7^{TP}} TF : VIIa_i + Xa : TFPI$	**S**
$TF : VIIa + IX \xrightarrow{k_9, K_{9M}} TF : VIIa + IXa$	**M**
$IXa + ATIII \xrightarrow{h_9} IXi + ATIII$	**S**
$TF : VIIa + X \xrightarrow{k_{7,10}, K_{7,10M}} TF : VIIa + Xa$	**M**

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Table (b) – continued from previous page

Reaction	Mechanism
$Xa + ATIII \xrightarrow{h_{10}} Xi + ATIII$	**S**
$Xa + TFPI \underset{h_{10}^{TP-}}{\overset{h_{10}^{TP+}}{\rightleftharpoons}} Xi + TFPI$	**S**
$II + Xa \xrightarrow{k_{2t}} IIa + Xa$	**S**
$IIa + ATIII \xrightarrow{h_2} IIi + ATIII$	**S**
$VIII + IIa \xrightarrow{k_8, K_{8M}} VIIIa + IIa$	**M**
$VIIIa \xrightarrow{h_8} VIIIi$	**F**
$V + IIa \xrightarrow{k_5, K_{5M}} Va + IIa$	**M**
$Va \xrightarrow{h_5} Vi$	**F**
$I + IIa \xrightarrow{k_f, K_{fM}} Ia$	**M**

(c) For enzymatic reactions on membrane surface

Reaction	Mechanism
$IX/IXa + AP_9 \underset{k_9^-}{\overset{k_9^+}{\rightleftharpoons}} IX^m/IXa^m$	**S**
$IXa^m + VIIIa^m \underset{k_{TEN}^-}{\overset{k_{TEN}^+}{\rightleftharpoons}} VIIIa^m : IXa^m$	**S**
$X/Xa + AP_{10} \underset{k_{10}^-}{\overset{k_{10}^+}{\rightleftharpoons}} X^m/Xa^m$	**S**
$X^m + VIIIa^m : IXa^m \xrightarrow{k_{10}, K_{10M}} Xa^m + VIIIa^m : IXa^m$	**M**
$Xa^m + Va^m \underset{k_{PRO}^-}{\overset{k_{PRO}^+}{\rightleftharpoons}} Va^m : Xa^m$	**S**
$II/IIa + AP_2 \underset{k_2^-}{\overset{k_2^+}{\rightleftharpoons}} II^m/IIa^m$	**S**
$II^m + Xa^m : Va^m \xrightarrow{k_2, K_{2M}} IIa^m + Xa^m : Va^m$	**M**
$VIII/VIIIa + AP_8 \underset{k_8^-}{\overset{k_8^+}{\rightleftharpoons}} VIII^m/VIIIa^m$	**S**
$VIII^m + IIa^m \xrightarrow{k_8^m, K_{8M}^m} VIIIa^m + IIa^m$	**M**
$VIII^m + Xa^m \xrightarrow{k_{8t}^m, K_{8tM}^m} VIIIa^m + Xa^m$	**M**
$V/Va + AP_5 \underset{k_5^-}{\overset{k_5^+}{\rightleftharpoons}} V^m/Va^m$	**S**

Continued on next page

Table (c) – continued from previous page

Reaction	Mechanism
$V^m + IIa^m \xrightarrow{k_5^m, K_{5M}^m} Va^m + IIa^m$	**M**
$V^m + IIa^m \xrightarrow{k_{5t}^m, K_{5tM}^m} Va^m + Xa^m$	**M**

3.1. *Kinetic constants and their effect on model predictions*

Kinetic constants used in mechanistic models are obtained from various sources and hence vary extensively. Small, seemingly minor, variations in conditions (like pH, temperature, [Ca^{2+}], etc) between experiments in each study are but one of the reasons for discrepancies in the values of kinetic constants reported. Further, the kinetic constants are obtained for purified proteins *in vitro* so that, the loss or enhancement of specific activity of the enzymes when combined in plasma is overlooked: this is another reason for the debate between Hemker et al. [2012] and Mann [2012]. Further, our constants are based on depletion of a reactant the way it is secreted. For a computational model to be applicable in testing hypothesis, the parameters need to be representative of the physiologically relevant network, but must have low levels of measurement errors. A minimal consensus on the kinetic constants and initial concentrations that can be used in mechanistic models is important not only in population-specific studies but also in patient-specific studies (Brummel-Ziedins [2013]).
However, at present there is no such consensus, and different workers use different kinetic constants for the same reaction which are sometimes not meant for use in the conditions being simulated. We illustrate the effect of such differences on the predictions of the model developed in Susree and Anand [2016]. We select the following cases:

- *variation due to change in kinetic constants for ATIII inhibition (whether of IXa, Xa, or IIa)* (Figures 2 and 3),
- *variation due to change in kinetic constants for activation of fX by tenase,* (Figure 4a) *and*
- *variation due to change in kinetic constants for activation of thrombin by prothrombinase* (Figure 4b).

Kinetic constants for ATIII inhibition of IXa, Xa and IIa from three different sources were used. The predictions of the model in Susree and Anand [2016] are documented for each set while keeping the rest of the constants unchanged. The thrombin concentration curve predicted by the model for constants from Hockin et al. [2002] shows a percentage difference of 123.6% and 8.05% compared to the original model (which has constants from Wiebe et al. [2003]) in terms of peak thrombin concentration and peak thrombin time, respectively (Figure 2). However, the concentration profile obtained using constants taken from Anand et al. [2008] shows -99.28% and 559.34% difference compared to the original model in terms of peak thrombin concentration and peak thrombin time (within the time of simulation), respectively. This difference is because Anand et al. [2008] use constants that have been reported for inhibition by ATIII in the presence of heparan sulfate (HS) which are far higher than those in the absence of HS.

Similarly, TF:VIIa production curves (Figure 3), under the conditions mentioned above, show significant disparity in peak concentration (67.28%) and time (-3.8%) when comparing the predictions with constants from Wiebe et al. [2003] (original model) with those from Hockin et al. [2002], while with the constants from Anand et al. [2008] no peak is observed within the time of simulation.

When constants for intrinsic tenase activation of factor X from three different sources (Anand et al. [2008] and Kuharsky and Fogelson [2001] vs Mann et al. [1990]) are used, there is a 26.62% (Anand et al. [2008]) and 11.73% (Kuharsky and Fogelson [2001]) rise in peak thrombin concentration, when compared

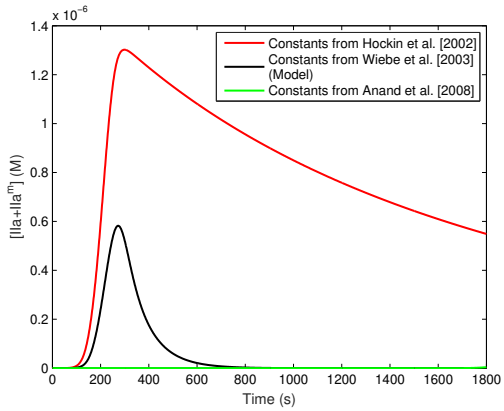


FIGURE 2. Variation in [IIa+IIa^m] for ATIII inhibition constants from different sources

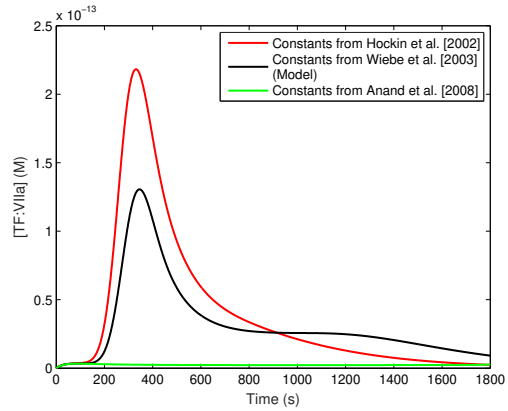
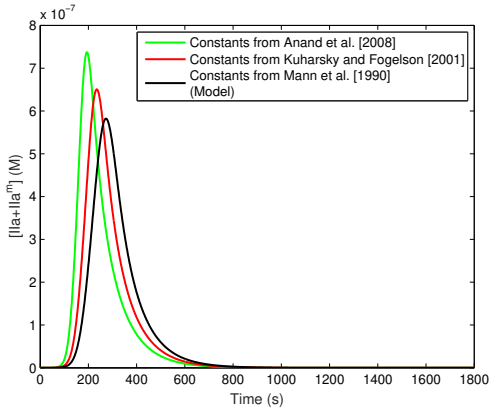
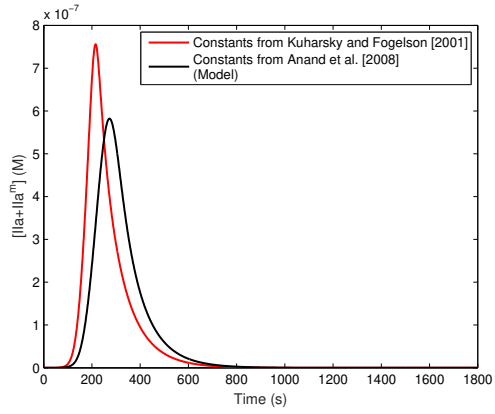


FIGURE 3. Variation in [TF:VIIa] for ATIII inhibition constants from different sources



(a)



(b)

FIGURE 4. Thrombin production w.r.t. variation in constants for (a) IXa^m :VIIIa^m activation of X^m, and (b) Xa^m :Va^m activation of II^m.

to the original model, respectively, as seen in Figure 4(a).
Constants for prothrombinase activation of prothrombin (reaction mechanism being Michaelis-Menten) from two different sources are used (Kuharsky and Fogelson [2001] vs Anand et al. [2008] (which is the source for the model)). As with intrinsic tenase activation of factor X, a similar difference in peak thrombin production of 29.83% is seen in Figure 4(b). These large differences prompted us to review the studies for each kinetic constant so as to identify a single value for a given condition (synthetic, *in vitro*, or *in vivo*).

4. Mechanism and Kinetic constants for Hemostatic reactions

In the following sections, we detail the studies which record the kinetics of individual reactions, involved in hemostasis, under conditions that mimic *in vivo*, or for *in vitro* plasma, or for synthetic plasma media.

4.1. *Activation of Resting Platelets*

The tissue factor and collagen exposed due to disruption of sub-endothelium lead to activation of platelets (Furie and Furie [2008]). Also, platelets are constitutively activated once they come in contact with other activated platelets in the plasma or on the sub-endothelial surface (Kuharsky and Fogelson [2001]).

4.2. *Activation of factor VII, binding to TF, and fVIIa inhibition by TFPI*

Vascular damage exposes the integral membrane protein, tissue factor, to the components of the clotting cascade in flowing blood. Binding of TF and factor VII/VIIa initiates coagulation via the extrinsic pathway (Shobe et al. [1999]). Activation of free and TF-bound factor VII has been studied earlier (Bajaj et al. [1981],O'Brien et al. [1994]). A methodical assessment of the kinetics of factor VII activation by factor Xa, thrombin (IIa) and TF:VIIa is given in Butenas and Mann [1996]. Regulation of the extrinsic pathway of coagulation occurs by the action of TFPI on TF:VIIa via factor Xa (Rapaport [1991]). It is observed that initially TFPI inhibits factor Xa (free or TF:VIIa-bound) and then proceeds to inhibit TF:VIIa. ATIII also inhibits TF:VIIa (at a rate much greater than that for factor VIIa alone) following a second-order kinetic mechanism (Lawson et al. [1993]). The model developed in Susree and Anand [2016] uses constants for TF binding to factor VII/VIIa, activation of factor VII and TF:VII as well as for inhibition of TF:VIIa (by Xa:TFPI and ATIII) from Hockin et al. [2002] which are those for synthetic plasma.

4.3. *Activation of factor XII and Prekallikrein, and fXIIa inhibition by ATIII, α2AP, and C1INH*

The contact pathway is triggered by auto-activation of factor XII in blood when it comes into contact with a negatively charged surface. The cofactor for this reaction is high molecular weight kininogen. Activated factor XII activates prekallikrein to kallikrein which, in a feedback action, activates factor XII at a much faster rate (2000 times) than auto-activation (Tankersley and Finlayson [1984]). XIIa production is inhibited by ATIII, α2AP, and C1INH, each of the reactions following second-order kinetics (Pixley et al. [1985]). C1INH also inhibits kallikrein (Van der Graaf et al. [1983]).

4.4. *Activation of factor XI, and fXIa inhibition by ATIII and α1AT*

In the contact pathway, XIIa activates factor XI to XIa. Factor XI is known to be activated by thrombin *in vivo* on the platelet surface. The reaction follows Michaelis-Menten kinetics (Hoffman et al. [2012], Monroe et al. [2002]). And inactivation of factor XIa is due to the action of ATIII (Soons et al. [1987]) and α_1AT (Scott et al. [1982]). The inactivation of XIa is a second-order reaction.

4.5. *Activation of factor IX, and fIXa inhibition by ATIII*

The extrinsic pathway proceeds by the activation of factor IX by the extrinsic tenase complex (TF:VIIa). This is augmented by factor XIa activation of factor IX via the intrinsic pathway (Bauer et al. [1990]). While the former reaction occurs on the sub-endothelial surface (Hoffman et al. [2012]), the latter reaction is now believed to occur on platelet surfaces (Gailani et al. [2001]). fIX activation by either TF:VIIa or fXIa follows Michaelis-Menten kinetics. Kinetic studies of fIX activation by TF:VIIa reveal that when compared to fX, fIX shows faster conversion (Zur and Nemerson [1979]) even at different cofactor concentrations (Komiyama et al. [1990]). Physiologically, activation of fIX by fXIa is also significant; a catalytic constant about 20-50 times higher than that for TF:VIIa activation of fIX establishes this fact (Walsh et al. [1984]). fIXa is inhibited *in vivo* by ATIII in a heparin-mediated manner (Wiebe et al. [2003]) and the reaction follows second-order kinetics.

4.6. *Activation of factor X, and fXa inhibition by TFPI and ATIII*

Factor X is activated by the extrinsic tenase complex (TF:VIIa) via the extrinsic pathway on the sub-endothelial cell surface. It is also activated by the intrinsic tenase complex (VIIIa:IXa) via the intrinsic pathway on platelet membrane surface, and this reaction is 50 times faster than activation by TF:VIIa (Mann [2003]). Factor X activation by both extrinsic (Krishnaswamy et al. [1992]) and intrinsic tenases (Rawala-Sheikh et al. [1990]) shows apparent Michaelis-Menten kinetics. TFPI is the main inhibitor of fXa. TFPI binds to fXa that is not bound to TF:VIIa and inhibits the progress of extrinsic pathway of coagulation via feedback inhibition of TF:VIIa (Baugh et al. [1998]). Like fIXa, fXa inhibition by ATIII also follows second-order kinetics for the heparin-catalyzed reaction (van't Veer and Mann [1997]); however fXa remains shielded while in complex with fVa (Rosenberg and Rosenberg [1984]).

4.7. *Activation of prothrombin (fII), and fIIa inhibition by ATIII*

Thrombin is the last enzyme of the coagulation cascade and it catalyzes the conversion of soluble fibrinogen into insoluble fibrin polymer. The zymogen prothrombin is activated to the enzyme thrombin on the surface of activated platelets by the prothrombinase (Xa:Va) complex (Butenas and Mann [2002],Rosing et al. [1985]). The mechanism of prothrombin activation by prothrombinase, which follows Michaelis-Menten kinetics, proceeds via formation of the intermediate, meizothrombin (Krishnaswamy et al. [1987]). Though not quantitatively significant, prothrombin is also activated in plasma by factor Xa (Esmon et al. [1974]). Thrombin is primarily inhibited by ATIII in the presence of heparan sulphate *in vivo*, and this is a second order reaction (Wiebe et al. [2003]).

4.8. *Activation of factor VIII, and fVIIIa inhibition/inactivation*

Both thrombin and factor Xa activate factor VIII. The Michaelis-Menten kinetics of these reactions have been documented in Hill-Eubanks and Lollar [1990], and De Cristofaro and De Filippis [2003]. While activation of factor VIII by factor Xa occurs on the surface of activated platelets, activation of factor VIII by thrombin is independent of any such requirement (Neuenschwander and Jesty [1988]). FVIIIa is inhibited by proteolytic cleavage by activated protein C (APC) on the endothelial membrane surface (O'Brien et al. [2000], Varfaj et al. [2006]). A reduction in k_{cat} shows that IXa protects VIIIa from APC in the intrinsic tenase complex. The presence of Protein S has been shown to enhance the rate of factor VIIIa inactivation (O'Brien et al. [2000]). FVIIIa also undergoes spontaneous decay in plasma following first-order kinetics (Neuenschwander and Jesty [1992]).

4.9. *Activation of factor V, and fVa inhibition/inactivation*

Activation of factor V proceeds in a manner similar to that of factor VIII, i.e., by thrombin and factor Xa (Monkovic and Tracy [1990a]). Thrombin activation of fV doesn't require a platelet surface, while that by fXa requires activated platelets: both reactions follow Michaelis-Menten kinetics. The Michaelis-Menten kinetics of fVa inactivation by APC, and the mitigation of protection of fVa (in complex with fXa) by protein S has been studied in Freyssinet et al. [1991], Solymoss et al. [1988].

4.10. *Formation and dissociation of Intrinsic Tenase complex (IXa:VIIIa)*

The fX activating complex of the intrinsic pathway, IXa:VIIIa, is formed by assembly of the clotting factors IXa and VIIIa on the membrane surface of activated platelets (Mann et al. [1990], Ahmad et al. [2003]). The presence of cofactor fVIIIa enhances the specific binding of fIXa to activated platelets for subsequent formation of intrinsic tenase (Ahmad et al. [1989]). The second-order kinetics for binding of fIXa and fVIIIa on platelet surfaces is studied well (van Dieijen et al. [1985]), as is the depletion of intrinsic-tenase activity due to instability of fVIIIa in the complex (Fay et al. [1996]).

4.11. *Formation and dissociation of Prothrombinase complex (Xa:Va)*

The platelet membrane-bound complex responsible for activation of prothrombin to thrombin is formed by a 1:1 stoichiometric interaction between fXa and (platelet membrane-bound) fVa (Tracy and Mann [1983]). The second-order kinetics of this reaction mechanism have been described in Mann et al. [1990], Krishnaswamy et al. [1987], Swords and Mann [1993].

4.12. *Kinetics of inhibition reactions involving ATIII, TFPI, and PC/APC*

ATIII is a major physiologic inhibitor of almost all serine proteases produced during the process of blood coagulation, while TFPI mainly targets factor Xa and the TF-VIIa-Xa complex. It is evident from kinetic studies that the presence of heparin accelerates the inhibitory action of ATIII (Wiebe et al. [2003], Rosenberg and Rosenberg [1984]). Inactivation by ATIII and TFPI occurs once the clotting enzymes escape into the plasma from the site of thrombus formation (Butenas and Mann [2002]). Apart from ATIII and TFPI, activated protein C (APC) also attenuates blood coagulation by inactivating factor Va and factor VIIIa present in their respective complexes, prothrombinase and tenase. While ATIII and TFPI are stoichiometric inhibitors, the protein C system is a dynamic inhibitory system (Butenas and Mann [2002]). Activation of protein C occurs when thrombin binds to its cofactor thrombomodulin on the endothelial surface. α_1AT brings about the inactivation of APC in plasma (Kolev et al. [1994]).

4.13. *Fibrinolysis*

Fibrinolysis begins following the activation of plasminogen (PLS), a circulating plasma zymogen, to plasmin (PLA) when it binds with fibrin and a tissue-type plasminogen activator (tPA) secreted by endothelial cells (Longstaff and Thelwell [2005]). Fibrinolysis is inhibited by the action of various enzymes like α_2 anti-plasmin (α_2AP) (second-order kinetics), and thrombin activable fibrinolysis inhibitor (TAFI) (Michaelis-Menten kinetics) (Rijken and Lijnen [2009]).

5. Discussion

We present here a list of reaction constants and the conditions that they best correspond to based on our review of single-scale models (developed in Kuharsky and Fogelson [2001], Hockin et al. [2002], Anand et al. [2008], and Susree and Anand [2016]): these constants have been used in the model developed in Susree and Anand [2016]. We propose that mechanistic models, which incorporate the reactions in Section 4, select the constants from among those in Table 2 with due care being given as to whether the constants are for *in vivo*, or *in vitro* conditions, or for synthetic plasma. The table, based on our review of the aforementioned models, lists kinetic constants with the correct references along with the conditions that they can be used in. The list of reactions involved in the process of coagulation as it occurs *in vivo* (platelet adhesion and activation, zymogen activation and inactivation, and clot lysis) have been outlined in the tables below along with the kinetics of the reactions and conditions under which the kinetic constants can be measured. Note that F represents 'First-order reaction mechanism', M represents 'Michaelis-Menten mechanism', and S represents 'Second-order mechanism'. The condition *'in vivo'* refers to kinetic constants measured for proteins derived from human plasma at conditions of 37°C, pH of 7.4-7.5, and $[Ca^{2+}]$ = 2.1-2.8mM. The condition *'in vitro'* also refers to kinetic constants measured for proteins derived from human plasma, and kept in test tube at conditions of 37°C, pH of 7.4-7.5, and $[Ca^{2+}]$ = 2.1-2.8mM. The condition *'synthetic'* refers to kinetic constants measured for purified proteins that are combined *in vitro* at conditions of 37°C, pH of 7.4-7.5, and $[Ca^{2+}]$ = 2.1-2.8mM so as to get concentrations that are same as those in human plasma.

Table 2: **Kinetic Constants and conditions where usable**

(a) For reactions involving platelets

Reaction	Kinetic Constant (Mechanism)	References	Conditions of applicability
platelet binding to and dissociation from subendothelium	k_P^+=$20nM^{-1}s^{-1}$ (S), k_P^-=$0.0s^{-1}$ (F)	Kuharsky and Fogelson [2001]	*in vivo*
platelet-activation of platelets	kpP= 0.3 $nM^{-1}s^{-1}$ (S)	Kuharsky and Fogelson [2001]	*in vitro*, *in vivo*
IIa-activation of platelets	$kp2$= 0.37 s^{-1} (S)	Kuharsky and Fogelson [2001]	*in vitro*, *in vivo*

(b) For enzymatic reactions in plasma

Reaction	Kinetic Constant (Mechanism)	References	Conditions of applicability
binding of TF and VII	$k_{T7}^+ = 3.2 \times 10^{-03} nM^{-1}s^{-1}$ (S)	O'Brien et al. [1994] Krishnaswamy [1992]	synthetic, *in vitro*
dissociation of TF:VII	$k_{T7}^- = 3.1 \times 10^{-03} s^{-1}$ (S)	O'Brien et al. [1994] Krishnaswamy [1992]	synthetic, *in vitro*
binding of TF and VIIa	$k_{T7a}^+ = 0.023nM^{-1}s^{-1}$ (S)	O'Brien et al. [1994] Shobe et al. [1999]	synthetic, *in vitro*
dissociation of TF:VIIa	$k_{T7a}^- = 3.1 \times 10^{-03} s^{-1}$ (S)	O'Brien et al. [1994] Shobe et al. [1999]	synthetic, *in vitro*
auto-activation of VII	$k_{TF7} = 4.4 \times 10^{-04} nM^{-1}s^{-1}$ (S)	Butenas and Mann [1996]	synthetic, *in vitro*
Xa-activation of VII	$k_{10,7} = 0.013nM^{-1}s^{-1}$ (S)	Butenas and Mann [1996]	synthetic, *in vitro*
IIa-activation of VII	$k_{2,7} = 2.3 \times 10^{-05} nM^{-1}s^{-1}$ (S)	Butenas and Mann [1996]	synthetic, *in vitro*
ATIII inactivation of TF:VIIa	$h_7^{AT} = 4.5 \times 10^{-07} nM^{-1}s^{-1}$ (S)	Lawson et al. [1993]	synthetic, *in vitro*
Xa:TFPI inactivation of TF:VIIa	$h_7^{TP} = 0.05nM^{-1}s^{-1}$ (S)	Baugh et al. [1998]	synthetic, *in vitro*
auto-activation of XII	$k = 3.3 \times 10^{-02} s^{-1}$ (M) $K_M = 7500nM$	Chatterjee et al. [2010]	synthetic, *in vitro*
kallikrein-activation of XII	$k_{12} = 7.25s^{-1}$ (M) $K_{12M} = 780.0nM$	Tankersley and Finlayson [1984]	synthetic, *in vitro*
inactivation of Kallikrein	$h_{kal} = 1.1 \times 10^{-02} s^{-1}$ (F)	Van der Graaf et al. [1983]	synthetic, *in vitro*
XIIa-activation of Kallikrein	$k_{kal} = 40.0s^{-1}$ (M) $K_{kal} = 37000.0nM$	Tankersley and Finlayson [1984]	synthetic, *in vitro*
inactivation of XIIa	$h_{12} = 1.417 \times 10^{-02} s^{-1}$ (F)	Silverberg and Kaplan [1982]	synthetic, *in vitro*
C1INH-inactivation of XIIa	$h_{12}^{C1} = 3.67 \times 10^{-06} nM^{-1}s^{-1}$ (S)	Pixley et al. [1985]	synthetic, *in vitro*
ATIII-inactivation of XIIa	$h_{12}^{AT} = 2.167 \times 10^{-08} nM^{-1}s^{-1}$ (S)	Pixley et al. [1985]	synthetic, *in vitro*
α2AP-inactivation of XIIa	$h_{12}^{AP} = 1.83 \times 10^{-07} nM^{-1}s^{-1}$ (S)	Pixley et al. [1985]	synthetic, *in vitro*
XIIa-activation of XI	$k_{11i} = 5.7 \times 10^{-04} s^{-1}$ (M) $K_{11iM} = 2000.0nM$	Gailani and Broze [1991]	synthetic, *in vitro*
ATIII-inactivation of XIa	$h_{11(A3)} = 2.67 \times 10^{-05} nM^{-1}s^{-1}$ (S)	Soons et al. [1987]	synthetic, *in vitro*, *in vivo*
α1AT-inactivation of XIa	$h_{11(L1)} = 2.16 \times 10^{-05} nM^{-1}s^{-1}$ (S)	Scott et al. [1982]	synthetic, *in vitro*, *in vivo*
TF:VIIa-activation of IX	$k_{7,9} = 0.26s^{-1}$ (M) $K_{7,9M} = 243.0nM$	Mann et al. [1990]	synthetic, *in vitro*, *in vivo*
XIa-activation of IX	$k_9 = 0.183s^{-1}$ (M) $K_{9M} = 160.0nM$	Sun and Gailani [1996]	synthetic, *in vitro*, *in vivo*

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Table (b) – continued from previous page

Reaction	Kinetic Constant (Mechanism)	References	Conditions of applicability
ATIII-inactivation of IXa	$h_9 = 2.223 \times 10^{-09} nM^{-1} s^{-1}$ (S)	Wiebe et al. [2003]	synthetic, *in vitro*, *in vivo*
TF:VIIa-activation of X	$k_{7,10} = 1.15 s^{-1}$ (M) $K_{7,10M} = 450.0 nM$	Mann et al. [1990]	synthetic, *in vitro*, *in vivo*
ATIII-inactivation of Xa	$h_{10}^{AT} = 5.78 \times 10^{-03} nM^{-1} s^{-1}$ (S)	Wiebe et al. [2003]	synthetic, *in vitro*, *in vivo*
binding of Xa with TFPI	$h_{10}^{TP+} = 9.0 \times 10^{-04} nM^{-1} s^{-1}$ (S)	Baugh et al. [1998]	synthetic, *in vitro*, *in vivo*
dissociation of Xa:TFPI	$h_{10}^{TP-} = 3.6 \times 10^{-04} nM^{-1} s^{-1}$ (S)	Baugh et al. [1998]	synthetic, *in vitro*, *in vivo*
Xa-activation of II	$k_{2t} = 7.5 \times 10^{-06} nM^{-1} s^{-1}$ (S)	Hockin et al. [2002]	synthetic, *in vitro*, *in vivo*
ATIII-inactivation of IIa	$h_2 = 4.817 \times 10^{-06} nM^{-1} s^{-1}$ (S)	Wiebe et al. [2003]	synthetic, *in vitro*, *in vivo*
IIa-activation of VIII	$k_8 = 0.9 s^{-1}$ (M) $K_{8M} = 147 nM$	De Cristofaro and De Filippis [2003]	synthetic, *in vitro*, *in vivo*
spontaneous decay of VIIIa	$h_8 = 0.0037 s^{-1}$ (F)	Neuenschwander and Jesty [1992]	synthetic, *in vitro*, *in vivo*
APC-inactivation of VIIIa	$h_{C8} = 0.66 s^{-1}$ (M) $H_{C8M} = 102.3 nM$	Varfaj et al. [2006]	synthetic, *in vivo*
IIa-activation of V	$k_5 = 0.233 s^{-1}$ (M) $K_{5M} = 71.7 nM$	Monkovic and Tracy [1990a]	synthetic, *in vitro*, *in vivo*
spontaneous decay of Va	$h_5 = 0.0028 s^{-1}$ (F)	Freyssinet et al. [1991]	synthetic, *in vitro*, *in vivo*
APC-inactivation of Va	$h_{C5} = 0.17 s^{-1}$ (M) $H_{C5M} = 14.6 nM$	Solymoss et al. [1988]	synthetic, *in vivo*

(c) For enzymatic reactions on membrane surface

Reaction	Kinetic Constant (Mechanism)	References	Conditions of applicability
XI/XIa binding to and dissociation from platelets	$k_{11}^{+} = 0.01 nM^{-1} s^{-1}$ (S) $k_{11}^{-} = 0.1 s^{-1}$ (F)	Fogelson et al. [2012]	*in vitro*, *in vivo*
IIa^m-activation of XI^m	$k_{11} = 1.3 \times 10^{-04} s^{-1}$ (M) $K_{11M} = 50.0 nM$	Gailani and Broze [1991]	*in vitro*, *in vivo*
IX/IXa binding to and dissociation from platelets	$k_9^{+} = 0.01 nM^{-1} s^{-1}$ (S) $k_9^{-} = 0.0257 s^{-1}$ (F)	Ahmad and Walsh [1994]	*in vitro*, *in vivo*
XIa^m-activation of IX^m	$k_9 = 0.183 s^{-1}$ (M) $K_{9M} = 160.0 nM$	Sun and Gailani [1996]	*in vitro*, *in vivo*
binding of IXa^m and $VIIIa^m$	$k_{TEN}^{+} = 0.01 nM^{-1} s^{-1}$ (S)	De Moerloose et al. [1997]	*in vitro*, *in vivo*
dissociation of IXa^m:$VIIIa^m$	$k_{TEN}^{-} = 5.0 \times 10^{-03} s^{-1}$ (F)	De Moerloose et al. [1997]	*in vitro*, *in vivo*
X/Xa binding to and dissociation from platelets	$k_{10}^{+} = 0.029 nM^{-1} s^{-1}$ (S) $k_{10}^{-} = 3.3 s^{-1}$ (F)	Krishnaswamy et al. [1988]	*in vitro*, *in vivo*
IXa^m:$VIIIa^m$-activation of X^m	$k_{10} = 8.33 s^{-1}$ (M) $K_{10M} = 63.0 nM$	Mann et al. [1990]	*in vitro*, *in vivo*
binding of Xa^m and Va^m	$k_{PRO}^{+} = 0.4 nM^{-1} s^{-1}$ (S)	De Moerloose et al. [1997]	*in vitro*, *in vivo*
dissociation of Xa^m:Va^m	$k_{PRO}^{-} = 0.2 s^{-1}$ (F)	De Moerloose et al. [1997]	*in vitro*, *in vivo*
II/IIa binding to and dissociation from platelets	$k_2^{+} = 0.01 nM^{-1} s^{-1}$ (S) $k_2^{-} = 14.0 s^{-1}$ (F)	Kuharsky and Fogelson [2001]	*in vitro*, *in vivo*

Continued on next page

Table (c) – continued from previous page

Reaction	Kinetic Constant (Mechanism)	References	Conditions of applicability
Xa^m:Va^m-activation of II^m	$k_2 = 22.4s^{-1}$ (M) $K_{2M} = 1060.0nM$	Krishnaswamy et al. [1987]	*in vitro*, *in vivo*
VIII/VIIIa binding to and dissociation from platelets	$k_8^+ = 4.3 \times 10^{-03} nM^{-1}s^{-1}$ (S) $k_8^- = 2.46 \times 10^{-03} s^{-1}$ (F)	Raut et al. [1999]	*in vitro*, *in vivo*
IIa^m-activation of $VIII^m$	$k_8^m = 0.9s^{-1}$ (M) $K_{8M}^m = 200nM$	De Cristofaro and De Filippis [2003]	*in vitro*, *in vivo*
Xa^m-activation of $VIII^m$	$k_{8t}^m = 0.023s^{-1}$ (M) $K_{8tM}^m = 20.0nM$	Lollar et al. [1985]	*in vitro*, *in vivo*
V/Va binding to and dissociation from platelets	$k_5^+ = 0.057nM^{-1}s^{-1}$ (S) $k_5^- = 0.17s^{-1}$ (F)	Krishnaswamy et al. [1988]	*in vitro*, *in vivo*
IIa^m-activation of V^m	$k_5^m = 0.23s^{-1}$ (M) $K_{5M}^m = 71.7nM$	Monkovic and Tracy [1990b]	*in vitro*, *in vivo*
Xa^m-activation of V^m	$k_{5t}^m = 0.046s^{-1}$ (M) $K_{5tM}^m = 10.4nM$	Monkovic and Tracy [1990a]	*in vitro*, *in vivo*
IIa-activation of I	$k_f = 59.0s^{-1}$ (M $K_{fM} = 3160.0nM$	Tsiang et al. [1996]	synthetic, *in vitro*, *in vivo*

(d) For reactions involving Protein C system

Reaction	Kinetic Constant (Mechanism)	References	Conditions of applicability
IIa-activation of protein-C	$k_{PC} = 0.65s^{-1}$ (M) $K_{PCM} = 3190.0nM$	Tsiang et al. [1996]	*in vivo*
α1AT-inactivation of APC	$h_{PC} = 1.1 \times 10^{-08} nM^{-1}s^{-1}$ (S)	Heeb et al. [1990]	synthetic, *in vitro*, *in vivo*

(e) For reactions involved in Fibrinolysis

Reaction	Kinetic Constant (Mechanism)	References	Conditions of applicability
tPA-activation of plasminogen	$k_{pla} = 0.2s^{-1}$ (M) $K_{mpl} = 18.0nM$	Madison et al. [1995]	*in vivo*
plasmin-inactivation of Ia	$h_1 = 25.0s^{-1}$ (M) $H_{1M} = 250000nM$	Diamond and Anand [1993]	synthetic, *in vitro*, *in vivo*
α2AP-inactivation of plasmin	$h_{pla} = 1.6 \times 10^{-03} nM^{-1}s^{-1}$ (S)	Kolev et al. [1994]	synthetic, *in vitro*, *in vivo*
IIa-activation of TAFI	$k_{TAFI(2)} = 2.1 \times 10^{-03} s^{-1}$ (M) $K_{TAFIM(2)} = 2140.0nM$	Bajzar [2000]	synthetic, *in vitro*, *in vivo*
plasmin-activation of TAFI	$k_{TAFI(P)} = 4.4 \times 10^{-04} s^{-1}$ (M) $K_{TAFIM(P)} = 55.0nM$	Bajzar [2000]	synthetic, *in vitro*, *in vivo*
TAFIa-inactivation of plasmin (Glu-residue)	$k_{Glu} = 2.34s^{-1}$ (M) $K_{GluM} = 142.0nM$	Foley et al. [2011]	synthetic, *in vitro*, *in vivo*
TAFIa-inactivation of plasmin (Lys-residue)	$k_{Lys} = 0.89s^{-1}$ (M) $K_{LysM} = 96.0nM$	Foley et al. [2011]	synthetic, *in vitro*, *in vivo*

The initial concentrations of the zymogens, inhibitors and platelets (Table 3) have been obtained from literature. They have been used previously in Anand et al. [2008] and Hockin et al. [2002]. The initial concentrations of activated zymogens have been set to 0.01% of that of the respective zymogens.

Table 3: **Initial Concentrations of the Proteins and Platelets**

Component	Normal Conc.(nM)	Component	Normal Conc.(nM)
TF	variable	$VIIIa^m$	0.0
VII	10.0	$VIII^m$	0.0
VII^m	0.0	VIIIa	0.00007
VIIa	0.1	VIII	0.7
$VIIa^m$	0.0	Va^m	0.0
IXa^m	0.0	V	20.0
IX^m	0.0	APC	0.006
IXa	0.009	PC	60.0
IX	90.0	ATIII	3400.0
Xa^m	0.0	TFPI	2.5
X^m	0.0	Xa:TFPI	0.0
Xa	0.017	$\alpha 1AT$	45000.0
X	170.0	Ia	0.70
IIa^m	0.0	I	7000.0
II^m	0.0	tPA	0.08
IIa	0.140	PLA	0.218
II	1400.0	PLS	2180.0
PL	10.0	$\alpha 2AP$	105.0
AP	0.001	SE	0.22
AP^s	0.0		

5.1. *Limitations & Extensions*

Note that the aforementioned reactions in Table 2 are those that pertain to the view of extrinsic coagulation espoused in Furie and Furie [2008]. A mathematical modeler who is exposed to the view of a different group, for example Walsh [2004], could select only some of the reactions mentioned in Table 2. The modeler could yet come up with a legitimate model of coagulation and use the parameters in Table 2. However, the modeler will then have to be aware that the reaction mechanisms, for the same reaction, could themselves differ in different views of coagulation. Our study does not address this aspect of hemostasis modeling, namely, the variable choices available in the reaction network. We refer the reader to the review by Diamond [2013], where the four possible factors (reaction network, reaction mechanism, kinetic parameters, initial condition) in a kinetic model of coagulation are mentioned. In the context of a particular biochemical view, we have addressed three factors (reaction mechanism, kinetic parameters, initial condition) in our study.

In addition to the reactions discussed using the sample model, there are other reactions that are important and understudied. Such reactions need further investigation or parameterization. These mainly include inhibition of platelet-activating agents, inhibition of fibrinolysis, platelet-platelet interaction, and fibrin polymerization and network formation. Further, integration of the various sub-processes like platelet interaction and aggregation, fibrin network formation, etc, occurring at different scales (not discussed in this review) can deliver a more elaborate understanding of the biological phenomenon of clotting.

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