

Mathematical Approaches to Modelling and Controlling Blood Thrombin Formation

Von der Fakultät für Mathematik, Informatik und Naturwissenschaften
der Rheinisch-Westfälischen Technischen Hochschule Aachen
zur Erlangung des akademischen Grades einer Doktorin
der Naturwissenschaften genehmigte Dissertation

vorgelegt von

Sandra Maria da Cunha Órfão

aus Quelimane (Mosambik)

Berichter: Universitätsprofessor Dr. Gerhard Jank
Universitätsprofessor Dr. Sebastian Walcher
Professor Dr. Khosrhow Mottaghy

Tag der mündlichen Prüfung: 26.02.2007

Diese Dissertation ist auf den Internetseiten der Hochschulbibliothek online verfügbar.

Danksagung

Angefangen hat alles mit der Weihnachtsfeier 2004 des Masterstudienganges Biomedical Engineering. Prof. Mottaghy kam zu Prof. Jank und mir und sagte, er habe eine Idee, bei dem er hoffe, wir können ihm helfen. Dass aus diesem Gespräch eine Dissertation in Mathematik der Grösse XL entstehen würde, war mir anfangs nicht klar. Verantwortlich dafür, dass daraus meine Arbeit entstanden ist, sind Prof. Dr. Gerhard Jank, Prof. Dr. Khosrow Mottaghy und Prof. Dr. Sebastian Walcher. Diesen drei Personen bin ich zu besonderem Dank verpflichtet.

Prof. Dr. G. Jank danke ich für seine ständige Unterstützung, nicht nur bei der Betreuung meiner Arbeit, sondern auch für alles, was er für mich getan hat seit ich in Aachen bin. Für mich ist er wie ein Mentor des Lebens und der Mathematik.

Prof. Dr. K. Mottaghy danke ich für die faszinierende Fragestellung und für die ständige Unterstützung und Bereitschaft immer dann, wenn ich ihn gebraucht habe.

Prof. Dr. S. Walcher ist später eingestiegen. Ohne sein Interesse, seine Unterstützung und Bereitschaft hätte meine Arbeit diese Tiefe in so kurzer Zeit nicht erreicht. Vielen Dank!

Mein persönlicher Dank gilt auch Gehrt Hartjen und Daniel Robertz für die MAPLE Beratung und Dirk Meierling bei Hilfestellungen in Graphentheorie. Nicht zu vergessen Wolfgang Kromen, Victor Schneider, Filipe Miranda und Miguel Alonso: Muito obrigada por terem lido e comentado o meu trabalho! Konstantin Weiß danke ich sowohl für die Unterstützung bei graphischen Darstellungen als auch für die Hilfe beim Poster, das ich in Bologna präsentiert habe. Für die unbezahlbare Unterstützung in allen Lagen des Lebens sage ich Dir Konstantin: Spassiba!

An meine restlichen Freunde, die mehr oder weniger von überall her kommen und die überall wohnen, die aber meine Odyssee immer begleitet haben, sage ich einfach: Obrigada! Gracias! Merci! Thanks! Danke!

An Frau Volkmann, die Professoren, Arbeitskollegen und Freunde des Lehrstuhl II für Mathematik sage ich Danke für die schöne Zeit mit Euch/Ihnen, insbesondere

für die angenehme Atmosphäre, und die netten Feierabende, wo ich immer gerne mitgemacht habe.

Aos meus irmãos e aos restantes familiares agradeço a atenção, o carinho e o apoio que me deram ao longo destes anos.

Bem hajam.

Aachen, Januar 2007

Sandra Órfão

Preface

The title of this thesis reveals the interdisciplinary nature of my investigation, where different mathematical methods were used to handle the questions of modelling, analyzing and controlling thrombin formation in blood coagulation systems.

Different scientific disciplines have different languages and different ways to build structures and processes, so that the flow between knowledge and new findings is usually restricted to one particular field. As a consequence, disciplines of exact, empirical or experimental nature can hardly communicate with each other. On the other hand, systems like the blood coagulation system, cannot be explained or understood only by using the laws or axioms of a single science. Thus, one of the aims of this thesis was to create a science to science interface and a common platform of knowledge based in different mathematical approaches. Everything started therefore by collecting the necessary information from Mathematics, Medicine and Biochemistry. So, there must be a proper way of stating the right questions and getting the right answers. Thereby, one of the essential points was to pick up a simple but by no means simplistic form to communicate contents.

The process of collecting relevant information from scientists with different backgrounds is not a simple matter. However, the process of filtering and synthesizing information from foreign disciplines is in general more demanding. In this phase, the first task is to select information by rhetoric asking: what is really important? what do I really need from the foreign disciplines? shall I go deeper into some of these subjects? is there some piece missing? where do I get it? The second task is to translate the information and state the problem in mathematical terms. The later here, one has to decide which mathematical branch or branches are more suitable to built the model and make further analysis. Thereby, it is crucial to investigate whether there are known approaches in the literature and to keep in mind that there are some natural relevant parameters with a very specific meaning that cannot be changed. So, the model has to reflect at least some of the most important characteristics of the system. Whenever possible, it should provide new insights about the mechanism that is the object of study. Last but not least, the results of the investigation should be made intelligible to all potential different

readers with variable scientific backgrounds. This is of major importance for the different scientists to use the information gained and to progress in their own area. In particular, mathematicians experience whether the approach can be fully based on classical results or whether there is still the need of new results.

Thrombin is the essential enzyme product of the blood coagulation process. Since the early investigations on this field, the blood coagulation process has been represented as a cascade of enzymatic reactions. Nowadays it is known to respond in a threshold manner, involving numerous intermeshed controls including feedforward and feedback loops. The regulation of the production of thrombin is vital to the maintenance of the hemostatic balance in humans. However, uncontrolled generation of this enzyme can lead to physiological disaster. From the medical literature it is known that foreign surface contact, e. g. during application of artificial organs, is a strong activator of whole of the system. Thus, intelligibility of the roles of the system components in this regulation is important for a therapeutic control of thrombotic and bleeding disorders. Chapter 1 contains the physiological background, where the principal procoagulatory and anticoagulatory factors and the two pathways leading to the formation of a fibrin clot are introduced. Furthermore, some aspects concerning the kinetics of coagulation are discussed together with a brief description of the contribution of mathematical modelling to the understanding of the dynamics inherent to this physiological system.

On the one hand, it is important for instance to know how biochemists deduce from a reaction scheme the differential equations they work with. On the other hand, it is also important to present contents in a terminology and formalism more standard in control and in dynamical systems theories. Thus, we put together the forthcoming information from the different literature sources in such a way that references in subsequent chapters are easily made. Terms like *stoichiometry* or *deficiency* of a network arise and help to gain insights into the structure of a chemical network and to understand how they influence its dynamics. All this is done in the first part of Chapter 2. Furthermore, since the blood coagulation system involves a series of enzymatic reactions, the second part of this chapter explains kinetic aspects of enzyme-catalyzed reactions. In particular, concepts like *quasi-steady assumption*, *Michaelis-Menten equation* or *enzyme inhibition* are briefly introduced.

Several attempts of modelling a part of the blood coagulation system include a stiff system of nonlinear differential equations with unknown parameters. These parameters are the reaction constants and the initial concentration of the coagulation factors, which are normally estimated by fitting experimental data. The number of parameters and equations is however considerably large, what might result in low parameter sensitivity. Moreover, the reaction mechanism is also not precisely known. In fact, there might be reactions that have been shown to exist in principle but do not occur in reality [SHH97]. Although kinetic analysis of

the individual reactions have been reported in the literature, an analysis of the complete reaction network has been given less attention. Altogether, another aim for this thesis was to make a careful mathematical analysis of two of the most cited models among the scientific community investigating the mechanisms of the blood coagulation cascade. One model is due to Stortelder, Hemker and Hemker [SHH97] and the other one is due to Jones and Mann [JoMa94].

These models are presented in Chapter 3. With the tools provided in Chapter 2, an analysis of the stoichiometry of both models is made and the numerical solution provided. Besides stoichiometry, we interpreted the reaction scheme of Jones and Mann in terms of homologies of graphs. Due to inconsistent information contained in the original model, the equations of the system are traced back by using the law of mass action and concluded that there is a term that has been introduced *a posteriori* to provoke a decay. The authors did not carefully analyze the consequences of this ansatz, though. This is done in the following chapter of this thesis.

An important characteristic of biochemical models is that variables representing chemical concentrations take only nonnegative values. So, positive invariance of both systems was checked. It turned out that the original system from Mann and Jones is not positively invariant. Stability is another natural mathematical requirement with clear biological significance. After checking that for any choice of initial nonnegative values the system converges to an equilibrium, one may ask furthermore about the structure of that equilibrium. This is done after identifying first integrals and therewith conserved quantities. The number of equations of both models could like this be reduced by the same amount of previously identified first integrals. The stability of the corrected version of the model from Mann and Jones was characterized in the sense of Lyapunov. Since zero is a simple eigenvalue of the Jacobian matrix of the function defined by the right-hand side of the system at a nonisolated equilibrium, local stability of Stortelder's model was concluded with a result given in Bibikov's lecture notes. This is accomplished in Chapter 4. A survey of known results of the qualitative theory of ordinary differential equations is given in Appendix A.

The equilibria depend in particular on the value of the constants that represent total concentrations. The questions to pose before proceeding are: if we are allowed to manipulate some of the variables, can the equilibria be changed at will and preserve stability? How many variables need to be manipulated? This kind of questions may impact approaches to therapy and rational drug design. The answer to some of these questions are given in Chapter 5 for Stortelder's model by using results from mathematical control theory. Since the linearized system is not completely controllable, we checked the flatness of the nonlinear system in the sense of M. Fließ and we identified a flat output. The results from control theory used are presented in Appendix B.

In Chapter 6, the governing equations of the reaction scheme published in [JoMa94] by using Michaelis and Menten relation are deduced followed by a qualitative analysis and model reduction of the new system. This system is responsive to changes in the concentration of the factors associated to hemophilic disorders. Moreover, based on this model for the extrinsic pathway we propose a new approach to model and simulating thrombin formation by the intrinsic pathway and do, as before, a qualitative analysis and a model reduction. To motivate the construction of a model only comprising a part of the intrinsic haemostasis, we give the example of a patient subject to heart valve replacement. This valves are normally artificial and therefore foreign substances may trigger the blood coagulation system. As a consequence, such patients have to take anti-coagulant drugs all their life time. So we wished to steer the system by using anti-coagulant drugs in order to prevent the formation of a thrombus. However, these two models do not account for the role of physiological inhibitory substances like anti-thrombin in the course of thrombin concentration with time. On the other hand, drugs like heparin only act in the presence of anti-thrombin. So, before studying the controllability of the systems presented in this chapter, one should extend them by including the action of inhibitors.

In Chapter 7 we propose a first approach to model the action of platelets on the common pathway by slightly modifying the model from Stortelder et al and extend the truncated model to a model by substituting *RVV* by the plasmatic factors leading to thrombin formation by the extrinsic pathway. As usual, this is accomplished together with a qualitative analysis of both systems and model reduction.

The last chapter is a summary of the main results and contains also some final remarks.

Unfortunately, due to the absence of experimental data, the models presented in this thesis could not be validated nor be rejected. But, one has to keep in mind that a mathematical model is useful to gain some understanding of the underlying dynamics and mechanisms governing the phenomena. So, we hope that this thesis will provide new insights into the process of thrombin formation towards a more systematical way to gain information from these and other models concerning the blood coagulation system.

Aachen, January 2007

Sandra Órfão

Contents

Preface	iii
1 The Blood Coagulation System	1
1.1 Coagulation factors. Coagulation cascade	2
1.1.1 Coagulation factors and related coagulation disorders . . .	2
1.1.2 Coagulation cascade	5
1.2 Regulation of thrombin levels	11
1.2.1 The role of anticoagulants	11
1.2.2 Pharmacological intervention in bleeding and in thrombosis	12
1.3 Kinetic aspects and mathematical modelling	14
1.3.1 Kinetic aspects	14
1.3.2 Mathematical modelling of the blood coagulation system .	14
2 Chemical Reaction Networks	19
2.1 Definition of a reaction network	20
2.1.1 Notation and terminology	20
2.1.2 Defining reaction networks	23
2.2 Kinetics	24
2.2.1 The law of mass action	24
2.2.2 The differential equations for a reaction system	25

2.2.3	Reaction network structure and nature of composition trajectories	27
2.2.4	Linkage classes. Weak reversibility. Deficiency	29
2.2.5	Two theorems	32
2.3	Enzyme kinetics	33
2.3.1	The quasi-steady state assumption	35
2.3.2	Enzyme inhibition	37
2.3.3	Cooperativity	39
2.3.4	Biological systems and feedback controls	41
3	Two Mathematical Models for Thrombin Formation	43
3.1	The model by Stortelder, Hemker and Hemker	44
3.1.1	Stoichiometric analysis	47
3.1.2	Numerical integration results and remarks	47
3.2	The model by Mann and Jones	51
3.2.1	Stoichiometric analysis	57
3.2.2	Model correction and numerical integration	65
4	Qualitative Analysis of Models for Thrombin Generation	75
4.1	The model by Stortelder, Hemker and Hemker	75
4.1.1	Positivity analysis	75
4.1.2	Invariance principle applied to Hemker's model	77
4.2	Model reduction	83
4.2.1	Linearization around an equilibrium point	85
4.2.2	Heuristic approach for reducing the number of equations	86
4.3	Model from Jones and Mann	90
4.3.1	Positivity analysis	90
4.3.2	Positivity of the corrected model	92
4.3.3	Linear first integrals and boundedness of the solutions	94
4.3.4	Model reduction	99

4.3.5	Linearization around an equilibrium point. Parameter identification by using the Jacobian	103
5	Steering Stortelder's Model	107
5.1	Stating the control problem of the linearized system	107
5.2	Flatness. Application to motion planning	112
6	Modelling Blood Thrombin Generation	117
6.1	Building the model for the extrinsic pathway	118
6.1.1	Numerical integration	129
6.1.2	Influence of changing complex $TFVII_a$ concentration	132
6.1.3	The effect of changing factor $VIII$ concentration	132
6.1.4	Qualitative analysis	134
6.2	Building the model for the intrinsic pathway	141
6.2.1	Numerical solution	146
6.2.2	Qualitative analysis	148
6.3	Discussion	155
7	Extending Stortelder's Model	157
7.1	Modelling platelet's contribution	157
7.1.1	The model	159
7.1.2	Numerical integration	160
7.1.3	Qualitative analysis	162
7.2	From the common to the extrinsic pathway	170
7.2.1	Qualitative analysis	174
8	Summary and Concluding Remarks	183
A	General Methods of Qualitative Theory	187
A.1	Basic definitions and criteria	187
A.2	Lyapunov's stability theory	189

A.2.1	Linearization principle for stability	190
A.2.2	Lyapunov functions	190
A.2.3	Critical case of one zero eigenvalue	191
A.3	Invariance. Limit sets	193
A.3.1	Invariance	193
A.3.2	Limit sets	195
A.4	LaSalle's invariance principle	196
B	Results from Control Theory	197
B.1	Basic definitions and criteria	197
B.1.1	Controllability and observability	199
B.1.2	Stabilizability and detectability	202
B.2	Second order Taylor expansions	202
B.3	About flat systems	203
C	Technical Remarks	207
C.1	Solving systems of stiff differential equations using SciLab	207
C.2	The modules TriSer and Tsolve	208
D	Original Model from Stortelder and Hemker	209
E	Platelets Contribution - An Extension of Stortelder's Model	215
F	Corrected Model from Mann and Jones	221
	Bibliography	235

Chapter 1

The Blood Coagulation System

One of the most peculiar and remarkable properties of *blood* is its ability to solidify or clot [Davie05]. The blood coagulation system maintains the integrity of the mammalian *circulatory system* and the balance of blood fluidity in response to vascular injury. The *hemostatic response* involves a complex series of events that require the interaction of blood and vascular cellular elements and *blood plasma*¹ proteins [JoMa94] [B-ZVBMR05]. It has been characterized as a series of *proteolytic* reactions in each of which an inactive precursor (*zymogen*) of a proteolytic enzyme is converted to the active enzyme (*protease*) in a cascade or waterfall pattern. The origin of this concept can be traced back to the early studies of the blood coagulation system [Davie05]. Nowadays, it is characterized as a network of feedback controlled reactions. The physiological response to vascular injury culminates in the rapid generation of *thrombin*, at the site of injury, which cleaves the plasma protein *fibrinogen* into insoluble *fibrin monomers* via *limited proteolysis* [Jesty05] [FoKu98] [JoMa94]. Thus, the response of the coagulation process is generally limited to the site of injury and is proportional in magnitude to the extent of vascular damage [MNCHK90]. Moreover, because each step in the series is enzyme catalyzed, and one enzyme molecule can theoretically catalyze the formation of a very large number of molecules of product, the cascade has the capacity of enormous amplification [Jesty05]. Qualitative or quantitative alterations in this hemostatic balance determines one of three possible outcomes: *hemorrhage*, *controlled hemostasis* or *thrombosis*. Thus, comprehensibility of the roles of the system components in this regulation is important for a therapeutic control of thrombotic and bleeding disorders.

Over the past 25 years, reasonably comprehensive insights of the stock of proteins and the associated biophysical and enzymatic processes involved in blood clotting

¹Fluid of the blood with its clotting mechanisms intact and ready to go

has been developed through the efforts of numerous investigators. A large amount of rigorously obtained data describes association states, membrane binding thermodynamics, enzyme complex assembly kinetics, and reaction kinetics for the role of processes [HJEM02]. However, the understanding of the dynamics is still poor [FoKu98].

This chapter is dedicated to the description of the blood coagulation cascade and its components. Aspects regarding the regulation of thrombin levels, clinical significance of the clotting cascade and pharmacological intervention are also discussed. Finally, an overview of how mathematical modelling has been contributing and can still contribute for the understanding of biological processes and in particular of the blood coagulation system is given.

1.1 Coagulation factors. Coagulation cascade

When a blood vessel is injured, a number of physiological mechanisms are activated that promote *hemostasis*, or the cessation of bleeding (*hemo*=blood; *stasis*=steady-state). Breakage of the *endothelial* lining of a vessel exposes *collagen* proteins from the *subendothelial connective tissue* to the blood.

The mechanisms for initiating and regulating blood coagulation in humans include the following three general processes [Davie05]:

- the immediate contraction of blood vessels at the site of injury, which limits the flow to the area of injury;
- formation of the *platelet*² plug;
- generation of a *fibrin* mesh or clot to stabilize the platelet plug. If the plug contains only platelets we speak about a *white thrombus*; if red blood cells are present, we call it a *red thrombus*.

Phospholipids that are exposed on the platelet membrane participate in the action of clotting factors.

1.1.1 Coagulation factors and related coagulation disorders

Most of the clotting proteins, or clotting factors, are precursors of proteolytic enzymes and are also known as *zymogens*. The second major group is the *cofactor proteins*, which accelerate reactions. Although it is known that some of the clotting

²Platelets or thrombocytes are cell fragments circulating in the blood that are involved in the mechanisms of primary hemostasis (see Figure 1.1)

proteins are synthesized in other tissues, the liver is the major site of synthesis for probably all the plasma proteins [Jesty05].

The nomenclature of the proteins involved in clotting is complicated and almost arbitrary. Actually, in many cases, there are at least two designations for the same protein. However, it is general practice to represent the inactive form of the coagulation factors by Roman numerals. A lower case "a" appearing as a subscript means "activated". For example, the activation of the zymogen factor X produces the protease factor X_a .

There are several ways to introduce the coagulation factors and related diseases. The choice of the most adequate among them depends actually on the reader. This thesis is written for audiences with different scientific backgrounds that include specialists on blood coagulation, like physiologists, and non-specialists, like mathematicians and engineers. So, we had to find a compromise. As coagulation factors were identified through patients with different coagulation disorders, historical aspects are added to help a non-specialist to understand better the relationship between the factor's nomenclature and the diseases to which they are related. For more historical aspects exploring these and other events and presenting the people involved in unraveling the basic mechanisms leading to the clotting of blood from it beginning on see [Davie05] and [Lin95].

John Hageman was a patient of Prof. Rattnoff, a professor of medicine at the Western Reserve University that lead a project on blood coagulation. Hageman had a rather strange clotting abnormality in that his blood did not clot when in contact to a glass test tube. However, this could be corrected by the addition of a small amount of plasma or *serum*³ from normal individuals or from patients with other known coagulation disorders such as *hemophilia*. The most interesting was that Hageman did not experience any bleeding tendency. From these studies it was concluded that in normal plasma there must be a protein in an inactive form that is activated in a test tube when bound to a glass surface or crushed glass or *kaolin*. This was consistent with the idea that this plasma protein could trigger fibrin formation in blood collected in a glass container in the absence of tissue extracts. This assumption was proved after several purification steps. The protein was not present in Hageman's blood and received the name *Hageman factor*, now called *factor XII* [Davie05].

In 1936, Patek and Stetson found that patients with hemophilia were lacking a factor present in normal plasma. They called it *anti-hemophilic factor* (AHF) or *anti-hemophilic globulin* (AHG). This deficiency is now called *hemophilia A* or *factor VIII deficiency*[Davie05].

Factor *XIII* was first called *Laki-Lorand* or *fibrin-stabilizing* factor. In the presence of this plasma protein and calcium fibrin became rather insoluble. Years later,

³Clotted plasma

it was found that activated factor *XIII* cross-links fibrin monomers [Davie05].

A rare disorder resulting in bruising and bleeding after minor lacerations or dental extraction has at its origin on the lack of a plasma protein factor *V*, also called *proaccelerin* or *Labile factor*. The disease was referred to as *parahemophilia*. Factor *V* is furthermore where we find the most common hereditary risk factor for *thrombosis*. Here, factor *V*, while it is normally converted to active factor *V*, is defective in its ability to be inactivated by protein *C* yielding abnormally high levels of *thrombin* generation [Davie05][Jesty05].

Thrombin is the essential enzyme product of the blood coagulation enzymatic cascade. The regulation of the production of this enzyme is vital for the maintenance of the hemostatic balance in humans. As a matter of fact, genetic and acquired deficiencies that cause reduction in, or the absence of, thrombin generation lead to hemorrhagic syndromes. Defects in the regulatory and dynamic processes that down-regulate thrombin generation are associated with thrombotic risk [BvVM99].

Alexander and co-workers described another factor in serum that accelerated the conversion of *prothrombin* to thrombin. This factor was called *serum prothrombin conversion accelerator (SPCA)* and the defect *factor VII deficiency*[Davie05].

Another clotting disorder called *hemophilia B* was described in 1952 among others by Biggs and co-workers. The protein lacking was known as *plasma thromboplastin component (PTC)*, *Christmas factor* or *factor IX* [Davie05].

In 1953, Rosenthal and co-workers described a clotting disease that they called *plasma thromboplastin antecedent (PTA) deficiency* or *factor XI deficiency*. Patients with PTA deficiency present mild or moderate bleeding symptoms that often became evident only after surgery or injury [Davie05].

Stuart factor deficiency or *factor X deficiency* was first described by Hougie and co-workers. In patients suffering from this bleeding disorder, factor *X* fails to readily convert prothrombin to thrombin, resulting consequently in the formation of an abnormal or delayed fibrin clot [Davie05].

Tissue factor is an integral membrane *glycoprotein* and functions as a receptor for factor *VII* or *VII_a* (circulating in the blood). It is normally expressed at only very low levels in the endothelial cells, which line the blood vessel [Davie05] [Jesty05].

While it is a fact that much of the existing knowledge of how coagulation works *in vivo* comes from clinical data on bleeding disorders of patients with hereditary deficiencies of clotting factors, it must be emphasized that such diseases are very rare. In fact, for each single person with hemophilia (A or B) - the most common hereditary bleeding defect- about 4 to 5000 other people will suffer a thrombotic

³Endothelial cells play a number of roles in the hemostatic process in addition to binding coagulation factors

episode during their lifetime [Jesty05]. Furthermore, it has been recently found that hereditary abnormalities are in fact much more common in thrombotic than in bleeding disorders.

As already mentioned, most clotting proteases require *cofactor* proteins to make the reactions that they catalyze go fast enough. A cofactor is a protein that has no catalytic site, but regulates the activity of an accompanying protease [Jesty05]. More specifically: factor VII_a requires tissue factor; factor IX_a requires factor $VIII_a$; factor X_a (acting on prothrombin) requires factor V_a ; and thrombin activation of protein C action on factor V and factor $VIII$ requires protein S .

Table 1.1 summarizes some of the most important aspects related to the coagulation factors, like their physiological concentration and their function. Note that the concentrations of the different factors are not exact. In the literature, the values vary rather widely. The values given in this table are the most common. The last column makes the correspondence between the coagulation factors and the *clotting pathway* within which they are activated. In the next section we describe the blood coagulation cascade and therewith explain the meaning of both the *intrinsic* and the *extrinsic* pathways.

1.1.2 Coagulation cascade

Already at the early times of investigation, the blood coagulation system was represented as a cascade of enzymatic reactions, first by Ratnoff and Davie and then by MacFarlane in 1964. These two models helped to clarify the sequence in which clotting factors interacted and provided concepts that were readily tested in laboratory environments [Davie05].

Historical reasons also justify the existence of two pathways (see Table 1.1) leading to the formation of a fibrin clot. One is called the *intrinsic pathway* or the *contact pathway* and the other the *extrinsic pathway* or the *tissue factor pathway* [Davie05]. Although initiated through different mechanisms, it is believed that the two pathways merge at the level of activated factor X to a final *common pathway* that results in the formation of insoluble fibrin polymers via the *prothrombinase complex*. The intrinsic pathway is initiated either by exposure of plasma to a negatively charged surface, such as that provided by collagen at the side of injury or by the glass of a test tube. The extrinsic pathway is initiated upon contact of circulating factor VII_a with the transmembrane protein tissue factor, which becomes available after vascular injury.

Furthermore, the coagulation process is now known to also involve numerous intermeshed controls including feedforward and feedback loops, in which an enzyme produced in one step promotes or inhibits earlier or later reactions [Jesty05]. Thus, the system is said to respond in a threshold manner [FoKu98], where an activation

Table 1.1: Coagulation factors and functional classification

	Name	Concentration [$\mu\text{mol/l}$]	Place Source	Properties Function	Pathway
Pro-coagulant factors	Factor <i>I</i> <i>Fibrinogen</i>	8.8	Liver Platelets	Soluble protein; Cofactor; Precursor of fibrin	Both
	Factor <i>II</i> <i>Prothrombin</i>	1.4	Liver(vit. K) Plasma	Precursor of thrombin; zymogen of serine protease; enzyme	Both
	Factor <i>III</i> <i>Tissue Factor (TF)</i>	–	Tissue cells	Lipoprotein; cofactor; enzyme	Extrinsic
	Factor <i>IV</i> <i>Calcium (Ca^{2+})</i>	2500	–	Necessary for the activation of the most coagulation factors	Both
	Factor <i>V</i> <i>Proaccelerin</i>	0.03	Liver Plasma/Platelets	Binds to platelet's membrane; cofactor	Both
	Factor <i>VI</i> <i>Act. factor V</i>	–	–	Part of the <i>prothrombinase</i> complex	Both
	Factor <i>VII</i> <i>Proconvertin</i>	0.03	Liver (vit. K) Plasma	Zymogen of serine protease; proenzyme	Extrinsic
	Factor <i>VIII</i> <i>Antihaemophilic factor</i>	< 0.0004	Plasma	Cofactor in the activation of factor <i>X</i>	Intrinsic
	Factor <i>IX</i> <i>Christmas factor, PTC*</i>	0.09	Liver (vit. K) Plasma	Zymogen of serine protease; proenzyme	Intrinsic
	Factor <i>X</i> <i>Stuarts factor</i>	0.2	Liver (vit. K) Plasma	Zymogen of serine protease; proenzyme	Intrinsic
	Factor <i>XI</i> <i>PTA**</i>	0.034	Plasma	Zymogen of serine protease; proenzyme	Intrinsic
	Factor <i>XII</i> <i>Hageman factor</i>	0.45	Plasma	Zymogen of serine protease activated by kallikerein; proenzyme	Intrinsic
	Factor <i>XIII</i> <i>Fibrin stab. factor</i>	0.1	Platelets Plasma	Transglutaminase; produces fibrin cross-linking	Both

*PTC - plasma thromboplastin component; **PTA - plasma thromboplastin antecedent;
prothrombinase-enzyme complex that converts prothrombin into thrombin.

signal below a certain level does not trigger the system [AtPa05].

The intrinsic pathway

The components of the intrinsic system are all in blood plasma and comprise the coagulation factors *VIII*, *IX*, *X*, *XI* and *XII*. Other participants are *Prekallikerein*, *kininogen* as well as *calcium ions* and *phospholipids* secreted from *platelets*.

Blood plasma gets in contact with "foreign" substances such as glass or other negatively charged substances. This activates factor *XII*. This is termed *contact phase*. Active factor *XII_a* in turn activates another clotting factor, factor *XI_a*, which activates in turn factor *IX*.

The next steps in the sequence require the presence of Ca^{2+} and phospholipids, which are provided by activated platelets [Davie05].

The role of factor *VIII* in this process is to act as a receptor, in the form of factor *VIII_a*, for the factors *IX_a* and *X*. The activation of factor *VIII* occurs in the presence of minute concentrations of thrombin in a feedback reaction. This small quantity of thrombin is available due to factor *IX_a* influence. Meanwhile, activated factor *VIII_a* forms a complex with activated factor *IX_a*, also known as *intrinsic tenase* and denoted by *IX_aVIII_a*. This complex activates factor *X* at a very high rate [BuMa02].

A physiological role for the Hageman factor in the coagulation pathway has not been clearly established yet because individuals lacking this factor have no bleeding complications. As a consequence, factor *XII* has been deleted from most coagulation schemes [Davie05]. However, contact activation may be sometimes related to pathological situations that cause abnormal activation [Jesty05], being the reactions well characterized *in vitro*. Still, the physiological activator of factor *XII* has not been identified [LKSM94].

The extrinsic pathway

The activation of the extrinsic pathway occurs, in comparison with the intrinsic, faster. Moreover, it is generally accepted that this pathway plays a major role in the initiation of blood coagulation [Davie05].

It is believed that the extrinsic cascade is initiated upon vascular injury, which leads to the exposure of tissue factor (*TF*) that binds phospholipids. The first procoagulant event is the combination of TF and factor *VII* to form a 1:1 complex in the presence of calcium, with little or no enzymatic/proteolytic activity, denoted by *TF.VII*. Thus, no significant generation of factor *X* can be realized until the bulk of *TF.VII* complex is activated. There are two known physiological

significant activators of $TF.VII$, the complex $TF.VII_a$ itself and the factor X_a . The factor $TF.VII_a$ enzyme complex, also known as *extrinsic tenase*, results out of the binding of small amounts of pre-existing circulating factor VII_a and besides factor $TF.VII$ and factor X_a , it activates as well factor IX by limited proteolysis [BvVM99] [Davie05], linking the two pathways at this stage. However, activated factor X_a is a potent activator of factor VII and this constitutes an example of surface-dependent positive feed-back loop [FoKu98]. Nevertheless, the ability of factor X_a to activate factor VII creates another link between the intrinsic and the extrinsic pathways.

Current literature supports the notion that the physiologic hemostatic response is initiated by the extrinsic pathway [LKSM94] - see for instance [Lin95] for two models supporting this theory. However, this would make the activation of factor X by the complex $VIII_aIX_a$ superfluous, what happens to be a paradox because this complex is thought to be one of the most strongest activators of factor X .

At this point, the key observation is that people who lack factor XII are clinically normal. Thus, if the intrinsic pathway functions normally, its contribution to haemostasis is small. In contrast, a deficiency in TF has never been observed in humans, and is probably lethal [Jesty05].

The common pathway. Activation of prothrombin to thrombin

As referred before, the two pathways merge. From the current literature, it is generally accepted that they merge at the activation of factor X . The common pathway can therefore be divided in three segments:

- activation of factor X ;
- thrombin formation from prothrombin;
- fibrinogen to fibrin.

After activation of factor X , the next step is to activate prothrombin to form thrombin, and then convert fibrinogen to fibrin and cross-link it, completing the basic clotting pathways.

From the limited amount of factor X_a produced by the complex $TF.VII_a$, picomolar concentrations of thrombin are produced, during an *initiation phase*, which partially activates platelets and cleaves factors V and $VIII$, generating their active forms V_a and $VIII_a$ by positive feedback [BvVM99]. It is furthermore defined as the time needed to generate approximately $2nM$ of thrombin [HJEM02].

Subsequently, during a *propagation phase*, factor X_a forms the so called *prothrombinase complex* with cofactor V_a , in the presence of calcium and phospholipids,

which is the primary activator of prothrombin. This yields the bulk of thrombin generation, (about 96 % of the total amount during the propagation phase [B-ZVBMR05]). Moreover, prothrombin activation by the complex X_aV_a is identical in form with the activation of factor X by the complex IX_aVIII_a . And, furthermore, it is generally accepted that the interaction of factor X_a with factor V_a enhances the turnover number of factor X_a about 2800 to 3000- fold [BWHL95] [NeTrMa84].

The thrombin further amplifies its own generation by activating factor XI and completing activation of platelets, factors V and $VIII$. Additionally, thrombin also cleaves fibrinogen and factor $XIII$ to form the insoluble cross-linked fibrin-clot [BuMa02].

The formation of thrombin may occur more rapidly as result of the release of *tissue thromboplastin*⁴ from damaged tissue cells.

As the concentration of thrombin increases, factor $VIII_a$ is cleaved by thrombin and inactivated [Davie05].

Figure 1.1 summarizes the most important reactions occurring. For completeness see for instance [Lin95], [Pru00].

Thrombocytes and procoagulatory activity

Fibrin formation is just one part of the hemostatic system. The other components are the platelets, and the system by which damaged vessels contract under sympathetic nervous control.

Platelet or *thrombocyte* function and coagulation are often separated for didactic reasons, but the two systems are closely interconnected, each requiring the other for its function. Nevertheless, a normal clot consists of a nested structure of aggregated platelets and fibrin [Jesty05].

In the absence of injury, platelets are repelled from each other and from the endothelial lining of vessels. In contrast, damage of the endothelium of vessels makes platelets stick to exposed collagen proteins starting the so called *primary hemostasis* (see Figure 1.1).

To some extent the platelets can function without the clotting system and vice-versa, but the platelets require products of the clotting system to aggregate properly, and the clotting system requires platelets to form fibrin properly. In other words, the primary platelet clot is relatively unstable. Therefore, an efficient hemostasis asks for the consolidation of the platelet rich thrombus. This starts with the activation of the blood coagulation cascade and with the building of

⁴Protein in the surface of endothelial cells

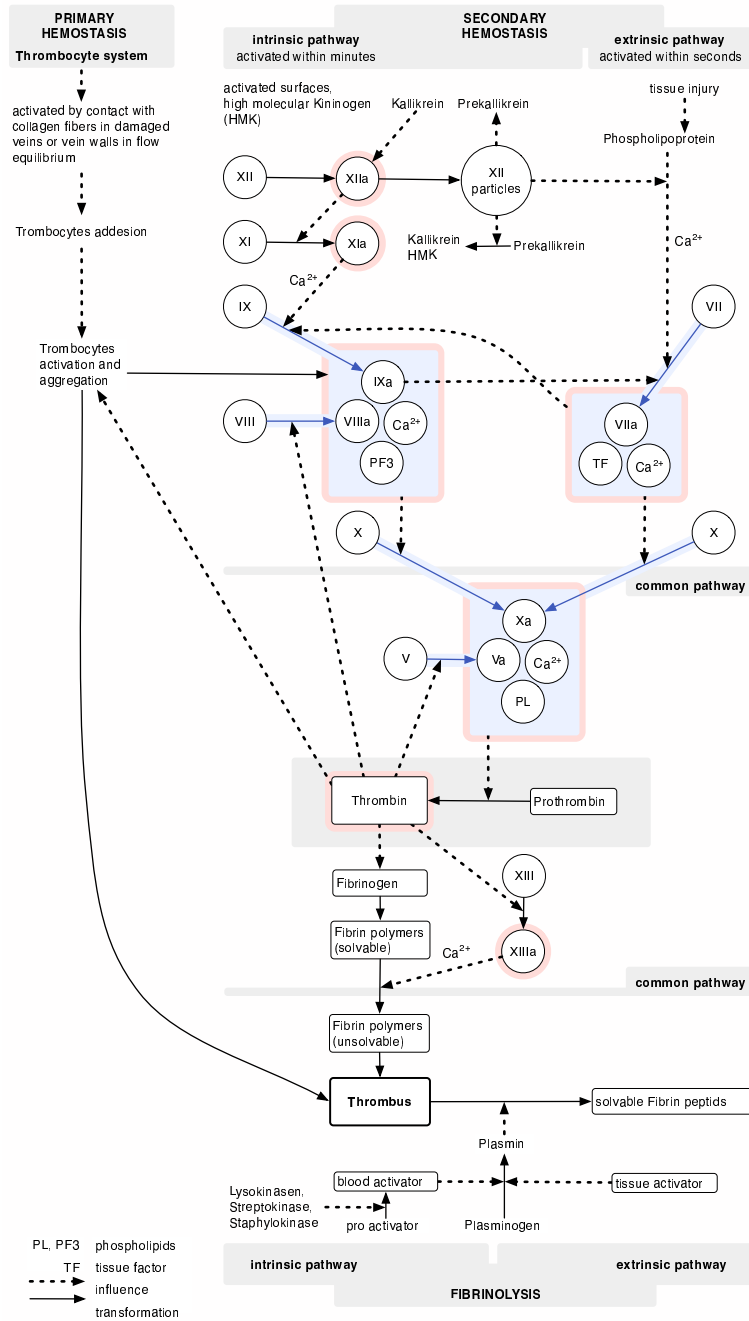


Figure 1.1: Blood coagulation cascade

thrombin and fibrin next to the area occupied by the aggregated platelets. So, we speak about the *secondary hemostasis* (see Figure 1.1).

Decisive for the procoagulatory effect of the thrombocytes are the *activated blood platelets*. In fact, during activation, negatively charged phospholipids on the thrombocytes surface are exposed, increasing the binding activity for plasmatic coagulation factors like factors *V*, *VIII_a*, *IX_a* and *X_a* while catalyzing both the tenase and prothrombinase complexes.

Additionally, platelets are also storage components of proteins involved in blood coagulation and its regulation. A deficiency in functional platelets, i. e., *thrombocytopenia*, is associated with bleeding complications. In "synthetic plasma" and in whole blood, thrombin generation profiles observed at platelet concentration below $0.1 \times 10^8/ml$ ($< 5\%$ of mean plasma value), the thrombin generation profile is similar to that observed in severe hemophilia blood [Jesty05].

More detailed description about thrombocytes, their morphology and function can be found in [Gaw99], [Lin95] and [Pru00].

1.2 Regulation of thrombin levels

1.2.1 The role of anticoagulants

There are two principal mechanisms by which thrombin activity is regulated.

The predominant form of thrombin in circulation is the inactive prothrombin, whose activation requires the pathways of proenzyme activity described above. So, the balance between active and inactive enzymes is assured by feedback mechanisms occurring at each step in the cascade. On the other hand, the initiation and propagation phases of the coagulation system are differentially regulated by substances called *inhibitors* or *anti-coagulants* as well, the principal being *antithrombin III (ATIII)*, activated proteins *C* and *S*, and *tissue factor pathway inhibitor (TFPI)* [BvVM99]. Besides these, we still may point out α_2 -*macroglobulin* and *heparin cofactor II* as thrombin inhibitors. Altogether, the major task is to prevent blood from clotting.

From the studies done about the process of the blood coagulation system, it behaves such that the interaction between procoagulants and inhibitors produces threshold responses with respect to *stimuli*, and the whole process functions altogether in a "yes / no" configuration in which the procoagulant initiating stimulus must be at a certain level [BvVM99].

ATIII is perhaps the most important coagulation inhibitor. It controls the activities of thrombin, and of factors *IX_a*, *X_a*, *XI_a* and *XII_a*. Moreover, it is in

significant molar excess to its target enzymes and is said to damp the propagation phase [BvVM99].

A number of epidemiological studies have shown that concentration variations of blood coagulation proteins, respectively prothrombin, *ATIII*, protein *C* and *S*, factors *VII*, *VIII* and *IX* within the 50 % to 150 % of their mean value concentration are associated with thrombotic risk [BvVM99].

TFPI inhibits the extrinsic pathway very quickly. In fact, this protein is a factor X_a dependent inhibitor of the complex $TF.VII_a$, blocking additional thrombin generation. The inability of *TFPI* to bind factor VII_a in the absence of factor X_a probably explains why factor VII_a is able to circulate for a relatively long period of time [Lin95].

It is also known from the literature (see for instance [BvVM99]) that if all procoagulants factors and stoichiometric inhibitors are at their mean plasma concentrations, thrombin generation occurs and after an initiation it reaches a maximum concentration of approximately 300nmol/L or $0.3\mu\text{mol/L}$ and it can vary between 100 and 400nmol/L depending upon the experimental circumstances. The formation and inhibition rates are equal at 2,5 minutes after the process has started. Subsequently, thrombin is completely inhibited in about 10 minutes. Two more scenarios were considered and analyzed: the first where *ATIII* and *TFPI* were present at 150 % and procoagulants reduced to 50 % of their mean values concentration and the second where the concentration of the anticoagulants by 50 % combined with an increase of the concentration of all procoagulants to 150 %. In the first case, the total thrombin concentration was reduced approximately 25 % of the normal profile; in the second case an 700 % increase in total thrombin generation was observed, the maximum concentration being not larger than $1\mu\text{mol/L}$.

Thrombin also plays an important regulatory role in coagulation as its activity initiates numerous positive and negative feedback loops [AtPa05]. It binds to thrombomodulin, another endothelial transmembrane protein, to activate protein *C*. The activated form of protein *C* degrades factors $VIII_a$ and V_a , limiting the activity of these two factors. Thereby, protein *S* acts as a cofactor of protein *C*.

Table 1.2 summarizes inhibitors and some properties.

1.2.2 Pharmacological intervention in bleeding and in thrombosis

One of the objectives of this thesis is to study the possibility of steering the system by means of an external control modelling the action of a drug on thrombin formation. So, as a motivation, we refer briefly some of the most common clotting disorders and their treatment.

	Name	Concentration [$\mu\text{mol/l}$]	Place Source	Properties Function
Anti-coagulant factors	<i>ATIII</i> <i>Antithrombin III</i>	3.4	Plasma	Mediated inactivation of factors II_a , VII_a , IX_a , X_a
	Protein <i>S</i>	0.3	vit. K Plasma	Cofactor for act. protein <i>C</i> in factors V_a and $VIII_a$ inactivation
	Protein <i>C</i>	0.06	vit. K Plasma	Zymogen of serine protease
	TFPI <i>Tissue factor pathway inhibitor</i>	0.0025	Platelets; plasma; tissue cells	Mediated inactivation of complex $TF : VII_a$ and its product complexes

Table 1.2: Inhibitors and some of their properties

The hemophilia resulting from a deficiency in factor *VIII* can be treated by infusion of factor *VIII* concentrates prepared from either human plasma or by recombinant *DNA* technology.

The activity of antithrombin *III* can become more effective by the use of *heparin*. Heparin can be given intravenously during certain medical procedures. What happens is that heparin binds to a specific site of antithrombin *III*, producing an altered form of the protein with higher affinity to thrombin and to the other factors also inhibited by antithrombin. Heparin is the most used anticoagulant drug for the immediate treatment of thrombosis (heart attack, thromboembolic stroke, pulmonary embolism, etc) and it is also used for anticoagulation in surgical procedures involving a significant risk of postoperative thrombosis. However, heparin only prevents clotting in the presence of *ATIII*. Thus, patients lacking this protein have a greater risk of suffering a thrombotic episode during their life time [Jesty05].

Other kind of drugs with anticoagulatory effect are *coumarin* based drugs. They inhibit the vitamin *K* dependent reactions, but it takes several days for their maximum effect to be realized.

1.3 Kinetic aspects and mathematical modelling

1.3.1 Kinetic aspects

Kinetic analysis of the individual reactions in the extrinsic pathway have been reported, but a mathematical analysis of the complete reaction network has been given less attention.

Although there is some controversy regarding the existence of some reaction or factor, we can enumerate the following information about kinetic aspects of the blood coagulation cascade:

- (i) Factor X_a is an enzyme with different catalytic properties in the presence or absence of its active cofactor factor V_a [LKSM94] [NTM79].
- (ii) According to [NeMa79], factor X_a in the presence of phospholipids and thrombin can activate factor V and factor $VIII$. But, in [MoTr90] it is shown that this reaction does not seem to play a role in clotting plasma, although it happens to be important under certain experimental circumstances.
- (iii) Once formed, factor $VIII_a$ is subject to spontaneous inactivation [LoPa91].
- (iv) Factor IX_a is virtually inactive without factor $VIII_a$ [vDTRH81].
- (v) Factors IX and X serve as competitive substrates for $TF.VII_a$ complex [LaMa91].
- (vi) In the presence of phospholipids, factor X_a catalyzes the formation of factor IX_a [LaMa91].

All these aspects make the interpretation of the kinetics of coagulation less than obvious. Other difficulties arise if we consider that there is an absence of steady state conditions; that concentration of all substrates, cofactors, and enzymes change throughout the reaction; that enzyme/activator concentrations exceed substrate concentrations and that the natural concentrations of the coagulation proteins in blood vary over an extremely wide range [JoMa94]. In other words, it is not known whether the reaction scheme deduced from experiments is indeed the one operative in plasma. In fact, there might exist unknown factors or reactions and reactions that have been shown to be possible in principle may not occur in reality [SHH97].

1.3.2 Mathematical modelling of the blood coagulation system

Although the cascade model of sequential reactions has provided enormous insights into the general process of hemostatic reactions *in vitro*, it has not explained in a

satisfactory way the dynamic regulation of blood coagulation reactions occurring *in vivo* [JoMa94].

A number of research groups have recognized the usefulness of mathematical modelling in trying to understand the coagulation process. Mathematical modelling is a valuable tool because it provides a rapid and a less expensive way to simulate planned experiments.

Nesheim et. al in [NeTrMa84] explore a steady-state model of prothrombin activity involving two phases. One phase corresponding to the bulk solution and other the thin atells surrounding phospholipid membranes. Without including explicit spatial dependencies, the steady state of thrombin production in both phases is examined as a function of phospholipid concentration.

Another more recent model from the same laboratory, see [JoMa94], considers dynamic interactions between a number of the coagulation enzymes and zymogens from the extrinsic pathway. Although inhibition is ignored and the possible regulatory role played by phospholipid surfaces in controlling the coagulation reactions is not addressed, this paper is one of the most cited in the blood coagulation literature and because of that it will deserve in this thesis a special treatment regarding mathematical aspects. In 2002, this model was extended and the results are published in [HJEM02]. This extension includes among others the TFPI-mediated inactivation of the complex $TF.VII_a$, the inactivation of factors II_a, VII_a, IX_a and X_a by $ATIII$, the initial activation of cofactors V and $VIII$ by thrombin generated by factor X_a membrane, factor $VIII$ dissociation, the bind competition and kinetic activation steps that exist between TF and factors VII and VII_a and the activation of factor VII by thrombin, factor X_a and factor IX_a . However, although the reaction scheme was published, the set of differential equations governing the system was omitted. In fact, a software package was written to enable rapid transformation of the chemical reaction schemes to the necessary time-dependent partial differential equations required for this model and their solution. The software package is said to use an internet based interface with a generally applicable Runge-Kutta⁵ solver that provides solutions to a family of time-dependent differential equations. Unfortunately, the information published is not enough to make a mathematical analysis.

Beltrami, Jesty and Willems take a different tack in analyzing a system made up of multiple interacting feedback loops [JeBeWi93] for solution-phase reactions. The main result is that the system responds in a threshold manner. However, they do not match any part of the coagulation pathway and no account is taken of surface binding reactions or transport of reactants. Fogelson et al. made an extension of this model including both solution-phase and membrane-phase reactions (see

⁵Belongs to the so called *one step methods*, where the basic principle is to collect information around the last approximation to define the next iteration step

[FoKu98]). In this approach, the concentrations of the membrane-binding site are limited and they were treated as control variables. The results were obtained by linearized stability analysis for the numerical solution with methods for stiff ordinary differential equations implemented in a software package. Still, the model system does not match any part of the coagulation system exactly. So, the reported results need to be tested and refined in more realistic models.

Stortelder, Hemker and Hemker presented in [SHH97] a truncated model comprising a system of non-linear differential equations with unknown parameters, namely the reaction constants and the initial concentration of the factors. Parameter estimation was used in order to determine these in such a way that the experimental data were well fitted by the theoretical model. This model concerns the common pathway; factor X was activated by a purified enzyme RVV , factor V and prothrombin activation in the presence of phospholipids is considered and it also includes the inactivation of thrombin by $ATIII$ and α_2 -macroglobulin.

Furthermore, according to Leipold in [LBRD95], the main limitations of some published models are:

- consideration of only a small part of the coagulation cascade;
- empirical description of interactions for which molecular mechanisms were known;
- determination of some or all of the parameters from experimental data by curve fitting without comparing the model predictions to experimental data.

Even if what is written in the first item is somehow desirable from the physiological point of view, one must keep in mind that there is not a single mathematical model describing all the possible mechanisms that occur in the blood coagulation system. In fact, any attempt in doing this will result in a very complex model from which it will be almost impossible either to gain some new insights or to understand and interpret unexpected experimental observations. Therefore, to reduce the dimension of the models without losing qualitative information seems to be a good approach. This is also one of the goals of this thesis.

For a review on the development of theoretical research in hemostasis and thrombosis using mathematical modelling and computer simulation see [AtPa05].

As a final remark, one may say that although activation of the clotting cascade is crucial, uncontrolled generation of thrombin can lead to disaster. Foreign surface contact, e.g. during the implant of artificial organs, is a strong activator of whole of the system and therefore anticoagulatory measures are unavoidable. The interaction of all these parameters is still the subject of numerous investigations, however they are mainly of experimental nature. In spite of great progress in this direction, it seems still to be justified to introduce mathematical models which

enable predicting e.g. the sequential enzymatic reaction leading to the formation of thrombin. Besides non-linearity, these systems also have the property of stiffness, including fast and slow modes, and therefore they should be also carefully analyzed.

In this work we deal with the models published in [SHH97] and in [JoMa94] and analyze them mathematically in order to be able to reduce the dimension in a rational way. As a result of the analysis performed, both models are extended and new aspects are introduced and studied. Moreover, mathematical approaches for modelling thrombin formation in both pathways are given. Because of the importance of pharmacological intervention in different clinical scenarios we analyze the system from [SHH97] regarding mathematical controllability aspects. Since the model from [JoMa94] does not include the action of inhibitors we do not study the controllability of this system, because there are drugs like heparin which efficiency depends on the presence of a physiological inhibitor.

Although the identification of some parameters from experimental data would be desirable, no experimental data is available that allows us to accomplish this task.

Chapter 2

Chemical Reaction Networks

This chapter is a self-contained exposition of some fundamental concepts of chemical reaction networks theory. It provides among others the necessary information for a non-specialist to understand how chemists and biochemical engineers derive the differential equations they work with and to explain how these differential equations are tied to a reaction network structure. Moreover, it is important for what follows in the next chapters to present things in a terminology and formalism more standard in control and in dynamical systems theories, for it is not a simple matter to put together and to refer the forthcoming information from the different literature sources. In the first part of the chapter we start with the definition of a chemical reaction network and end by giving some definitions and properties relating the reaction network structure and the nature of composition trajectories based on the papers [Aris65a] and [Aris65b] written by Rutherford Aris, on the lecture notes [Fein79] from Martin Feinberg and on the paper [Son01] from Eduardo Sontag. Thereby we motivate the formalism by working through some examples. We will see that for the classes of deficiency zero and deficiency one chemical reaction networks the existence and uniqueness of equilibria, and local asymptotic stability are well characterized. But, for other kind of classes there are still lot of questions open regarding these and other dynamical properties. Since the blood coagulation system is a biochemical network where inactive proenzymes are converted to active enzymes, in the second part we turn our attention to enzyme catalyzed reactions. Regarding this subject, we refer in particular to [KeSn98], [SeSl89], [BriHal25], [Seg88], [Son05b] and [Mur93].

2.1 Definition of a reaction network

In [Aris65a] and [Aris65b] Aris examines the foundations of a rational analysis of chemical systems and their reactions whereas Feinberg in [Fein79] makes a first attempt to develop a more general theory about chemical reaction networks by providing theorems which tie qualitative aspects directly to the reaction network structure.

2.1.1 Notation and terminology

The algebra of finite dimensional vector spaces lies at the basis of formal reaction kinetics of the simplest representation of chemical species [Aris65a].

Elementary atomic species or *chemical elements* are the basic unit of structure available when we consider a reaction system. Atomic species can combine to form *molecular species*. We have the following definition:

Definition 2.1.1. A *molecular species* \mathcal{S}_s is an entity of the form

$$\mathcal{S}_s = \beta_s^1 \mathcal{B}_1 + \beta_s^2 \mathcal{B}_2 + \dots + \beta_s^T \mathcal{B}_T, \quad s = 1, \dots, S$$

where $\beta_s^t, s = 1, \dots, S$ are integers. The $\mathcal{B}_t, t = 1, \dots, T$ are symbols for the chemical elements.

Besides the set of molecular species or *chemical species* \mathcal{S} , we can associate to each reaction network two more sets. The first is the set of objects appearing before and after the reaction arrows. These objects are called *complexes*¹ of the network and the set of complexes is designated by \mathcal{C} . The second set is the set \mathcal{R} of *reactions*.

¹The word *complex* is also used for instance while describing enzyme catalyzed reactions (see Section 2.3). So, be aware of the context to avoid confusion

As a preliminary motivation and to illustrate these we consider the following example taken from [Fein79]:

Example 2.1.2. Suppose that A, B, C, D and E are chemical species and that the chemical reactions occurring among them are well reflected in the following diagram

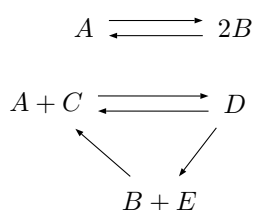


Figure 2.1: Example of a reaction scheme.

Then $\mathcal{S} = \{A, B, C, D, E\}$, $\mathcal{C} = \{A, 2B, A + C, D, B + E\}$, and $\mathcal{R} = \{A \rightarrow 2B, 2B \rightarrow A, A + C \rightarrow D, D \rightarrow A + C, D \rightarrow B + E, B + E \rightarrow A + C\}$.

In the sequel, the idea is to associate to each of these sets a finite-dimensional vector space, so that we may speak about the *vector of species concentrations* or about the *vector of reaction rate constants*.

Before proceeding, we need to introduce some notation and give some more definitions.

$\mathbb{R}_{\geq 0}$ (resp. \mathbb{R}_+) represent the set of nonnegative (resp. positive) real numbers.

Let I be a finite set. Then, \mathbb{R}^I represents the vector space of real valued functions with domain I , where the addition of functions and the multiplication of a function by a real number are defined in the usual way. Likewise, by $\mathbb{R}_{\geq 0}^I$ (resp. \mathbb{R}_+^I) it is meant the set of \mathbb{R}^I , which elements are functions that take non-negative (resp. exclusively positive) values.

For $\mathbf{x} \in \mathbb{R}^I$ the subset of I defined by

$$\text{supp } \mathbf{x} = \{i \in I : x_i \neq 0\}$$

is called *support* of \mathbf{x} .

If J is a subset of I , the symbol ω_J is used to indicate the characteristic function on J . That is, ω_J is the vector of \mathbb{R}^I such that

$$(\omega_J)_i = \begin{cases} 1 & \text{if } i \in J \\ 0 & \text{if } i \notin J. \end{cases}$$

In particular, if $J = \{j\}$ then we shall write only ω_j and

$$(\omega_j)_i = \begin{cases} 1 & \text{if } i = j \\ 0 & \text{if } i \neq j. \end{cases}$$

The *standard basis* of \mathbb{R}^I is given by

$$\{\omega_j \in \mathbb{R}^I : j \in I\},$$

and we have that the dimension of \mathbb{R}^I is equal to the number of elements of the finite set I .

Hence, each $\mathbf{x} \in \mathbb{R}^I$ has the representation

$$\mathbf{x} = \sum_{j \in I} x_j \omega_j.$$

\mathbb{R}^I is taken to be endowed with the *standard scalar product* as follows: if \mathbf{x} and \mathbf{z} are vectors of \mathbb{R}^I then

$$\langle \mathbf{x}, \mathbf{z} \rangle = \sum_{i \in I} x_i z_i.$$

Let us now interpret the latter considerations in terms of reaction network terminology.

With each species $s \in \mathcal{S}$ there is associated a (non-negative) molar concentration c_s . That is to say that there is a function $c : \mathcal{S} \rightarrow \mathbb{R}_{\geq 0}$ assigning to each species its molar concentration. Note that, $c \in \mathbb{R}_{\geq 0}^{\mathcal{S}} \subset \mathbb{R}^{\mathcal{S}}$ is the *composition state vector*.

If our reactor is in composition state c , then

$$\text{supp } c = \{s \in \mathcal{S} : c_s \neq 0\}.$$

Hence, the support of c is the set of species present in the reactor.

Suppose that the network is endowed with *mass action kinetics* (see Section 2.2.1 for more details) so that with each reaction in \mathcal{R} there is associated a (positive) rate constant. Then, there exists a function $k : \mathcal{R} \rightarrow \mathbb{R}_+$ and $k \in \mathbb{R}_+^{\mathcal{R}} \subset \mathbb{R}^{\mathcal{R}}$ is a *vector of rate constants*.

Remark 2.1.3. Let us suppose that the species $\omega_A \in \mathbb{R}^{\mathcal{S}}$ combines with the species $\omega_B \in \mathbb{R}^{\mathcal{S}}$. Then we write $\omega_A + \omega_B \in \mathbb{R}^{\mathcal{S}}$. By convention one could write instead $A + B \in \mathbb{R}^{\mathcal{S}}$. So, the complexes of a network are regarded as vectors in $\mathbb{R}^{\mathcal{S}}$ or, in particular, as vectors in $\mathbb{R}_{\geq 0}^{\mathcal{S}}$. Thus, it makes sense to add two complexes, to multiply a complex by a number and to calculate the scalar product of a complex with any other vector of $\mathbb{R}^{\mathcal{S}}$. Altogether, $\mathcal{C} \in \mathbb{R}_{\geq 0}^{\mathcal{S}}$.

Remark 2.1.4. If m species and r reactions constitute the reaction system then

$$\dim \mathbb{R}_{\geq 0}^{\mathcal{S}} = m \text{ and } \dim \mathbb{R}_+^{\mathcal{R}} = r.$$

2.1.2 Defining reaction networks

We supply the formal definition of a chemical reaction network presented in [Fein79].

Definition 2.1.5. A *chemical reaction network* consists of three sets:

- (i) a finite set \mathcal{S} , which elements are called *species* of the network.
- (ii) a finite set \mathcal{C} of distinct vectors in $\mathbb{N}_{\geq 0}^{\mathcal{S}}$ such that

$$\bigcup_{\mathbf{p} \in \mathcal{C}} \text{supp } \mathbf{p} = \mathcal{S}.$$

The elements of \mathcal{C} are called the *complexes* of the network.

- (iii) a relation $\mathcal{R} \subset \mathcal{C} \times \mathcal{C}$ such that
 - (a) $(\mathbf{p}, \mathbf{p}) \notin \mathcal{R}$, for all $\mathbf{p} \in \mathcal{C}$ (i. e. no complex reacts to itself).
 - (b) For each $\mathbf{p} \in \mathcal{C}$ there exists $\mathbf{p}' \in \mathcal{C}$ such that $(\mathbf{p}', \mathbf{p}) \in \mathcal{R}$ or such that $(\mathbf{p}, \mathbf{p}') \in \mathcal{R}$ (i. e. no complex is isolated).

The elements of \mathcal{R} are called the *reactions* of the network. For each pair $(\mathbf{p}, \mathbf{p}') \in \mathcal{R}$ we say that the complex \mathbf{p} *reacts to* the complex \mathbf{p}' . We can use instead a more suggestive notation $\mathbf{p} \rightarrow \mathbf{p}'$ to say that \mathbf{p} reacts to \mathbf{p}' . Then, the vector \mathbf{p} is called the *reactant complex* and \mathbf{p}' is called *product complex* of the reaction $\mathbf{p} \rightarrow \mathbf{p}'$.

Remark 2.1.6. A chemical reaction on the set of species $\{\mathbf{s}_i\}, i = 1, \dots, n$ can be written as

$$R \equiv \nu_1 \mathbf{s}_1 + \dots + \nu_n \mathbf{s}_n. \quad (2.1.1)$$

The multipliers $\nu_i \in \mathbb{R}$ are called *stoichiometric² coefficients* and they represent the relative molar proportions of each molecular species in the reaction. If all the stoichiometric coefficients are zero then the reaction is said to be *trivial*.

The condition for a reaction to be proper is known in chemistry as *balancing the equation* and it implies that

$$\nu_1 \mathbf{s}_1 + \dots + \nu_n \mathbf{s}_n = \mathbf{0}.$$

²From the Greek, "stoicheion"= element. The word *Stoichiometry* is part of the common vocabulary of chemists and chemical engineers. According to [Aris65b] "Stoichiometry literally means measurement of the elements but the word is commonly used to refer to all manner of calculations regarding the components of a chemical system...Stoichiometry is essentially the bookkeeping of material components of the chemical system."

Conventionally, molecular species regarded as *product complexes* have a positive stoichiometric coefficient. *Reactant complexes* are the molecular species with negative coefficient [Aris65a].

Remark 2.1.7. If we have a set of r chemical reactions on $\{\mathbf{s}_i\}$ then each reaction can be written as

$$R_j \equiv \sum_{i=1}^n \nu_{ij} \mathbf{s}_i, \quad j = 1, \dots, r,$$

where $\nu = (\nu_{ij})$ is the so called *matrix of stoichiometric coefficients* of the set $\{\mathbf{s}_i\}$ or simply *stoichiometric matrix*.

The number of linearly independent reactions is equal to the rank of ν .

The next section is dedicated to the highlight of important aspects concerning the kinetics for a reaction network.

2.2 Kinetics

One of the major aims of studying the kinetics of chemical networks is to analyze systems of differential equations which describe the time evolution of the concentration of the n chemical species involved. Let us start by defining the kinetics for a reaction network.

Definition 2.2.1. A *kinetics* for a reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ is an assignment to each reaction $\mathbf{p} \rightarrow \mathbf{p}' \in \mathcal{R}$ of a continuous *rate function* $\mathcal{K}_{\mathbf{p} \rightarrow \mathbf{p}'} : \mathbb{R}_{\geq 0}^{\mathcal{S}} \rightarrow \mathbb{R}_{\geq 0}$ such that

$$\mathcal{K}_{\mathbf{p} \rightarrow \mathbf{p}'}(\mathbf{c}) > 0 \text{ if and only if } \text{supp } \mathbf{p} \subset \text{supp } \mathbf{c}.$$

Definition 2.2.2. A *reaction system* $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K}\}$ is the reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ endowed with a kinetics \mathcal{K} .

2.2.1 The law of mass action

The *law of mass action* describes the rate at which chemicals, whether large macromolecules or simple ions, collide and interact to form different chemical combinations³. Thus, the number of collisions per unit of time is taken to be proportional to the product of the concentrations of the chemicals involved and the factor of proportionality depends on the geometrical shapes and sizes of the reactant molecules, and on the temperature of the mixture [KeSn98]. This law describes

³Collision model

well-stirred reactions with no special inhomogeneities and is not generally valid at high concentrations.

Formally we have the following definition:

Definition 2.2.3. A kinetics \mathcal{K} for a reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ is of *mass action* type if, for each $\mathbf{p} \rightarrow \mathbf{p}' \in \mathcal{R}$, there exists a positive number $k_{\mathbf{p} \rightarrow \mathbf{p}'}$ such that

$$\mathcal{K}_{\mathbf{p} \rightarrow \mathbf{p}'}(\mathbf{c}) \equiv k_{\mathbf{p} \rightarrow \mathbf{p}'} \prod_{s \in \mathcal{S}} c_s^{\nu_s}, \quad (2.2.1)$$

where ν_s is the stoichiometric coefficient of species s in the reactant complex \mathbf{p} of the reaction $\mathbf{p} \rightarrow \mathbf{p}'$. The number $k_{\mathbf{p} \rightarrow \mathbf{p}'}$ is called the *rate constant* of the reaction $\mathbf{p} \rightarrow \mathbf{p}'$.

Example 2.2.4. Let us consider the reactions $A + C \rightarrow D$ and $2B \rightarrow A$ of the diagram in Example 2.1.2. For the reaction $A + C \rightarrow D$, the higher the concentration of A , the more occurrences of the reactions will be (similarly for C). That is, it is presumed that the occurrence rate of the reaction $A + C \rightarrow D$ is proportional to the probability of A and C to meet, which in turn, it is proportional, at low concentrations, to the value of $c_A c_C$. For the reaction $2B \rightarrow A$ one says that 2 molecules of B are needed to form A , but we still have the same chemical interpretation as before. We respectively write:

$$\mathcal{K}_{A+C \rightarrow D}(\mathbf{c}) \equiv k_{A+C \rightarrow D} (c_A)^1 (c_B)^0 (c_C)^1 (c_D)^0 (c_E)^0 = k_{A+C \rightarrow D} c_A c_C$$

and

$$\mathcal{K}_{2B \rightarrow A}(\mathbf{c}) \equiv k_{2B \rightarrow A} (c_A)^0 (c_B)^2 (c_C)^0 (c_D)^0 (c_E)^0 = k_{2B \rightarrow A} (c_B)^2.$$

Remark 2.2.5. For \mathbf{c} and \mathbf{p} in $\mathbb{R}_{\geq 0}^{\mathcal{S}}$ let us define $\mathbf{c}^{\mathbf{p}}$ as follows:

$$\mathbf{c}^{\mathbf{p}} := \prod_{s \in \mathcal{S}} c_s^{\nu_s}.$$

Then, mass action rate functions take the form

$$\mathcal{K}_{\mathbf{p} \rightarrow \mathbf{p}'}(\mathbf{c}) \equiv k_{\mathbf{p} \rightarrow \mathbf{p}'} \mathbf{c}^{\mathbf{p}}.$$

The behavior of a homogeneous chemical system can be described by a system of differential equations obtained from the reaction mechanism by applying the law of mass action. Therefore, these equations are also known as *mass balance equations*. In the next section we describe how to achieve this.

2.2.2 The differential equations for a reaction system

Definition 2.2.6. Let $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ be a reaction network. The *reaction vector* corresponding to the reaction $\mathbf{p} \rightarrow \mathbf{p}' \in \mathcal{R}$ is the vector $\mathbf{p}' - \mathbf{p} \in \mathbb{R}^{\mathcal{S}}$.

Remark 2.2.7. Note that the component of $\mathbf{p}' - \mathbf{p}$ relative to the standard basis of $\mathbb{R}^{\mathcal{S}}$ corresponding to species $s \in \mathcal{S}$ is just $\nu'_p - \nu_p$, i. e. the difference between the stoichiometric coefficient of s in the product component \mathbf{p}' and its stoichiometric coefficient in the reactant complex \mathbf{p} .

Definition 2.2.8. For a reaction system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K}\}$ the *species formation rate function* $f : \mathbb{R}_{\geq 0}^{\mathcal{S}} \rightarrow \mathbb{R}^{\mathcal{S}}$ is defined by

$$f(\mathbf{c}) \equiv \sum_{\mathcal{R}} \mathcal{K}_{\mathbf{p} \rightarrow \mathbf{p}'}(\mathbf{c})(\mathbf{p}' - \mathbf{p}).$$

That is, $f(\cdot)$ is obtained by summing the reaction vectors for the network, each multiplied by the corresponding reaction rate function. Moreover it fulfills the following positivity requirement:

Lemma 2.2.9. *Let $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K}\}$ be a reaction system with species formation rate function $f(\cdot)$. Then, for every $s \in \mathcal{S}$ and every $\mathbf{c} \in \mathbb{R}_{\geq 0}^{\mathcal{S}}$, $c_s = 0$ implies that $f_s(\mathbf{c}) \geq 0$.*

Remark 2.2.10. The species formation for a mass action system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, k\}$ takes the form:

$$f(\mathbf{c}) \equiv \sum_{\mathcal{R}} k_{\mathbf{p} \rightarrow \mathbf{p}'} \mathbf{c}^{\mathbf{p}}(\mathbf{p}' - \mathbf{p}).$$

By the *differential equation of a reaction system* $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K}\}$ we mean

$$\dot{\mathbf{c}} = f(\mathbf{c}) = \sum_{\mathcal{R}} \mathcal{K}_{\mathbf{p} \rightarrow \mathbf{p}'}(\mathbf{c})(\mathbf{p}' - \mathbf{p}). \quad (2.2.2)$$

Equation (2.2.2) is a vector differential equation which encodes the system of scalar functions

$$\dot{c}_s = \sum_{\mathcal{R}} \mathcal{K}_{\mathbf{p} \rightarrow \mathbf{p}'}(\mathbf{c})(\mathbf{p}' - \mathbf{p})_s, \text{ for all } s \in \mathcal{S}.$$

In particular, for a mass action system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, k\}$ the corresponding vector differential equation is given by

$$\dot{\mathbf{c}} = f(\mathbf{c}) = \sum_{\mathcal{R}} k_{\mathbf{p} \rightarrow \mathbf{p}'} \mathbf{c}^{\mathbf{p}}(\mathbf{p}' - \mathbf{p}).$$

Example 2.2.11. Consider the reaction: with k_+ and k_- denoting the forward and

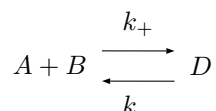


Figure 2.2: Second order reaction scheme.

the reverse rate constants of the reaction, respectively. The quantities A and B are consumed by the forward reaction and produced by the reverse reaction, therefore the rate of change of $[A]$ and of $[B]$ for the bidirectional reaction is respectively given by:

$$\frac{d[A]}{dt} = k_-[D] - k_+[A][B]; \quad \frac{d[B]}{dt} = k_-[D] - k_+[A][B].$$

For $[D]$ we have,

$$\frac{d[D]}{dt} = -k_-[D] + k_+[A][B].$$

Remark 2.2.12. If there exists an equilibrium, concentrations are not changing so that

$$[D]_{\text{eq}} = \frac{k_+}{k_-}[A]_{\text{eq}}[B]_{\text{eq}}.$$

If there are no other reactions involving A and D then $[A] + [D] = A_0$ is constant and

$$[D] = A_0 \frac{[B]}{K_{\text{eq}} + [B]}. \quad (2.2.3)$$

The number $K_{\text{eq}} = \frac{k_-}{k_+}$ is called the *equilibrium constant*, and relates to the relative preference for the chemicals to be in the combined state D compared to the dissociated state. The equilibrium constant has units of concentration. If K_{eq} is small, then there is a high affinity between A and B [KeSn98].

In the next section, some considerations are given that help to gain some geometric insight into the way phase portraits of the differential equations governing the reaction scheme are structured.

2.2.3 Reaction network structure and nature of composition trajectories

Regardless of the kinetics, reaction network structure imposes restrictions on the shape and behavior of the composition trajectories. In particular, a trajectory

that passes through a composition $c \in \mathbb{R}_{\geq 0}^S$ can eventually reach a composition $c' \in \mathbb{R}_{\geq 0}^S$ only if the pair (c', c) is compatible with certain "stoichiometrical" conditions the reaction network imposes [Fein79]. Roughly speaking, due to existence of conservation laws, composition trajectories are not completely free to wander in an arbitrary fashion through $\mathbb{R}_{\geq 0}^S$ since there are only certain directions in which the species formation rate vector can point. In other words, the species formation rate $f(c)$ must point along the cone generated by the reaction vectors and belongs to the linear subspace of \mathbb{R}^S generated by them.

Definition 2.2.13. The *stoichiometric subspace* for a reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ is the linear subspace $S \subset \mathbb{R}^S$ defined by

$$S = \text{span}\{y' - y \in \mathbb{R}^S : y \rightarrow y' \in \mathcal{R}\}.$$

The dimension of the stoichiometric subspace for a reaction network is equal to the rank of the network.

The following lemma is proved in [Fein79] states that there are conservation laws, respectively first integrals⁴, arising from the stoichiometry.

Lemma 2.2.14. Let $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K}\}$ be a reaction system and let $c : I \rightarrow \mathbb{R}_{\geq 0}^S$ be a solution of

$$\dot{c} = \sum_{\mathcal{R}} \mathcal{K}_{y \rightarrow y'}(c)(y' - y), \quad (2.2.4)$$

where $I \subset \mathbb{R}$. Then, for arbitrary $t_1, t_2 \in I$ such that $t_2 > t_1$ the solution c satisfies

$$c(t_2) - c(t_1) = \sum_{\mathcal{R}} \int_{t_1}^{t_2} \mathcal{K}_{y \rightarrow y'}(c(\tau))(y' - y) d\tau, \text{ for all } y \rightarrow y' \in \mathcal{R}. \quad (2.2.5)$$

Remark 2.2.15. A composition $c_1 \in \mathbb{R}_{\geq 0}^S$ can follow a composition $c_2 \in \mathbb{R}_{\geq 0}^S$ along a solution of (2.2.4). Thus, if a solution $c(t)$ of (2.2.4) passes through a composition c_0 then

$$c(t) \in (c_0 + S) \cap \mathbb{R}_{\geq 0}^S,$$

where S is the stoichiometric subspace and

$$c_0 + S = \{c_0 + \gamma; \gamma \in S\} \subset \mathbb{R}^S$$

is the parallel translate of S that passes through c_0 and $(c_0 + S) \cap \mathbb{R}_{\geq 0}^S$ referred to as *stoichiometric compatibility class* [Son01].

Now we are able to define the *stoichiometric compatibility of two vectors*.

Definition 2.2.16. Let $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ be a reaction network, and let $S \subset \mathbb{R}^S$ be its stoichiometric subspace. Two vectors $c_1 \in \mathbb{R}_{\geq 0}^S$ and $c_2 \in \mathbb{R}_{\geq 0}^S$ are stoichiometrically compatible if $c_1 - c_2$ lies in S .

⁴See Definition A.1.4

Remark 2.2.17. Stoichiometric compatibility is an equivalence relation that induces a partition of $\mathbb{R}_{\geq 0}$, respectively of \mathbb{R}_+^S into equivalence classes, the already mentioned *stoichiometric compatibility classes* for the network. Moreover, since $\dot{\mathbf{c}} \in S$, trajectories remain in classes, that is, classes are positive invariant manifolds for the dynamical system [Son01]. For the definition of positively invariant subsets see Appendix A.

Following Horn and Jackson, one says that a network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ is conservative if there exists a positive vector⁵ $\mathbf{m} \in S^\perp$, the orthogonal complement of the stoichiometric subspace for the network. Moreover, it can be proved that a network is conservative if and only if all its stoichiometric compatibility classes are compact.

For other concepts and examples of application, we refer to [Fein79].

2.2.4 Linkage classes. Weak reversibility. Deficiency

Reaction network structure may be discussed in terms of concepts like *linkage classes*, *weak reversibility* and *deficiency* of a network. While the first two depend essentially on a network's character as a graph and not on the precise nature of the complexes, the last one is influenced by the algebraic nature of the complexes, what is the same as to say that the stoichiometry of the network plays no role. The basic ideas behind these concepts are easy to understand in an intuitive way. Therefore, we introduce them in an informal way following [Fein79].

⁵I.e. with all entries greater than zero

Linkage classes

Consider the following reaction diagram:

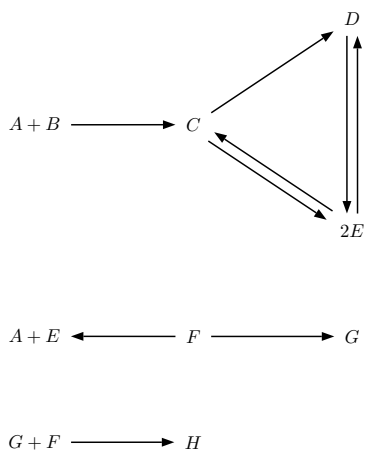


Figure 2.3: Network with 3 linkage classes.

We observe that it is composed by three disjoint pieces. One containing the complexes $\{A + B, C, D, 2E\}$, other containing the complexes $\{A + E, F, G\}$ and the third one contains the complexes $\{G + F, H\}$.

These sets are called *linkage classes*.

Weak reversibility

A *reversible* network is one in which each reaction is accompanied by its "anti-reaction". I. e., if the reaction $y \rightarrow y'$ is considered in a reversible network, so it is $y \rightarrow y'$.

Example 2.2.18. The diagram of Figure 2.4 is reversible and that of Figure 2.5 not.

If a network is not reversible then it may be *weakly reversible*. This is an important case to consider because both networks have almost the same properties. Moreover, every reversible network is also weakly reversible. A network is then weakly reversible if, whenever there is a directed arrow path leading from complex y to

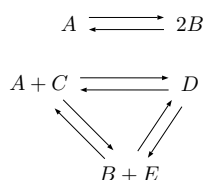


Figure 2.4: Reversible network.

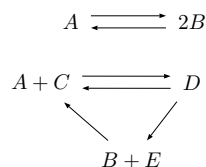


Figure 2.5: Not reversible but weakly reversible network.

complex y' , there is also a directed arrow path leading from y' back to y . Both networks of Example 2.2.18 are weakly reversible.

In the following figure, we give two examples of networks which are not weakly reversible.

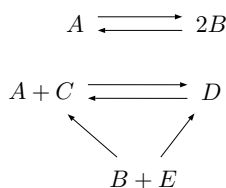


Figure 2.6: Not weakly reversible network.

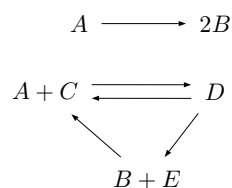


Figure 2.7: Not weakly reversible network.

Deficiency of a network

Since stoichiometry influences the rank of a network it plays a role by discussing the deficiency of a network.

The deficiency amounts to a *non-negative* integer index, which helps to classify networks. Let n be the number of complexes, l the number of linkage classes and s the rank of the network. Denoting the deficiency by δ we have the following relation:

$$\delta := n - l - s.$$

Example 2.2.19. The network of Figure 2.5 has 5 complexes, 2 linkage classes and rank 3. Thus, $\delta = 0$. The same for the networks in Figures 2.4, 2.6 and 2.7.

2.2.5 Two theorems

The following theorems are proved in [Fein79] and state the existence and uniqueness of equilibria⁶, and local asymptotic stability for deficiency zero and deficiency one networks. For the definitions of an equilibrium point and of asymptotic stability see Appendix A.

Theorem 2.2.20 (Deficiency Zero Theorem). *Let $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ be any reaction network of deficiency zero.*

- (i) *If the network is not weakly reversible then, for arbitrary kinetics \mathcal{K} , the differential equations for the reaction system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K}\}$ cannot admit a strictly positive equilibrium.*
- (ii) *If the network is not weakly reversible then, for arbitrary kinetics \mathcal{K} , the differential equations for the reaction system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K}\}$ cannot admit a periodic composition trajectory containing a positive composition.*
- (iii) *If the network is weakly reversible then, for any mass action kinetics $k \in \mathbb{R}_+^{\mathcal{R}}$, the differential equations for the mass action system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, k\}$ have the following properties: There exists within each positive stoichiometric compatibility class precisely one equilibrium; that equilibrium is asymptotically stable; and there cannot exist a nontrivial cyclic composition trajectory in $\mathbb{R}_+^{\mathcal{R}}$.*

Theorem 2.2.21 (Deficiency One Theorem). *Let $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ be a reaction network with l linkage classes. Let δ denote the deficiency of the network, δ_θ the deficiency of the θ -th linkage class, $\theta = 1, 2, \dots, l$. Suppose furthermore that:*

- (i) $\delta_\theta \leq 1, \quad \theta = 1, 2, \dots, l$
- (ii) $\delta = \sum_{\theta=1}^l \delta_\theta.$

If the network is weakly reversible then, for any mass action kinetics $k \in \mathbb{R}_+^{\mathcal{R}}$, the differential equations for the mass action system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, k\}$ admit precisely one equilibrium in each positive stoichiometric compatibility class.

Remark 2.2.22. The absence of weak reversibility condition might exclude the existence of positive equilibria or affect the uniqueness of equilibria within a positive stoichiometric compatibility class.

⁶It is noteworthy to point out that when communicating with biologists and physicists the words *equilibrium* and *steady state* do not have the same meaning. A steady state is one in which the concentration is constant in the macroscopic sense, but this does not mean that chemical reactions are not taking place. An equilibrium means something much stronger. So, never use the word equilibrium while talking about steady states [Son05a]

2.3 Enzyme kinetics

Reactions that do not follow mass action kinetics are usually preceded by a complex mechanism consisting of two or more elementary reaction steps. Complicated reaction schemes may arise for instance if we are in the presence of a reaction catalyzed by an enzyme. Typically, one obtains systems of highly nonlinear differential equations with many kinetic and stoichiometric parameters. These systems are stiff, have multiple timescales and are computationally demanding to solve numerically, making data fitting difficult as well. Since the blood coagulation system comprises biochemical reactions involving enzyme activation we discuss briefly this topic.

Enzymes are catalysts (generally proteins) that help convert other molecules called *substrates* into *products*, but they themselves are not changed by the reaction. Three of the most important features of enzymes are *catalytic power*, *specificity* and *regulation*. In fact, enzymes accelerate the conversion of substrate into product by given increases in speed of up to 10 million times; they are specific as they catalyze the reaction of only a particular substrate or closely related substrates and complicated positive and negative feedback systems allow precise control over the set of reactions [KeSn98].

In the nineteenth century, the first scientists studying enzyme kinetics of the single enzyme-substrate reaction experienced a number of difficulties. The experimental practice was to follow the reaction over an extended period of time and to explain observations in terms of the solutions of second-order rate equations. Brown and other workers found that enzyme reactions do not follow directly the law of mass action [SchMai03]. For, as the concentration of the substrate S is increased, the rate of the reaction increases only to a certain extent, reaching a maximal reaction velocity at high substrate concentrations. In 1903, V. Henri proposed the following reaction scheme:

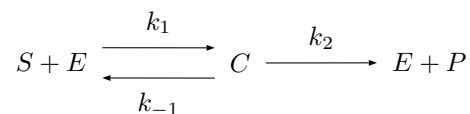


Figure 2.8: Reaction scheme for an enzymatic reaction proposed by Henri.

Using the formalism given above, we have:

$$\mathcal{S} = \{S, E, C, P\}; \mathcal{C} = \{S + E, C, E + P\} \text{ and } \mathcal{R} = \{S + E \rightarrow C, C \rightarrow S + E, C \rightarrow E + P\}.$$

Here, the enzyme E converts the substrate S into the product P through a two step process. First, E combines reversibly with S to form a complex C , which then breaks down irreversibly with a certain probability per unit time k_2 into the product, P , releasing E in the process. The reverse reaction between E and P to reform the complex is often slow enough to be neglected [Seg91].

According to the theory of enzymatic reactions of Leonor Michaelis and Maud Leonora Menten in 1913, enzymes can be studied by measuring initial rate of product formation under certain conditions. The established mathematical model helps to understand the deviation from the law of mass action. In 1925, Briggs and Haldane made another extension of Henri's formulation [Son05b].

Let $s = [S]$, $c = [C]$, $e = [E]$ and $p = [P]$. Applying the law of mass action and what has been said in Section 2.2 to the reaction mechanism in Figure 2.8 yields four differential equations for the rate of change of s , c , e and p as

$$\begin{aligned}\frac{ds}{dt} &= k_{-1}c - k_1se \\ \frac{de}{dt} &= (k_{-1} + k_2)c - k_1se \\ \frac{dc}{dt} &= k_1se - (k_{-1} + k_2)c \\ \frac{dp}{dt} &= k_2c.\end{aligned}\tag{2.3.1}$$

These equations are also known as Briggs-Haldane equations. They were derived in [BriHal25], which constitutes the first mathematical discussion of the so-called *quasi-steady state assumption*.

Notice that p can be found by direct integration and there is a conserved quantity since

$$\frac{de}{dt} + \frac{dc}{dt} = 0,\tag{2.3.2}$$

so that $e + c = e_0$, where $e_0 \neq 0$ is the total amount of available enzyme. Moreover, at time $t = 0$ we have $s = s_0$, $c = 0$ and $p = 0$.

System (2.3.1) reduces to

$$\begin{aligned}\frac{ds}{dt} &= -k_1(e_0 - c)s + k_{-1}c \\ \frac{dc}{dt} &= k_1(e_0 - c)s - (k_{-1} + k_2)c.\end{aligned}\tag{2.3.3}$$

2.3.1 The quasi-steady state assumption

Following [SeSl89], the hypothesis for the quasi-steady state approximation are:

- (i) experimental measurements of the reaction rate should be performed after a short initial phase, called the *pre-steady state* period but before the concentration of the substrate decays considerably;
- (ii) if the rate of product formation is approximately constant over the time interval of observation then c is also approximately constant (see the 4th equation of (2.3.1));
- (iii) while s is high enough, the free enzyme E combines immediately with another molecule of the substrate S . Then a quasi-steady-state is achieved in which the enzyme is always saturated with its substrate.
- (iv) from items (ii) and (iii) we have $\frac{dc}{dt} \simeq 0$ for an appreciable period of time.

Applying the quasi-steady state assumption reduces the order of the system of differential equations by an amount equal to the number of chemical species to which the assumption was applied [ChaRu94].

Let us rewrite the system (2.3.3) as

$$\begin{aligned}\frac{ds}{dt} &= -k_1(e_0 - c)s + k_{-1}c \\ \frac{dc}{dt} &= k_1(e_0 - c)s - (k_{-1} + k_2)c = k_1[se_0 - (K_m + s)c],\end{aligned}\tag{2.3.4}$$

where $K_m = \frac{k_{-1} + k_2}{k_1}$.

If c is approximately constant then we can solve the second equation for c , yielding:

$$c = \frac{e_0 s}{K_m + s}.$$

Quoting [BdBS96], a differential equation for s , valid after the transient, can be easily derived by realizing that if $\frac{dc}{dt}$ is effectively zero then the two equations of (2.3.4) can be added and

$$\frac{ds}{dt} = -k_2 c,$$

i.e.,

$$\frac{ds}{dt} = -k_2 \frac{e_0 s}{K_m + s}.\tag{2.3.5}$$

By convention, the substrate level changes negligibly during the fast transient so that $s(0) = s_0$. This initial condition and the differential equation (2.3.5) constitute the quasi-steady state assumption. The constant K_m is also known as *Michaelis constant*.

By the method of separation of variables, we have:

$$s + K_m \ln \frac{s}{s_0} = s_0 - k_2 e_0 t. \quad (2.3.6)$$

One then hopes that these quasi-steady state approximations will provide a good approximation for calculating the post-transient development of the system under consideration.

Despite of being a useful approximation, the Michaelis-Menten law is not universally applicable. One of the problems is to derive analytical approximations by using the quasi-steady state assumption and identifying parameter regimens in which they hold.

The original Michaelis-Menten condition requires $\frac{e_0}{s_0}$ small, which was also the basic assumption in [BriHal25].

The validity of the quasi-steady state was then first discussed by Laidler (1955). He suggested that an excess of substrate concentration is the main prerequisite and found out that the initial concentration of substrate must greatly exceed that of the enzyme such that

$$\frac{e_0}{s_0} \ll 1.$$

This condition can be derived mathematically after nondimensionalize⁷ the system of differential equations by taking:

$$x = \frac{s}{s_0}; \quad y = \frac{c}{e_0} \quad \text{and} \quad \tau = k_1 e_0 t.$$

A proof can be found in [SeSl89].

Stayton and Fromm found the quasi-steady state assumption to generally hold for

$$\frac{s_0}{e_0} > 100$$

by means of simulation modelling on a digital computer [StFr79].

More recently, Segel and Slemrod showed in [SeSl89] that a more general condition is

$$\frac{e_0}{K_m + s_0} \ll 1.$$

⁷The aim is to identify independent parameters and to determine their relative magnitude. An overview about scaling and nondimensionlization can be found in [LinSe88] or in [Seg88]

They estimated two different timescales, the duration of the pre-steady-state period (i. e. the *fast time scale*) and the duration of the period during which the substrate is converted to product according to the quasi-steady state assumption (i.e. the *slow time scale*). Knowledge of these scales is prerequisite to choose suitable dimensionless independent variables. This approach is also described in [Mur93].

This assumption guarantees that there is not a significant fraction of the substrate bound to the enzyme during the assay [Seg88] [SeSl89]. Moreover, the final confirmation that smallness of the appropriate parameter is necessary and sufficient for the quasi-steady state assumption to be valid have been given only recently (see [NoeWa05]).

The quasi-steady state approximation can provide a good approximation even if $s_0 \approx e_0$ as long as e_0 is small compared to K_m [Seg88][SeSl89]. In [SchMai00] and in [SchMen97] the case of high enzyme concentration is treated.

Another important aspect of enzyme kinetics in complex biochemical pathways and, in particular, of the blood coagulation mechanism, is the effect of inhibitors and activators of the enzyme. This topic is handled in the next section.

2.3.2 Enzyme inhibition

An *enzyme inhibitor* is a substance that inhibits the catalytic action of the enzyme.

Studies of reactions involving enzyme inhibition contribute to the understanding of enzyme mechanisms, including control processes in the cell and the mode of action of various drugs.

Loss of activity may be either *reversible*, where the activity of the enzyme may be restored by removing the inhibitor, or *irreversible*, where the loss of activity is time dependent and cannot be recovered during the timescale of interest. Furthermore, if the inhibited enzyme is totally inactive, irreversible inhibition behaves as a time-dependent loss of enzyme concentration. Irreversible inhibitors are also known as *catalytic poisons*.

In the class of reversible inhibitors we may distinguish between *competitive* and *allosteric* inhibitors. To understand the distinction keep in mind that an enzyme is usually a large protein, considerably larger than the substrate molecule whose reaction is catalyzed. Embedded in the large enzyme protein are one or more active sites, to which the substrate can bind in a "lock-and-key" fashion to form the complex [KeSn98]. If another molecule has a shape similar enough to that of the substrate molecule, it may also bind to the active site, preventing the binding of a substrate molecule, thus inhibiting the reaction. The inhibition is then called competitive because the inhibitor competes with the substrate molecule for the

active site. In most cases, drugs act by competitive inhibition [Son05b].

Enzymes also have other binding sites different from the active site, the binding of which regulates the activity of the enzyme at the active site. These binding sites are structurally different from the catalytic active sites and are called *allosteric* or *regulatory* sites [KeSn98]. The inhibition is also said to be *noncompetitive*. If the formation of the complex *Enzyme – Substrate – Inhibitor* is excluded then the inhibition is competitive.

We illustrate the competitive inhibition by presenting an example given in [Son05b].

The chemical model is given by:

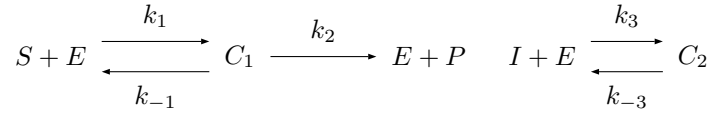


Figure 2.9: Example of a reaction scheme for competitive inhibition.

Using the same notation as above and letting $[I] = i$; $[C_1] = c_1$ and $[C_2] = c_2$. In terms of ODE's, we have:

$$\begin{aligned} \frac{ds}{dt} &= k_{-1}c_1 - k_1se \\ \frac{de}{dt} &= (k_{-1} + k_2)c_1 + k_{-3}c_2 - k_1se - k_3ie \\ \frac{dc_1}{dt} &= k_1se - (k_{-1} + k_2)c_1 \\ \frac{dc_2}{dt} &= k_3ie - k_{-3}c_2 \\ \frac{di}{dt} &= k_{-3}c_2 - k_3ie \\ \frac{dp}{dt} &= k_2c_1. \end{aligned} \tag{2.3.7}$$

Note that $c_1 + c_2 + e = e_0$. Moreover, $i + c_2 = i_0$, where $i(0) = i_0$. This allows us to eliminate e and i from the equations. Discarding at first the equation for p , we are left:

$$\begin{aligned} \frac{ds}{dt} &= k_{-1}c_1 - k_1s(e_0 - c_1 - c_2) \\ \frac{dc_1}{dt} &= k_1s(e_0 - c_1 - c_2) - (k_{-1} + k_2)c_1 \\ \frac{dc_2}{dt} &= k_3(i_0 - c_2)(e_0 - c_1 - c_2) - k_{-3}c_2. \end{aligned} \tag{2.3.8}$$

Assuming that the enzyme concentrations are small relative to substrate, one may furthermore perform a quasi-steady state approximation followed by a singular perturbation analysis.

Setting $\frac{dc_i}{dt} = 0$, $i = 1, 2$ without eliminating i , we obtain

$$c_1 = \frac{K_i e_0 s}{K_m i + K_i s + K_m K_i} \text{ and } c_2 = \frac{K_m e_0 i}{K_m i + K_i s + K_m K_i},$$

where K_m is the Michaelis constant and $K_i = \frac{k_{-3}}{k_3}$.

The product formation rate is $\frac{dp}{dt} = k_2 c_1$. Therefore, with $V_{max} = k_2 e_0$,

$$\frac{dp}{dt} = \frac{V_{max} s}{s + K_m(1 + i/K_i)}.$$

If $i = 0$ then this formula reduces to the case where there is no inhibition.

Observe that the rate of product formation is smaller, at least for $i \gg 1$, $k_3 \gg 1$ and $k_3 \ll 1$.

An example of allosteric inhibition can also be found in [Son05b]. More detailed information is provided in [Mur93]. For a more general theory about equilibrium binding of macromolecules with ligands see [Seg91].

2.3.3 Cooperativity

For many enzymes, the reaction velocity is not a simple hyperbolic curve, as predicted by the Michaelis-Menten model, but it has often a sigmoid like form. This can result from cooperative effects, in which the enzyme can bind more than one substrate molecule, but the binding of one substrate molecule affects the binding of subsequent ones [KeSn98]. Hill (1910) was one of the first to appreciate a sigmoid like behavior of protein while studying the binding of oxygen to hemoglobin.

In [Mur93], the case in which an enzyme can bind two substrate molecules was treated. There are three possibilities for the state of the enzyme. Namely, as free molecule, E , as a complex with an occupied center, C_1 , and as a complex with two occupied centers, C_2 . The reaction mechanism is given by

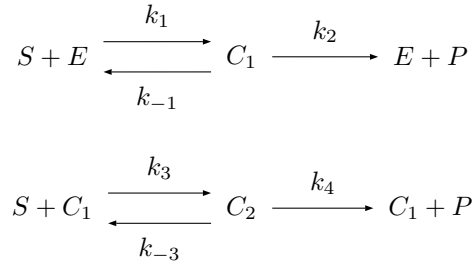


Figure 2.10: Reaction scheme in which an enzyme binds to two substrate molecules.

With lower case letters denoting concentrations, the mass action law gives

$$\begin{aligned}
 \frac{ds}{dt} &= -k_1se + k_{-1}c_1 - k_3sc_1 + k_{-3}c_2 \\
 \frac{dc_1}{dt} &= k_1se - (k_{-1} + k_2)c_1 - k_3sc_1 + (k_4 + k_{-3})c_2 \\
 \frac{dc_2}{dt} &= k_3sc_1 - (k_4 + k_{-3})c_2 \\
 \frac{de}{dt} &= -k_1se + (k_{-1} + k_2)c_1 \\
 \frac{dp}{dt} &= k_2c_1 + k_4c_2.
 \end{aligned} \tag{2.3.9}$$

Appropriate initial conditions are $s(0) = s_0; e(0) = e_0; c_1(0) = c_2(0) = p(0) = 0$. Moreover, $e + c_1 + c_2 = e_0$. Since, p can be obtained by quadrature and the total amount of enzyme is conserved, the number of equations needed reduces to three.

$$\begin{aligned}
 \frac{ds}{dt} &= -k_1e_0s + (k_{-1} + k_1s - k_3s)c_1 + (k_1s + k_{-3})c_2 \\
 \frac{dc_1}{dt} &= -k_1e_0s + (k_{-1} + k_2 + k_1s + k_3s)c_1 + (k_4 - k_1s + k_{-3})c_2 \\
 \frac{dc_2}{dt} &= k_3sc_1 - (k_4 + k_{-3})c_2,
 \end{aligned} \tag{2.3.10}$$

A singular perturbation approach is used in [Mur93] to get

$$\frac{dp}{dt} = -\frac{ds}{dt} = k_2c_1 + k_4c_2 = \frac{(k_2K'_m + k_4s)e_0s}{K_mK'_m + k_2s + s^2},$$

where $K_m = \frac{k_{-1} + k_2}{k_1}$ and $K'_m = \frac{k_4 + k_{-3}}{k_3}$ are the Michaelis constants for the reaction scheme of Figure 2.10.

When a cooperative phenomenon is suspected in an enzymatic reaction, a *Hill plot* is often made. The underlying assumption is that

$$\frac{dp}{dt} = \frac{V_{max}s^n}{K_m + s^n},$$

where $n > 0$ is not usually an integer. This is called the *Hill equation* and n is called *Hill coefficient*.

Remark 2.3.1. An integer Hill coefficient may in some cases have a mechanistic explanation and be interpreted as the number of substrate molecules that can bind simultaneously to the enzyme.

Remark 2.3.2. Although the Hill equation may be a reasonable quantitative form to describe a reaction's velocity in a Michaelis-Menten sense the detailed reactions which give rise to it are not too realistic [Son05b] [Mur93]. However, empirical rate forms like the Hill equation are extremely useful in modelling.

2.3.4 Biological systems and feedback controls

In this section, we go through some of the most important aspects regarding feedback controls and biological systems by giving some examples. The information provided here is essentially taken from [Mur93].

Many biological systems, as is the case of the blood coagulation system, have feedback controls built into them and it is fundamental to know how to model them. In rough terms, feedback is when the product of one step in a reaction sequence has an effect on other reaction steps. Three specific examples are those of *autocatalysis*, *activation* and *inhibition*. Autocatalysis is the process whereby a chemical is involved in its own production. For an example and subsequent discussion of the model equations see [Mur93]. For the particular case of the blood coagulation system, we are more interested in the processes of activation and inhibition.

Feedback inhibition

Suppose we have a system of differential equations that has been reduced to two key elements yielding the dimensionless mechanism:

$$\begin{aligned} \frac{du}{dt} &= \frac{k_1}{k_2 + v} - k_3u \\ \frac{dv}{dt} &= k_4u - k_5v, \end{aligned} \tag{2.3.11}$$

where k_1, k_2, k_3, k_4 and k_5 are positive constants. The biological interpretation is

that u activates v , through the term k_4u , and both u and v are degraded linearly proportional to their concentrations; these are the k_3u and k_4v , respectively. This linear degradation is referred to as *first order kinetics removal*. The term $\frac{k_1}{k_2 + v}$ shows a negative feedback by v on the production of u , since an increase in v decreases the production of u .

In [Mur93] it is shown that there is a stable positive stationary point. For the concepts of stability and stationary point see Appendix A.

Two further examples one exhibiting substrate inhibition and the other considering an activator-inhibitor system are also briefly discussed. The competitive inhibition described above with some detail could also have been taken here as an example.

For a generalization of a system of differential equations to describe the kinetics of enzyme amplifier systems based on the presence of a negative feedback loop see [MaMo74].

Remark 2.3.3 (Final comment). We will see in the next chapter that the formalism presented in this chapter cannot be applied 100 % to the models considered in this thesis, in particular for the analysis of stoichiometry. The reason is essentially that the formalism by Aris, Feinberg and others do not include the situation in which one substance is activated by another in a network. This would require a much more general theory. Nevertheless, the information summarized in this chapter helps for instance to understand some of the principal concepts related to the modelling and analysis of complex networks in a simple way. In the next chapter we make an interpretation of our problem using this language and try to get some structural information about the networks we are dealing with.

Chapter 3

Two Mathematical Models for Thrombin Formation

As already mentioned in Chapter 1, the plasma coagulation system is a biochemical chain reaction where inactive proenzymes are converted to active enzymes involving several positive and negative feedbacks. Although the cascade model has provided enormous insights into the general process, it does not satisfactorily explain the dynamic regulation of blood coagulation reaction. In fact, one of the problems encountered in the study of complicated biochemical processes like thrombin generation in plasma is that neither the reaction mechanism nor the reaction constants and initial concentrations are precisely known. Therefore, these quantities are usually taken as unknown parameters in the theoretical model and are estimated by fitting experimental data. In the literature there are several mathematical models for approaching a part of the blood coagulation mechanism. The models normally comprise stiff systems of non-linear differential equations with unknown parameters, namely the reaction constants and/or the initial concentration of the factors.

In this work, we restrict ourselves to the analysis of two of the most cited models among the scientific community investigating the mechanisms of the blood coagulation system. One of the models is due to Stortelder, Hemker and Hemker [SHH97] and the other is due to Jones and Mann [JoMa94]. In this chapter we are mainly concerned with the description of the models and the analysis comprises at the first stoichiometric aspects of the networks and secondly the numerical integration. Noteworthy is that, the model from [JoMa94] had to be corrected before because the set of differential equations does not match the reaction scheme given.

The qualitative mathematical analysis including kinetic and dynamical aspects is performed in Chapter 4.

3.1 The model by Stortelder, Hemker and Hemker

The model proposed in [SHH97] concerns the common pathway; factor X was activated by a purified enzyme RVV , factor V and prothrombin activation in the presence of phospholipids is considered and also includes the inactivation of thrombin by $ATIII$ and α_2 -macroglobulin.

The reaction scheme for thrombin formation postulated by Stortelder et al. is represented in Figure 3.1:

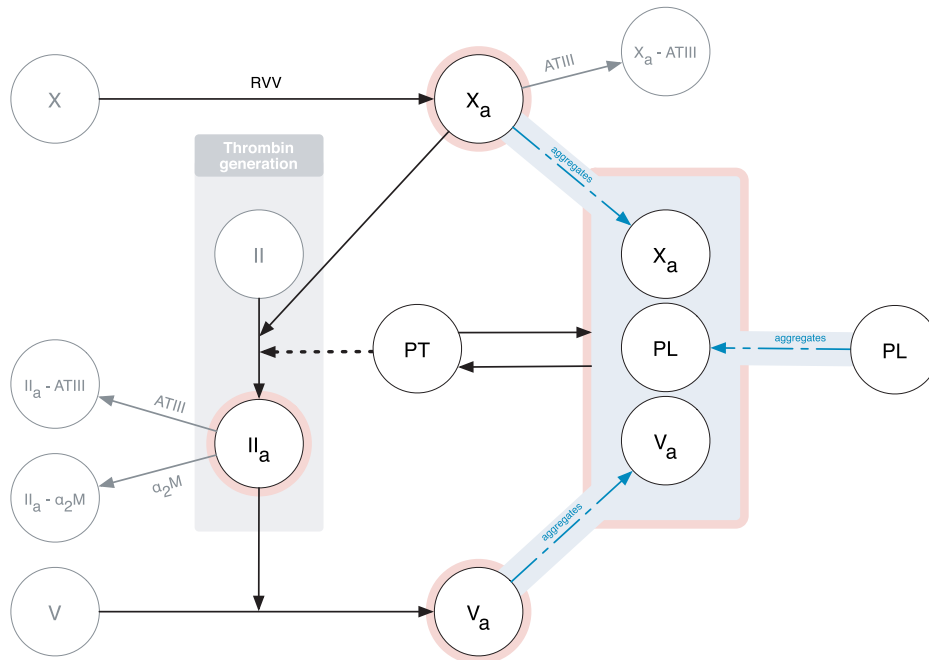


Figure 3.1: Reaction scheme for thrombin formation by Stortelder et. al.

The coagulation factors are denoted by Roman numerals, the subscript a denoting their activated form. The chain of reactions starts with the activation of factor X by the purified enzyme from Russel's Viper Venom (RVV), kept constant in each

experiment performed, followed by the activation of factor V , the production of prothrombinase in the presence of phospholipids and the activation of prothrombin. The inactivation of factor X_a by $ATIII$ and of thrombin by $ATIII$ and α_2 -macroglobulin (α_2M) are also taken into account.

Based on the existing biochemical knowledge, in particular using the Michaelis and Menten equation, the reaction scheme was transformed into a set of 9 non-linear differential equations with unknown parameters, namely the reaction constants and the initial concentrations of the reactants.

$$\begin{aligned}
\frac{d[X]}{dt} &= -\frac{kcat_X[X][RVV]}{km_X + [X]} \\
\frac{d[X_a]}{dt} &= \frac{kcat_X[X][RVV]}{km_X + [X]} - ki_{X_a}[X_a] - k_{PT}[V_a][X_a][PL] + k_{PL}[PT] \\
\frac{d[V]}{dt} &= \frac{kcat_V[V][II_a]}{km_V + [V]} \\
\frac{d[V_a]}{dt} &= \frac{kcat_V[V][II_a]}{km_V + [V]} - k_{PT}[V_a][X_a][PL] + k_{PL}[PT] \\
\frac{d[PL]}{dt} &= -k_{PT}[V_a][X_a][PL] + k_{PL}[PT] \\
\frac{d[PT]}{dt} &= k_{PT}[V_a][X_a][PL] - k_{PL}[PT] \\
\frac{d[II]}{dt} &= -\frac{kcat_{II}[II][PT]}{km_{II} + [II]} - \frac{kcat_2[II][X_a]}{km_2 + [II]} \\
\frac{d[II_a]}{dt} &= \frac{kcat_{II}[II][PT]}{km_{II} + [II]} + \frac{kcat_2[II][X_a]}{km_2 + [II]} - ki_{II_a\alpha_2M}[II_a] - ki_{II_aATIII}[II_a] \\
\frac{d[II_a\alpha_2M]}{dt} &= ki_{II_a\alpha_2M}[II_a].
\end{aligned} \tag{3.1.1}$$

The noncatalytic reactions were modelled by using the law of mass action and inhibition was characterized as being of first order kinetics. Notice that the total mass is not constant and that the system is not balanced.

Although the reaction scheme was not explicitly published in the usual way, we infer from (3.1.1) that following reactions occur:

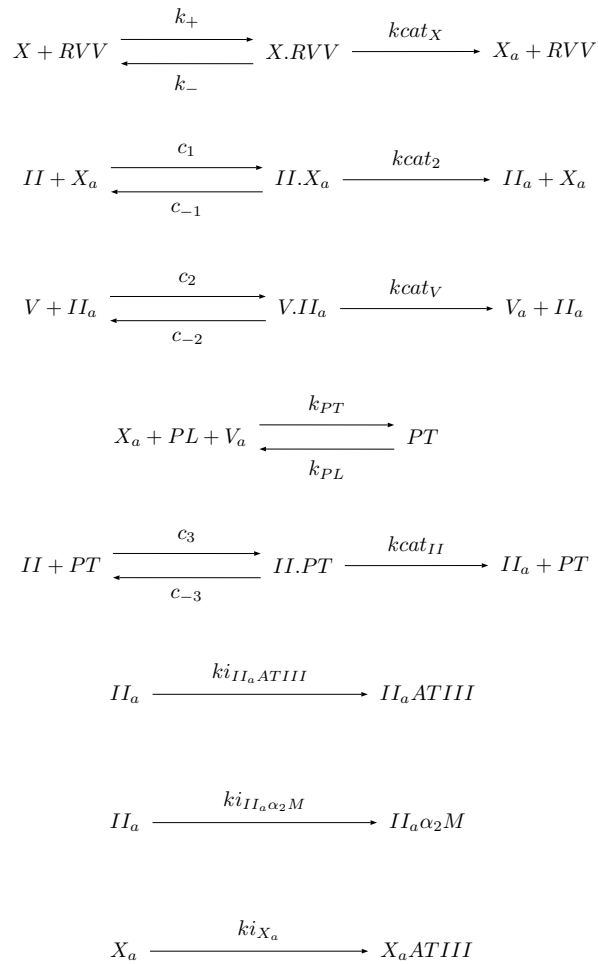


Figure 3.2: Reaction scheme corresponding to (3.1.1).

In addition, the course of the concentration of the amidolytic activity of the thrombin was also modelled and the corresponding equation was:

$$AmAct = [II_a] + 0.556[II_a\alpha_2M]. \quad (3.1.2)$$

Estimates for the 13 reaction constants were obtained by fitting the model to experimental data:

$kcat_X$	km_X	ki_{X_a}	k_{PT}	k_{PL}	$kcat_V$		
239.1	23.65	4.531	122.9	801.4	7.844		
km_V	$kcat_{II}$	km_{II}	$kcat_2$	km_2	$ki_{II_a\alpha_2M}$	ki_{II_aATIII}	
149.7	43.87	62.25	12.4	0.06148	0.1762	0.7859	

Table 3.1: Dimensionless constants estimated by Stortelder et al. after fitting the model to experimental data.

3.1.1 Stoichiometric analysis

Although this analysis should proceed that of the kinetics, the model proposed by Stortelder et al. does not satisfy the law of mass action and the concepts introduced in Chapter 2 do not apply. Furthermore, the system includes first order reactions and it is neither reversible nor weakly reversible. Consequently, a stoichiometric analysis like the one made by Sontag in [Son01] is also not possible.

3.1.2 Numerical integration results and remarks

Since the last equation of (3.1.1) is a simple integration, it was at first not considered. The remaining stiff system of eight equations was then solved numerically for $t \in [0, 30]$ minutes using the ODE solver from SCILAB (see Appendix C for some technical details), allowing implicit integration of the system. We set $RVV=0.03$ and considered the following set of initial values:

$$X(0) = 0.2, X_a(0) = 0, V(0) = 0.03, V_a(0) = 0, PL(0) = 0.05, PT(0) = 0, \\ II(0) = 1.4, II_a(0) = 0.$$

These values correspond to the physiological concentrations of the different factors in blood in $\mu mol/L$ and the substances to be activated have initial concentration equal to zero.

The algorithm converged without major problems. We present the graphic corresponding to the course of thrombin concentration versus the course of prothrombin concentration with time in Figure 3.3 and the one corresponding to the course of the concentration of the amidolytic activity of the thrombin modelled by (3.1.2) in Figure 3.4.

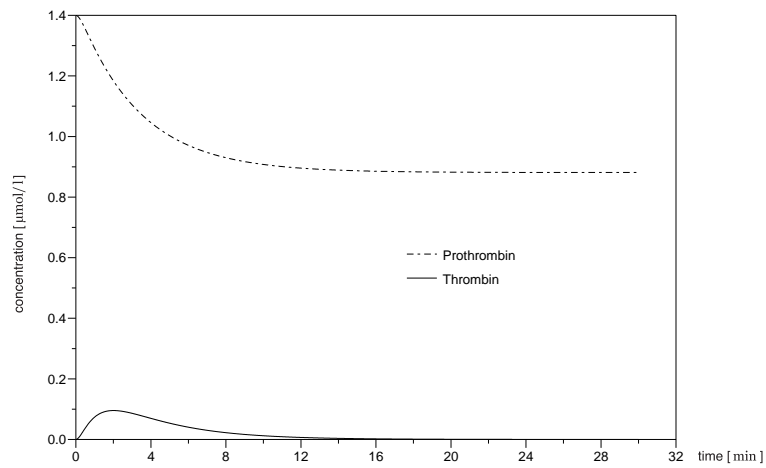


Figure 3.3: Thrombin versus prothrombin concentration.

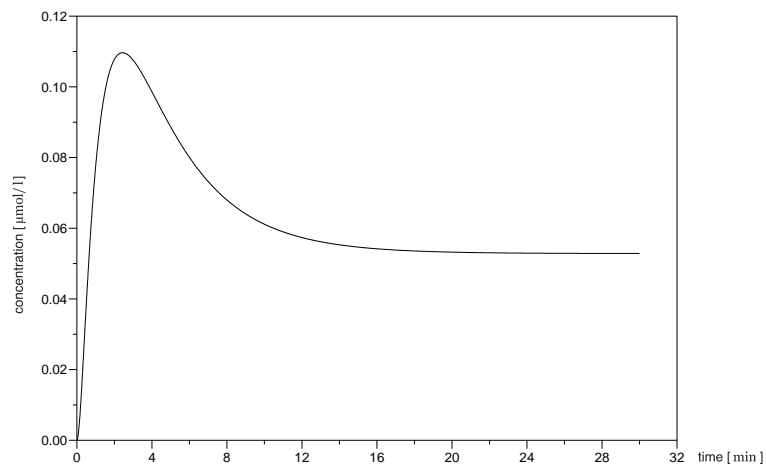


Figure 3.4: Course of the amidolytic activity of thrombin.

The SCILAB code and the remaining graphics can be found in Appendix D¹.

From the graphics, we are finally able to draw some conclusions and make some remarks.

Factor X is not completely activated, but the amount of factor X_a is enough to start the production of thrombin. This influence is modelled in the reaction term

$$r_\tau = \frac{k_{cat2}[II][X_a]}{k_{m2} + [II]},$$

present in equations 7 and 8 of (3.1.1). Therefore, as Stortelder et al. stated, this reaction cannot be neglected specially at the beginning, where a small percentage of prothrombin is transformed into thrombin. This little amount of thrombin is enough to activate factor V to build the prothrombinase complex, which is expected by the physiologists to be the major activator of prothrombin making the influence of factor X_a insignificant after an initial period of time. As this fact is not reflected in the model, Stortelder and Hemker [SHH97] pointed out that both factor X_a and PT complex contribute similarly in percentage for thrombin generation, not excluding however that this might only be the case where RVV is taken as factor X activator.

Prothrombinase is a Ca^{2+} -dependent, 1:1, enzymatic complex of factor X_a and factor V_a that assemble in a reversible association on the surface of negatively charged phospholipid vesicles or platelets and the course of prothrombinase concentration with time was modelled by using a third order term also appearing in equations 2, 4 and 5 of (3.1.1). This term seems to be responsible for the concentration of PT to be almost constant except perhaps at the beginning. Apparently, it varies much more rapidly than the concentrations of the remaining factors yielding some numerical uncertainties also reflected in the graphical representation, see Figures D.5 and D.6. In Section 7.1 we analyze this term more carefully and make an analogy with the work done by Nesheim and co-workers in [NeTrMa84].

In fact, only about 35 % of the whole amount of prothrombin is used to form thrombin, which is simultaneously inactivated by the action of $ATIII$ and of α_2 -macroglobulin, keeping the whole amount of thrombin at a concentration level less or equal to $0.1 \mu mol/L$. If the system is controllable, one may think of influencing the system with an external control and obtain a desired amount of thrombin in a given time interval. In Chapter 5 we prove that the linearized system is not completely controllable, but we will be able to find a controllable subspace.

Although being very compact, the model of Stortelder et al. meets in several points the established knowledge in the field and constitutes a good starting point for ongoing research. So, later in Chapter 7 we propose a new approach for modelling

¹All the concentrations are in $\mu mol/L$ and the time is given in minutes

the common pathway of the blood coagulation system based on a small modification of this model, giving special relevance to the action of thrombocytes in the process of thrombin formation and their influence in the amount of thrombin formed. In addition, we substitute RVV by introducing plasmatic components of the clotting system that do not play a role in the truncated mechanism studied by Stortelder and Hemker and extend the model. As starter we use the complex $TF.VII_a$

But, first we analyze mathematical aspects like stability and controllability of the system (3.1.1). This is done in Chapters 4 and 5.

In the sequel we show by representing the numerical solution graphically that by changing the value of some constants we may influence the values of the concentration at the equilibrium. This will provide some hints that are useful for analysing controllability aspects.

The influence of changing the values of $k_{iII_a\alpha_2M}$ and k_{iII_aATIII} on the course of thrombin concentration with time

In the next figure we illustrate the influence of changing the values of the constants k_{iII_aATIII} on the course of thrombin concentration with time. It is easy to see that increasing the value of $k_{12} = k_{iII_aATIII}$ results in a decrease in concentration.

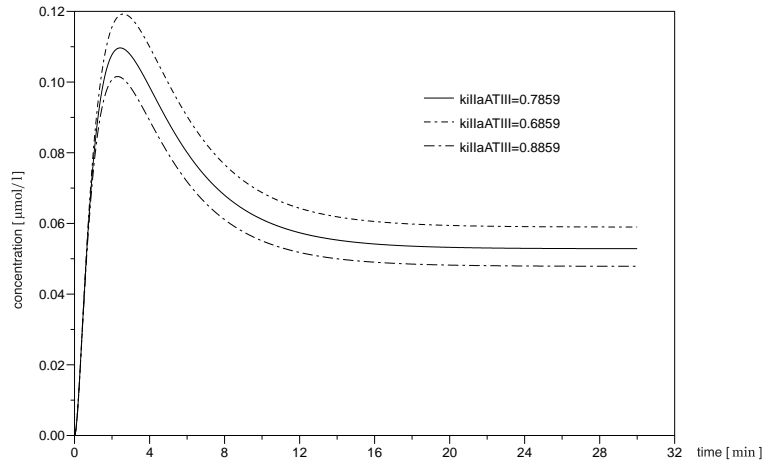


Figure 3.5: Influence of changing the value of k_{iII_aATIII} .

In Figure 3.6 we illustrate the influence of changing the values of the constants

$k_{iII_a\alpha_2M}$ on the course of thrombin concentration with time. This time we observe that the maximal concentration does not change, but the value of the concentration at the equilibrium decreases with decreasing values of $k_{13} = k_{iII_a\alpha_2M}$.

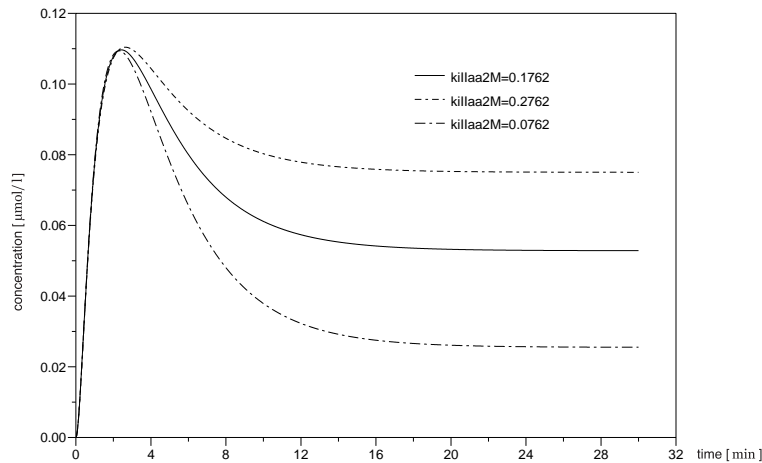


Figure 3.6: Influence of changing the value of $k_{iII_a\alpha_2M}$.

Notice that the initial lag characteristic of thrombin generation curves is almost not perceptible.

3.2 The model by Mann and Jones

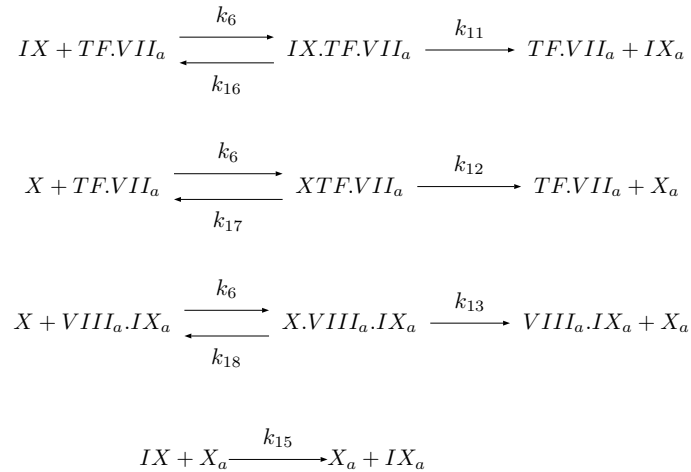
The model proposed in [JoMa94] describes the tissue factor pathway, also extrinsic pathway, to thrombin and it involves the activation of the factors *IX*, *X* and *VIII*. Here, all protein-lipid binding reactions were saturated. Hence, lipid binding equilibria are not included as reactions steps and therefore there was no need to include a factor for the concentration of lipid, in contrast with the model proposed in [SHH97]. Moreover, Mann and Jones claim that their model is responsive to alterations in the concentrations of factors *VIII* and *V*, as well as in their activated forms *VIII_a* and *V_a*. Thus, the effect of thrombin generation in the presence of disease can be studied.

The model comprises however 19 differential equations, which means an increase in complexity by comparison with the model presented in [SHH97], making a straight forward interpretation of the results more difficult. Furthermore, by the process of developing the model there are some constructive and qualitative aspects

deserving our special attention. In particular, the authors derive a set of differential equations using the law of mass action and it turns out that it does not correspond to the reaction scheme published in the same paper. Moreover, since we are in the presence of enzyme catalyzed reactions, the Michaelis-Menten approach should preferably be applied. So, there is no motivation to use the law of mass action instead.

In this section we start by making a stoichiometric analysis for the reaction scheme published in [JoMa94] (see also Figure 3.7). Analogously as we did in the previous section for the model of Stortelder and Hemker, and to gain more insights we also tried to calculate the numerical solution of the set of equations published by Mann and Jones in [JoMa94] (see also (3.2.1)). However, the addition of the term $-|I - [VIII_a IX_a]| + (I - [VIII_a IX_a])$ seems to be made *a posteriori* and the set of equations does not match the reaction scheme. So, this model asks for some correction and this will be done in Section 3.2.2. First, like the authors did, we derive the set of equations using the law of mass action and make the qualitative analysis in Chapter 4. Other kinetic aspects will be discussed in Chapter 4 and in Chapter 6.

The reaction scheme published in [JoMa94] including the relevant reactions in coagulation that are consistent with the experimental results presented by Lawson et. al. in [LKSM94] is given by:



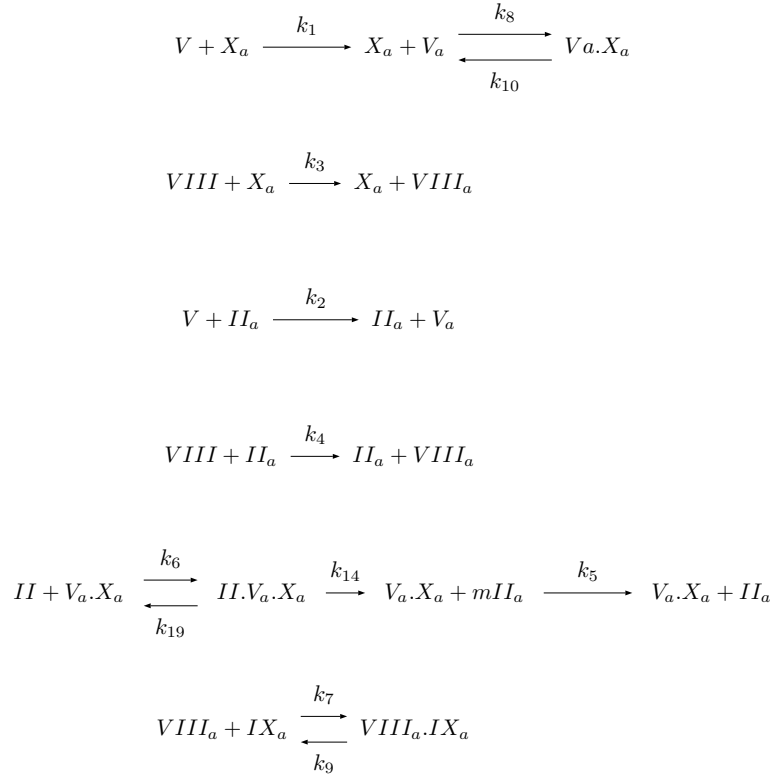


Figure 3.7: Reaction scheme from [JoMa94], where each complex is represented only once.

By applying the law of mass action, the corresponding set of differential equations governing the system was set to be as follows.

$$\begin{aligned}
\frac{d[TFVII_a]}{dt} &= k_{11}[TFVII_a IX] - k_6[TFVII_a][IX] + k_{16}[TFVII_a IX] + \\
&\quad + k_{12}[TFVII_a X] - k_6[TFVII_a][X] + k_{17}[TFVII_a X] \\
\frac{d[IX]}{dt} &= k_{16}[TFVII_a IX] - k_6[TFVII_a][IX] - \\
&\quad - k_{15}[IX][X_a] - k_{15}[IX][V_a X_a] \\
\frac{d[X]}{dt} &= k_{17}[TFVII_a X] - k_6[X][TFVII_a] - k_6[X][VIII_a IX_a] + \\
&\quad + k_{18}[VIII_a IX_a X]
\end{aligned}$$

$$\begin{aligned}
\frac{d[V]}{dt} &= -k_1[V][X_a] - k_2[V][II_a] - k_2[V][mII_a] \\
\frac{d[VIII]}{dt} &= -k_3[VIII][X_a] - k_4[VIII][II_a] - k_4[VIII][mII_a] \\
\frac{d[II]}{dt} &= k_{19}[V_a X_a II] - k_6[II][V_a X_a] \\
\frac{d[VIII_a IX_a]}{dt} &= k_7[VIII_a][IX_a] - k_9[VIII_a IX_a] - k_6[VIII_a IX_a][X] + \\
&\quad + k_{18}[VIII_a IX_a X] + k_{13}[VIII_a IX_a X] - \\
&\quad - |I - [VIII_a IX_a]| + (I - [VIII_a IX_a]) \quad !! \\
\frac{d[V_a X_a]}{dt} &= k_8[X_a][V_a] - 2k_{10}[V_a X_a] + k_{19}[V_a X_a II] - k_6[V_a X_a][II] + \\
&\quad + k_{14}[V_a X_a II] \\
\frac{d[II_a]}{dt} &= k_5[V_a X_a][mII_a] \\
\frac{d[V_a X_a II]}{dt} &= k_6[V_a X_a][II] - k_{19}[V_a X_a II] - k_{14}[V_a X_a II] \\
\frac{d[mII_a]}{dt} &= k_{14}[V_a X_a II] - k_5[V_a X_a][mII_a] \\
\frac{d[TFVII_a IX]}{dt} &= k_6[TFVII_a][IX] - k_{16}[TFVII_a IX] - k_{11}[TFVII_a IX] \\
\frac{d[TFVII_a X]}{dt} &= k_6[TFVII_a][X] - k_{17}[TFVII_a X] - k_{12}[TFVII_a X] \\
\frac{d[VIII_a IX_a X]}{dt} &= k_6[VIII_a IX_a][X] - k_{18}[VIII_a IX_a X] - k_{13}[VIII_a IX_a X] \\
\frac{d[IX_a]}{dt} &= k_9[VIII_a IX_a] - k_7[VIII_a][IX_a] + k_{11}[TFVII_a IX] + \\
&\quad + k_{15}[IX][X_a] + k_{15}[IX][V_a X_a] \\
\frac{d[X_a]}{dt} &= k_{10}[V_a X_a] - k_6[X_a][V_a] + k_{12}[TFVII_a X] + k_{13}[VIII_a IX_a X] \\
\frac{d[V_a]}{dt} &= k_{10}[V_a X_a] - k_6[X_a][V_a] + k_1 x_4[X_a] + k_2[V][II_a] + k_2[V][mII_a] \\
\frac{d[VIII_a]}{dt} &= k_9[VIII_a IX_a] - k_7[VIII_a][IX_a] + k_3[VIII][X_a] + \\
&\quad + k_4[VIII][II_a] + k_4[VIII][mII_a] \\
\frac{dI}{dt} &= (-|I - [VIII_a IX_a]| + (I - [VIII_a IX_a]))k_{20} \quad !!
\end{aligned} \tag{3.2.1}$$

The mathematical model was developed in [JoMa94] by following three steps:

- (i) identification of the enzymatic reactions that are integral part to the complete coagulation cascade.

- (ii) empirical restricted approximations for the rate constants.
- (iii) adjustment of the rate constants in such a way that the theoretical model simulates the results derived from laboratory experiments.

The value of the rate constants used to model the activation of thrombin were based upon published rate constants determined in earlier work and they are listed in Table 3.2.

Constant	Value	Description
k_1	$2 \times 10^7 M^{-1} s^{-1}$	Activation of V by X_a
k_2	$2 \times 10^7 M^{-1} s^{-1}$	Activation of V by II_a
k_3	$1 \times 10^7 M^{-1} s^{-1}$	Activation of $VIII$ by X_a
k_4	$2 \times 10^7 M^{-1} s^{-1}$	Activation of $VIII$ by II_a
k_5	$1 \times 10^7 M^{-1} s^{-1}$	Conversion of mII_a to II_a by $V_a X_a$
k_6	$1 \times 10^8 M^{-1} s^{-1}$	On-rate for rapidly formed complexes
k_7	$1 \times 10^7 M^{-1} s^{-1}$	On-rate for $VIII_a IX_a$ complex
k_8	$4 \times 10^8 M^{-1} s^{-1}$	On-rate for $V_a X_a$ complex
k_9	$0.005 s^{-1}$	Off-rate for $VIII_a IX_a$ complex
k_{10}	$0.4 s^{-1}$	Off-rate for $V_a X_a$ complex
k_{11}	$0.3 s^{-1}$	V_{max} for activation of IX by $TF.VIII_a$
k_{12}	$1.15 s^{-1}$	V_{max} for activation of X by $TF.VIII_a$
k_{13}	$8.2 s^{-1}$	V_{max} for activation of X by $VIII_a IX_a$
k_{14}	$32 s^{-1}$	V_{max} for mII_a formation by $V_a X_a$
k_{15}	$1 \times 10^5 M^{-1} s^{-1}$	Activation of IX by X_a
k_{16}	$24 s^{-1}$	Off-rate for IX on $TF.VII_a$ complex
k_{17}	$44 s^{-1}$	Off-rate for X on $TF.VII_a$ complex
k_{18}	$0.001 s^{-1}$	Off-rate for X on $VIII_a IX_a$ complex
k_{19}	$70 s^{-1}$	Off-rate for II on $V_a X_a$ complex
k_{20}	$0.02 s^{-1}$	Constant for the slow degradation of $VIII_a IX_a$ complex

Table 3.2: Rate constants, their dimensionalized value and respective description.

For doing parameter identification it is important to say a word about how the constants were obtained and to mention that Jones and Mann did not exclude other sets of rate constants that matched the experimental results because mea-

measurements are sometimes made under different circumstances and different values are obtained.

The values of k_1 and k_2 were first obtained from the kinetics of factor V activation, published in a work of Monkovic and Tracy [MoTr90], and then readjusted to match the empirical results of Lawson et. al in [LKSM94].

The constants k_3 and k_4 were initially approximated by analogy and then refined based on the empirical results in [LKSM94]. Moreover, the estimation suffered another adjustment after fitting the model to experimental data.

Because no scientific papers reporting estimates were available, the value of the constants k_5 and k_{20} were solely identified by fitting the model to experimental data.

The value of k_6 was assumed for all rapidly formed complexes.

The values of k_7, k_8 are known from the literature, as well as the ratios $\frac{k_{10}}{k_8}, \frac{k_9}{k_7}$.

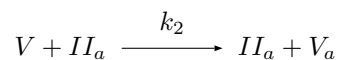
k_{11}, k_{12}, k_{13} and k_{14} were tacked directly from previously published values of the k_{cat} and K_m values for the reactions.

The constant k_{15} was estimated based on the results published in [LaMa91], while k_{16}, k_{17}, k_{18} and k_{19} were calculated from the Michaelis-Menten relationship.

Jones and Mann reported what they classified as encouraging results after different sets for the rate constants were tested and the implications of the changes made were discussed while providing the validity of the model through graphical evidence of numeric solutions obtained from Runge-Kutta methods. Furthermore, they established a reaction profile characterized by:

- a lag or initiation phase;
- a propagation phase;
- a decay of meizothrombin to thrombin observed between 140s and 180s

Before regarding the kinetic aspects inherent to the model (3.2.1) let us first perform the stoichiometric analysis. Noteworthy is that, we are not going to be able to apply the concepts given in Chapter 2 in a straight forward way. One of the reasons is that not all reaction mechanisms of Figure 3.7 have a unambiguous interpretation. For example consider the reaction scheme



It could be interpreted als a catalytic reaction, however the term $k_2[V][II_a]$ appears in the fourth equation with negative sign and in equation 17 with positive sign. So, we infer that the law of mass action was used to model a second order reaction corresponding to the reaction scheme



The set of species involved remains unchanged, though. The same is not true if the authors refer to a first order reaction.

3.2.1 Stoichiometric analysis

According to the notations and definitions given in Chapter 2 lets us make the stoichiometric analysis for this model.

The set of chemical species for the network given in Figure 3.7 is given by:

$$\mathcal{S} = \{IX, TF.VII_a, IX.TF.VII_a, IX_a, X, X.TF.VII_a, X_a, VIII_a.IX_a, X.VIII_a.IX_a, V, V_a, V_a.X_a, VIII, VIII_a, II_a, II, II.V_aII_a, mII_a\}.$$

Thus, $m := \#\mathcal{S} = 18$.

The set of complexes is given by

$$\mathcal{C} := \{IX + TF.VII_a, IX.TF.VII_a, TF.VII_a + IX_a, X + TF.VII_a, X.TF.VII_a, TF.VII_a + X_a, X + VIII_a.IX_a, X.VIII_a.IX_a, VIII_a.IX_a + X_a, IX + X_a, X_a + IX_a, V + X_a, X_a + V_a, V_a.X_a, VIII + X_a, X_a + VIII_a, V + II_a, II_a + V_a, VIII + II_a, II_a + VIII_a, II + V_a.X_a, II.V_a.X_a, V_a.X_a + mII_a, V_a.X_a + II_a, VIII_a + IX_a, VIII_a.IX_a\}$$

and $n := \#\mathcal{C} = 26$.

Considering the whole reaction system as a graph, whose nodes are the complexes and the edges labeled by the reaction rate constants we associate with this graph its 26×26 incidence matrix $A = a_{l,m}$, listing all the edge labels (for instance, $a_{21} = k_1$, to indicate a reaction with the rate constant k_1 , from the first node to the second node). In this case, the matrix has the representation (3.2.2).

Notice that this matrix is reducible. As a consequence, the graph is not strongly connected. This means that, given any two complexes there is not necessarily a path linking them. Furthermore, the reaction network is neither reversible nor weakly reversible.

By using the notation of Definition 2.1.5, let the i -th element of \mathcal{C} by the order of appearance be designated by \mathbf{p}_i , $i = 1, \dots, 26$. Then, the set \mathcal{R} of reactions is given by:

$$\begin{aligned} \mathcal{R} = \{ & \mathbf{p}_1 \rightarrow \mathbf{p}_2, \mathbf{p}_2 \rightarrow \mathbf{p}_1, \mathbf{p}_2 \rightarrow \mathbf{p}_3, \mathbf{p}_4 \rightarrow \mathbf{p}_5, \mathbf{p}_5 \rightarrow \mathbf{p}_4, \mathbf{p}_5 \rightarrow \mathbf{p}_6, \mathbf{p}_7 \rightarrow \mathbf{p}_8, \\ & \mathbf{p}_8 \rightarrow \mathbf{p}_7, \mathbf{p}_8 \rightarrow \mathbf{p}_9, \mathbf{p}_{10} \rightarrow \mathbf{p}_{11}, \mathbf{p}_{12} \rightarrow \mathbf{p}_{13}, \mathbf{p}_{13} \rightarrow \mathbf{p}_{14}, \mathbf{p}_{14} \rightarrow \mathbf{p}_{13}, \\ & \mathbf{p}_{15} \rightarrow \mathbf{p}_{16}, \mathbf{p}_{17} \rightarrow \mathbf{p}_{18}, \mathbf{p}_{19} \rightarrow \mathbf{p}_{20}, \mathbf{p}_{21} \rightarrow \mathbf{p}_{22}, \mathbf{p}_{22} \rightarrow \mathbf{p}_{21}, \mathbf{p}_{22} \rightarrow \mathbf{p}_{23}, \\ & \mathbf{p}_{23} \rightarrow \mathbf{p}_{24}, \mathbf{p}_{25} \rightarrow \mathbf{p}_{26}, \mathbf{p}_{26} \rightarrow \mathbf{p}_{25} \}. \end{aligned}$$

Thus, $r := \#\mathcal{R} = 22$.

Hence, by Remark 2.1.7, each reaction can be written as

$$R_j \equiv \sum_{i=1}^{26} \nu_{ij} \mathbf{p}_i, \quad j = 1, \dots, 22,$$

where $\nu = (\nu_{ij}) \in \mathbb{R}^{(26,22)}$ is the stoichiometric matrix.

Our first aim is now to calculate the dimension of the stoichiometric subspace. So, it is sufficient to determine how many linearly independent reactions are in the network.

To calculate the coordinates of the reaction vectors we make use of the definition of the support of a complex (see Section 2.1.1). For the sake of simplicity, let us first denote each element of \mathcal{S} respectively by S_k , $k = 1, \dots, 18$. Then, we construct a $(26, 18)$ -matrix with entries 0 or 1. Thus, entry $(i, k) = 1$ means that

$$S_k \in \text{supp } \mathbf{p}_i, \quad i = 1, \dots, 26, k = 1, \dots, 18.$$

The matrix has the following representation:

$$\begin{pmatrix} 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}. \quad (3.2.3)$$

Each line defines a vector $\mathbf{b}_i, i = 1, \dots, 26$ for each complex. Then, the reaction vectors are

$$\begin{aligned} \mathbf{s}_1 &:= \mathbf{b}_2 - \mathbf{b}_1 = (-1, -1, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)^T; \\ \mathbf{s}_2 &:= \mathbf{b}_1 - \mathbf{b}_2 = (1, 1, -1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)^T; \\ \mathbf{s}_3 &:= \mathbf{b}_3 - \mathbf{b}_2 = (0, 1, -1, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)^T; \\ \mathbf{s}_4 &:= \mathbf{b}_5 - \mathbf{b}_4 = (0, -1, 0, 0, -1, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)^T; \\ \mathbf{s}_5 &:= \mathbf{b}_4 - \mathbf{b}_5 = (0, 1, 0, 0, 1, -1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)^T; \\ \mathbf{s}_6 &:= \mathbf{b}_6 - \mathbf{b}_5 = (0, 1, 0, 0, 0, -1, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)^T; \\ \mathbf{s}_7 &:= \mathbf{b}_8 - \mathbf{b}_7 = (0, 0, 0, 0, -1, 0, 0, -1, 1, 0, 0, 0, 0, 0, 0, 0, 0)^T; \\ \mathbf{s}_8 &:= \mathbf{b}_7 - \mathbf{b}_8 = (0, 0, 0, 0, 1, 0, 0, 1, -1, 0, 0, 0, 0, 0, 0, 0, 0)^T; \end{aligned}$$

$$\begin{aligned}
\mathbf{s}_9 &:= \mathbf{b}_9 - \mathbf{b}_8 = (0, 0, 0, 0, 0, 0, 1, 1, -1, 0, 0, 0, 0, 0, 0, 0, 0, 0)^T; \\
\mathbf{s}_{10} &:= \mathbf{b}_{11} - \mathbf{b}_{10} = (-1, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)^T; \\
\mathbf{s}_{11} &:= \mathbf{b}_{13} - \mathbf{b}_{12} = (0, 0, 0, 0, 0, 0, 0, 0, 0, -1, 1, 0, 0, 0, 0, 0, 0, 0)^T; \\
\mathbf{s}_{12} &:= \mathbf{b}_{14} - \mathbf{b}_{13} = (0, 0, 0, 0, 0, 0, -1, 0, 0, 0, -1, 1, 0, 0, 0, 0, 0, 0)^T; \\
\mathbf{s}_{13} &:= \mathbf{b}_{13} - \mathbf{b}_{14} = (0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 1, -1, 0, 0, 0, 0, 0, 0)^T; \\
\mathbf{s}_{14} &:= \mathbf{b}_{16} - \mathbf{b}_{15} = (0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, -1, 1, 0, 0, 0, 0, 0)^T; \\
\mathbf{s}_{15} &:= \mathbf{b}_{18} - \mathbf{b}_{17} = (0, 0, 0, 0, 0, 0, 0, 0, 0, -1, 1, 0, 0, 0, 0, 0, 0, 0)^T; \\
\mathbf{s}_{16} &:= \mathbf{b}_{20} - \mathbf{b}_{19} = (0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, -1, 1, 0, 0, 0, 0, 0)^T; \\
\mathbf{s}_{17} &:= \mathbf{b}_{22} - \mathbf{b}_{21} = (0, 0, 0, 0, 0, 0, 0, 0, 0, 0, -1, 0, 0, 0, -1, 1, 0, 0)^T; \\
\mathbf{s}_{18} &:= \mathbf{b}_{21} - \mathbf{b}_{22} = (0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 1, -1, 0, 0)^T; \\
\mathbf{s}_{19} &:= \mathbf{b}_{23} - \mathbf{b}_{22} = (0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, -1, 1, 0)^T; \\
\mathbf{s}_{20} &:= \mathbf{b}_{24} - \mathbf{b}_{23} = (0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0, -1)^T; \\
\mathbf{s}_{21} &:= \mathbf{b}_{26} - \mathbf{b}_{25} = (0, 0, 0, -1, 0, 0, 0, 1, 0, 0, 0, 0, 0, -1, 0, 0, 0, 0)^T; \\
\mathbf{s}_{22} &:= \mathbf{b}_{25} - \mathbf{b}_{26} = (0, 0, 0, 1, 0, 0, 0, -1, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0)^T.
\end{aligned}$$

Thus, the stoichiometric subspace is the submanifold

$$\mathcal{D} := \text{span}\{\mathbf{s}_1, \dots, \mathbf{s}_{22}\}.$$

Since there are 12 linearly independent vectors, $\dim S = 12$. We may say,

$$\mathcal{D} := \langle \mathbf{s}_1, \mathbf{s}_3, \mathbf{s}_4, \mathbf{s}_6, \mathbf{s}_7, \mathbf{s}_{11}, \mathbf{s}_{12}, \mathbf{s}_{14}, \mathbf{s}_{17}, \mathbf{s}_{19}, \mathbf{s}_{20}, \mathbf{s}_{22} \rangle.$$

This submanifold can also be described as the set of solutions of:

$$\begin{aligned}
S_1 + S_3 &= S_2 + S_3 = S_3 + S_4 = S_2 + S_6 = S_5 + S_6 = S_6 + S_7 = S_5 + S_9 = \\
S_8 + S_9 &= S_{10} + S_{11} = S_7 + S_{11} = S_{11} + S_{12} = S_{13} + S_{14} = S_{12} + S_{17} = S_{16} + S_{17} = \\
S_{17} + S_{18} &= S_{15} + S_{18} = S_4 + S_8 = S_{14} + S_8 = 0.
\end{aligned}$$

Moreover, by Remark 2.2.15 and Definition 2.2.16, each intersection between a parallel translate of \mathcal{D} and the positive orthant is a stoichiometric compatibility class.

So, each stoichiometric class is given by the nonnegative points in

$$\begin{aligned}
\{S_1 + S_3 = c_1; S_2 + S_3 = c_2; S_3 + S_4 = c_3; S_2 + S_6 = c_4; S_5 + S_6 = c_5; S_6 + S_7 = \\
c_6; S_5 + S_9 = c_7; S_8 + S_9 = c_8; S_{10} + S_{11} = c_9; S_7 + S_{11} = c_{10}; S_{11} + S_{12} = \\
c_{11}; S_{13} + S_{14} = c_{12}; S_{12} + S_{17} = c_{13}; S_{16} + S_{17} = c_{14}; S_{17} + S_{18} = c_{15}; S_{15} + S_{18} =
\end{aligned}$$

$$c_{16}; S_4 + S_8 = c_{17}; S_{14} + S_8 = c_{18}\},$$

where $c_i, i = 1, \dots, 18$ are nonnegative constants.

Furthermore, the rank of the network is as well 12 and the network has deficiency $\delta = 26 - 10 - 12 = 4$.

However, since the network is not weakly reversible, by using the theorems given previously we cannot conclude the existence and uniqueness of an equilibrium point in a stoichiometric class. The same can be said regarding the asymptotic stability with respect to initial conditions.

Remark 3.2.1. We can associate to the matrix (3.2.3) the following graph:

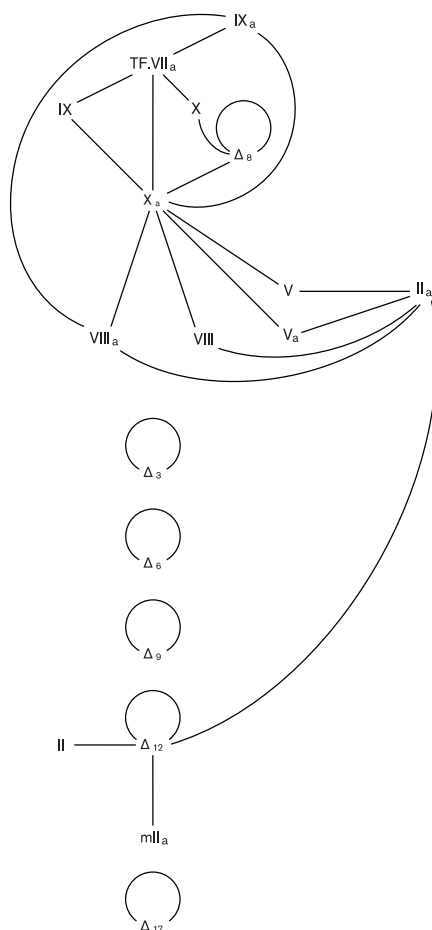


Figure 3.8: Graph corresponding to the matrix (3.2.3).

Each node is denoted with the respective chemical specie. An edge connecting two different species represents a complex of the network. The number of simple loops is equal to the number of intermediate complexes formed, in the figure they are denoted by Δ_i , $i = 3, 6, 9, 8, 12, 17$. We observe that the graph is not strongly connected, but if we eliminate $\Delta_3, \Delta_6, \Delta_9$ and Δ_{17} we obtain a connected graph. In Chapter 4 we will identify exactly 6 linear first integrals and see that this elimination is possible.

The previous calculation can also be interpreted in terms of homologies of graphs. We summarize the principal aspects in the following remark, where the nomenclature is adapted from [Zer00].

Remark 3.2.2. Let $\Gamma = (V, E, \varphi)$ be a directed finite graph with V representing the set of vertices, E representing the set of edges and $\varphi : E \rightarrow V \times V, e \rightarrow \varphi(e) = (\varphi_1(e), \varphi_2(e))$ is the incidence map. Suppose that $\#V = p$ and that $\#E = q$. If $\varphi(e) = (v_1, v_2)$, then v_1 is called the *initial vertex* and v_2 the *terminal vertex* of e .

In our case $V = \mathcal{C}$ and $E = \mathcal{R}$. Then $p = 26$ and $q = 22$.

Let R be a ring with unity.

We define a function $f : V \rightarrow R$ as the *vertex weight function*.

Let the function $g : E \rightarrow \mathbb{R}$ be the *edge weight function*, which in our case assigns to the reaction $R_l, l = 1, \dots, 22$ its rate constant.

We define furthermore the *boundary operator* $\partial : R^E \rightarrow R^V; i \rightarrow \partial(i)$, where R is a ring. This operator assigns a vertex weight function to each edge weight function $i : E \rightarrow R$ as follows:

$$\partial(i)(v) = \sum_{e \in E(v, -)} i(e) - \sum_{e \in E(-, v)} i(e),$$

where $E(v, -) = \varphi_1^{-1} = \{e \in E, \varphi_1(e) = v\}$ is the set of all edges with initial vertex v and $E(-, v) = \varphi_2^{-1} = \{e \in E, \varphi_2(e) = v\}$ is the set of all edges with terminal vertex v .

In our case, the matrix representation of ∂ is the stoichiometric matrix $\nu \in \mathbb{Z}^{p \times q}$ where

$$\nu_{ij} = \begin{cases} 1 & \text{if } \varphi_1(e_j) = v_i \neq \varphi_2(e_j) \\ -1 & \text{if } \varphi_2(e_j) = v_i \neq \varphi_1(e_j) \\ 0 & \text{otherwise.} \end{cases}$$

Moreover, we have the following exact sequence:

$$\mathbf{0} \rightarrow R^{b_1} \xrightarrow{B} R^q \xrightarrow{\nu} R^p \xrightarrow{C} R^{b_0} \rightarrow \mathbf{0},$$

b_0 is the number of connected components, or the number of linkage classes if we want to use the designation of Feinberg, and b_1 is the number of fundamental loops

or the number of cycles existing in Γ . These numbers are called *Betti numbers*² of the graph.

By definition it holds

$$\text{Im}(B) = \text{Ker}(\nu) \text{ and } \text{Im}(\nu) = \text{Ker}(C).$$

Thus,

$$\text{rank}(\nu) + b_1 = q \text{ and } \text{rank}(\nu) + b_0 = p$$

which implies $q - b_1 = p - b_0$ or $b_1 = q - p + b_0$. In our case $\text{rank}(\nu) = 16$. Thus, $b_0 = 10$ and $b_1 = 6$. So, we have 10 connected components and 6 cycles.

Furthermore, the deficiency of the network can also be defined by the number $\chi = b_0 - b_1$, also known as *Euler-Poincaré characteristic of the graph*.

Let $C = \begin{pmatrix} 1 & \dots & 1 \\ \vdots & & \vdots \\ 1 & \dots & 1 \end{pmatrix} \in \mathbb{Z}^{10 \times 18}$. Since $C\nu = \mathbf{0}$, we may discard 10 lines of the

stoichiometric matrix without losing information. We obtain a matrix $\nu' \in \mathbb{Z}^{12 \times 26}$.

Finally, let $B \in \mathbb{Z}^{26 \times 6}$ be such that

$$B_{ij} = \begin{cases} 1 & \text{if the } j^{\text{th}} \text{ loop contains the edge } e_i \\ -1 & \text{if the } j^{\text{th}} \text{ loop contains the edge } -e_i \\ 0 & \text{otherwise.} \end{cases},$$

where $-e$ is defined for each e to be the edge that is directed from the terminal to the initial vertex of e . It holds $\nu B = \mathbf{0}$. The matrices ν' and B^T can be used to define a transformation that allow us to reduce the dimension of a system, what is very useful in practice, in particular for systems of very high dimension. For a more general theory and an example of application to electrical networks we refer to [Zer00].

Remark 3.2.3. The mass action dynamics can be also summarized by the system

$$\dot{\mathbf{x}} = \sum_{i=1}^n \sum_{j=1}^n a_{ij} x_1^{b_{1j}} x_2^{b_{2j}} \dots x_m^{b_{mj}} (\mathbf{b}_i - \mathbf{b}_j),$$

where x_i represents the concentration of the specie i with $i = 1, \dots, 18, n = 26$.

As already mentioned in Remark 2.2.17, since $\dot{\mathbf{x}} \in \mathcal{D}$, trajectories remain in classes, that is, classes are positive-time invariant manifolds for the dynamical system.

²In algebraic topology, the Betti number is a topologic invariant associated to certain topological spaces, varieties, graphs, etc. E. g. in a topological space the Betti number gives the maximum number of cuts that can be made without dividing the space into two pieces, in a connected graph it represents the maximum number of edges that can be removed without violating the connectivity property of the graph

Remark 3.2.4. Many drugs work by interrupting either flux or metabolite levels to an extent that is harmful to the organism, either because they reduce an important flux to very low levels or because they increase the level of a metabolite to toxic proportions. Conservation constraints can impose hard limits to the extent to which fluxes can be altered. Thus, stoichiometric analysis is an important initial evaluation of whether manipulating a particular target might be effective or not. Besides this practical implication, there is also a theoretical implication concerning the analysis of biochemical control which is dependent on the identification of conserved quantities [SaIn04].

3.2.2 Model correction and numerical integration

As it is given in [JoMa94], SciLab was not able to integrate the system of differential equations (3.2.1). Differentiability of the right-hand side is an important feature to be satisfied, since for implicit integration methods, like the ones included in routines for solving systems of stiff ODE's with SciLab, it is necessary to supply the Jacobian matrix of the right-hand side of the system. Otherwise, the program computes the Jacobian numerically with all disadvantages of a numerical calculation, in particular if the system is not stable. In this case, small changes in the parameters or in the initial data provided another solution or nonconvergence followed.

Remark 3.2.5. Observing the right-hand side more carefully and taking the law of mass action into account and the stoichiometric analysis previously performed, we see that there are terms included in some equations describing reactions that cannot arise from the reaction scheme presented in the same paper. Without excluding the possibility that some of these incorrections may follow from several typographic errors, we point out more specifically that

- (i) the term $k_{15}[IX][V_aX_a]$, appears in the second equation of the system (3.2.1) with a minus and equation 15 with a plus. This means that both factors IX and complex V_aX_a interact in a second order reaction to form factor IX_a and only the concentration of IX changes. Physiologically, this would mean activation of factor IX by the complex V_aX_a , that acts as enzyme and catalyzes the reaction. However, this situation is not contemplated in the reaction scheme published in [JoMa94].
- (ii) Similarly, the terms $k_2[V][mII_a]$ and $k_4[VIII][mII_a]$ appear respectively in equations 4 and 5 of the system (3.2.1) with a minus sign and, with a plus sign in equations 17 and 18, respectively. The kind of reaction is the same as the one described in item (i). Physiologically, this should model the activation of factors V and $VIII$ by mII_a .
- (iii) In equation number 8 of (3.2.1), the stoichiometric coefficient of $[V_aX_a]$ is

equal to 2, but the corresponding reaction scheme only includes one molecule of the factor represented by the variable $[V_a X_a]$ (see Figure 3.9).

(iv) In equations 16 and 17, the reaction constant k_6 should be replaced by k_8 .

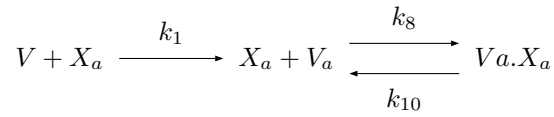


Figure 3.9: Reaction scheme number 5 in [JoMa94].

The last remarks, together with the stoichiometric analysis, ask for a correction of the model. There are two possibilities, either to assume that the set of equations is correct and the reaction scheme from Figure 3.7 must be completed or to assume that the reaction scheme is valid and settle the new system of equations. In physiological context, picking up the first alternative means that factor IX can also be activated by the action of the complex $V_a X_a$ with rate k_{15} and that the meizothrombin (factor mII_a) also activates factor V and factor $VIII$. So, using the same notation as the authors, the following reactions would occur:

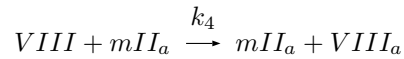
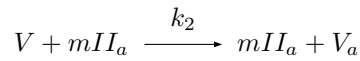
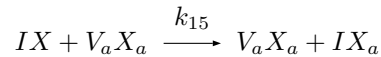


Figure 3.10: Reaction scheme to complete the one of Figure 3.7 in terms of the coagulating factors.

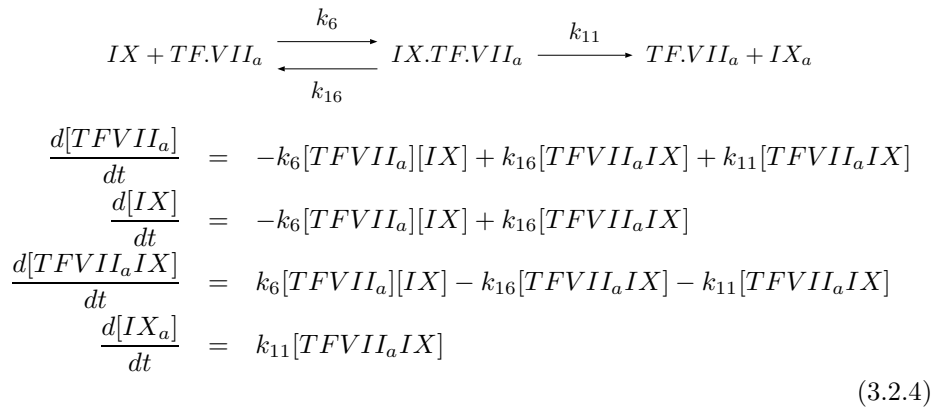
As a matter of fact, there is no guarantee that these three reactions do really happen. Furthermore, in the actual medical literature (see Chapter 1, Figure 1.1 or [Pru00]) these reactions are not included.

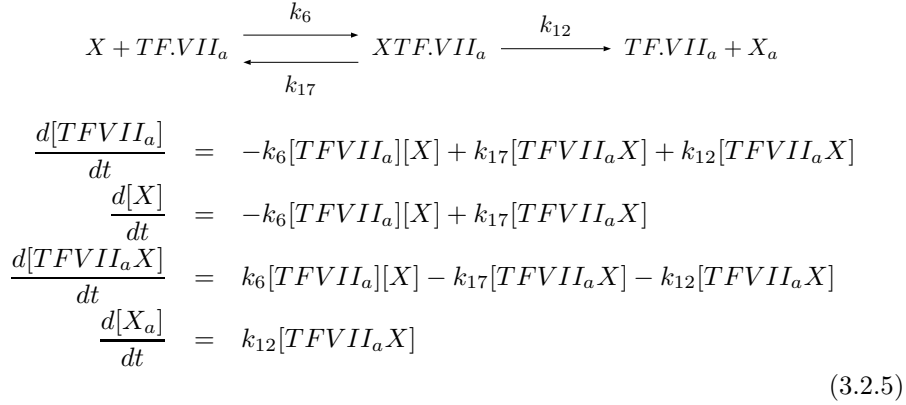
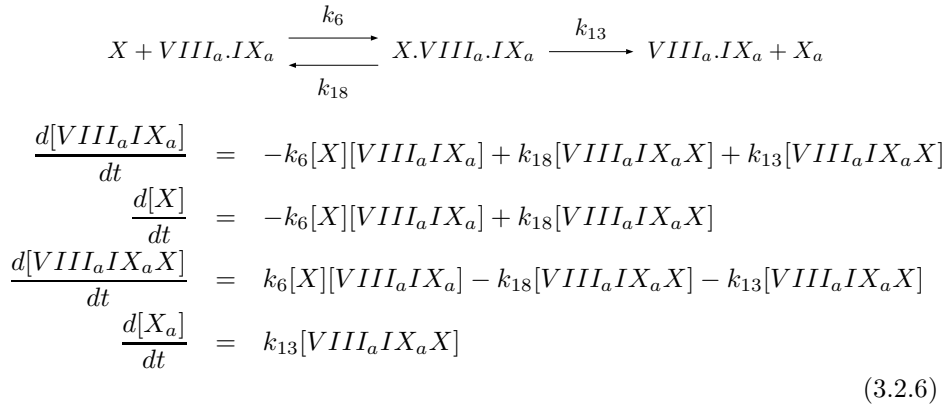
Remark 3.2.6. It is also noteworthy to point out that a physical constraint associated with the stability of the factor $VIII_a$ - factor IX_a complex has been incorporated into the model based on the empirically established decay of this complex with time by adding the term $-|I - [VIII_a IX_a]| + (I - [VIII_a IX_a])$, a kind of *switch function*, to the differential equation that describes the course of the concentration of this complex with time (see equation 7 of (3.2.1)). This term appears again multiplied by the constant k_{20} in the last equation, what leads one to suspect about the way the model was constructed. Furthermore, it is not clear why the authors have chosen a nondifferentiable function to cause the desired effect. Moreover, neither the numerical nor structural implications of these constraints were discussed in [JoMa94]. In Section 4.3, we treat with more detail the consequence of adding this term.

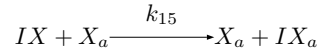
In the sequel, we restrict ourselves to the reaction scheme of Figure 3.7, settle the new equations and try to obtain the solution numerically. In a first attempt, we use, like Mann and Jones did, the law of mass action. But, later in Chapter 6 we propose an approach that uses the Michaelis-Menten equation since, actually, we are in the presence of enzyme catalyzed reactions. At the same time, we look critically at some reactions of the reaction scheme proposed by Mann and Jones in [JoMa94].

Using the law of mass action and taking into account the stoichiometric analysis, we take the rates of the reactions to be proportional to the concentrations of the reactants. We thereby obtain systematically the nonlinear autonomous differential equation system as it follows.

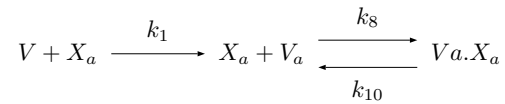
Reaction scheme 1 and respective contribution to rates of change



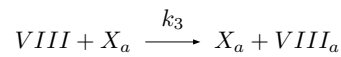
Reaction scheme 2 and respective contribution to rates of change**Reaction scheme 3 and respective contribution to rates of change**

Reaction scheme 4 and respective contribution to rates of change

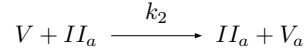
$$\begin{aligned} \frac{d[IX]}{dt} &= -k_{15}[IX][X_a] \\ \frac{d[IX_a]}{dt} &= k_{15}[IX][X_a] \end{aligned} \quad (3.2.7)$$

Reaction scheme 5 and respective contribution to rates of change

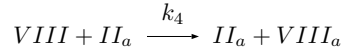
$$\begin{aligned} \frac{d[X_a]}{dt} &= -k_8[X_a][V_a] + k_{10}[V_a X_a] \\ \frac{d[V]}{dt} &= -k_1[V][X_a] \\ \frac{d[V_a]}{dt} &= k_1[V][X_a] + k_{10}[V_a X_a] - k_8[X_a][V_a] \\ \frac{d[V_a X_a]}{dt} &= k_8[X_a][V_a] - k_{10}[V_a X_a] \end{aligned} \quad (3.2.8)$$

Reaction scheme 6 and respective contribution to rates of change

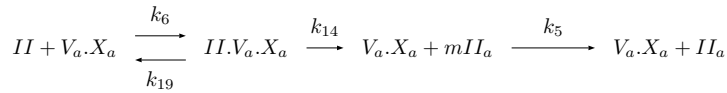
$$\begin{aligned} \frac{d[VIII]}{dt} &= -k_3[VIII][X_a] \\ \frac{d[VIII_a]}{dt} &= k_3[VIII][X_a] \end{aligned} \quad (3.2.9)$$

Reaction scheme 7 and respective contribution to rates of change

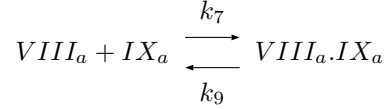
$$\begin{aligned} \frac{d[V]}{dt} &= -k_2[V][II_a] \\ \frac{d[V_a]}{dt} &= k_2[V][II_a] \end{aligned} \quad (3.2.10)$$

Reaction scheme 8 and respective contribution to rates of change

$$\begin{aligned} \frac{d[VIII]}{dt} &= -k_4[VIII][II_a] \\ \frac{d[VIII_a]}{dt} &= k_4[VIII][II_a] \end{aligned} \quad (3.2.11)$$

Reaction scheme 9 and respective contribution to rates of change

$$\begin{aligned} \frac{d[II]}{dt} &= -k_6[II][V_a X_a] + k_{19}[V_a X_a II] \\ \frac{d[V_a X_a]}{dt} &= -k_6[II][V_a X_a] + k_{19}[V_a X_a II] + k_{14}[V_a X_a II] \\ \frac{d[II_a]}{dt} &= k_5[V_a X_a][mII_a] \\ \frac{d[V_a X_a II]}{dt} &= -k_{19}[V_a X_a II] - k_{14}[V_a X_a II] + k_6[II][V_a X_a] \\ \frac{d[mII_a]}{dt} &= k_{14}[V_a X_a II] - k_5[V_a X_a][mII_a] \end{aligned} \quad (3.2.12)$$

Reaction scheme 10 and respective contribution to rates of change

$$\begin{aligned} \frac{d[VIII_a]}{dt} &= -k_7[VIII_a][IX_a] + k_9[VIII_a IX_a] \\ \frac{d[IX_a]}{dt} &= -k_7[VIII_a][IX_a] + k_9[VIII_a IX_a] \\ \frac{d[VIII_a IX_a]}{dt} &= -k_9[VIII_a IX_a] + k_7[VIII_a][IX_a] \end{aligned} \quad (3.2.13)$$

Notice that we obtained only 18 equations instead of 19. This is a hint that the term $-|I - [VIII_a IX_a]| + (I - [VIII_a IX_a])$ was added *a posteriori*. Thus the introduction of such a term cannot be justified neither by the structural aspects of the network nor by the implicit kinetics. The 18 equations correspond to the 18 species contained in the set \mathcal{S} of species and the second-hand part of the system suffered some little changes. The changes reflect furthermore the aspects discussed above in Remark 3.2.5.

The numerical integration was performed in SciLab. As initial values, we used the vector

$$\mathbf{x}_0 = [0.000005; 0.09; 0.2; 0.032; 0.0007; 1.4; 0; 0; 0; 0; 0; 0; 0; 0; 0; 0].$$

All variables corresponding to activated substances have initial value zero and for the remaining variables we took the physiological concentrations in blood of the different factors in $\mu M/L$.

The values of the constants in [JoMa94] were adjusted regarding the dimension. The integration interval was taken to be of 16 minutes. Since some constants are expressed in s^{-1} , we integrate over the interval [0,960] seconds.

The activity of meizothrombin is known to be about 120 % that of α -thrombin³. Thus, a relative specific activity of 1.2 is assigned to meizothrombin and 1 to α -thrombin in order to model thrombin activity in experimental results [JoMa94].

³In the model this corresponds solely to factor *II*

In the next figure we visualize the whole amount of thrombin in blood for the first 4 minutes:

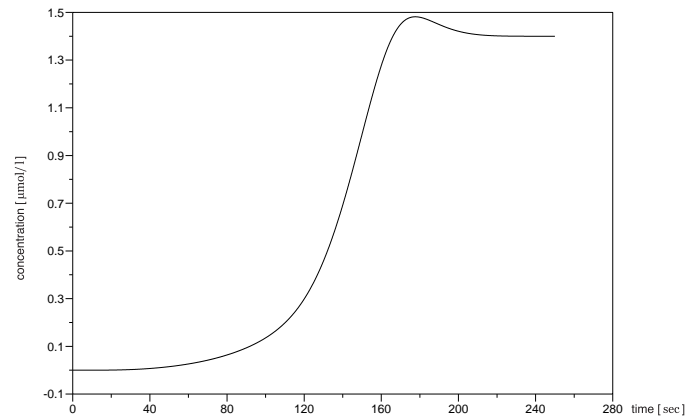


Figure 3.11: Activated thrombin (time=250 seconds \approx 4 min).

This graphic has the same behavior as the one published in [JoMa94]. The SciLab code and the remaining graphics are given in Appendix F with concentrations expressed in $\mu\text{mol}/L$ and time in s .

The experimentally observed decay of the complex $VIII_aIX_a$ was also not reflected in the corrected model and this we confirm visually in Figure 3.12.

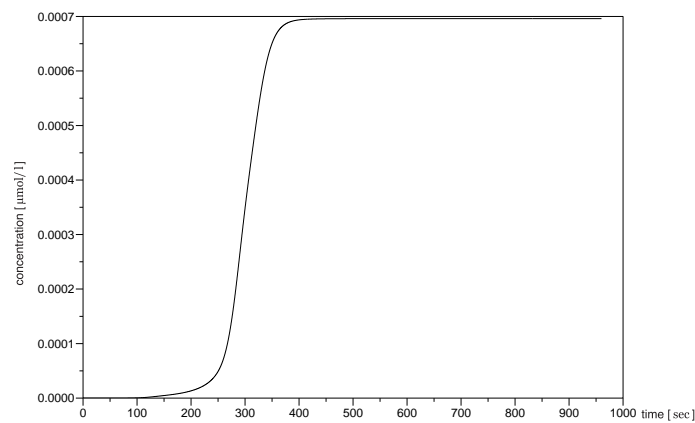


Figure 3.12: Complex $VIII_aIX_a$ does not decay, contrary to the experimental results.

A possible explanation for this result is that this model does not take into account the action of inhibitors. As a matter of fact and as already mentioned in Chapter 1, thrombin is responsible for generating a protease called activated protein *C*, which then permanently inactivates the two cofactors, factor V_a and factor $VIII_a$, with second order kinetics [BeJe95]. So, it is very likely that the complex $VIII_aIX_a$ will decay in a natural fashion. In 2002, the model from Mann and Jones was extended and the results are published in [HJEM02]. It includes among others the *TFPI*-mediated inactivation of the complex $TF.VII_a$, the inactivation of factors II_a , VII_a , IX_a and X_a by *ATIII*, the initial activation of cofactors V and $VIII$ by thrombin generated by factor X_a membrane, factor $VIII$ dissociation, the bind competition and kinetic activation steps that exist between *TF* and factors VII and VII_a and the activation of factor VII by thrombin, factor X_a and factor IX_a . However, although the reaction scheme was published, the set of differential equations governing the process was omitted. Moreover, the information published is neither enough to make a cautious mathematical analysis nor to verify if some of the aspects discussed in this section were corrected or not.

A challenge for future investigation would be to trace back the set of differential equations published in [HJEM02] by using the actual knowledge about the modelling of chemical reactions networks involving enzymes and compare the simulation to the experimental results obtained in order to see if it reflects the principal properties of blood coagulation accepted by the scientific community as been true. This would provide a more deep understanding of the blood coagulation process in a more systematic approach in the analysis of such kind of systems.

Chapter 4

Qualitative Analysis of Models for Thrombin Generation

The aim of this chapter is to perform a qualitative analysis of two mathematical models for thrombin generation in blood. The first is due to Stortelder, Hemker and Hemker [SHH97], and the second due to Mann and Jones [JoMa94].

The analysis is carried out applying the concepts given in Appendix A.

4.1 The model by Stortelder, Hemker and Hemker

4.1.1 Positivity analysis

We first write the system of ordinary differential equations (3.1.1) in vector form:

$$\frac{d\mathbf{x}}{dt} = \mathbf{f}(\mathbf{x}), \text{ where } \mathbf{f} \text{ is a vector function and } \mathbf{x} \text{ is a vector.}$$

In our case, the vector $\mathbf{x} = \mathbf{x}(t)$ has 9 components, each of which is a function of time. Let its components be written as $x_i \geq 0, i = \{1, \dots, 9\}$ and they represent the concentrations of the different factors involved in the blood coagulation process. Respectively, we have

$$x_1 = [X], \quad x_2 = [X_a], \quad x_3 = [V], \quad x_4 = [V_a], \quad x_5 = [PL], \quad x_6 = [PT], \quad x_7 = [II], \\ x_8 = [II_a], \quad \text{and} \quad x_9 = [II_a\alpha_2M].$$

For simplicity, we also rename the constants present in the model:

$$\begin{aligned} k_1 &:= kcat_X, & k_2 &:= km_X, & k_3 &:= ki_{X_a}, & k_4 &:= k_{PT}, & k_5 &:= k_{PL}, & k_6 &:= kcat_V, \\ k_7 &:= km_V, & k_8 &:= kcat_{II}, & k_9 &:= km_{II}, & k_{10} &:= kcat_2, & k_{11} &:= km_2, \\ k_{12} &:= ki_{II_a\alpha_2M} \text{ and } k_{13} &:= ki_{II_aATIII}. \end{aligned}$$

Then, the system (3.1.1) can now be written as follows:

$$\begin{aligned} \frac{dx_1}{dt} &= -\frac{k_1x_1RVV}{k_2+x_1} \\ \frac{dx_2}{dt} &= \frac{k_1x_1RVV}{k_2+x_1} - k_3x_2 - k_4x_4x_2x_5 + k_5x_6 \\ \frac{dx_3}{dt} &= -\frac{k_6x_3x_8}{k_7+x_3} \\ \frac{dx_4}{dt} &= \frac{k_6x_3x_8}{k_7+x_3} - k_4x_4x_2x_5 + k_5x_6 \\ \frac{dx_5}{dt} &= -k_4x_4x_2x_5 + k_5x_6 \\ \frac{dx_6}{dt} &= k_4x_4x_2x_5 - k_5x_6 \\ \frac{dx_7}{dt} &= -\frac{k_8x_7x_6}{k_9+x_7} - \frac{k_{10}x_7x_2}{k_{11}+x_7} \\ \frac{dx_8}{dt} &= \frac{k_8x_7x_6}{k_9+x_7} + \frac{k_{10}x_7x_2}{k_{11}+x_7} - k_{12}x_8 - k_{13}x_8 \\ \frac{dx_9}{dt} &= k_{12}x_8. \end{aligned} \tag{4.1.1}$$

Furthermore, the components $f_i(\mathbf{x})$ of $\mathbf{f}(\mathbf{x})$ are defined for $i = 1, \dots, 9$ by

$$\begin{aligned} f_1(\mathbf{x}) &:= -\frac{k_1x_1RVV}{k_2+x_1}, & f_2(\mathbf{x}) &:= \frac{k_1x_1RVV}{k_2+x_1} - k_3x_2 - k_4x_4x_2x_5 + k_5x_6, \\ f_3(\mathbf{x}) &:= -\frac{k_6x_3x_8}{k_7+x_3}, & f_4(\mathbf{x}) &:= \frac{k_6x_3x_8}{k_7+x_3} - k_4x_4x_2x_5 + k_5x_6, \\ f_5(\mathbf{x}) &:= -k_4x_4x_2x_5 + k_5x_6, & f_6(\mathbf{x}) &:= k_4x_4x_2x_5 - k_5x_6, \\ f_7(\mathbf{x}) &:= -\frac{k_8x_7x_6}{k_9+x_7} - \frac{k_{10}x_7x_2}{k_{11}+x_7}, & f_8(\mathbf{x}) &:= \frac{k_8x_7x_6}{k_9+x_7} + \frac{k_{10}x_7x_2}{k_{11}+x_7} - k_{12}x_8 - k_{13}x_8 \end{aligned}$$

and $f_9(\mathbf{x}) := k_{12}x_8$, respectively.

In the sequel we prove that the positive orthant of \mathbb{R}^9 and its closure are positively invariant sets for the system (4.1.1) by applying Proposition A.3.10. This shows in particular that any orbit starting with positive initial values will remain positive for all times.

Let $\mathcal{P} = \{\mathbf{x} \in \mathbb{R}^9 : x_1 > 0, \dots, x_9 > 0\}$ be the positive orthant of \mathbb{R}^9 .

Proposition 4.1.1. \mathcal{P} and $\overline{\mathcal{P}}$ are positively invariant for the system (4.1.1).

Proof. The vector \mathbf{x} represents a vector of concentrations, this means that $x_i \geq 0$, $i = 1, \dots, 9$, then the system is defined in a relative open subset of $\overline{\mathcal{P}}$ with $\mathbf{f}(\mathbf{x}) = (f_1(\mathbf{x}), \dots, f_9(\mathbf{x}))^T$, where the function \mathbf{f} is C^∞ .

For $\mathbf{x} \in \partial\mathcal{P}$, define $C_{\mathbf{x}} = \mathcal{P}$. Setting furthermore $x_k = 0$ in f_k , $k = 1, \dots, 9$ we obtain:

$$\begin{aligned} f_1(0, x_2, \dots, x_9) &= 0 \quad \text{for all } x_2 \geq 0, \dots, x_9 \geq 0; \\ f_2(x_1, 0, x_3, \dots, x_9) &= \frac{k_1 x_1 R V V}{k_2 + x_1} + k_5 x_6 \geq 0 \quad \text{for all } x_j \geq 0, j \neq 2; \\ f_3(x_1, x_2, 0, \dots, x_9) &= 0 \quad \text{for all } x_j \geq 0, j \neq 3; \\ f_4(x_1, x_2, x_3, 0, \dots, x_9) &= \frac{k_6 x_3 x_8}{k_7 + x_3} + k_5 x_6 \geq 0 \quad \text{for all } x_j \geq 0, j \neq 4; \\ f_5(x_1, x_2, x_3, x_4, 0, \dots, x_9) &= k_5 x_6 \geq 0 \quad \text{for all } x_j \geq 0, j \neq 5; \\ f_6(x_1, \dots, x_5, 0, x_7, x_8, x_9) &= k_4 x_4 x_2 x_5 \geq 0 \quad \text{for all } x_j \geq 0, j \neq 6; \\ f_7(x_1, \dots, x_6, 0, x_8, x_9) &= 0 \quad \text{for all } x_j \geq 0, j \neq 7; \\ f_8(x_1, \dots, x_7, 0, x_9) &= \frac{k_8 x_7 x_6}{k_9 + x_7} + \frac{k_{10} x_7 x_2}{k_{11} + x_7} \geq 0 \quad \text{for all } x_j \geq 0, j \neq 8; \\ f_9(x_1, \dots, x_8, 0) &= k_{12} x_8 \geq 0 \quad \text{for all } x_j \geq 0, j \neq 9. \end{aligned}$$

Thus, for every $i \in \{1, \dots, 9\}$, $f_i(x_1, \dots, x_{i-1}, 0, x_{i+1}, \dots, x_9) \geq 0$, and therefore $\mathbf{f}(\mathbf{x}) \in \overline{C_{\mathbf{x}}}$, for all $\mathbf{x} \in \partial\mathcal{P}$. Hence, \mathcal{P} and $\overline{\mathcal{P}}$ are positively invariant for the system (4.1.1). \square

4.1.2 Invariance principle applied to Hemker's model

Since

$$\begin{cases} \dot{x}_5 + \dot{x}_6 = 0 \\ \dot{x}_3 + \dot{x}_4 + \dot{x}_6 = 0 \\ \dot{x}_7 + \dot{x}_8 + \left(1 + \frac{k_{13}}{k_{12}}\right) \dot{x}_9 = 0, \end{cases} \quad (4.1.2)$$

we conclude that there are at least three conserved quantities in the system (4.1.1). In fact, we have the following proposition:

Proposition 4.1.2. The scalar valued functions $\varphi_1(\mathbf{x}) = x_6 + x_5$, $\varphi_2(\mathbf{x}) = x_3 + x_4 + x_6$ and $\varphi_3(\mathbf{x}) = x_7 + x_8 + \left(1 + \frac{k_{13}}{k_{12}}\right) x_9$ defined on \mathbb{R}^9 are first integrals of the system (4.1.1).

Corollary 4.1.3. *Given any solution of (4.1.1) with nonnegative initial values, the components $x_3(t), x_4(t), x_5(t), x_6(t), x_7(t), x_8(t), x_9(t)$ are bounded.*

Proof. By directly applying the last theorem and Remark A.1.5 we conclude that the solutions of the system (4.1.1) remain in the level set of φ_1, φ_2 and of φ_3 , in which they start. Hence, $\varphi_1(x_i(t)), \varphi_2(x_i(t))$ and $\varphi_3(x_i(t))$ are constant functions of t for all solutions and therefore bounded. Thus, the components $x_i(t), i = 3, 4, 5, 6, 7, 8, 9$ are bounded. \square

Moreover, for the components $x_1(t)$ and $x_2(t)$ a similar proposition holds.

Corollary 4.1.4. *Given any solution of (4.1.1) with nonnegative initial values, the components $x_1(t), x_2(t)$ are bounded.*

Proof. Consider $\varphi_4(\mathbf{x}) = x_1 + x_2 + x_6$ and $\varphi_5(\mathbf{x}) = x_1$ defined on \mathbb{R}^9 . Let us calculate the Lie derivative of these functions. By applying Theorem A.1.2 we have

$$\begin{aligned} L_{\mathbf{f}}(\varphi_4)(\mathbf{x}) &= D_{\mathbf{x}}(\varphi_4)\mathbf{f}(\mathbf{x}) = (1, 1, 0, 0, 0, 1, 0, 0, 0) \begin{pmatrix} f_1(\mathbf{x}) \\ \vdots \\ f_9(\mathbf{x}) \end{pmatrix} \\ &= f_1(\mathbf{x}) + f_2(\mathbf{x}) + f_6(\mathbf{x}) = -k_3x_2 \end{aligned}$$

and

$$\begin{aligned} L_{\mathbf{f}}(\varphi_5)(\mathbf{x}) &= D_{\mathbf{x}}(\varphi_5)\mathbf{f}(\mathbf{x}) = (1, 0, 0, 0, 0, 0, 0, 0, 0) \begin{pmatrix} f_1(\mathbf{x}) \\ \vdots \\ f_9(\mathbf{x}) \end{pmatrix} \\ &= f_1(\mathbf{x}) = -\frac{k_1x_1RVV}{k_2 + x_1}. \end{aligned}$$

Since $x_2 \geq 0$ and $x_1 \geq 0$ we have

$$L_{\mathbf{f}}(\varphi_4)(\mathbf{x}) \leq 0, \text{ and } L_{\mathbf{f}}(\varphi_5)(\mathbf{x}) \leq 0 \quad (4.1.3)$$

As sum of one or more nonnegative functions $x_i(t), i = 1, \dots, 9$, the functions $\varphi_j(\mathbf{x}) \geq 0, j = 4, 5$. Together with (4.1.3), we conclude that the functions φ_j are non increasing and bounded for $j = 4, 5$ along a trajectory. That is, there are constants β_j such that $0 \leq \varphi_j(\mathbf{x}) \leq \beta_j, j = 4, 5$. Hence every trajectory remains in the subset of \mathcal{P} defined by $\varphi_j(\mathbf{x}) \leq \beta_j$, which is bounded. Consequently, the components $x_i(t), i = 1, 2$ are defined and bounded. \square

It would be desirable to find out more first integrals before proceeding. However, there is no general principle for finding first integrals of a nonlinear system of first order differential equations. So, we try to do it first by visual inspection.

A closer look at the right-hand side of the system (4.1.1) reveals that the third equation is actually of separable variables and as a consequence x_9 can be written explicitly as a function of x_3 . In fact, for $x_3 > 0$, we have

$$\frac{\dot{x}_3(k_7 + x_3)}{x_3} = -k_6 x_8 = -\frac{k_6}{k_{12}} \dot{x}_9.$$

Integrating now both sides with respect to t and knowing that $x_3 > 0$, because of its physiological meaning, we obtain:

$$k_7 \ln x_3 \Big|_0^t + x_3 \Big|_0^t = -\frac{k_6}{k_{12}} x_9 \Big|_0^t$$

or

$$\frac{d}{dt} \left(k_7 \ln x_3 + x_3 + \frac{k_6}{k_{12}} x_9 \right) = 0.$$

Therefore

$$\frac{k_6}{k_{12}} x_9 + k_7 \ln x_3 + x_3 = k_7 \ln x_3(0) + x_3(0).$$

So, the following proposition holds.

Proposition 4.1.5. *The scalar valued function defined on \mathbb{R}^9 by*

$$\psi(\mathbf{x}) = \frac{k_6}{k_{12}} x_9 + k_7 \ln x_3 + x_3$$

is a first integral of the system (4.1.1).

Theorem 4.1.6. *The positive limit set $\omega(\mathbf{y})$ of (4.1.1) is contained in*

$$N := \{ \mathbf{x} \in \mathcal{P} : x_1 = 0, x_2 = 0, x_4 + x_3 + x_6 = x_3(0), x_6 + x_5 = x_5(0), x_8 = 0,$$

$$x_7 + x_8 + \left(1 + \frac{k_{13}}{k_{12}} \right) x_9 = x_7(0) \}.$$

Furthermore, every solution starting in N is stationary and the equilibrium point is stable.

Proof. The two previous results together with Theorem A.3.11 imply that the compact sets $M_\alpha := \{ \mathbf{x} \in \mathcal{P} : \varphi_j(\mathbf{x}) \leq \alpha, j = 1, \dots, 5 \}$ are positively invariant for all $\alpha \in [0, \beta_j)$. Theorem A.3.15 guarantees that the solution of the initial value problem exists on $[0, \infty)$. Moreover, this solution approaches its positive limit set $\omega(\mathbf{y})$, as $t \rightarrow \infty$. So, $\omega(\mathbf{y})$ is nonempty, compact and connected. By Theorem A.3.16 $\omega(\mathbf{y})$ is also invariant.

Hence, by applying LaSalle's principle stated in Theorem A.4.1 we conclude that

$$\omega(\mathbf{y}) \subset N.$$

Let furthermore $z(t) = (z_1(t), \dots, z_9(t))^T$ be a solution of (4.1.1) in N . We prove that $z(t)$ is stationary. It holds, $z(t) = (0, 0, z_3(t), z_4(t), z_5(t), z_6(t), z_7(t), 0, z_9(t))^T$. Thus, together with (4.1.1) we have

$$\dot{z}(t) = \begin{pmatrix} 0 \\ 0 \\ \dot{z}_3 \\ \dot{z}_4 \\ \dot{z}_5 \\ \dot{z}_6 \\ \dot{z}_7 \\ 0 \\ \dot{z}_9 \end{pmatrix} \stackrel{!}{=} \begin{pmatrix} 0 \\ k_5 z_6 \\ 0 \\ k_5 z_6 \\ k_5 z_6 \\ -k_5 z_6 \\ \frac{k_8 z_7 z_6}{k_9 + z_7} \\ \frac{k_8 z_7 z_6}{k_9 + z_7} \\ 0 \end{pmatrix}.$$

This means, in particular, that $\dot{z}_2 = 0$ and $\dot{z}_8 = 0$, since $z_8 = 0$ and $z_2 = 0$. This implies $z_6 = 0$ and

$$\dot{z}(t) = (0, 0, 0, 0, 0, 0, 0, 0, 0)^T.$$

That is, $z(t)$ is constant and the solution is stationary.

The ω -limit set of (4.1.1) is nonempty. However, we still do not know exactly its representation. To calculate the equilibrium points we set the right-hand side of the system equal to zero and solve for \mathbf{x} . The set of stationary points belongs to the set \mathcal{E} given by:

$$E = \alpha_3 \begin{pmatrix} 0 \\ 0 \\ 1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix} + \alpha_4 \begin{pmatrix} 0 \\ 0 \\ 0 \\ 1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix} + \alpha_5 \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix} + \alpha_7 \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 0 \\ 0 \end{pmatrix} + \alpha_9 \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \end{pmatrix}, \quad \alpha_3, \alpha_4, \alpha_5, \alpha_7, \alpha_9 \in \mathbb{R}_0^+.$$

The points of \mathcal{E} belong to N if $\alpha_3 + \alpha_4 = 0.03 = x_3(0)$, $\alpha_7 = 1.4 - \left(\frac{k_{12} + k_{13}}{k_{12}}\right) \alpha_9$

and $\alpha_5 = 0.05 = x_5(0)$. In other words, they belong to the triangle Π with parametric equation

$$P = \begin{pmatrix} 0 \\ 0 \\ 0 \\ x_3(0) \\ x_5(0) \\ 0 \\ x_7(0) \\ 0 \\ 0 \end{pmatrix} + \beta_3 \begin{pmatrix} 0 \\ 0 \\ 1 \\ -1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix} + \beta_9 \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ -(1 + \frac{k_{13}}{k_{12}}) \\ 0 \\ 0 \end{pmatrix}, \quad (4.1.5)$$

where $0 \leq \beta_3 \leq 0.03$ and $0 \leq \beta_9 \leq 1.4 \frac{k_{12}}{k_{12} + k_{13}}$ to exclude negative concentrations.

To study the nature of the equilibrium we first take a look at the general representation of the Jacobian at any point of Π :

$$\mathbf{J} = \begin{pmatrix} -\frac{k_1[RVV]}{k_2} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{k_1[RVV]}{k_2} & -k_3 - k_4\alpha_4\alpha_5 & 0 & 0 & 0 & k_5 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & \frac{-k_6\alpha_3}{k_7 + \alpha_3} & 0 \\ 0 & -k_4\alpha_4\alpha_5 & 0 & 0 & 0 & k_5 & \frac{k_6\alpha_3}{k_7 + \alpha_3} & 0 \\ 0 & -k_4\alpha_4\alpha_5 & 0 & 0 & 0 & k_5 & 0 & 0 \\ 0 & k_4\alpha_4\alpha_5 & 0 & 0 & 0 & -k_5 & 0 & 0 \\ 0 & -\frac{k_{10}\alpha_7}{k_{11} + \alpha_7} & 0 & 0 & 0 & -\frac{k_8\alpha_7}{k_9 + \alpha_7} & 0 & 0 \\ 0 & \frac{k_{10}\alpha_7}{k_{11} + \alpha_7} & 0 & 0 & 0 & \frac{k_8\alpha_7}{k_9 + \alpha_7} & 0 & -k_{12} - k_{13} \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & k_{12} \end{pmatrix}.$$

We observe that the Jacobian at any stationary point has 5 zero columns. In particular, this means that zero is an eigenvalue of the matrix with algebraic and

geometric multiplicities equal to 5 and that there are conserved quantities present. However, by Proposition 4.1.5 and Proposition 4.1.2 the order of the system may be reduced from 9 to 5. The reduced system has furthermore a non-isolated singularity that can be deduced from (4.1.5).

The Jacobian of the reduced system at the equilibrium has the representation

$$J_r := \begin{pmatrix} a_1 & 0 & 0 & 0 & 0 \\ -a_1 & a_2 & 0 & a_3 & 0 \\ 0 & 0 & 0 & 0 & a_4 \\ 0 & a_5 & 0 & a_3 & 0 \\ 0 & a_6 & 0 & a_7 & a_8 \end{pmatrix}. \quad (4.1.6)$$

It holds however that $a_2 = -k_3 + a_5$, with $k_3 > 0$. Under this condition we may write

$$J_r := \begin{pmatrix} a_1 & 0 & 0 & 0 & 0 \\ -a_1 & -k_3 + a_5 & 0 & a_3 & 0 \\ 0 & 0 & 0 & 0 & a_4 \\ 0 & a_5 & 0 & a_3 & 0 \\ 0 & a_6 & 0 & a_7 & a_8 \end{pmatrix}. \quad (4.1.7)$$

In this case, the eigenvalues of the matrix (4.1.7) can be calculated explicitly as:

$$\lambda_1 = 0,$$

$$\lambda_2 = a_1 = -\frac{k_1 R V V}{k_2},$$

$$\lambda_3 = a_8 = -k_{12} - k_{13},$$

$$\lambda_4 = \frac{1}{2}(a_3 + (a_5 - k_3)) + \frac{1}{2}\sqrt{(a_3 + (a_5 - k_3))^2 + 4a_3k_3},$$

$$\lambda_5 = \frac{1}{2}(a_3 + (a_5 - k_3)) - \frac{1}{2}\sqrt{(a_3 + (a_5 - k_3))^2 + 4a_3k_3}.$$

Since k_1, k_2, k_{12} and k_{13} are positive follows $\lambda_2, \lambda_3 < 0$.

From the physiological meaning of the constants involved we also have immediately $a_3 = -k_5 < 0$.

Since $0 < y_3 \leq 0.03$ then $0.03 - y_3 \geq 0$. I. e., $a_2 = -k_3 - 0.05k_4(0.03 - y_3) < 0$. And the same argument holds to justify that $a_5 = -0.05k_4(0.03 - y_3) < 0$.

Thus, λ_4 and λ_5 have negative real parts.

Since the reduced system has a non-isolated singularity we are in the conditions of Theorem A.2.8 and the equilibrium point of the reduced system is stable but not asymptotically stable. Moreover, the system converges to a single point where the coordinate corresponding to $\lambda_1 = 0$ is a constant depending on the initial value problem and the remaining ones are equal zero.

Furthermore, because of the stationarity of every solution starting in N , we conclude immediately that the system converges to a nonisolated equilibrium point where the coordinates corresponding to the eigenvalues equal zero are constants depending on the initial value problem and the remaining ones are equal zero. Then the equilibrium point of the system (4.1.1) is stable. \square

In the sequel we reduce the order of the system (4.1.1).

4.2 Model reduction

The aim of this section is to reduce the dimension of system (4.1.1) in different steps. Three of the conserved quantities can immediately be eliminated by means of the three first integrals given already in Proposition 4.1.2 and a coordinate transformation. For this purpose, we consider first the initial values

$$x_3(0) = 0.03, x_4(0) = 0, x_5(0) = 0.05, x_6(0) = 0, x_7(0) = 1.4, x_8(0) = 0 \text{ and } x_9(0) = 0.$$

We obtain

$$\varphi_1(\mathbf{x}) = x_6 + x_5, \quad \varphi_2(\mathbf{x}) = x_3 + x_4 + x_6 \text{ and}$$

$$\varphi_3(\mathbf{x}) = x_7 + x_8 + \left(1 + \frac{k_{13}}{k_{12}}\right) x_9.$$

The number of equations will be reduced to 6 by setting

$$x_4 = x_5 - x_3 - 0.02; \quad x_6 = -x_5 + 0.05 \quad x_7 = -x_8 - \left(1 + \frac{k_{13}}{k_{12}}\right) x_9 + 1.4.$$

Hence, the 4th, the 6th and the 7th equations can be omitted. By Proposition 4.1.5

every occurrence of x_9 can be substituted by

$$\frac{k_{12}}{k_6}(k_7 \ln x_3(0) - k_7 \ln x_3 + x_3(0) - x_3)$$

and, since the last equation is a simple integration of x_8 , we can omit the 9th equation.

Remark 4.2.1. As $\dot{x}_3 \leq 0$, then x_3 is monotonically decreasing. Thus, $x_3(0) - x_3 \geq 0$, which implies $k_7 \ln x_3(0) - k_7 \ln x_3(0) \geq 0$. Hence, $x_9 \geq 0$.

Altogether we are now able to reduce the dimension of the system to 5 after performing the following coordinate transformation

$$y_1 := x_1; y_2 := x_2; y_3 := x_3; y_4 := x_5; y_5 := x_8,$$

and each occurrence of x_4, x_6, x_7 and x_9 is substituted respectively by:

$$x_4 = y_4 - y_3 - 0.02;$$

$$x_6 = -y_4 + 0.05;$$

$$x_7 = 1.4 - y_5 - \frac{k_{12} + k_{13}}{k_6}(c - k_7 \ln y_3 - y_3);$$

$$x_9 = \frac{k_{12}}{k_6}(c - k_7 \ln y_3 - y_3), \text{ where } c = k_7 \ln x_3(0) + x_3(0).$$

The reduced system comprises the following system of differential equations:

$$\begin{aligned} \frac{dy_1}{dt} &= -\frac{k_1 y_1 R V V}{k_2 + y_1} \\ \frac{dy_2}{dt} &= \frac{k_1 y_1 R V V}{k_2 + y_1} - k_3 y_2 - k_4 (y_4 - y_3 - 0.02) y_2 y_4 + k_5 (-y_4 + 0.05) \\ \frac{dy_3}{dt} &= -\frac{k_6 y_3 y_5}{k_7 + y_3} \\ \frac{dy_4}{dt} &= -k_4 (y_4 - y_3 - 0.02) y_2 y_4 + k_5 (-y_4 + 0.05) \\ \frac{dy_5}{dt} &= \frac{k_8 \left(1.4 - y_5 - \left(\frac{k_{12} + k_{13}}{k_6}\right)\right) (c - k_7 \ln y_3 - y_3) (-y_4 + 0.05)}{k_9 + \left(1.4 - y_5 - \left(\frac{k_{12} + k_{13}}{k_6}\right)\right) (c - k_7 \ln y_3 - y_3)} \\ &\quad + \frac{k_{10} \left(1.4 - y_5 - \left(\frac{k_{12} + k_{13}}{k_6}\right)\right) (c - k_7 \ln y_3 - y_3) y_2}{k_{11} + \left(1.4 - y_5 - \left(\frac{k_{12} + k_{13}}{k_6}\right)\right) (c - k_7 \ln y_3 - y_3)} - k_{12} y_5 - k_{13} y_5 \end{aligned} \tag{4.2.1}$$

Setting the right-hand side of the system (4.2.1) to zero and solving to \mathbf{y} we verify

that all the equilibrium points of this system belong to the segment of line:

$$P = \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0.05 \\ 0 \end{pmatrix} + \mu_3 \begin{pmatrix} 0 \\ 0 \\ 1 \\ 0 \\ 0 \end{pmatrix}, \text{ where } 0 \leq \mu_3 \leq 0.03. \quad (4.2.2)$$

Remark 4.2.2. Notice that $0.05 = y_4(0)$ and that $0.03 = y_3(0)$.

4.2.1 Linearization around an equilibrium point

At any point of the segment of line (4.2.2), the Jacobian matrix of (4.2.1) has the following representation:

$$J_r = \begin{bmatrix} -\frac{k_1 RVV}{k_2} & 0 & 0 & 0 & 0 \\ \frac{k_1 RVV}{k_2} & -k_3 - 0.05 k_4 (0.03 - y_3) & 0 & -k_5 & 0 \\ 0 & 0 & 0 & 0 & -\frac{k_6 y_3}{k_7 + y_3} \\ 0 & -0.05 k_4 (0.03 - y_3) & 0 & -k_5 & 0 \\ 0 & a_6 & 0 & a_7 & -k_{13} - k_{12} \end{bmatrix}, \quad (4.2.3)$$

where $a_6 = \frac{k_{10}(1.4k_6 - (k_{12} + k_{13})(c - k_7 \ln(y_3) - y_3))}{k_{11}k_6 + 1.4k_6 - (k_{12} + k_{13})(c - k_7 \ln(y_3) - y_3)}$ and

$$a_7 = -\frac{k_8(1.4k_6 - (k_{12} + k_{13})(c - k_7 \ln(y_3) - y_3))}{k_6k_9 + 1.4k_6 - (k_{12} + k_{13})(c - k_7 \ln(y_3) - y_3)}.$$

We see that 0 is an eigenvalue of the Jacobian matrix which eigenvector is the direction vector of (4.2.2). This eigenvector is in particular the canonical vector pointing in the same direction as the Cartesian nonnegative axis corresponding to the variable represented by y_3 .

Remark 4.2.3. We can say more about the nature of the reaction mechanism according to the signs of the entries of the Jacobian matrix. So, a positive entry in the Jacobian matrix \mathbf{J}_{ij} indicates that near steady state an increase in a substance y_j gives rise to production of y_i , whereas negative elements in the matrix indicate that y_i is removed as a direct result of an increase in y_j , indicating an inhibitory interaction. If the elements in the principal diagonal are positive then we can speak about autocatalysis. A zero element of the matrix indicates that there is no

direct interaction between y_j and y_i . Indirect interactions can nevertheless also be inferred: if y_i acts on y_j which acts on y_k , then y_i acts indirectly on y_k whereas the sign of this indirect influence is determined by the signs of the direct steps. Moreover, these steps can be interpreted in terms of positive and negative feedback loops [CSM04]. Thus, it is also of interest to investigate the signs of the entries denoted by a_6 and a_7 .

The first observation is that a_6 and a_7 have signs, which will uniquely depend on the sign of the term

$$1.4k_6 - (k_{12} + k_{13})(c - k_7 \ln(y_3) - y_3). \quad (4.2.4)$$

Let us suppose that (4.2.4) is positive. Then it holds,

$$1.4 > \frac{k_{12} + k_{13}}{k_6} (c - k_7 \ln(y_3) - y_3),$$

which is equivalent to

$$\mathbf{x}_7^{eq} < \mathbf{x}_7(0).$$

This last inequality is always true since x_7 is monotonically decreasing, because $\dot{\mathbf{x}}_7 \leq 0$ (see equation 7 of (4.1.1)).

Hence, $a_6 > 0$ and $a_7 < 0$.

4.2.2 Heuristic approach for reducing the number of equations

For each $0 < y_3 \leq 0.03$ fixed, we obtain a subsystem of (4.2.1) in the variables y_1, y_2, y_4 and y_5 with a single equilibrium point.

Since $0.05 = y_4(0) := c_1$, the subsystem in the variables y_1, y_2, y_4 and y_5 may be written in a more general form and comprises the following equations:

$$\begin{aligned}
\frac{dy_1}{dt} &= -\frac{k_1 y_1 R V V}{k_2 + y_1} \\
\frac{dy_2}{dt} &= \frac{k_1 y_1 R V V}{k_2 + y_1} - k_3 y_2 - k_4 (y_4 - y_3 - 0.02) y_2 y_4 + k_5 (-y_4 + c_1) \\
\frac{dy_4}{dt} &= -k_4 (y_4 - y_3 - 0.02) y_2 y_4 + k_5 (-y_4 + c_1) \\
\frac{dy_5}{dt} &= \frac{k_8 \left(1.4 - y_5 - \left(\frac{k_{12} + k_{13}}{k_6}\right)\right) (c - k_7 \ln y_3 - y_3) (-y_4 + c_1)}{k_9 + \left(1.4 - y_5 - \left(\frac{k_{12} + k_{13}}{k_6}\right)\right) (c - k_7 \ln y_3 - y_3)} \\
&\quad + \frac{k_{10} \left(1.4 - y_5 - \left(\frac{k_{12} + k_{13}}{k_6}\right)\right) (c - k_7 \ln y_3 - y_3) y_2}{k_{11} + \left(1.4 - y_5 - \left(\frac{k_{12} + k_{13}}{k_6}\right)\right) (c - k_7 \ln y_3 - y_3)} - k_{12} y_5 - k_{13} y_5
\end{aligned} \tag{4.2.5}$$

the equilibrium point being $E := (0, 0, c_1, 0)$.

The general expression of the Jacobian at E can be directly deduced from (4.2.3) and is given by:

$$J = \begin{bmatrix} -\frac{k_1 R V V}{k_2} & 0 & 0 & 0 \\ \frac{k_1 R V V}{k_2} & -k_3 - 0.05 k_4 (0.03 - y_3) & -k_5 & 0 \\ 0 & -0.05 k_4 (0.03 - y_3) & -k_5 & 0 \\ 0 & a_5 & a_6 & -k_{13} - k_{12} \end{bmatrix}, \tag{4.2.6}$$

where $a_5 = \frac{k_{10} (1.4 k_6 - (k_{12} + k_{13})) (c - k_7 \ln(y_3) - y_3)}{k_{11} k_6 + 1.4 k_6 - (k_{12} + k_{13}) (c - k_7 \ln(y_3) - y_3)}$ and

$a_6 = -\frac{k_8 (1.4 k_6 - (k_{12} + k_{13})) (c - k_7 \ln(y_3) - y_3)}{k_6 k_9 + 1.4 k_6 - (k_{12} + k_{13}) (c - k_7 \ln(y_3) - y_3)}$ with $0 < y_3 \leq 0.03$ fixed.

All eigenvalues of the Jacobian (4.2.6) have real part less than zero and they are equal to the nonzero eigenvalues of J_r . By using Lyapunov's direct method described in Section A.2 we conclude that the system (4.2.5) is asymptotically stable.

Let us now evaluate numerically the result of the linearization of (4.2.5) at E for the set of constants published in [SHH97].

Evaluating the results with concrete values for the reaction constants published in [SHH97].

To illustrate numerically the results of the analysis performed, let us substitute the values of the constants published in [SHH97], perform the linearization of the system (4.2.5) at E and make further conclusions.

After substituting the values of the constants, the Jacobian matrix is given by:

$$A = \begin{pmatrix} -0.3033 & 0 & 0 & 0 \\ 0.3033 & -4.536 & -801.4 & 0 \\ 0 & -0.00541 & -801.4 & 0 \\ 0 & 11.54 & -0.593 & -0.9621 \end{pmatrix}.$$

The matrix A is stable and all the solutions can be written explicitly. As an example, we present the solution of the initial value problem $\dot{\mathbf{x}}(t) = A\mathbf{x}(t)$, $\mathbf{x}(0) = (0.2, 0, 0.05, 0)$ and compare it graphically with the solution obtained while solving numerically the non-linear system (4.2.5).

This step was performed in SciLab by using the following code:

```
T=[0:0.001:30];- integration interval
x0=[0.2;0;0.05;0]; - starting values
xeq=[0;0;0.05;0]; - equilibrium point
```

The function representing the right hand-side of the linear system

$$\dot{\mathbf{x}} = BCoagHemk4jv(xeq) * \mathbf{x},$$

where $BCoagHemk4jv(xeq)$ is the function defined by the Jacobian of the system evaluated at the equilibrium point.

```
function xdot=lincoag4(t,x)
xdot=BCoagHemk4jv(xeq)*x
endfunction
sm=x0-xeq; - shifting the equilibrium to the origin.
ylin=ode(sm,0,T,lincoag4); - storing all the solutions in ylin
```

Plotting simultaneously the solutions of thrombin generation of both the non-linear and the linear system we obtain Figure 4.1.

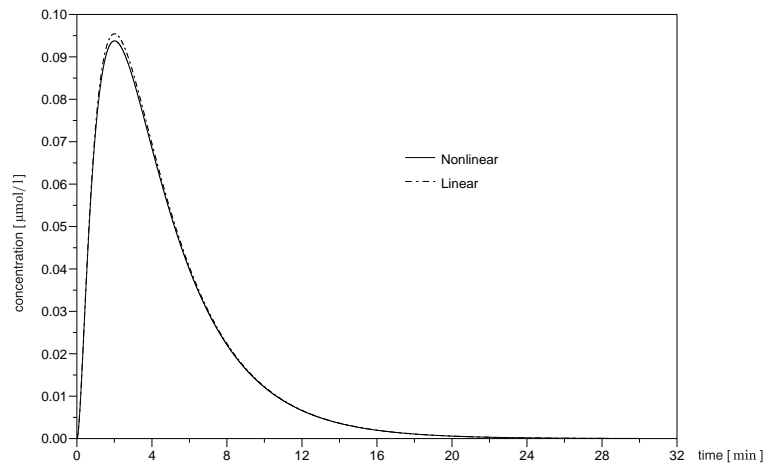


Figure 4.1: Course of thrombin from the solution of the non-linear system versus the solution from the linear system.

We observe that both graphics can hardly be distinguished from each other. Together with the theoretical analysis already performed, we conclude that the linear approximation preserved the global behavior of the system. Since it is more convenient for mathematical analysis and parameter estimation, it constitutes a helpful tool in addition to experimental blood coagulation investigation and can be considered as a good basis for model extension.

4.3 Model from Jones and Mann

The model proposed by Jones and Mann in [JoMa94] comprises a system of 19 nonlinear differential equations and is reproduced in this thesis in Chapter 3 in the equation set (3.2.1). The qualitative analysis of this system should follow the same pattern as the one done for the model of Stortelder et. al. However, from the analysis made in Chapter 3 we concluded that given the reaction scheme (3.2.1), the system proposed by Jones and Mann in [JoMa94] does not satisfy the law of mass action and some correction was done. In this section we first prove that the mathematical property of positivity that must be inherent to this kind of systems is not satisfied by the system published in [JoMa94]. Moreover, we prove that the corrected model with mass action kinetics does have this property and further analysis regarding stability of the corrected system was made.

4.3.1 Positivity analysis

Like we did before for Stortelder's model, we start by writing the system (3.2.1) in vector form as $\frac{d\mathbf{x}}{dt} = \mathbf{f}(\mathbf{x})$. Now the vector \mathbf{x} has 19 components $x_i, i = 1, \dots, 19$. For that matter, let

$$\begin{aligned}
 x_1 &= [TFVIIa]; & x_2 &= [IX]; & x_3 &= [X]; & x_4 &= [V]; & x_5 &= [VIII]; & x_6 &= [II]; \\
 x_7 &= [VIIIaIXa]; & x_8 &= [VaXa]; & x_9 &= [IIa]; & x_{10} &= [VaXaII]; & x_{11} &= [mIIa]; \\
 x_{12} &= [TFVIIaIX]; & x_{13} &= [TFVIIaX]; & x_{14} &= [VIIIaIXaX]; & x_{15} &= [IXa]; \\
 x_{16} &= [Xa]; & x_{17} &= [Va]; & x_{18} &= [VIIIa] & \text{and } x_{19} &= [I].
 \end{aligned}
 \tag{4.3.1}$$

The autonomous system of nonlinear differential equations (3.2.1) can now be written as:

$$\begin{aligned}
 \frac{dx_1}{dt} &= k_{11}x_{12} - k_6x_1x_2 + k_{16}x_{12} + k_{12}x_{13} - k_6x_1x_3 + k_{17}x_{13} \\
 \frac{dx_2}{dt} &= k_{16}x_{12} - k_6x_1x_2 - k_{15}x_2x_{16} - k_{15}x_2x_8 \\
 \frac{dx_3}{dt} &= k_{17}x_{13} - k_6x_3x_1 - k_6x_3x_7 + k_{18}x_{14} \\
 \frac{dx_4}{dt} &= -k_1x_4x_{16} - k_2x_4x_9 - k_2x_4x_{11} \\
 \frac{dx_5}{dt} &= -k_3x_5x_{16} - k_4x_5x_9 - k_4x_5x_{11}
 \end{aligned}$$

$$\begin{aligned}
\frac{dx_6}{dt} &= k_{19}x_{10} - k_6x_6x_8 \\
\frac{dx_7}{dt} &= k_7x_{18}x_{15} - k_9x_7 - k_6x_7x_3 + k_{18}x_{14} + k_{13}x_{14} - |x_{19} - x_7| + (x_{19} - x_7) \\
\frac{dx_8}{dt} &= k_8x_{16}x_{17} - 2k_{10}x_8 + k_{19}x_{10} - k_6x_8x_6 + k_{14}x_{10} \\
\frac{dx_9}{dt} &= k_5x_8x_{11} \\
\frac{dx_{10}}{dt} &= k_6x_8x_6 - k_{19}x_{10} - k_{14}x_{10} \\
\frac{dx_{11}}{dt} &= k_{14}x_{10} - k_5x_8x_{11} \\
\frac{dx_{12}}{dt} &= k_6x_1x_2 - k_{16}x_{12} - k_{11}x_{12} \\
\frac{dx_{13}}{dt} &= k_6x_1x_3 - k_{17}x_{13} - k_{12}x_{13} \\
\frac{dx_{14}}{dt} &= k_6x_7x_3 - k_{18}x_{14} - k_{13}x_{14} \\
\frac{dx_{15}}{dt} &= k_9x_7 - k_7x_{18}x_{15} + k_{11}x_{12} + k_{15}x_2x_{16} + k_{15}x_2x_8 \\
\frac{dx_{16}}{dt} &= k_{10}x_8 - k_6x_{16}x_{17} + k_{12}x_{13} + k_{13}x_{14} \\
\frac{dx_{17}}{dt} &= k_{10}x_8 - k_6x_{16}x_{17} + k_1x_4x_{16} + k_2x_4x_9 + k_2x_4x_{11} \\
\frac{dx_{18}}{dt} &= k_9x_7 - k_7x_{18}x_{15} + k_3x_5x_{16} + k_4x_5x_9 + k_4x_5x_{11} \\
\frac{dx_{19}}{dt} &= (-|x_{19} - x_7| + (x_{19} - x_7))k_{20}.
\end{aligned} \tag{4.3.2}$$

The positive orthant of \mathbb{R}^{19} is defined as $\mathcal{P} = \{\mathbf{x} \in \mathbb{R}^{19} : x_1 > 0, \dots, x_{19} > 0\}$.

Proposition A.3.10 is again applied to prove that there are orbits starting with positive initial values not remaining positive for all times. The following proposition holds:

Proposition 4.3.1. \mathcal{P} is not positively invariant for the system (4.3.2).

Proof. It is sufficient to prove that the solutions of $\frac{dx_{19}}{dt} = f_{19}(\mathbf{x})$ do not remain positive for all times.

The term $-|x_{19} - x_7| + (x_{19} - x_7)$ is in both the 7th and 19th equations. In fact, we have:

$$-|x_{19} - x_7| + (x_{19} - x_7) = \begin{cases} 0 & , x_{19} \geq x_7 \\ 2(x_{19} - x_7) & , x_{19} < x_7 \end{cases}$$

Setting furthermore $x_k = 0$, in f_k , $k = 7, 19$ we obtain:

$f_7(x_1, \dots, x_6, 0, x_8, \dots, x_{19}) = k_7 x_{18} x_{15} + k_{18} x_{14} + k_{13} x_{14}$, because $x_{19} \geq x_7 = 0$.
Thus, $f_7(x_1, \dots, x_6, 0, x_8, \dots, x_{19}) \geq 0$ for all $x_j \geq 0$, $j \neq 7$.

But

$f_{19}(x_1, \dots, x_{18}, 0) = -2k_{20} x_7 < 0$, for all $x_j \geq 0$, $j \neq 19$, because $x_7 > x_{19} = 0$ by definition.

This means that the solutions of $\frac{dx_{19}}{dt} = f_{19}(\mathbf{x})$ do not remain positive for all times. \square

Remark 4.3.2. We recall that, in the paper [JoMa94], Jones and Mann report that, based on empirical data, there is a decay in the activity of the factor denoted by the variable x_7 . To model this decay, they introduced *a posteriori* a new variable, x_{19} that represents the maximal concentration of the variable x_7 and claim that the corresponding equation should cause a decreasing maximal concentration of the variable x_7 with time. So, a similar factor is also used in determining the changing concentration of the factor $VIII_a$ - factor IX_a complex. However, it is not clear why they used a nondifferentiable function to produce this kind of behavior. In addition, positivity of the system is also not preserved for all times. In Chapter 6 we propose another function to model such effect.

In the sequel, we make the same analysis as before for the corrected model presented in Section 3.2.2.

4.3.2 Positivity of the corrected model

The concentrations of the substrates, enzymes, complexes and products involved are denoted as in (4.3.1).

Altogether, we obtain the following system of 18 differential equations:

$$\begin{aligned}
\frac{dx_1}{dt} &= k_{11}x_{12} - k_6x_1x_2 + k_{16}x_{12} + k_{12}x_{13} - k_6x_1x_3 + k_{17}x_{13} \\
\frac{dx_2}{dt} &= k_{16}x_{12} - k_6x_1x_2 - k_{15}x_2x_{16} \\
\frac{dx_3}{dt} &= k_{17}x_{13} - k_6x_3x_1 - k_6x_3x_7 + k_{18}x_{14} \\
\frac{dx_4}{dt} &= -k_1x_4x_{16} - k_2x_4x_9 \\
\frac{dx_5}{dt} &= -k_3x_5x_{16} - k_4x_5x_9 \\
\frac{dx_6}{dt} &= k_{19}x_{10} - k_6x_6x_8 \\
\frac{dx_7}{dt} &= k_7x_{18}x_{15} - k_9x_7 - k_6x_7x_3 + k_{18}x_{14} + k_{13}x_{14} \\
\frac{dx_8}{dt} &= k_8x_{16}x_{17} - k_{10}x_8 + k_{19}x_{10} - k_6x_8x_6 + k_{14}x_{10} \\
\frac{dx_9}{dt} &= k_5x_8x_{11} \\
\frac{dx_{10}}{dt} &= k_6x_8x_6 - k_{19}x_{10} - k_{14}x_{10} \\
\frac{dx_{11}}{dt} &= k_{14}x_{10} - k_5x_8x_{11} \\
\frac{dx_{12}}{dt} &= k_6x_1x_2 - k_{16}x_{12} - k_{11}x_{12} \\
\frac{dx_{13}}{dt} &= k_6x_1x_3 - k_{17}x_{13} - k_{12}x_{13} \\
\frac{dx_{14}}{dt} &= k_6x_7x_3 - k_{18}x_{14} - k_{13}x_{14} \\
\frac{dx_{15}}{dt} &= k_9x_7 - k_7x_{18}x_{15} + k_{11}x_{12} + k_{15}x_2x_{16} \\
\frac{dx_{16}}{dt} &= k_{10}x_8 - k_8x_{16}x_{17} + k_{12}x_{13} + k_{13}x_{14} \\
\frac{dx_{17}}{dt} &= k_{10}x_8 - k_8x_{16}x_{17} + k_1x_4x_{16} + k_2x_4x_9 \\
\frac{dx_{18}}{dt} &= k_9x_7 - k_7x_{18}x_{15} + k_3x_5x_{16} + k_4x_5x_9
\end{aligned} \tag{4.3.3}$$

The positive orthant of \mathbb{R}^{18} is defined as $\mathcal{P} = \{\mathbf{x} \in \mathbb{R}^{18} : x_1 > 0, \dots, x_{18} > 0\}$. The following proposition states that the system (4.3.3) only admits positive solutions for positive starting values. Since the proof has the same structure as the proof of Proposition 4.1.1 we keep it short by indicating only the major steps.

Proposition 4.3.3. \mathcal{P} and $\overline{\mathcal{P}}$ are positively invariant for the system (4.3.3).

Proof. The vector \mathbf{x} represents again a vector of concentrations, this means that $x_i \geq 0$, $i = 1, \dots, 18$. Then the system is defined in a relative open subset of $\overline{\mathcal{P}}$ with $\mathbf{f}(\mathbf{x}) = (f_1(\mathbf{x}), \dots, f_{18}(\mathbf{x}))^T$, where the function \mathbf{f} is C^∞ .

For $\mathbf{x} \in \partial\mathcal{P}$, define $C_{\mathbf{x}} = \mathcal{P}$. Set $x_k = 0$, in $f_k, k = 1, \dots, 18$.

For every $i \in \{1, \dots, 18\}$, $f_i(x_1, \dots, x_{i-1}, 0, x_{i+1}, \dots, x_{18}) \geq 0$, and therefore $\mathbf{f}(\mathbf{x}) \in \overline{C_{\mathbf{x}}}$, for all $\mathbf{x} \in \partial\mathcal{P}$. Hence, \mathcal{P} and $\overline{\mathcal{P}}$ are positively invariant for the system (4.3.3) and, as a consequence, for positive initial values it admits only positive solutions. \square

4.3.3 Linear first integrals and boundedness of the solutions

Corollary 4.3.4. Given any solution of (4.3.3) with nonnegative initial values, all the components $x_i(t), i = 1, \dots, 18$ are bounded.

Proof. In this case, since all variables are involved, it is sufficient to prove that the scalar valued functions defined on \mathbb{R}^{18} by

$$\varphi_1(\mathbf{x}) = x_1 + x_{12} + x_{13};$$

$$\varphi_2(\mathbf{x}) = x_2 + x_{12} + x_{15} + x_7 + x_{14};$$

$$\varphi_3(\mathbf{x}) = x_3 + x_{13} + x_{14} + x_{16} + x_8 + x_{10};$$

$$\varphi_4(\mathbf{x}) = x_4 + x_{17} + x_8 + x_{10};$$

$$\varphi_5(\mathbf{x}) = x_5 + x_{18} + x_7 + x_{14};$$

$$\varphi_6(\mathbf{x}) = x_6 + x_{11} + x_{10} + x_9$$

are first integrals of the system (4.3.3). Indeed by applying again Definition A.1.4:

$$\begin{aligned} L_{\mathbf{f}}(\varphi_1)(\mathbf{x}) &= D_{\mathbf{x}}(\varphi_1)\mathbf{f}(\mathbf{x}) = (1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 0, 0, 0, 0, 0) \begin{pmatrix} f_1(\mathbf{x}) \\ \vdots \\ f_{18}(\mathbf{x}) \end{pmatrix} \\ &= f_1(\mathbf{x}) + f_{12}(\mathbf{x}) + f_{13}(\mathbf{x}) = 0. \end{aligned}$$

$$\begin{aligned} L_{\mathbf{f}}(\varphi_2)(\mathbf{x}) &= D_{\mathbf{x}}(\varphi_2)\mathbf{f}(\mathbf{x}) = (0, 1, 0, 0, 0, 0, 1, 0, 0, 0, 0, 1, 0, 0, 1, 0, 0, 0) \begin{pmatrix} f_1(\mathbf{x}) \\ \vdots \\ f_{18}(\mathbf{x}) \end{pmatrix} \\ &= f_2(\mathbf{x}) + f_7(\mathbf{x}) + f_{12}(\mathbf{x}) + f_{15}(\mathbf{x}) = 0. \end{aligned}$$

$$\begin{aligned}
L_{\mathbf{f}}(\varphi_3)(\mathbf{x}) &= D_{\mathbf{x}}(\varphi_3)\mathbf{f}(\mathbf{x}) = (0, 0, 1, 0, 0, 0, 0, 1, 0, 1, 0, 0, 1, 1, 0, 1, 0, 0) \begin{pmatrix} f_1(\mathbf{x}) \\ \vdots \\ f_{18}(\mathbf{x}) \end{pmatrix} \\
&= f_3(\mathbf{x}) + f_8(\mathbf{x}) + f_{10}(\mathbf{x}) + f_{13}(\mathbf{x}) + f_{14}(\mathbf{x}) + f_{16}(\mathbf{x}) = 0.
\end{aligned}$$

$$\begin{aligned}
L_{\mathbf{f}}(\varphi_4)(\mathbf{x}) &= D_{\mathbf{x}}(\varphi_4)\mathbf{f}(\mathbf{x}) = (0, 0, 0, 1, 0, 0, 0, 1, 0, 1, 0, 0, 0, 0, 0, 0, 1, 0) \begin{pmatrix} f_1(\mathbf{x}) \\ \vdots \\ f_{18}(\mathbf{x}) \end{pmatrix} \\
&= f_4(\mathbf{x}) + f_8(\mathbf{x}) + f_{10}(\mathbf{x}) + f_{17}(\mathbf{x}) = 0.
\end{aligned}$$

$$\begin{aligned}
L_{\mathbf{f}}(\varphi_5)(\mathbf{x}) &= D_{\mathbf{x}}(\varphi_5)\mathbf{f}(\mathbf{x}) = (0, 0, 0, 0, 1, 0, 1, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 1) \begin{pmatrix} f_1(\mathbf{x}) \\ \vdots \\ f_{18}(\mathbf{x}) \end{pmatrix} \\
&= f_5(\mathbf{x}) + f_7(\mathbf{x}) + f_{14}(\mathbf{x}) + f_{18}(\mathbf{x}) = 0.
\end{aligned}$$

$$\begin{aligned}
L_{\mathbf{f}}(\varphi_6)(\mathbf{x}) &= D_{\mathbf{x}}(\varphi_6)\mathbf{f}(\mathbf{x}) = (0, 0, 0, 0, 0, 1, 0, 0, 1, 1, 1, 0, 0, 0, 0, 0, 0, 0, 0) \begin{pmatrix} f_1(\mathbf{x}) \\ \vdots \\ f_{18}(\mathbf{x}) \end{pmatrix} \\
&= f_6(\mathbf{x}) + f_9(\mathbf{x}) + f_{10}(\mathbf{x}) + f_{11}(\mathbf{x}) = 0.
\end{aligned}$$

By directly applying Proposition 4.3.3 and Remark A.1.5, we conclude that the solutions of the system (4.3.3) remain in the level set of $\varphi_j, j = 1, \dots, 6$ in which they start. Hence, $\varphi_j(x_i(t)), j = 1, \dots, 6$ are constant functions of t for all solutions and therefore bounded. Thus, the components $x_i(t), i = 1, \dots, 18$ are bounded. \square

Proposition 4.3.5. *Let $\varphi_7(\mathbf{x}) = x_6 + x_{10}, \varphi_8(\mathbf{x}) = x_3 + x_{13} + x_{14}, \varphi_9(\mathbf{x}) = x_4$ and $\varphi_{10}(\mathbf{x}) = x_5$ be scalar functions defined on \mathbb{R}^{18} . Then $L_{\mathbf{f}}(\varphi_i)(\mathbf{x}) \leq 0, i = 7, \dots, 10$.*

Proof.

$$\begin{aligned} L_{\mathbf{f}}(\varphi_7)(\mathbf{x}) &= D_{\mathbf{x}}(\varphi_7)\mathbf{f}(\mathbf{x}) = (0, 0, 0, 0, 0, 1, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0) \begin{pmatrix} f_1(\mathbf{x}) \\ \vdots \\ f_{18}(\mathbf{x}) \end{pmatrix} \\ &= f_6(\mathbf{x}) + f_{10}(\mathbf{x}) = -k_{14}x_{10} \leq 0, \text{ since } x_{10} \geq 0; \end{aligned}$$

$$\begin{aligned} L_{\mathbf{f}}(\varphi_8)(\mathbf{x}) &= D_{\mathbf{x}}(\varphi_8)\mathbf{f}(\mathbf{x}) \\ &= (0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 0, 0, 0, 0) \begin{pmatrix} f_1(\mathbf{x}) \\ \vdots \\ f_{18}(\mathbf{x}) \end{pmatrix} \\ &= -(k_{12}x_{13} + k_{13}x_{14}) \leq 0, \text{ since } x_{13}, x_{14} \geq 0; \end{aligned}$$

$$\begin{aligned} L_{\mathbf{f}}(\varphi_9)(\mathbf{x}) &= D_{\mathbf{x}}(\varphi_9)\mathbf{f}(\mathbf{x}) \\ &= (0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0) \begin{pmatrix} f_1(\mathbf{x}) \\ \vdots \\ f_{18}(\mathbf{x}) \end{pmatrix} \\ &= x_4(-k_1x_{16} - k_2x_9) \leq 0, \text{ since } x_4, x_9, x_{16} \geq 0; \end{aligned}$$

$$\begin{aligned} L_{\mathbf{f}}(\varphi_{10})(\mathbf{x}) &= D_{\mathbf{x}}(\varphi_{10})\mathbf{f}(\mathbf{x}) \\ &= (0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0) \begin{pmatrix} f_1(\mathbf{x}) \\ \vdots \\ f_{18}(\mathbf{x}) \end{pmatrix} \\ &= x_5(-k_3x_{16} - k_4x_9) \leq 0, \text{ since } x_5, x_9, x_{16} \geq 0. \end{aligned}$$

□

Remark 4.3.6. As sum of one or more nonnegative functions $x_i(t)$, $i = 1, \dots, 18$, the functions $\varphi_j(\mathbf{x}) \geq 0$, $j = 7, \dots, 10$. Together with the previous result, we conclude that the functions φ_j are non increasing and bounded for $j = 7, \dots, 10$ along a trajectory. That is, there are constants β_j such that $0 \leq \varphi_j(\mathbf{x}) \leq \beta_j$, $j = 7, \dots, 10$. Hence every trajectory remains in the subset of \mathcal{P} defined by $\varphi_j(\mathbf{x}) \leq \beta_j$, which is bounded.

Proposition 4.3.7. *The positive limit set $\omega(\mathbf{y})$ of (4.3.3) is contained in the set*

$$\begin{aligned} N := \{ \mathbf{x} \in \mathcal{P} : x_{10} = 0, \quad x_1 + x_{12} + x_{13} = x_1(0), \quad x_2 + x_{12} + x_{15} + x_7 + x_{14} = x_2(0), \\ x_6 + x_{11} + x_{10} + x_9 = x_6(0), \quad x_3 + x_{13} + x_{14} + x_{16} + x_8 + x_{10} = x_3(0), \\ x_4 + x_{17} + x_8 + x_{10} = x_4(0), \quad x_5 + x_{18} + x_7 + x_{14} = x_5(0), x_{13} = 0, x_{14} = 0 \}. \end{aligned}$$

Proof. The two previous results together with Theorem A.3.11 imply that the compact sets $M_\alpha := \{\mathbf{x} \in \mathcal{P} : \varphi_j(\mathbf{x}) \leq \alpha, j = 1, \dots, 10\}$ are positively invariant for all $\alpha \in [0, \beta_j)$. Theorem A.3.15 guarantees that the solution of the initial value problem exists on $[0, \infty)$. Moreover, this solution approaches its positive limit set $\omega(\mathbf{y})$, as $t \rightarrow \infty$. So, $\omega(\mathbf{y})$ is nonempty, compact and connected. By Theorem A.3.16 $\omega(\mathbf{y})$ is also invariant.

Hence, by applying LaSalle's principle stated in Theorem A.4.1 we conclude that

$$\omega(\mathbf{y}) \subset N.$$

□

Remark 4.3.8. The conditions defining N are not enough to prove that any solution in N is stationary. This set contains invariant sets other than the set of stationary points.

In the sequel we derive further conditions to be satisfied by the set of equilibrium points.

Deriving further conditions for the set of equilibrium points

While trying to find new conditions to be satisfied by an equilibrium point, one can easily see that the value of some variables at the equilibrium depend on other variables and their value at the equilibrium.

In order to gain more insights, we set the right-hand side of the system (4.3.3) equal to zero and solve for $x_i, i = 1, \dots, 18$ by using the modules `TriSer` and `Tsolve` implemented in the software package `epsilon` of MAPLE 10 (see Appendix C for a brief explanation). Altogether, 34 different sets were obtained but only 4 of them were relevant for further analysis. The excluded sets contained either negative values for some variable $x_i, i = 1, \dots, 18$ or $x_1 = 0$. The sets with $x_1 = 0$ are those for which the constant solution is obtained and this justifies the exclusion.

The selected sets are:

$$E_1 := \{\mathbf{x} \in \overline{\mathcal{P}} : x_2 = 0, x_3 = 0, x_4 = 0, x_5 = 0, x_6 = 0, x_7 = \frac{k_7 x_{18} x_{15}}{k_9},$$

$$x_8 = \frac{k_8 x_{16} x_{17}}{k_{10}}, x_{10} = 0, x_{11} = 0, x_{12} = 0, x_{13} = 0, x_{14} = 0\}$$

$$E_2 := \{\mathbf{x} \in \overline{\mathcal{P}} : x_2 = 0, x_3 = 0, x_4 = 0, x_5 = 0, x_7 = \frac{k_7 x_{18} x_{15}}{k_9},$$

$$x_8 = 0, x_{10} = 0, x_{12} = 0, x_{13} = 0, x_{14} = 0, x_{17} = 0\}.$$

$$E_3 := \{\mathbf{x} \in \overline{\mathcal{P}} : x_2 = 0, x_3 = 0, x_4 = 0, x_5 = 0, x_7 = \frac{k_7 x_{18} x_{15}}{k_9}, \\ x_8 = 0, x_{10} = 0, x_{12} = 0, x_{13} = 0, x_{14} = 0, x_{16} = 0\}.$$

$$E_4 := \{\mathbf{x} \in \overline{\mathcal{P}} : x_2 = 0, x_3 = 0, x_7 = \frac{k_7 x_{18} x_{15}}{k_9}, x_8 = 0, \\ x_9 = 0, x_{10} = 0, x_{12} = 0, x_{13} = 0, x_{14} = 0, x_{16} = 0\}.$$

The only set that matches the numerical results is E_1 . In the other three sets $x_8 = 0$ and this only happens at $t = 0$. However, at the beginning we have $x_2 \neq 0$. This yields a contradiction and constitutes the first sign of instability.

The following proposition holds:

Proposition 4.3.9. *Any solution $z(t)$ starting in N given in Proposition 4.3.7 is stationary if $z_{15}(t), z_{16}(t), z_{17}(t)$ and $z_{18}(t)$ are stationary.*

Proof. Let $z(t)$ be a solution in N satisfying the given conditions, i. e.

$$z(t) = (z_1(t), z_2(t), z_3(t), z_4(t), z_5(t), z_6(t), z_7(t), z_8(t), z_9(t), 0, z_{11}(t), z_{12}, 0, 0, \\ z_{15}(t), z_{16}(t), z_{17}(t), z_{18}(t))^T.$$

Thus,

$$\dot{z}(t) = (\dot{z}_1, \dot{z}_2, \dot{z}_3, \dot{z}_4, \dot{z}_5, \dot{z}_6, \dot{z}_7, \dot{z}_8, \dot{z}_9, 0, \dot{z}_{11}, \dot{z}_{12}, 0, 0, 0, 0, 0, 0)^T.$$

$\dot{z}_{10}(t) = 0$ implies $k_6 z_8 z_6 = 0$. Since $z_8 \neq 0$ it follows $z_6 = 0$ and $\dot{z}_6(t) = 0$.

Conditions $\dot{z}_{13}(t) = 0$ and $\dot{z}_{14}(t) = 0$ imply $k_6 z_1 z_3 = 0$ and $k_6 z_7 z_3 = 0$.

Since $z_1 \neq 0$ it holds $z_3(t) = 0$ and consequently $\dot{z}_3(t) = 0$.

$z_{18}(t), z_{15}(t)$ stationary imply $z_7(t)$ stationary and $\dot{z}_7(t) = 0$.

As $z_5 + z_{18} + z_7 = c_5$ then $z_5(t)$ is constant and therefore $\dot{z}_5(t) = 0$.

On the other hand, $\dot{z}_{15}(t) = 0$ implies $k_{11} z_{12} + k_{15} z_2 z_{16} = 0$. That is $z_{12} = 0$ and $z_2 z_{16} = 0$ because of positivity. But, $z_{12} = 0$ implies $\dot{z}_{12}(t) = 0$ and $z_2 = 0$ as a consequence. I. e., $\dot{z}_2(t) = 0$. Furthermore, z_1 must be constant because $z_{12} = 0$ and $\dot{z}_1(t) = 0$ follows.

$z_{16}(t), z_{17}(t)$ stationary imply z_8 constant and $\dot{z}_8(t) = 0$. Moreover, z_4 is also constant and $\dot{z}_4(t) = 0$ holds.

A positivity argument can be used to justify $z_9 = 0$ and $\dot{z}_9(t) = 0$. This implies in particular that $k_5 z_8 z_{11} = 0$ or $\dot{z}_{11}(t) = 0$.

Altogether, $\dot{z}(t) = (0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)^T$ and $z(t)$ is stationary.

□

For practical reasons, the stability question will be handled after reducing the number of equations to 12, since the Jacobian at the equilibrium will have eigenvalues only with real part different from zero.

4.3.4 Model reduction

Let us now reduce the number of equations of the system (4.3.3) to 12. From the first integrals given in Proposition 4.3.4 we eliminate the variables x_1, x_2, x_3, x_4, x_6 and x_7 . This yields a system of differential equations where all the variables correspond to activated substances and this is important, because we can only control activated substances. On the other hand, the equation for factor *VIII* is included, so we are able to study one of the types of haemophilia from the model more directly.

Setting

$$\begin{aligned} x_1 &= -x_{12} - x_{13} + c_1; \\ x_2 &= -x_{12} - x_{15} + x_5 + x_{18} + c_2; \\ x_3 &= -x_{13} - x_{14} - x_{16} - x_8 - x_{10} + c_3; \\ x_4 &= -x_{17} - x_8 - x_{10} + c_4; \\ x_6 &= -x_{11} - x_{10} - x_9 + c_6; \\ x_7 &= -x_5 - x_{18} - x_{14} + c_5, \end{aligned}$$

where $c_1 = x_1(0); c_2 = x_2(0); c_3 = x_3(0); c_4 = x_4(0); c_5 = x_5(0)$ and $c_6 = x_6(0)$, are different from zero,

and making the following coordinate transformation:

$$y_1 := x_5; y_2 := x_8; y_3 := x_9; y_4 := x_{10}; y_5 := x_{11}; y_6 := x_{12}; y_7 := x_{13}; y_8 := x_{14}; y_9 := x_{15}; y_{10} := x_{16}; y_{11} := x_{17}; y_{12} := x_{18};$$

we obtain the following system of differential equations:

$$\frac{dy_1}{dt} = -k_3 y_1 y_{10} - k_4 y_1 y_3 \tag{4.3.4}$$

$$\frac{dy_2}{dt} = k_8 y_{10} y_{11} - k_{10} y_2 + k_{19} y_4 - k_6 y_2 (-y_5 - y_4 - y_3 + c_6) + k_{14} y_4 \quad (4.3.5)$$

$$\frac{dy_3}{dt} = k_5 y_2 y_5 \quad (4.3.6)$$

$$\frac{dy_4}{dt} = k_6 y_2 (-y_5 - y_4 - y_3 + c_6) - k_{19} y_4 - k_{14} y_4 \quad (4.3.7)$$

$$\frac{dy_5}{dt} = k_{14} y_4 - k_5 y_2 y_5 \quad (4.3.8)$$

$$\begin{aligned} \frac{dy_6}{dt} = & k_6 (-y_6 - y_7 + c_1) (-y_6 - y_9 + y_1 + y_{12} - c_5 + c_2) - \\ & -k_{16} y_6 - k_{11} y_6 \end{aligned} \quad (4.3.9)$$

$$\begin{aligned} \frac{dy_7}{dt} = & k_6 (-y_6 - y_7 + c_1) (-y_7 - y_8 - y_{10} - y_2 - y_4 + c_3) - \\ & -k_{17} y_7 - k_{12} y_7 \end{aligned} \quad (4.3.10)$$

$$\begin{aligned} \frac{dy_8}{dt} = & k_6 (-y_1 - y_{12} - y_8 + c_5) (-y_7 - y_8 - y_{10} - y_2 - y_4 + c_3) - \\ & -k_{18} y_8 - k_{13} y_8 \end{aligned} \quad (4.3.11)$$

$$\begin{aligned} \frac{dy_9}{dt} = & k_9 (-y_1 - y_{12} - y_8 + c_5) - k_7 y_{12} y_9 + k_{11} y_6 + \\ & k_{15} (-y_6 - y_9 + y_1 + y_{12} - c_5 + c_2) y_{10} \end{aligned} \quad (4.3.12)$$

$$\frac{dy_{10}}{dt} = k_{10} y_2 - k_8 y_{10} y_{11} + k_{12} y_7 + k_{13} y_8 \quad (4.3.13)$$

$$\frac{dy_{11}}{dt} = k_{10} y_2 - k_8 y_{10} y_{11} + k_1 (-y_{11} - y_2 - y_4 + c_4) y_{10} +$$

$$k_2(-y_{11} - y_2 - y_4 + c_4)y_3 \quad (4.3.14)$$

$$\frac{dy_{12}}{dt} = k_9(-y_1 - y_{12} - y_8 + c_5) - k_7y_{12}y_9 + k_3y_1y_{10} + k_4y_1y_3 \quad (4.3.15)$$

Since this set of equations was obtained from a coordinate transformation of the system (4.3.3) it follows that the equilibrium point belongs to the set

$$E_5 := \{\mathbf{y} \in \mathbb{R}^{12} : y_1 = 0, y_2 = \frac{k_8}{k_{10}}y_{10}y_{11}, y_4 = 0, y_5 = 0, y_6 = 0, y_7 = 0, y_8 = 0\}.$$

Substituting these conditions in the system of equations (4.3.4) to (4.3.15) it is easy to see that we obtain a zero of the functions defined by the right-hand side of equations (4.3.4), (4.3.6), (4.3.8) and (4.3.13).

Further conditions can be deduced by setting to zero the right-hand side of the remaining equations evaluated at a point of E_5 . Systematically we obtain:

- from (4.3.7)

$$y_3 = c_6.$$

This condition is also necessary for the right-hand side of (4.3.5) to be equal zero;

- from (4.3.10)

$$\frac{dy_7}{dt} = 0 \Leftrightarrow y_6 = c_1 \text{ or } y_2 + y_{10} = c_3.$$

Since $c_1 \neq 0$, it holds $y_2 + y_{10} = c_3$ or $y_{10} = \frac{c_3k_{10}}{k_8y_{11} + k_{10}}$.

Furthermore, this condition also sets the right-hand side of (4.3.11) to zero.

- from (4.3.14) we have

$$\frac{dy_{11}}{dt} = 0 \Leftrightarrow (-y_{11} - y_2 + c_4)(k_1y_{10} + k_2y_3) = 0 \Leftrightarrow y_{11} + y_2 = c_4,$$

because $k_1y_{10} + k_2y_3 \neq 0$, since $k_1, k_2, k_3, y_{10} > 0$.

The equilibrium point must still set to zero the right-hand side of

$$\frac{dy_6}{dt} = k_6c_1(-y_9 + y_{12} - c_5 + c_2) \quad (4.3.16)$$

$$\frac{dy_9}{dt} = k_9(-y_{12} + c_5) - k_7y_{12}y_9 + k_{15}(-y_9 + y_{12} - c_5 + c_2)y_{10} \quad (4.3.17)$$

$$\frac{dy_{12}}{dt} = k_9(-y_{12} + c_5) - k_7 y_{12} y_9. \quad (4.3.18)$$

By (4.3.16) we have

$$\frac{dy_6}{dt} = 0 \Leftrightarrow y_{12} - y_9 = c_5 - c_2. \quad (4.3.19)$$

By (4.3.18) we obtain

$$\frac{dy_{12}}{dt} = 0 \Leftrightarrow y_{12} = \frac{k_9 c_5}{k_9 + k_7 y_9}. \quad (4.3.20)$$

If (4.3.19) and (4.3.20) are simultaneously satisfied it follows immediately $\frac{dy_9}{dt} = 0$.

Moreover, substituting (4.3.19) in (4.3.17) and solving for y_9 we obtain

$$y_9 = \frac{-(k_9 + k_7(c_5 - c_2)) \pm \sqrt{(k_9 + k_7(c_5 - c_2))^2 + 4k_7 k_9 c_2}}{2k_7}.$$

Taking the physiological meaning into account, $c_5 \leq c_2$ and $-(k_9 + k_7(c_5 - c_2))$ is a number between 0 and 1. Thus $y_9 > 0$.

Remark 4.3.10. From the considerations done in Chapter 1 we observe that if $c_5 = 0$, we are in the presence of hemophilia A and if $c_2 = 0$ of hemophilia B. So, some reactions will not take place.

Remark 4.3.11. Substituting the values given in Table 3.2 and $c_i, i = 1, \dots, 5$ by the respective initial concentrations we obtain for the equilibrium point the same values like the ones that can be deduced by visual inspection of the numerical solution.

Remark 4.3.12. Altogether we obtained the coordinates of the unique equilibrium point towards which the system comprising equations (4.3.4) to (4.3.15) converges. Thus, by applying Theorem A.4.1 follows asymptotic stability of the equilibrium point of this system.

In the sequel we substitute the values of the constants $k_i, i = 1, \dots, 20$ given in [JoMa94] in the system with the 12 equations and linearize the system. Since some of these constants were not measured (see Section 3.2), we could use the Jacobian matrix of the 12 equation system to make parameter identification. So, we give some information regarding this.

4.3.5 Linearization around an equilibrium point. Parameter identification by using the Jacobian

The general expression of the Jacobian at the equilibrium point of the system of equations (4.3.4) to (4.3.15) is given by (4.3.21).

Substituting the values of the constants, we obtain the representation of the matrix A given in (4.3.22).

The eigenvalues of this matrix are:

$-104,147; -67,64; -45.2; -31.38; -29.68; -25.29;$
 $-8.30; -0.90; -0.89; -0.3099; -0.031; -0.023.$

Thus, the matrix is stable, but there are reactions occurring in different time scales.

As before, we can additionally say more about the nature of the reaction mechanism, as a positive entry in the Jacobian matrix A_{ij} indicates that near steady state an increase in a substance y_j gives rise to production of y_i , whereas negative elements in the matrix indicate that y_i is removed as a direct result of an increase in y_j , indicating an inhibitory interaction. If the elements in the principal diagonal are positive then we can speak about autocatalysis. A zero element of the matrix indicates that there is no *direct* interaction from y_j to y_i . Indirect interactions can also be inferred: if y_i acts on y_j which acts on y_k , then y_i acts indirectly on y_k whereas the sign of this indirect influence is determined by the signs of the direct steps. Moreover, these steps can be interpreted in terms of positive and negative feedback loops [CSM04].

One of the possibilities to determine the properties of the Jacobian is to use perturbation to system parameters [CSM04]. But, before proceeding to parameter identification by using the Jacobian there are some important points to consider.

In that context, it should be clear whether it is necessary to measure the concentration of all species, or whether the measurement of a subset of the constituent species is sufficient to determine system properties. Furthermore, since the most direct approach to evaluating the Jacobian matrix for a biochemical network is to experimentally perturb one or more concentrations from steady state and monitor the response of each of the chemical species as the system relaxes, one should pick up a perturbation strategy. In order to achieve this, one should decide whether all or just a subset of the species need to be perturbed and, whether random perturbations are applied at regular intervals or a uniform perturbation is applied at irregular time intervals.

$$A = \begin{pmatrix} a_1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & a_3 & a_6 & a_8 & a_6 & 0 & 0 & 0 & 0 & a_{13} & a_{14} & 0 & 0 \\ 0 & 0 & 0 & 0 & \frac{k_5}{k_6}a_6 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -a_6 & -a_8 & -a_6 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & a_9 & -\frac{k_5}{k_6}a_6 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ a_2 & 0 & 0 & 0 & 0 & a_2 - k_{16} - k_{11} & 0 & 0 & -a_2 & 0 & 0 & 0 & a_2 \\ 0 & -a_2 & 0 & -a_2 & 0 & a_{11} & a_{18} & -a_2 & 0 & -a_2 & 0 & 0 & 0 \\ a_{11} & a_4 & 0 & a_4 & 0 & 0 & a_4 & a_{19} & 0 & a_4 & 0 & 0 & a_{11} \\ -k_9 + a_{10} & 0 & 0 & 0 & 0 & k_{11} - a_{10} & 0 & -k_9 & a_{12} - a_{10} & 0 & 0 & 0 & a_{16} \\ 0 & -a_3 & 0 & 0 & 0 & 0 & k_{12} & k_{13} & 0 & -a_{13} & -a_{14} & 0 & 0 \\ 0 & -a_3 + a_5 & a_7 & a_5 & 0 & 0 & 0 & 0 & 0 & -a_{13} + \frac{k_1}{k_2}a_7 & a_{15} & 0 & 0 \\ -k_9 - a_1 & 0 & 0 & 0 & 0 & 0 & 0 & -k_9 & -a_{12} & 0 & 0 & 0 & a_{17} \end{pmatrix}. \quad (4.3.21)$$

$$a_1 = -k_3 y_{10}(eq) - k_4 c_6;$$

$$a_2 = k_6 c_1;$$

$$a_3 = -k_{10};$$

$$a_4 = -k_6(-y_9(eq) + c_2);$$

$$a_5 = -k_1 y_{10}(eq) - k_2 c_6;$$

$$a_6 = k_6 \frac{k_8}{k_{10}} y_{10}(eq) y_{11}(eq);$$

$$a_7 = k_2(-y_{11}(eq) - \frac{k_8}{k_{10}} y_{10}(eq) y_{11}(eq) + c_4);$$

$$a_8 = k_{19} + a_6 + a_9;$$

$$a_9 = k_{14}$$

$$a_{10} = k_{15} y_{10}(eq);$$

$$a_{11} = -k_6(-y_{10}(eq) - \frac{k_8}{k_{10}} y_{10}(eq) y_{11}(eq) + c_3);$$

$$a_{12} = -k_7(y_9(eq) + c_5 - c_2);$$

$$a_{13} = k_8 y_{11}(eq);$$

$$a_{14} = \frac{k_8}{k_{15}} a_{10};$$

$$a_{15} = -\frac{k_8}{k_{15}} a_{10} - \frac{k_1}{k_{15}} a_{10} - k_2 c_6;$$

$$a_{16} = -k_9 - k_7 y_9(eq) + a_{10};$$

$$a_{17} = -k_9 - k_7 y_9(eq);$$

$$a_{18} = a_{11} - a_2 - k_{12} - k_{17};$$

$$a_{19} = a_{11} + a_4 - k_{18} - k_{13}.$$

⁰ $y_i(eq)$ denotes the concentration of the variable y_i at the equilibrium point

$$A = \begin{pmatrix} -29.68 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & -0.4 & 3.1 & 105.1 & 3.1 & 0 & 0 & 0 & 0 & 0.0776 & 67.2 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.31 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -3.1 & -105.1 & -3.1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 32 & -0.31 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0.0005 & 0 & 0 & 0 & 0 & -25.2995 & 0 & 0 & -0.0005 & 0 & 0 & 0 & 0.0005 \\ 0 & -0.0005 & 0 & -0.0005 & 0 & -0.1 & -45.25 & -0.0005 & 0 & -0.005 & 0 & 0 & 0 \\ -0.1 & -0.03 & 0 & -0.03 & 0 & 0 & -0.03 & -8.331 & 0 & -0.003 & 0 & 0 & -0.1 \\ 0.0118 & 0 & 0 & 0 & 0 & 0.28232 & 0 & -0.005 & -0.0208 & 0 & 0 & 0 & -0.8782 \\ 0 & 0.4 & 0 & 0 & 0 & 0 & 1.15 & 8.2 & 0 & -0.0776 & -67.2 & 0 & 0 \\ 0 & -30.96 & 0.01612 & -31.36 & 0 & 0 & 0 & 0 & 0 & -0.06148 & -98.56 & 0 & 0 \\ 29.675 & 0 & 0 & 0 & 0 & 0 & 0 & -0.005 & -0.004 & 0 & 0 & 0 & -0.895 \end{pmatrix}. \quad (4.3.22)$$

One in all, if we assume that a reaction mechanism involving n species is described by the system of equations

$$\frac{dy_i}{dt} = f_i(y_1, \dots, y_n; p_1, \dots, p_{n_p}), i = 1, \dots, n,$$

where y_i is the concentration of the i -th chemical species and f_i is the in general nonlinear function describing the production and consumption of y_i . Expanding in a Taylor series about the steady state \mathbf{y}^* , the system kinetics is determined by the system of equations

$$\frac{u_i}{dt} = \sum_{j=1}^n \left(\frac{\partial f_i}{\partial y_j} \right)_{\mathbf{y}^*} u_j + o(|\mathbf{u}|), i = 1, \dots, n, \quad (4.3.23)$$

where $u_i(t) = y_i(t) - y_i^*$. For the linear system

$$\frac{u_i}{dt} = \mathbf{J}_i \mathbf{u}(t)$$

arising from (4.3.23), where the vector \mathbf{J}_i is the i -th row of the Jacobian matrix, the identification problem is essentially an exercise in linear regression.

Notice that the steady state concentration must be known in order to calculate $\mathbf{u} = \mathbf{y} - \mathbf{y}^*$.

The system comprising equations (4.3.4) to (4.3.15) is polynomial. Thus, the parameters enter linearly and the model can be fitted to the data using least squares and singular value decomposition. Because of the large number of parameters to be fitted that multivariate polynomial models may comprise, this kind of model have limited usefulness. However, the analysis made in this chapter and the information given in Section 3.2 allows to reduce to three the number of constants to be identified, namely k_5 , k_{15} and k_6 . The remaining reaction constants have been already published and validated through experimental data. Moreover, given the representation (4.3.21) of the Jacobian at the equilibrium point, we observe that the entries of the matrix are influenced by the steady state concentration of the variables y_9 , y_{10} and y_{11} and initial concentration of 6 variables, that can be used as control variables since they represent the physiologic concentration of some of the factors involved in the process of blood coagulation system.

Chapter 5

Steering Stortelder's Model

As there are some substances like heparin capable of influencing the action of substances present in the blood, like *ATIII*, one of the goals of this work was to verify whether it is possible to steer or influence the blood coagulation system by means of a control function. Those variables are modelled as input variables of the system. It turned out that the linearized system is not completely controllable. So we investigate whether the system is flat and describe briefly how flatness can be used to solve the control problem.

5.1 Stating the control problem of the linearized system

In this section we state the control problem for the system (4.2.1) and prove that the linearized system around the equilibrium point is not completely controllable. However we could identify a controllable subspace and give an example using the constants given in Table 3.1. Nevertheless, the system is completely observable.

The variables $y_2(t)$ and $y_5(t)$ describe the behavior of the activated forms of factor *X* and factor *II*, respectively. These substances belong to the same stoichiometric class, so it is possible to influence the course of their concentrations with time by using drops of the same substance. In the sequel we denote the concentration of this substance by the scalar function u .

Let therefore $\mathbf{y} \in \mathbb{R}^5$ denote the state vector of the system (4.2.1) and $u \in \mathbb{R}$

denote the control variable. Consider furthermore the matrix $B \in \mathbb{R}^{(5,1)}$ given by

$$B = \begin{pmatrix} 0 \\ 1 \\ 0 \\ 0 \\ 1 \end{pmatrix}.$$

Let furthermore \mathbf{f} be the function defined by the right-hand side of (4.2.1). Then \mathbf{f} is of class C^∞ and the nonlinear initial value control problem can be stated as

$$\dot{\mathbf{y}} = \mathbf{f}(\mathbf{y}) + Bu, \text{ with } \mathbf{y}(0) = \mathbf{y}_0.$$

The equilibrium point \mathbf{y}^* can be shifted to the origin by introducing the new variable $\xi := \mathbf{y} - \mathbf{y}^*$.

The Taylor expansion around \mathbf{y}^* yields:

$$\dot{\mathbf{y}} = \dot{\xi} = \mathbf{f}(\mathbf{y}^* + \xi) + Bu = A\xi + Bu + o(\xi),$$

where A is the Jacobian of the system at \mathbf{y}^* having the representation given in (4.2.3).

By neglecting the terms of order $o(\xi)$ we obtain linear approximations ξ_l for ξ and the following initial value problem:

$$\dot{\xi}_l = A\xi_l + Bu, \text{ with } \xi_l(0) = \mathbf{y}_0 - \mathbf{y}^*. \quad (5.1.1)$$

Proposition 5.1.1. *The system (5.1.1) is not completely controllable.*

Proof. Since $\text{rank } B = 1$ we check the controllability of the system (5.1.1) by using Corollary B.1.8.

Let $U = [B, AB, A^2B, A^3B, A^4B]$ be the controllability matrix, where

$$A = \begin{pmatrix} a_1 & 0 & 0 & 0 & 0 \\ -a_1 & a_2 & 0 & a_3 & 0 \\ 0 & 0 & 0 & 0 & a_4 \\ 0 & a_5 & 0 & a_3 & 0 \\ 0 & a_6 & 0 & a_7 & a_8 \end{pmatrix} \text{ and } B = \begin{pmatrix} 0 \\ 1 \\ 0 \\ 0 \\ 1 \end{pmatrix}.$$

Then U has the following representation:

$$U = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 \\ 1 & a_2 & a_2^2 + a_3a_5 & (a_2^2 + a_3a_5)a_2 + (a_2a_4 + a_3^2)a_5 & \star \\ 0 & a_4 & a_4a_6 + a_8a_4 & a_4a_6a_2 + a_4a_7a_5 + a_4a_8a_6 + a_4a_8^2 & \star \\ 0 & a_5 & a_5a_2 + a_3a_5 & (a_5a_2 + a_3a_5)a_2 + (a_3a_5 + a_3^2)a_5 & \star \\ 1 & a_6 + a_8 & a_6a_2 + a_7a_5 + a_8a_6 + a_8^2 & (a_6a_2 + a_7a_5 + a_8a_6)a_2 + \\ & & & + (a_6a_3 + a_7a_3 + a_8a_7)a_5 + a_8^2a_6 + a_8^3 & \star \end{pmatrix}.$$

$\text{rank } U \leq 4 < n$ and as a consequence the system (5.1.1) is not completely controllable. \square

Remark 5.1.2. This result means in particular that there might be some reaction missing that influences the controllability of the system and that this linear system cannot be influenced only by using a single control substance.

Remark 5.1.3. MAPLE calculates the rank symbolically and generically yielding $\text{rank } U = 4$, but there are certain choices of the values of the constants $a_i, i = 1, \dots, 8$ such that $\text{rank } U < 4$. It is easy to find general conditions for $\text{rank } U \geq 2$, however to exclude the possibility that $\text{rank } U \neq 3$ is not a simple matter due to the complexity of some of the elements of this matrix. On the other hand, there are not so many constellations of values that would generate linearly dependent rows or columns. And, for a given set of starting values, the probability that this happens is almost zero. So, we may state that $\text{rank } U = 4$ almost surely.

If $\text{rank } U = 4$ then, by Lemma B.1.9, it is possible to find a controllable subspace of dimension 4.

Let $T := \begin{pmatrix} 0 & 0 & 0 & 0 & 1 \\ 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 1 & 0 & 0 & 0 & 0 \end{pmatrix} \in GL(5)$. Then $T^{-1} := T$ and we obtain

$$\tilde{A} = T^{-1}AT = \begin{pmatrix} a_8 & a_6 & 0 & a_7 & 0 \\ 0 & a_2 & 0 & a_3 & -a_1 \\ a_4 & 0 & 0 & 0 & 0 \\ 0 & a_5 & 0 & a_3 & 0 \\ 0 & 0 & 0 & 0 & a_1 \end{pmatrix} \quad \tilde{B} = T^{-1}B = \begin{pmatrix} 1 \\ 1 \\ 0 \\ 0 \\ 0 \end{pmatrix}.$$

Thus,

$$A_1 = \begin{pmatrix} a_8 & a_6 & 0 & a_7 \\ 0 & a_2 & 0 & a_3 \\ a_4 & 0 & 0 & 0 \\ 0 & a_5 & 0 & a_3 \end{pmatrix} \quad \text{and} \quad B_1 = \begin{pmatrix} 1 \\ 1 \\ 0 \\ 0 \end{pmatrix}.$$

By Lemma B.1.9 it follows immediately:

Proposition 5.1.4. *If $\text{rank } U = 4$ then the pair $[A_1, B_1]$ is controllable.*

This means, in particular, that there is a possibility of influencing the latter system through the inputs [Sw84].

Example 5.1.5. Suppose that we administrate a substance acting on the activated factors X_a and II_a in one unity dose and want to see how long does it take until

an equilibrium is reached. In other words, we apply several impulses to the system and want to know the response of the system to these impulses. Therefore, we consider as control function the unit pulse function

$$\delta_T(t) = \begin{cases} 0 & \text{if } t \leq 0 \\ \frac{1}{T} & \text{if } 0 < t < T \\ 0 & \text{if } t \geq T, \end{cases}$$

where T denotes the time at which an impulse is applied.

We may hypothetically be interested in increasing the maximal concentration of thrombin in about 20 % and change the value at the equilibrium. We achieve this by steering the system by an impulse of $0.045\mu\text{mol/L}$ of the control substance administrated in every minute. The course of thrombin concentration as a function of time corresponding to this situation is plotted in the following figure:

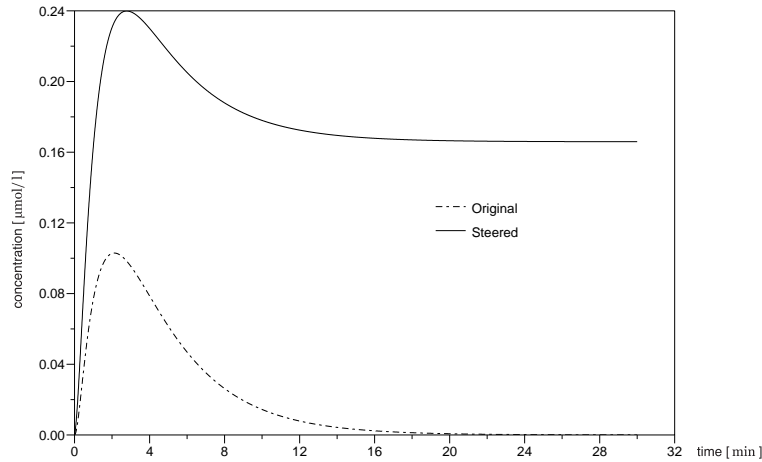


Figure 5.1: Comparison between the course of the concentration of thrombin as a function of time before and after the system was influenced by a control substance acting in the system.

The hypothetical inactivation of $ATIII$ and α_2M in one minute step and the increase of the maximum concentration of thrombin until a maximum of $1.4\mu\text{mol/L}$ is reached is illustrated in Figure 5.2. This effect can be achieved after steering the system by an impulse of $0.37\mu\text{mol/L}$ of the control substance administrated in every minute.

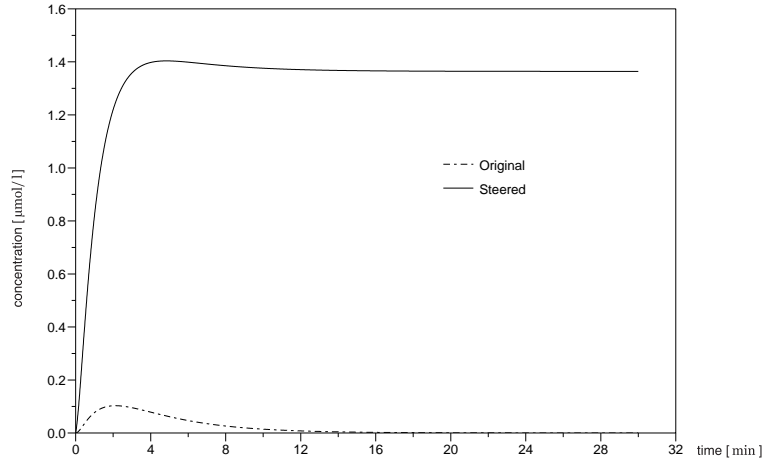


Figure 5.2: Comparison between the course of the concentration of thrombin as a function of time before and after hypothetical inactivation of $ATIII$ and α_2M .

Observability and detectability

Observability of the systems means that it is possible to reconstruct each state of the system from the outputs [Sw84].

Theorem 5.1.6. *The system (5.1.1) with output η*

$$\eta = C\xi_t, \quad C \in \mathbb{R}^{(4,5)}$$

with $C = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{pmatrix}$ is completely observable.

Proof. Because the system is time-invariant, we prove that the system is completely observable by using item (i) of Theorem B.1.1.

Let $V = \begin{pmatrix} C \\ CA \\ CA^2 \\ CA^3 \\ CA^4 \end{pmatrix}$ be the observability matrix.

Since

$$A = \begin{pmatrix} a_1 & 0 & 0 & 0 & 0 \\ -a_1 & a_2 & 0 & a_3 & 0 \\ 0 & 0 & 0 & 0 & a_4 \\ 0 & a_5 & 0 & a_3 & 0 \\ 0 & a_6 & 0 & a_7 & a_8 \end{pmatrix} \text{ and } C = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{pmatrix}$$

the observability matrix has size 20 by 5.

As $\text{rank } C = 4$ it holds $\text{rank } V \geq 4$. I. e. $\text{rank } V = 4$ or $\text{rank } V = 5$.

$$\text{Since } CA = \begin{pmatrix} a_1 & 0 & 0 & 0 & 0 \\ -a_1 & a_2 & 0 & a_3 & 0 \\ 0 & 0 & 0 & 0 & a_4 \\ 0 & a_6 & 0 & a_7 & a_8 \end{pmatrix} \text{ and } a_3 = -k_5 < 0 \text{ then } \text{rank } V = 5.$$

Thus, the system is observable and the assertion follows. \square

Remark 5.1.7. The pair $[A_1, B_1]$ with output $\tilde{\eta} = \tilde{C}\tilde{\xi}_l, \tilde{\xi}_l \in \mathbb{R}^4$ is also completely observable. Thereby, we may consider $\tilde{C} = I_4$.

5.2 Flatness. Application to motion planning

Since the linearization of the system (4.2.1) is not completely controllable, let us check if the system obtained from (4.1.1) after reduction by using the linear first integrals given in (4.1.2) is flat. Furthermore, we describe briefly how flatness can be used for solving the control problem.

For convenience, let the vector \mathbf{x} denote the state vector. Thus, $x_1 = [X]$; $x_2 = [X_a]$; $x_3 = [V]$; $x_4 = [PL]$; $x_5 = [II]$ and $x_6 = [II_a]$.

The coordinate transform yields the following system of 6 equations:

$$\begin{aligned} \frac{dx_1}{dt} &= -\frac{k_1 x_1 R V V}{k_2 + x_1} \\ \frac{dx_2}{dt} &= \frac{k_1 x_1 R V V}{k_2 + x_1} - k_3 x_2 - k_4 (x_4 - x_3 - 0.02) x_2 x_4 + k_5 (-x_4 + 0.05) \\ \frac{dx_3}{dt} &= -\frac{k_6 x_3 x_6}{k_7 + x_3} \\ \frac{dx_4}{dt} &= -k_4 (x_4 - x_3 - 0.02) x_2 x_4 + k_5 (-x_4 + 0.05) \\ \frac{dx_5}{dt} &= -\frac{k_8 x_5 (-x_4 + 0.05)}{k_9 + x_5} - \frac{k_{10} x_5 x_2}{k_{11} + x_5} \\ \frac{dx_6}{dt} &= \frac{k_8 x_5 (-x_4 + 0.05)}{k_9 + x_5} + \frac{k_{10} x_5 x_2}{k_{11} + x_5} - k_{12} x_6 - k_{13} x_6. \end{aligned} \tag{5.2.1}$$

In the sequel we state the control problem and model the influence of heparin and of an hypothetical drug D capable of influencing the activity of α_2M on the course of thrombin concentration with time. For that matter, let $\mathbf{u} \in \mathbb{R}_{\geq 0}^3$ denote the input vector.

The parameter RVV gives the first impulse to the system and can be considered as an input variable. So, $u_1 =: RVV$ is the first component of the vector \mathbf{u} .

As already mentioned, heparin works as a catalyst for $ATIII$, which in its turn inactivates factors X_a and II_a . The influence of $ATIII$ on the motion of these two factors is represented in the system (5.2.1) by the constants k_3 and k_{13} , respectively. So, let u_2 represent the action of heparin.

In the model (5.2.1), thrombin is also inhibited by α_2M and the inhibitory activity is represented by the constant k_{12} . As a matter of fact, we discussed already in Chapter 3 how changes in the value of this constant are reflected in the course of thrombin concentration with time. Therefore, let the third component of \mathbf{u} be denoted as u_3 and represent the action of an hypothetical drug D .

We obtain the following control system:

$$\begin{aligned}
\frac{dx_1}{dt} &= -\frac{k_1 x_1 u_1}{k_2 + x_1} \\
\frac{dx_2}{dt} &= \frac{k_1 x_1 u_1}{k_2 + x_1} - k_3(1 + u_2)x_2 - k_4(x_4 - x_3 - 0.02)x_2 x_4 + k_5(-x_4 + 0.05) \\
\frac{dx_3}{dt} &= -\frac{k_6 x_3 x_6}{k_7 + x_3} \\
\frac{dx_4}{dt} &= -k_4(x_4 - x_3 - 0.02)x_2 x_4 + k_5(-x_4 + 0.05) \\
\frac{dx_5}{dt} &= -\frac{k_8 x_5(-x_4 + 0.05)}{k_9 + x_5} - \frac{k_{10} x_5 x_2}{k_{11} + x_5} \\
\frac{dx_6}{dt} &= \frac{k_8 x_5(-x_4 + 0.05)}{k_9 + x_5} + \frac{k_{10} x_5 x_2}{k_{11} + x_5} - k_{12}(1 + u_3)x_6 - k_{13}(1 + u_2)x_6 \\
\frac{dx_7}{dt} &= u_1 \\
\frac{dx_8}{dt} &= u_2 \\
\frac{dx_9}{dt} &= u_3.
\end{aligned} \tag{5.2.2}$$

This system is of the form (B.3.1) with $\dim \mathbf{x} = 9$, and $\dim \mathbf{u} = 3$.

Proposition 5.2.1. *The system (5.2.2) is flat and $\mathbf{y} = (x_1, k_3(1 + u_2)x_2, x_4)$ is a flat output.*

Proof. Consider the parametrization:

$$\begin{aligned} y_1 &= x_1 = \varphi_1(x_1); \\ y_2 &= k_3(1+u_2)x_2 = \varphi_2(x_1, x_2, x_3, x_4, u_2); \\ y_3 &= x_4 = \varphi_3(x_2, x_3, x_4). \end{aligned} \quad (5.2.3)$$

Thus, the variables $y_i, i = 1, 2, 3$ can be expressed as a function $\varphi_i, i = 1, 2, 3$ of the state vector and of the input vector and item (i) of Definition B.3.1 is fulfilled.

The state and the input variables can also be expressed as a function of $y_i, i = 1, 2, 3$ and a finite number of its time derivatives as follows:

$$\begin{aligned} x_1 &= y_1 \\ x_2 &= \frac{y_2}{k_3(1+u_2)} \\ x_4 &= y_3. \end{aligned}$$

Substituting the parametrization (5.2.3) in the fourth equation of the system (5.2.2) we obtain:

$$\begin{aligned} -k_4(\dot{y}_3 - x_3 - 0.02)\frac{y_2y_3}{k_3(1+u_2)} + k_5(-y_3 + 0.05) &= \dot{y}_3 \Leftrightarrow \\ -k_4(\dot{y}_3 - x_3 - 0.02)y_2y_3 + k_5k_3(1+u_2)(-y_3 + 0.05) - \dot{y}_3k_3(1+u_2) &= 0. \end{aligned}$$

Solving for x_3 and considering $y_2 \neq 0$ and $y_3 \neq 0$ we obtain furthermore

$$x_3 = \frac{1}{k_4y_2y_3} [k_4(\dot{y}_3 - 0.02)y_2y_3 - k_5k_3(1+u_2)(-y_3 + 0.05) + k_5k_3(1+u_2)\dot{y}_3].$$

From the third equation of (5.2.2) we obtain for x_6 the following representation as a function of y_3 and of \dot{y}_3

$$x_6 = -\frac{(k_7 + y_3)\dot{y}_3}{k_6y_3}, \text{ with } y_3 \neq 0.$$

We still need to prove that the state variable x_5 such a representation also exists. From equation five of the system (5.2.2) we have

$$\dot{x}_5 = -\frac{k_8x_5(-x_4 + 0.05)}{k_9 + x_5} - \frac{k_{10}x_5x_2}{k_{11} + y_5}.$$

On the other hand, by equation number six it holds

$$\dot{x}_5 = -\dot{x}_6 - k_{12}(1+u_3)x_6 - k_{13}(1+u_2)x_6.$$

Using again the parametrization (5.2.3) and equating the right hand-side of these two last equations we obtain

$$\begin{aligned} \xi_1(\mathbf{y}, \mathbf{u})(k_9 + x_5)(k_{11} + x_5) &= \xi_2(\mathbf{y}, \mathbf{u})(k_{11} + x_5)x_5 - \xi_3(\mathbf{y}, \mathbf{u})(k_9 + x_5)x_5 \Leftrightarrow \\ \xi_1(k_9 k_{11} + (k_9 + k_{11})x_5 + x_5^2) - (k_{11}\xi_2 - k_9\xi_3)x_5 - (\xi_2 - \xi_3)x_5^2 &= 0 \Leftrightarrow \\ (\xi_1 - \xi_2 + \xi_3)x_5^2 + (\xi_1(k_9 + k_{11}) - k_{11}\xi_2 + k_9\xi_3)x_5 + \xi_1 k_9 k_{11} &= 0, \end{aligned}$$

where,

$$\xi_1(\mathbf{y}, \mathbf{u}) = \xi_1 = (-\dot{x}_6 - k_{12}(1 + u_3)x_6 - k_{13}(1 + u_2)x_6)k_3(1 + u_2);$$

$$\xi_2(\mathbf{y}, \mathbf{u}) = \xi_2 = -k_8(-y_3 + 0.05)k_3(1 + u_2);$$

$$\xi_3(\mathbf{y}, \mathbf{u}) = \xi_3 = k_{10}y_2.$$

By solving the second degree equation and taking into account that $x_5 > 0$ and $\dot{x}_5 < 0$ we obtain x_5 as a function of \mathbf{y} and a finite number of its time derivatives, as well as a function of \mathbf{u} .

Moreover, there is a function ψ such that $\mathbf{u} = \psi(\mathbf{y}, \dot{\mathbf{y}}, \ddot{\mathbf{y}})$ as

$$u_1 = -\frac{\dot{x}_1(k_2 + x_1)}{k_1 x_1} = -\frac{\dot{y}_1(k_2 + y_1)}{k_1 y_1}, \text{ with } y_1 \neq 0.$$

$$u_2 = \frac{-\dot{x}_1 - \dot{x}_2 + \dot{x}_4 - k_3 x_2}{k_3 x_2} = \frac{-\dot{y}_1 - \dot{y}_2 + \dot{y}_3 - k_3 y_2}{k_3 y_2}, \text{ with } y_2 \neq 0 \text{ and}$$

$$u_3 = \frac{-\dot{x}_5 - \dot{x}_6 - k_{13}(1 + u_2)x_6 - k_{12}x_6}{k_{12}x_6} = \zeta(\mathbf{y}, \dot{\mathbf{y}}, \ddot{\mathbf{y}}), \text{ with } y_3 \neq 0.$$

Thus, item (ii) of Definition B.3.1 is satisfied. Moreover, by Remark B.3.2 and since $\dim \mathbf{u} = \dim \mathbf{y}$, it follows immediately that item (iii) also holds and therefore the system is flat with flat output \mathbf{y} . \square

Remark 5.2.2. In the proof of Proposition 5.2.1, we assume the flat output to be everywhere nonsingular, so that we could invert it and express \mathbf{x} and \mathbf{u} as a function φ of \mathbf{y} and its derivatives,

$$(\mathbf{y}, \dot{\mathbf{y}}, \dots, \mathbf{y}^{(q)}) \mapsto (\mathbf{x}, \mathbf{u}) = \varphi(\mathbf{y}, \dot{\mathbf{y}}, \dots, \mathbf{y}^{(q)}).$$

However, it can happen that a singularity is an interesting point of operation while motion planning. Since, φ is not defined at such a point and the previous calculation does not apply. To overcome the problem one can for instance "blow up" the singularity by considering trajectories $t \rightarrow \mathbf{y}(t)$ such that

$$t \rightarrow \varphi(\mathbf{y}(t), \dot{\mathbf{y}}(t), \dots, \mathbf{y}^{(q)}(t))$$

can be extended into a smooth mapping at points where φ is not defined, which requires a detailed study of the singularity. The development of a general statement regarding motion planning with singularities is not in the scope of this work. For a more substantial application see [FLMR95].

Application to motion planning

Let us consider the problem of steering from an initial state $\mathbf{x}(\tau_0) = \mathbf{x}_0$ to a final state $\mathbf{x}(\tau_f) = \mathbf{x}_f$. We parametrize the components of the flat output $y_i, i = 1, 2, 3$ by

$$y_i(t) := \sum_j^N A_{ij} \lambda_j(t),$$

where the $\lambda_j(t), j = 1, \dots, N$ are basis functions. This reduces the problem from finding a function in an infinite dimensional space to finding a finite set of parameters. The values of the flat output and its derivatives from the desired points in state space and then solve for the coefficients A_{ij} in the following system of equations:

$$\begin{array}{ll} y_i(\tau_0) &= \sum_j A_{ij} \lambda_j(\tau_0) & y_i(\tau_f) &= \sum_j A_{ij} \lambda_j(\tau_f) \\ &\vdots & &\vdots \\ y_i^{(q)}(\tau_0) &= \sum_j A_{ij} \lambda_j^{(q)}(\tau_0) & y_i^{(q)}(\tau_f) &= \sum_j A_{ij} \lambda_j^{(q)}(\tau_f). \end{array}$$

This approach is merely algebraic in theory and yields efficient computationally algorithms in practice. Moreover, the number of states that are needed to know in order to find a reasonable state space representation can be considerably reduced in comparison to traditional approaches to trajectory generation, such as optimal control. This is a very relevant aspect for systems like the blood coagulation mechanism where it is not easy to obtain experimental data for all the states of the system.

Chapter 6

Modelling Blood Thrombin Generation

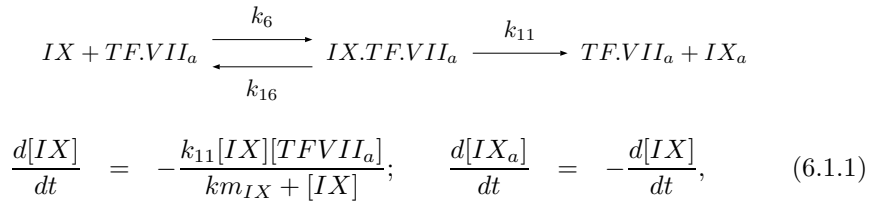
In this chapter we first rewrite the equations for the reaction scheme given in Figure 3.7 concerning the extrinsic pathway model published in [JoMa94] by using the Michaelis-Menten kinetics. This approach takes into consideration that we are in the presence of enzymatic reactions with positive and negative feedback loops. The constants that are not given directly in Jones and Mann's paper are deduced from the empirical research of Leipold et al. [LBRD95] that includes the reaction scheme in [JoMa94]. Moreover, we study the influence of changing the concentrations of the complex $TFVII_a$ and of factor $VIII$ in the new model. The qualitative analysis and model reduction followed the same pattern as in Chapter 4. Based on this new approach to modelling the extrinsic pathway we propose furthermore a new model for the intrinsic pathway leading to the formation of thrombin. The intrinsic pathway will start with the activation of factor XII and for the simulation we use for the reaction constants a value 20% inferior to the ones published in [JoMa94], since the intrinsic pathway for thrombin formation is slower in comparison to the extrinsic. As a motivation for considering a model only for the intrinsic pathway we give the following clinical situation. A patient is subjected to a valve replacement. These valves are usually artificial and the material used may trigger the intrinsic coagulation process. Such a kind of patients must take anticoagulatory medication for the rest of their lives.

6.1 Building the model for the extrinsic pathway

In Chapter 3 we described and made the stoichiometric analysis of the model developed by Mann and Jones published in [JoMa94]. Due to inconsistent information, we derived the set of differential equations governing the system of reactions by using the law of mass action. Thereby, the primary intention was to correct the given system following the same principle as the authors did. Like this, we obtained a set of differential equations that differ from the original one only in a few aspects with the advantage that it remains positive for positive initial values. The numerical solution was in all similar to the one in [JoMa94] so that the occurrence in this paper of several typographic mistakes cannot be excluded. In this section we focus on the reaction scheme itself and question whether the law of mass action is adequate since, after all, the blood coagulation cascade is a series of enzymatic reactions. As we have seen in Chapter 2, under certain circumstances, this kind of reactions are well described by the Michaelis-Menten equation.

By applying the concepts exposed in Chapter 2 we identify each factor in its inactive form as the substrate and we are interested in showing the role of the enzyme as an input to the transformation of this substrate into product, i. e., the activated form of the coagulation factor. The product has catalytic properties, therefore it will play the role of the enzyme in some subsequent reaction. The constants $k_i, i = 1, \dots, 19$ keep the same meaning as in [JoMa94] but sometimes it will be necessary to introduce some other constants.

Reaction scheme 1 and respective contribution to rates of change



where $km_{IX} = \frac{k_{11} + k_{16}}{k_6}$.

Substituting the values of the constants published in [JoMa94] after converting the units, we get $km_{IX} = 0.253 \mu mol/L$.

Reaction scheme 2 and respective contribution to rates of change

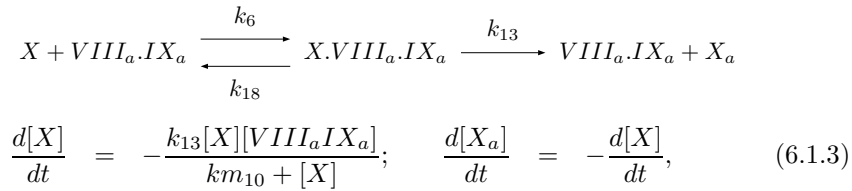


$$\frac{d[X]}{dt} = -\frac{k_{12}[X][TFVII_a]}{km_X + [X]}, \quad \frac{d[X_a]}{dt} = -\frac{d[X]}{dt}, \quad (6.1.2)$$

where $km_X = \frac{k_{12} + k_{17}}{k_6}$.

Substituting the values of the constants published in [JoMa94] after converting units, we get $km_X = 4.515 \mu\text{mol}/L$.

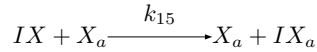
Reaction scheme 3 and respective contribution to rates of change



where $km_{10} = \frac{k_{13} + k_{18}}{k_6}$.

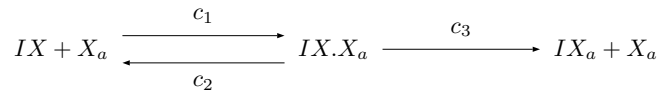
Substituting the values of the constants published in [JoMa94] after converting units, we get $km_{10} = 0.8201 \mu\text{mol}/L$.

Reaction scheme 4 and respective contribution to rates of change



This reaction scheme can be interpreted in several ways, depending on the reaction constants and on the concentration of the different factors involved.

If this reaction scheme represents a catalytic reaction it should have been written as:



The change in time of the concentration of factor IX can then be given by the Michaelis-Menten relation. Thus,

$$\frac{d[IX]}{dt} = -\frac{c_3[IX][X_a]}{km_9 + [IX]}, \quad \frac{d[IX_a]}{dt} = -\frac{d[IX]}{dt}, \quad (6.1.4)$$

where $km_9 = \frac{c_3 + c_2}{c_1}$.

As we have seen in Chapter 2, the expression (6.1.4) may be replaced by closely related expressions.

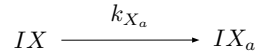
If $km_9 \gg [IX]$ or if $km_9 \ll [IX]$ then it transforms, without loss of accuracy, respectively into the alternatives

$$\frac{d[IX]}{dt} = -\frac{c_3[IX][X_a]}{km_9}; \quad \frac{d[IX_a]}{dt} = -\frac{d[IX]}{dt} \quad (6.1.5)$$

or

$$\frac{d[IX]}{dt} = -c_3[X_a]; \quad \frac{d[IX_a]}{dt} = -\frac{d[IX]}{dt}. \quad (6.1.6)$$

A third possibility follows when we are in the presence of a first order reaction. The reaction scheme should then be replaced by



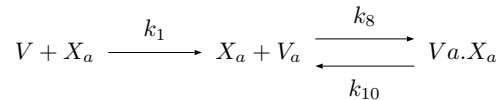
and

$$\frac{d[IX]}{dt} = -k_{X_a}[IX]; \quad \frac{d[IX_a]}{dt} = -\frac{d[IX]}{dt}. \quad (6.1.7)$$

Notice that (6.1.5) is the same as to assume that a second order reaction occurs. This means, in particular, that one could directly use the law of mass action as Mann and Jones did.

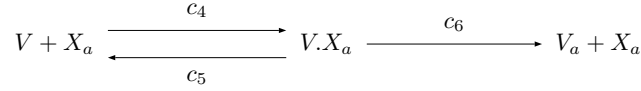
It is furthermore noteworthy that the activation of factor IX by factor X_a is not considered in the paper [LBRD95] written by Leipold, Bozarth, Racanelli, and Dicker of the same laboratory as Mann and Jones, which is prior to [JoMa94]. Moreover, this reaction is also not included in the reaction scheme published in [HJEM02], an article where both Mann and Jones are also two of the authors. So, it seems that there is some controversy regarding the existence or not of this reaction. For our purpose, we first assume that it exists and then observe what are the consequences of changing the value of the constant in the course of thrombin concentration with time. From the value of the constant $k_{X_a} := k_{15}$ in [JoMa94] and from [KKK01], we estimated $km_9 = 2 \mu\text{mol}/L$ and $c_3 = 0.2s^{-1}$.

Reaction scheme 5 and respective contribution to rates of change



We split this reaction scheme into two and set the governing differential equations as follows.

1-



$$\frac{d[V]}{dt} = -\frac{c_6[V][X_a]}{km_V + [V]}; \quad \frac{d[V_a]}{dt} = -\frac{d[V]}{dt}, \quad (6.1.8)$$

where $km_V = \frac{c_6 + c_5}{c_4}$ and $c_4 = k_1$. Then,

if $km_V \gg [V]$ or if $km_V \ll [V]$ we have respectively

$$\frac{d[V]}{dt} = -\frac{c_6[V][X_a]}{km_V}; \quad \frac{d[V_a]}{dt} = -\frac{d[V]}{dt} \quad (6.1.9)$$

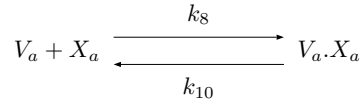
or

$$\frac{d[V]}{dt} = -c_6[X_a]; \quad \frac{d[V_a]}{dt} = -\frac{d[V]}{dt}. \quad (6.1.10)$$

The third possibility reads

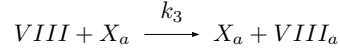
$$\frac{d[V]}{dt} = -k_1[V]; \quad \frac{d[V_a]}{dt} = -\frac{d[V]}{dt}. \quad (6.1.11)$$

2-

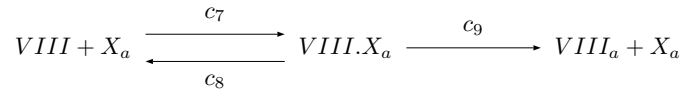


$$\begin{aligned} \frac{d[V_a]}{dt} &= -k_8[X_a][V_a] + k_{10}[V_a \cdot X_a] \\ \frac{d[X_a]}{dt} &= -k_8[X_a][V_a] + k_{10}[V_a \cdot X_a] \\ \frac{d[V_a \cdot X_a]}{dt} &= k_8[X_a][V_a] - k_{10}[V_a \cdot X_a]. \end{aligned} \quad (6.1.12)$$

In [LBRD95] one reads $c_4 = 100 \mu\text{mol}/L$; $c_5 = 1\text{s}^{-1}$ and $c_6 = 0.043\text{s}^{-1}$. Thus, $km_V = 0.0143 \mu\text{mol}/L$. Since km_V is about $\frac{1}{3}[V]$ one replace (6.1.8) by (6.1.9) without losing accuracy.

Reaction scheme 6 and respective contribution to rates of change

Following the same argument as for the reaction scheme number four, we have:



in the case of a catalytic reaction. Thus,

$$\frac{d[VIII]}{dt} = -\frac{c_9[VIII][X_a]}{km_{VIII} + [VIII]}; \quad \frac{d[VIII_a]}{dt} = -\frac{d[VIII]}{dt}, \quad (6.1.13)$$

where $km_{VIII} = \frac{c_9 + c_8}{c_7}$ and $c_7 = k_3$.

If $km_{VIII} \gg [VIII]$ or if $km_{VIII} \ll [VIII]$ then respectively we have

$$\frac{d[VIII]}{dt} = -\frac{c_9[VIII][X_a]}{km_{VIII}}; \quad \frac{d[VIII_a]}{dt} = -\frac{d[VIII]}{dt} \quad (6.1.14)$$

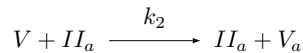
or

$$\frac{d[VIII]}{dt} = -c_9[X_a]; \quad \frac{d[VIII_a]}{dt} = -\frac{d[VIII]}{dt}. \quad (6.1.15)$$

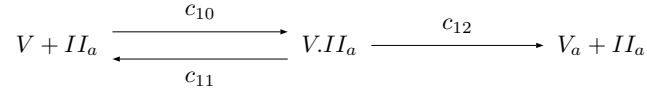
The third possibility reads

$$\frac{d[VIII]}{dt} = -k_3[VIII]; \quad \frac{d[VIII_a]}{dt} = -\frac{d[VIII]}{dt}. \quad (6.1.16)$$

In [LBRD95] one reads $c_7 = 100 \mu\text{mol}/L$; $c_8 = 2.1s^{-1}$ and $c_9 = 0.023s^{-1}$. Thus, $km_{VIII} = 0.02123 \mu\text{mol}/L$. Since $km_{VIII} \gg [VIII]$ one could replace (6.1.13) by (6.1.14) without losing accuracy.

Reaction scheme 7 and respective contribution to rates of change

Following the same argument as before we have



in the case of a catalytic reaction. Thus,

$$\frac{d[V]}{dt} = -\frac{c_{12}[V][II_a]}{km_5 + [V]}, \quad \frac{d[V_a]}{dt} = -\frac{d[V]}{dt}, \quad (6.1.17)$$

where $km_5 = \frac{c_{12} + c_{11}}{c_{10}}$ and $c_{10} = k_2$.

If $km_5 \gg [V]$ or if $km_5 \ll [V]$ then respectively we have

$$\frac{d[V]}{dt} = -\frac{c_{12}[V][II_a]}{km_5}, \quad \frac{d[V_a]}{dt} = -\frac{d[V]}{dt} \quad (6.1.18)$$

or

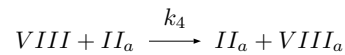
$$\frac{d[V]}{dt} = -c_{12}[II_a]; \quad \frac{d[V_a]}{dt} = -\frac{d[V]}{dt}. \quad (6.1.19)$$

The third possibility reads

$$\frac{d[V]}{dt} = -k_2[V]; \quad \frac{d[V_a]}{dt} = -\frac{d[V]}{dt}. \quad (6.1.20)$$

In [LBRD95] one reads $c_{10} = 100 \mu\text{mol}/L$; $c_{11} = 1\text{s}^{-1}$ and $c_{12} = 0.043\text{s}^{-1}$. Thus, $km_5 = 0.0746 \mu\text{mol}/L$. Since km_5 is about $3.7[V]$ one could possibly replace (6.1.17) by (6.1.18). Convergence problems arise while solving the system numerically, though. Therefore, for the set of constants and starting data available, one may discard this possibility from now on.

Reaction scheme 8 and respective contribution to rates of change



In case of a catalytic reaction, we have



Thus,

$$\frac{d[VIII]}{dt} = -\frac{c_{15}[VIII][II_a]}{km_8 + [VIII]}; \quad \frac{d[VIII_a]}{dt} = -\frac{d[VIII]}{dt}, \quad (6.1.21)$$

where $km_8 = \frac{c_{15} + c_{14}}{c_{13}}$ and $c_{13} = k_4$.

If $km_8 \gg [VIII]$ or if $km_8 \ll [VIII]$ then respectively we have

$$\frac{d[VIII]}{dt} = -\frac{c_{15}[VIII][II_a]}{km_8}; \quad \frac{d[VIII_a]}{dt} = -\frac{d[VIII]}{dt} \quad (6.1.22)$$

or

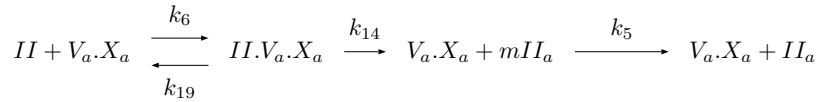
$$\frac{d[VIII]}{dt} = -k_4[II_a]; \quad \frac{d[VIII_a]}{dt} = -\frac{d[VIII]}{dt}. \quad (6.1.23)$$

The third possibility reads

$$\frac{d[VIII]}{dt} = -\frac{c_{15}[VIII]}{km_8}; \quad \frac{d[VIII_a]}{dt} = -\frac{d[VIII]}{dt}. \quad (6.1.24)$$

In [LBRD95] one reads $c_{13} = 100 \mu\text{mol}/L$; $c_{14} = 15s^{-1}$ and $c_{15} = 0.9s^{-1}$. Thus, $km_8 = 0.0143 \mu\text{mol}/L$. Since $km_8 \gg [VIII]$ one can replace (6.1.21) by (6.1.22) without losing accuracy.

Reaction scheme 9 and respective contribution to rates of change



Once again we split the reaction scheme into two.

1-



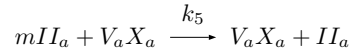
This is a typical reaction to apply Michaelis and Menten equation. Hence,

$$\frac{d[II]}{dt} = -\frac{k_{14}[II][V_aX_a]}{km_{II} + [II]}, \quad \frac{d[mII_a]}{dt} = -\frac{d[II]}{dt}, \quad (6.1.25)$$

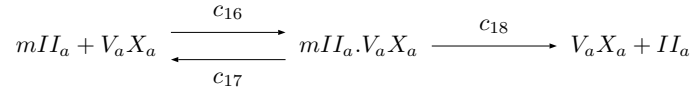
where $km_{II} = \frac{k_{14} + k_{19}}{k_6}$.

Substituting the values of the constants published in [JoMa94] after converting units, we get $km_{II} = 1.06 \mu\text{mol}/L$.

2-



We were already confronted with this kind of reaction. So, if we are in the presence of a catalytic reaction, the reaction scheme should be



Thus, the Michaelis and Menten equation is used to establish the equations of motion

$$\frac{d[mII_a]}{dt} = -\frac{c_{18}[mII_a][V_aX_a]}{km_{mII_a} + [mII_a]}, \quad \frac{d[II_a]}{dt} = -\frac{d[mII_a]}{dt}, \quad (6.1.26)$$

where $km_{mII_a} = \frac{c_{18} + c_{17}}{c_{16}}$, with $c_{16} = k_5$.

If $km_{mII_a} \gg [mII_a]$ or if $km_{mII_a} \ll [mII_a]$ then respectively we have

$$\frac{d[mII_a]}{dt} = -\frac{c_{18}[mII_a][V_aX_a]}{km_{mII_a}}, \quad \frac{d[II_a]}{dt} = -\frac{d[mII_a]}{dt} \quad (6.1.27)$$

or

$$\frac{d[mII_a]}{dt} = -c_{18}[V_aX_a]; \quad \frac{d[II_a]}{dt} = -\frac{d[mII_a]}{dt}. \quad (6.1.28)$$

The third possibility reads

$$\frac{d[mII_a]}{dt} = -k_5[mII_a]; \quad \frac{d[II_a]}{dt} = -\frac{d[mII_a]}{dt}. \quad (6.1.29)$$

In [LBRD95] one reads $c_{16} = 100 \mu\text{mol}/L$; $c_{17} = 15s^{-1}$ and $c_{18} = 0.9s^{-1}$. Thus, $km_{mII_a} = 0.81 \mu\text{mol}/L$. The relation (6.1.27) could possibly replace the relation (6.1.26), but only at the beginning of the reaction where $[mII_a] = 0$.

Reaction scheme 10 and respective contribution to rates of change

Reaction scheme and differential equations equal to (3.2.13).

In [LBRD95] it is assumed moreover that factor $VIII_a$ can be inactivated by factor IX_a . Since this may influence the final equilibrium state of the complex $VIII_aIX_a$ we consider in addition for our model the following reaction scheme:

Reaction scheme 11 and respective contribution to rates of change



$$\frac{d[VIII_a]}{dt} = -\frac{c_{21}[VIII_a][IX_a]}{km_{VIII_a} + [VIII_a]}; \quad \frac{d[VIII_a^*]}{dt} = -\frac{d[VIII_a]}{dt}, \quad (6.1.30)$$

where $km_{VIII_a} = \frac{c_{21} + c_{20}}{c_{19}}$.

If $km_{VIII_a} \gg [VIII_a]$ or if $km_{VIII_a} \ll [VIII_a]$ then respectively we have

$$\frac{d[VIII_a]}{dt} = -\frac{c_{21}[VIII_a][IX_a]}{km_{VIII_a}}; \quad \frac{d[VIII_a^*]}{dt} = -\frac{d[VIII_a]}{dt} \quad (6.1.31)$$

or

$$\frac{d[VIII_a]}{dt} = -c_{21}[IX_a]; \quad \frac{d[VIII_a^*]}{dt} = -\frac{d[VIII_a]}{dt}. \quad (6.1.32)$$

The third possibility reads

$$\frac{d[VIII_a]}{dt} = -ki_{VIII_a}[VIII_a]; \quad \frac{d[VIII_a^*]}{dt} = -\frac{d[VIII_a]}{dt}. \quad (6.1.33)$$

In [LBRD95] one reads $c_{19} = 100 \mu\text{mol}/L$; $c_{20} = 0.17s^{-1}$ and $c_{21} = 0.00008s^{-1}$. Thus, $km_{VIII_a} = 0.0017 \mu\text{mol}/L$.

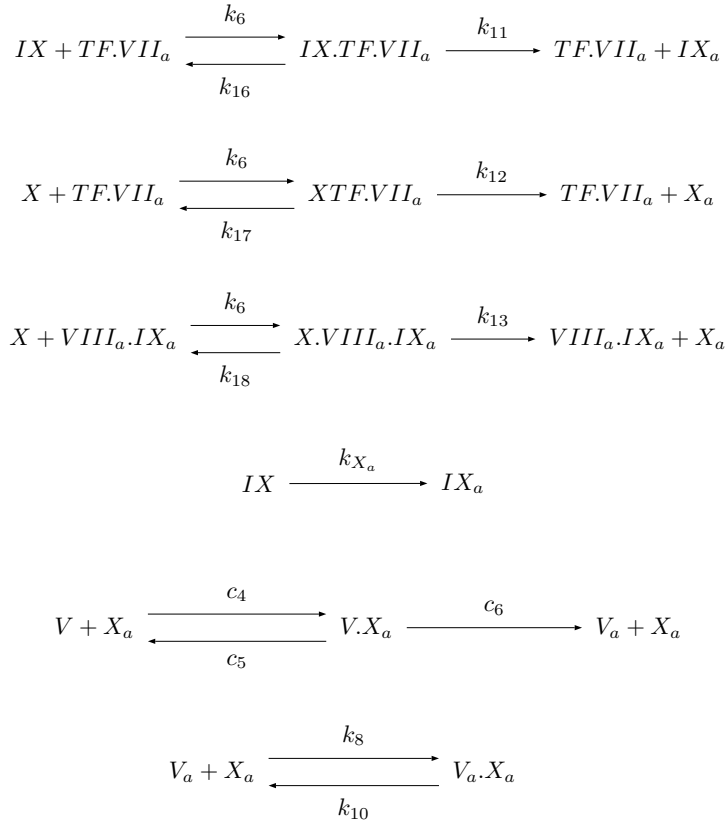
Altogether, this leads to a large number of candidate models, specially because the considerations made while setting the differential equations for reaction scheme 4 are theoretically valid each time we make use of the Michaelis Menten equation. However, from all the candidates one should select that model which:

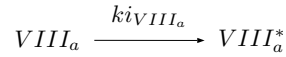
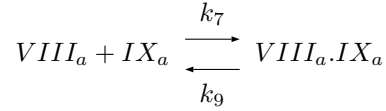
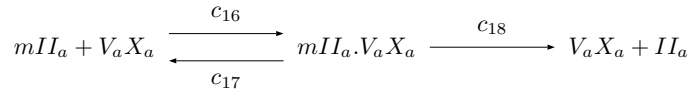
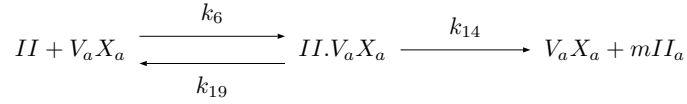
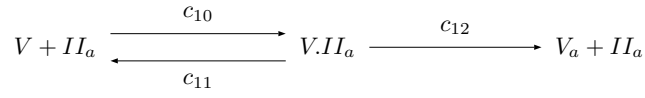
- (i) meets the established knowledge in the field;

- (ii) does not contain redundant steps;
- (iii) fits the phenomena observed.

In this thesis, due to the lack of experimental data, we will chose the model that best approximates the numerical results obtained by Mann and Jones in [JoMa94]. Thus, to decide which mathematical description is more adequate, we need to make use of the values of the constants $k_i, i = 1, \dots, 19$, listed in Table 3.2, and of the constants calculated above based on the published articles from [JoMa94] and [LBRD95].

While testing several combinations of possible reaction schemes and respective mathematical description, we concluded after adjusting the values of some constants that a another mathematical approach describing the extrinsic pathway is given if we consider the following reaction scheme and the following set of 14 differential equations:





$$\frac{d[IX]}{dt} = -\frac{k_{11}[IX][TFVII_a]}{km_{IX} + [IX]} - k_{15}[IX] \quad !$$

$$\frac{d[IX_a]}{dt} = \frac{k_{11}[IX][TFVII_a]}{km_{IX} + [IX]} + k_{15}[IX] - k_7[VIII_a][IX_a] + k_9[VIII_a IX_a]!$$

$$\frac{d[X]}{dt} = -\frac{k_{12}[X][TFVII_a]}{km_X + [X]} - \frac{k_{13}[X][VIII_a IX_a]}{km_{10} + [X]}$$

$$\frac{d[X_a]}{dt} = \frac{k_{12}[X][TFVII_a]}{km_X + [X]} + \frac{k_{13}[X][VIII_a IX_a]}{km_{10} + [X]} - k_8[V_a][X_a] + k_{10}[V_a X_a]$$

$$\begin{aligned}
\frac{d[V]}{dt} &= -\frac{c_6[V][X_a]}{km_V} - \frac{c_{12}[V][II_a]}{km_5 + [V]} && ! \\
\frac{d[V_a]}{dt} &= \frac{c_6[V][X_a]}{km_V} + \frac{c_{12}[V][II_a]}{km_5 + [V]} - k_8[V_a][X_a] + k_{10}[V_aX_a] && ! \\
\frac{d[V_aX_a]}{dt} &= k_8[V_a][X_a] - k_{10}[V_aX_a] \\
\frac{d[VIII]}{dt} &= -\frac{c_9}{km_{VIII}}[VIII][X_a] - \frac{c_{15}}{km_8}[VIII][II_a] && ! \\
\frac{d[VIII_a]}{dt} &= \frac{c_9}{km_{VIII}}[VIII][X_a] + \frac{c_{15}}{km_8}[VIII][II_a] && ! \\
&\quad -k_7[VIII_a][IX_a] + k_9[VIII_aIX_a] - ki_{VIII_a}[VIII_a] \\
\frac{d[II]}{dt} &= -\frac{k_{14}[II][V_aX_a]}{km_{II} + [II]} \\
\frac{d[II_a]}{dt} &= \frac{c_{18}[mII_a][V_aX_a]}{km_{mII_a} + [mII_a]} \\
\frac{d[mII_a]}{dt} &= \frac{k_{14}[II][V_aX_a]}{km_{II} + [II]} - \frac{c_{18}[mII_a][V_aX_a]}{km_{mII_a} + [mII_a]} \\
\frac{d[VIII_aIX_a]}{dt} &= k_7[VIII_a][IX_a] - k_9[VIII_aIX_a] \\
\frac{d[VIII_a^*]}{dt} &= ki_{VIII_a}[VIII_a] && !
\end{aligned} \tag{6.1.34}$$

The symbol ! at the end of the equation means that at least one term was simplified from the Michaelis and Menten equation taking into account the discussion above.

6.1.1 Numerical integration

The values of the constants used for the numerical integration are listed in Table 6.1. The values of some constants were adjusted in order to obtain a better agreement between the solutions of the two models, in particular regarding the course of the concentration of activated thrombin with time (see Figure 6.1). This adjustment was done within a range of possible values that can be justified by the many varied conditions under which the empirical rates are derived and such that the assumptions made above while constructing the model remain valid. Nevertheless, with the original values of the constants we would get a curve exactly with

the same qualitative behavior that would fit the experimental data presented in [JoMa94] with approximately the same error.

Constant	Value [$\mu M^{-1} s^{-1}$]	Constant	Value [s^{-1}]
km_{IX}	0.253	c_3	0.2
km_9	2	c_6	0.043
km_X	4.515	c_9	0.023
km_{10}	2.8901	c_{12}	0.26
km_V	1.43×10^{-3}	c_{15}	0.9
km_5	0.0746	c_{18}	15
km_{VIII}	0.02123		
km_8	2.159		
km_{II}	0.9		
km_{mII_a}	1.06		
ki_{VIII_a}	0.8		

Table 6.1: Rate constants used for the numerical integration of (6.1.34).

Based on the results from [LBRD95], we put $[TFVII_a] = 0.000048 \mu mol/L$ as starting input and considered the following vector of initial values corresponding to the physiologic concentrations in $\mu mol/L$ of the coagulation factors involved. Once again, the initial value of the activated factors was taken equal to zero:

$$[0.09; 0; 0.20; 0; 0.02; 0; 0; 0.0007; 0; 1.4; 0; 0; 0; 0]^T.$$

In Figure 6.1, we represent the first 4 minutes of the course of activated thrombin with time given by (6.1.34) versus the model by Mann and Jones given in [JoMa94], corrected in Section 3.2.2 and reduced to 12 equations in one of the subsections of Section 4.3.

The remaining solutions behave in the same manner as before excepting the one corresponding to the complex $VIII_aIX_a$. In fact, contrary to the original model from Mann and Jones, there is a decay in the course of the concentration of the complex $VIII_aIX_a$ with time after some maximal concentration was reached (see Figure 6.2). This result make us believe that the reaction of reaction scheme 11 above was missing in [JoMa94] and this may actually be the reason why there was a contradiction between the estimated curve and the experimental data.

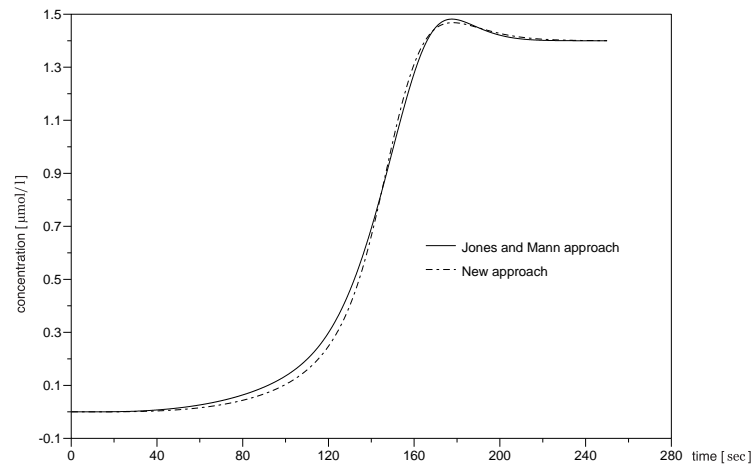
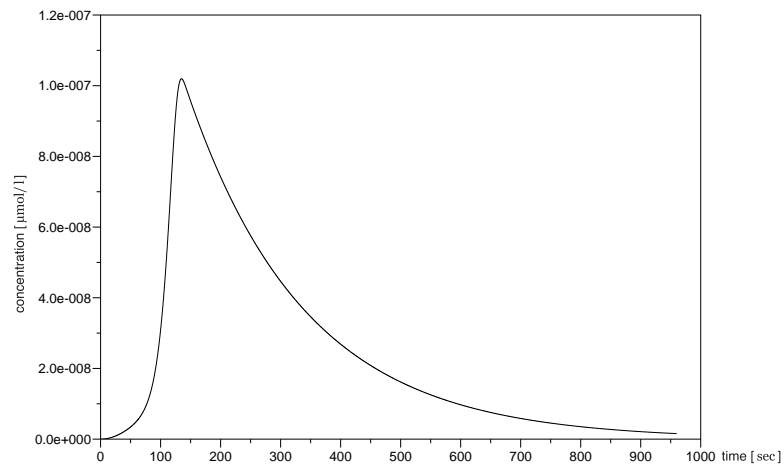


Figure 6.1: First 4 minutes of thrombin formation.

Figure 6.2: Decay of complex $VIII_aIX_a$ after (6.1.34).

In [JoMa94], the validation of the model proposed was done by comparing the model response to experimental results. Despite of the importance of this confrontation it would be out of the scope of this thesis to write down and discuss with our model all the aspects covered by Mann and Jones. However, some attention will be devoted in the sequel to two of them, namely the influence of changing the concentration of the complex $TFVII_a$ and the effect of factor $VIII$ on the reaction progress.

6.1.2 Influence of changing complex $TFVII_a$ concentration

Mann and Jones reported that changing the concentration of $TFVII_a$ has only minimal effects on the maximal rate of thrombin formation during the propagation phase of the reaction, while having the greatest influence on the initiation phase. As a consequence, the major effect observed was the propagation of the *lag* or initiation phase with decreasing values of the complex concentration. This effect can be observed also in the solution given by our model (see Figure 6.3).

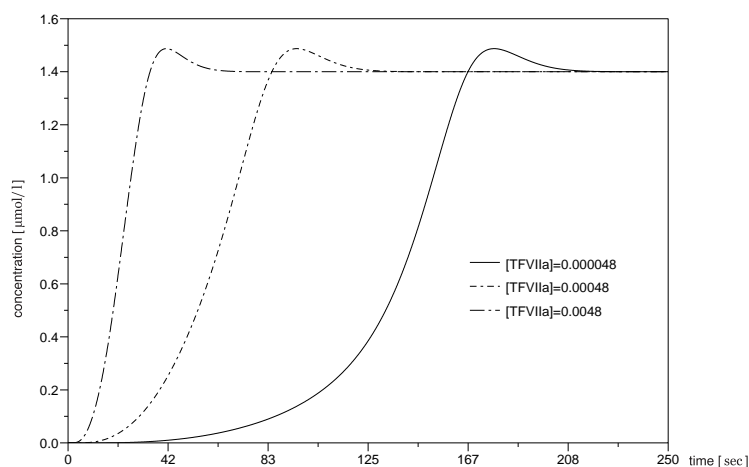


Figure 6.3: Course of thrombin formation for different $TFVII_a$ concentrations.

6.1.3 The effect of changing factor $VIII$ concentration

It is known from the literature (see [Lin95]) that factor $VIII$ plays a critical role in the initiation of the coagulation and that in absence of factor $VIII$, the ac-

tivated partial thromboplastin time (APT¹) is extended. However, factor *VIII* deficiency does not extend the prothrombin-time (PT²). The simulation of thrombin formation was modelled using the same rate constants and setting the initial concentration of factor *VIII* to zero. When lower concentrations of *TFVII_a* are used in experiments without factor *VIII*, the propagation phase is depressed. By contrast, at high concentrations of *TFVII_a*, omitting factor *VIII* from the reaction mixture has a minimal effect on the time course or form of the thrombin generation curve. This means that the complex *TFVII_a* form the threshold levels of factor *X_a* required to achieve explosive prothrombin activation without the need of the complex *VIII_aIX_a*. Our model can reproduce this happening and, as an example, the next figure illustrates the course of thrombin generation at $[TFVII_a] = 0.000048 \mu\text{mol}/L$ in normal blood and in hemophilic blood with factor *VIII* deficiency during the first 4 minutes. Time is given in seconds.

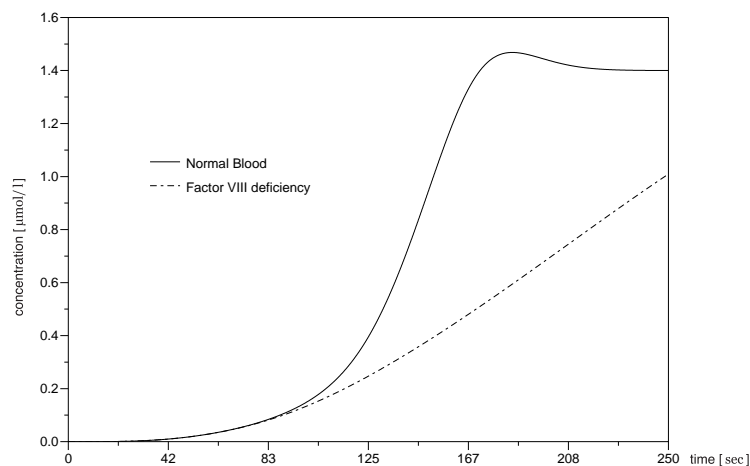


Figure 6.4: Course of thrombin formation: normal blood versus hemophilic blood.

¹Laboratory test specially sensitive to levels of factors *VIII* and *IX* below 25%

²Test of the coagulation system sensitive to deficiencies of factors *VII*, *X*, *V*, and *II*

In the next section we proceed with the qualitative analysis of (6.1.34). We will see furthermore that the order of the system can still be reduced because of the existence of first integrals.

6.1.4 Qualitative analysis

Positivity

Let us start as usual by rewriting the system (6.1.34) of ordinary differential equations in vector form:

$$\mathbf{x} = \mathbf{f}(\mathbf{x}),$$

where the components of \mathbf{x} are given by $x_1 = [IX]$, $x_2 = [IX_a]$, $x_3 = [X]$, $x_4 = [X_a]$, $x_5 = [V]$, $x_6 = [V_a]$, $x_7 = [V_a X_a]$, $x_8 = [VIII]$, $x_9 = [VIII_a]$, $x_{10} = [II]$, $x_{11} = [II_a]$, $x_{12} = [mII_a]$, $x_{13} = [VIII_a IX_a]$ and $x_{14} = [VIII_a^*]$.

The system (6.1.34) can now be written as

$$\begin{aligned} \frac{dx_1}{dt} &= -\frac{k_{11}x_1[TFVII_a]}{km_{IX} + x_1} - k_{15}x_1 \\ \frac{dx_2}{dt} &= \frac{k_{11}x_1[TFVII_a]}{km_{IX} + x_1} + k_{15}x_1 - k_7x_9x_2 + k_9x_{13} \\ \frac{dx_3}{dt} &= -\frac{k_{12}x_3[TFVII_a]}{km_X + x_3} - \frac{k_{13}x_3x_{13}}{km_{10} + x_3} \\ \frac{dx_4}{dt} &= \frac{k_{12}x_3[TFVII_a]}{km_X + x_3} + \frac{k_{13}x_3x_{13}}{km_{10} + x_3} - k_8x_6x_4 + k_{10}x_7 \\ \frac{dx_5}{dt} &= -\frac{c_6x_5x_4}{km_V} - \frac{c_{12}x_5x_{11}}{km_5 + x_5} \\ \frac{dx_6}{dt} &= \frac{c_6x_5x_4}{km_V} + \frac{c_{12}x_5x_{11}}{km_5 + x_5} - k_8x_6x_4 + k_{10}x_7 \\ \frac{dx_7}{dt} &= k_8x_6x_4 - k_{10}x_7 \\ \frac{dx_8}{dt} &= -\frac{c_9}{km_{VIII}}x_8x_4 - \frac{c_{15}}{km_8}x_8x_{11} \\ \frac{dx_9}{dt} &= \frac{c_9}{km_{VIII}}x_8x_4 + \frac{c_{15}}{km_8}x_8x_{11} - k_7x_9x_2 + k_9x_{13} - ki_{VIII_a}x_9 \end{aligned}$$

$$\begin{aligned}
\frac{dx_{10}}{dt} &= -\frac{k_{14}x_{10}x_7}{km_{II} + x_{10}} \\
\frac{dx_{11}}{dt} &= \frac{c_{18}x_{12}x_7}{km_{mII_a} + x_{12}} \\
\frac{dx_{12}}{dt} &= \frac{k_{14}x_{10}x_7}{km_{II} + x_{10}} - \frac{c_{18}x_{12}x_7}{km_{mII_a} + x_{12}} \\
\frac{dx_{13}}{dt} &= k_7x_9x_2 - k_9x_{13} \\
\frac{dx_{14}}{dt} &= ki_{VIII_a}x_9.
\end{aligned} \tag{6.1.35}$$

Let furthermore

$$\mathcal{P} = \{\mathbf{x} \in \mathbb{R}^{14} : x_1 > 0, \dots, x_{14} > 0\}$$

be the positive orthant of \mathbb{R}^{14} .

Proposition 6.1.1. \mathcal{P} and $\overline{\mathcal{P}}$ are positively invariant for the system (6.1.35).

Proof. Similarly as before, the vector \mathbf{x} represents a vector of concentrations, and this means that $x_i \geq 0, i = 1, \dots, 14$. Then, the system is defined in a relative open subset of \mathcal{P} with $\mathbf{f}(\mathbf{x}) = (f_1(\mathbf{x}), \dots, f_{14}(\mathbf{x}))^T$, where the function \mathbf{f} is C^∞ and the components $f_i, i = 1, \dots, 14$ are defined by the right-hand side of (6.1.35).

For $\mathbf{x} \in \partial\mathcal{P}$, define $C_x = \mathcal{P}$. Setting furthermore $x_k = 0$ in $f_k, k = 1, \dots, 14$ yields $f_i(x_1, \dots, x_{k-1}, 0, x_{k+1}, \dots, x_{14}) \geq 0$ for all $x_j \geq 0, j \neq k$ and $i = 1, \dots, 14$.

Therefore, $\mathbf{f}(\mathbf{x}) \in \overline{\mathcal{P}}$, for all $\mathbf{x} \in \partial\mathcal{P}$. Hence, \mathcal{P} and $\overline{\mathcal{P}}$ are positively invariant for the system (6.1.35). \square

As a consequence of the last proposition, any orbit starting with positive initial values will remain positive for all times.

Linear first integrals and boundedness of the solutions

Proposition 6.1.2. Given any solution of (6.1.35) with nonnegative initial values, all the components $x_i(t), i = 1, \dots, 14$ are bounded.

Proof. In this case, since all variables are involved, it is sufficient to prove that the scalar valued functions defined on \mathbb{R}^{14} by

$$\varphi_1(\mathbf{x}) = x_1 + x_2 + x_{13};$$

$$\varphi_2(\mathbf{x}) = x_3 + x_4 + x_7;$$

$$\varphi_3(\mathbf{x}) = x_5 + x_6 + x_7;$$

$$\varphi_4(\mathbf{x}) = x_8 + x_9 + x_{13} + x_{14};$$

$$\varphi_5(\mathbf{x}) = x_{10} + x_{11} + x_{12}$$

are first integrals of the system (6.1.35).

Indeed by applying again Definition A.1.4:

$$\begin{aligned} L_{\mathbf{f}}(\varphi_1)(\mathbf{x}) &= D_{\mathbf{x}}(\varphi_1)\mathbf{f}(\mathbf{x}) = (1, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0) \begin{pmatrix} f_1(\mathbf{x}) \\ \vdots \\ f_{14}(\mathbf{x}) \end{pmatrix} \\ &= f_1(\mathbf{x}) + f_2(\mathbf{x}) + f_{13}(\mathbf{x}) = 0. \end{aligned}$$

$$\begin{aligned} L_{\mathbf{f}}(\varphi_2)(\mathbf{x}) &= D_{\mathbf{x}}(\varphi_2)\mathbf{f}(\mathbf{x}) = (0, 0, 1, 1, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0) \begin{pmatrix} f_1(\mathbf{x}) \\ \vdots \\ f_{14}(\mathbf{x}) \end{pmatrix} \\ &= f_3(\mathbf{x}) + f_4(\mathbf{x}) + f_7(\mathbf{x}) = 0. \end{aligned}$$

$$\begin{aligned} L_{\mathbf{f}}(\varphi_3)(\mathbf{x}) &= D_{\mathbf{x}}(\varphi_3)\mathbf{f}(\mathbf{x}) = (0, 0, 0, 0, 1, 1, 1, 0, 0, 0, 0, 0, 0, 0, 0) \begin{pmatrix} f_1(\mathbf{x}) \\ \vdots \\ f_{14}(\mathbf{x}) \end{pmatrix} \\ &= f_5(\mathbf{x}) + f_6(\mathbf{x}) + f_7(\mathbf{x}) = 0. \end{aligned}$$

$$\begin{aligned} L_{\mathbf{f}}(\varphi_4)(\mathbf{x}) &= D_{\mathbf{x}}(\varphi_4)\mathbf{f}(\mathbf{x}) = (0, 0, 0, 0, 0, 0, 0, 1, 1, 0, 0, 0, 1, 1) \begin{pmatrix} f_1(\mathbf{x}) \\ \vdots \\ f_{14}(\mathbf{x}) \end{pmatrix} \\ &= f_8(\mathbf{x}) + f_9(\mathbf{x}) + f_{13}(\mathbf{x}) + f_{14}(\mathbf{x}) = 0. \end{aligned}$$

$$\begin{aligned} L_{\mathbf{f}}(\varphi_5)(\mathbf{x}) &= D_{\mathbf{x}}(\varphi_5)\mathbf{f}(\mathbf{x}) = (0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 1, 0, 0) \begin{pmatrix} f_1(\mathbf{x}) \\ \vdots \\ f_{14}(\mathbf{x}) \end{pmatrix} \\ &= f_{10}(\mathbf{x}) + f_{11}(\mathbf{x}) + f_{12}(\mathbf{x}) = 0. \end{aligned}$$

By directly applying Proposition 6.1.1 and Remark A.1.5, we conclude that the solutions of the system (6.1.35) remain in the level set of φ_j , $j = 1, \dots, 5$ in which they start. Hence, $\varphi_j(x_i(t))$, $j = 1, \dots, 5$ are constant functions of t for all solutions and therefore bounded. Thus, the components $x_i(t)$, $i = 1, \dots, 14$ are bounded. \square

Convergence to a stationary point

The next proposition gives us more conditions that define the set containing the positive limit set $\omega(\mathbf{y})$ of the system (6.1.35). However, since the proof follows exactly the same pattern as the proof of Proposition 4.3.5 it is immediate and there is no need to repeat it here.

Proposition 6.1.3. *Let $\varphi_6(\mathbf{x}) = x_1$, $\varphi_7(\mathbf{x}) = x_3$, $\varphi_8(\mathbf{x}) = x_5$, $\varphi_9(\mathbf{x}) = x_8$, and $\varphi_{10}(\mathbf{x}) = -x_9$ be scalar functions defined on \mathbb{R}^{14} . Then $L_{\mathbf{f}}(\varphi_i)(\mathbf{x}) \leq 0$, $i = 6, \dots, 10$. Equality follows only if $x_1 = x_3 = x_5 = x_8 = x_9 = 0$.*

Remark 6.1.4. Together with Proposition 6.1.1, we conclude that the functions φ_j are non increasing and bounded for $j = 6, \dots, 10$ along a trajectory. That is, there are constants β_j such that $0 \leq \varphi_j(\mathbf{x}) \leq \beta_j$, $j = 6, \dots, 10$. Hence every trajectory remains in the subset of \mathcal{P} defined by $\varphi_j(\mathbf{x}) \leq \beta_j$, which is bounded.

Proposition 6.1.5. *The positive limit set $\omega(\mathbf{y})$ of (6.1.35) is contained in the set*

$$N := \{\mathbf{x} \in \mathcal{P} : x_1 + x_2 + x_{13} = x_1(0), \quad x_3 + x_4 + x_7 = x_3(0), \quad x_5 + x_6 + x_7 = x_5(0), \\ x_8 + x_9 + x_{13} + x_{14} = x_8(0), \quad x_{10} + x_{11} + x_{12} = x_{10}(0), \quad x_1 = 0, \quad x_3 = 0, \\ x_5 = 0, \quad x_8 = 0, \quad x_9 = 0\}.$$

Proof. The two previous results together with Theorem A.3.11 imply that the compact sets $M_\alpha := \{\mathbf{x} \in \mathcal{P} : \varphi_j(\mathbf{x}) \leq \alpha, j = 1, \dots, 10\}$ are positively invariant for all $\alpha \in [0, \beta_j)$. Theorem A.3.15 guarantees that the solution of the initial value problem exists on $[0, \infty)$. Moreover, this solution approaches its positive limit set $\omega(\mathbf{y})$, as $t \rightarrow \infty$. So, $\omega(\mathbf{y})$ is nonempty, compact and connected. By Theorem A.3.16 $\omega(\mathbf{y})$ is also invariant.

Hence, by applying LaSalle's principle stated in Theorem A.4.1 we conclude that

$$\omega(\mathbf{y}) \subset N.$$

□

Remark 6.1.6. The conditions defining N are not enough to prove that any solution in N is stationary. This set, like before, contains invariant sets other than the set of stationary points.

In the sequel we derive further conditions to be satisfied by the set of equilibrium points by setting the right-hand side of the system (6.1.35) to zero and solving for \mathbf{x} .

Deriving further conditions for the set of equilibrium points

While trying to find new conditions to be satisfied by an equilibrium point, one observes that the value of the variable x_7 at the equilibrium depend on the variables x_6 and x_4 and their value at the equilibrium. We get

$$E_1 := \{\mathbf{x} \in \bar{\mathcal{P}} : x_1 = 0, x_3 = 0, x_5 = 0, x_7 = \frac{k_8 x_6 x_4}{k_{10}}, x_8 = 0, x_9 = 0, x_{10} = 0, x_{12} = 0, x_{13} = 0\}.$$

The following proposition holds:

Proposition 6.1.7. *If $z_7 = \frac{k_8}{k_{10}} z_6(t) z_4(t)$, $z_{10}(t) = 0$ and $z_{12}(t) = 0$ then any solution $z(t)$ starting in N given in Proposition 6.1.5 is stationary.*

Proof. Let $z(t)$ be a solution in N satisfying the given conditions, i. e.

$$z(t) = (0, z_2(t), 0, z_4(t), 0, z_6(t), \frac{k_8}{k_{10}} z_6(t) z_4(t), 0, 0, 0, z_{11}(t), 0, z_{13}(t), z_{14}(t))^T.$$

Thus,

$$\dot{z}(t) = (0, \dot{z}_2, 0, \dot{z}_4, 0, \dot{z}_6, \frac{k_8}{k_{10}} (\dot{z}_6 z_4 + z_6 \dot{z}_4), 0, 0, 0, \dot{z}_{11}, 0, \dot{z}_{13}, \dot{z}_{14})^T.$$

Since $z_9 = 0$ and $z_{12} = 0$ it holds $\dot{z}_{14} = 0$ and $\dot{z}_{11} = 0$.

On the other hand,

$$z_{13}(t) + z_{14}(t) = x_8(0) \Leftrightarrow \dot{z}_{13}(t) + \dot{z}_{14}(t) = 0. \text{ This implies } z_{13}(t) = 0 \text{ and thus } \dot{z}_{13} = 0 \text{ and } \dot{z}_2(t) = 0.$$

Furthermore, $x_7 = \frac{k_8}{k_{10}} z_6(t) z_4(t)$ implies $\dot{z}_4 = 0, \dot{z}_6 = 0$ and $z_7 = 0$.

Altogether, $\dot{z}(t) = (0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)^T$ and $z(t)$ is stationary.

□

For practical reasons, the stability question will be handled after reducing the number of equations to 9.

Model reduction

Let us now reduce the number of equations of the system (6.1.35) to 9. From the

first integrals given in Proposition 6.1.2 we eliminate the variables x_3, x_5, x_8, x_{10} and x_{13} .

Setting

$$\begin{aligned} x_{13} &= -x_1 - x_2 + d_1; & x_3 &= -x_4 - x_7 + d_2; \\ x_{10} &= -x_{11} - x_{12} + d_3; & x_5 &= -x_7 - x_6 + d_4; \\ x_8 &= -x_9 - x_{13} - x_{14} + d_5, \end{aligned}$$

where $d_1 = x_1(0); d_2 = x_3(0); d_3 = x_{10}(0); d_4 = x_5(0); d_5 = x_8(0)$ are different from zero,

and making the following coordinate transformation:

$$\begin{aligned} y_1 &:= x_1; & y_2 &:= x_2; & y_3 &:= x_4; & y_4 &:= x_6; & y_5 &:= x_7; & y_6 &:= x_9; & y_7 &:= x_{11}; & y_8 &:= x_{12}; \\ y_9 &= x_{14} \end{aligned}$$

we obtain the following system of differential equations:

$$\begin{aligned} \frac{dy_1}{dt} &= -\frac{k_{11}y_1[TFVII_a]}{km_{IX} + y_1} - k_{15}y_1 \\ \frac{dy_2}{dt} &= \frac{k_{11}y_1[TFVII_a]}{km_{IX} + y_1} + k_{15}y_1 - k_7y_6y_2 + k_9(d_1 - y_1 - y_2) \\ \frac{dy_3}{dt} &= \frac{k_{12}(d_2 - y_3 - y_5)[TFVII_a]}{km_X + (d_2 - y_3 - y_5)} + \frac{k_{13}(d_2 - y_3 - y_5)(d_1 - y_1 - y_2)}{km_{10} + (d_2 - y_3 - y_5)} \\ &\quad - k_8y_4y_3 + k_{10}y_5 \\ \frac{dy_4}{dt} &= \frac{c_6(d_4 - y_4 - y_5)y_3}{km_V} + \frac{c_{12}(d_4 - y_4 - y_5)y_7}{km_5 + (d_4 - y_4 - y_5)} - k_8y_4y_3 + k_{10}y_5 \\ \frac{dy_5}{dt} &= k_8y_4y_3 - k_{10}y_5 \\ \frac{dy_6}{dt} &= \frac{c_9}{km_{VIII}}(d_5 - y_6 - d_1 + y_1 + y_2 - y_9)y_3 - ki_{VIII_a}y_6 + \\ &\quad \frac{c_{15}}{km_8}(d_5 - y_6 - d_1 + y_1 + y_2 - y_9)y_7 - k_7y_6y_2 + k_9(d_1 - y_1 - y_2) \\ \frac{dy_7}{dt} &= \frac{c_{18}y_8y_5}{km_{mII_a} + y_8} \\ \frac{dy_8}{dt} &= \frac{k_{14}(d_3 - y_7 - y_8)y_5}{km_{II} + (d_3 - y_7 - y_8)} - \frac{c_{18}y_8y_5}{km_{mII_a} + y_8} \\ \frac{dy_9}{dt} &= ki_{VIII_a}y_6. \end{aligned} \tag{6.1.36}$$

This system has a single equilibrium point and its coordinates can be calculated explicitly by setting the right-hand side of (6.1.36) to zero and solving to \mathbf{y} . There are very complicated terms involved and surely lots of impossible solutions due to the physiological meaning of the variables, so we are going to make use of all the information available about the system to exclude some candidates.

From $\frac{dy_1}{dt} = 0$ and $\frac{dy_9}{dt} = 0$ we obtain $y_1 = 0$ and $y_6 = 0$. Substituting these in the second equation we have $\frac{dy_2}{dt} = 0$ only if $y_2 = d_1$.

The variable y_3 corresponds to factor X_a , the activated form of factor X , and the variable y_4 to factor V_a . Together they build the prothrombinase complex, which here is represented by variable y_5 . So the value at the equilibrium will depend on the value at the equilibrium of y_4 and y_3 . Thus the fifth equation will be equal zero if $y_5 = \frac{k_8}{k_{10}}y_4y_3$. From the numerical results, we see that this value is different from zero, so at equilibrium $y_4 > 0$ and $y_3 > 0$.

Hence, $\frac{dy_7}{dt} = 0 \Leftrightarrow y_8 = 0$. This implies furthermore that $\frac{dy_8}{dt} = 0$ only if $y_7 = 0$.

Moreover, after substituting the previous conditions in the remaining sixth, third and fourth equations we get the conditions $y_9 = d_5$, $y_3 + y_5 = d_2$ and $y_5 + y_4 = d_4$, respectively.

Substituting the value of y_5 in the two last equations we obtain a system of two equations on y_4 and y_3 .

Solving for y_3 we obtain following relation between y_3 and y_4 :

$$y_3 = \frac{d_2 - d_4}{k_{10}} + y_4.$$

Notice that $d_2 = [X](0)$ and $d_4 = [V](0)$ and $d_2 > d_4$, so $y_3 > 0$.

The value of y_4 at equilibrium is the positive solution of the following second degree equation:

$$k_{10}y_4^2 + (k_{10}^2 + k_8(d_2 - d_4))y_4 - k_{10}d_4 = 0.$$

Notice furthermore that this equation only admits real solutions.

Remark 6.1.8. Substituting the values of the constants involved we obtain for the equilibrium point the same values like the ones that can be deduced by visual inspection of the numerical solution.

Remark 6.1.9. Altogether we obtained the coordinates of the unique equilibrium point towards which the system (6.1.36) converges. Thus, by applying Theorem A.4.1 follows asymptotic stability of the equilibrium point of this system.

In comparison with the original model from Jones and Mann that was corrected and reduced to 12 equations in Section 4.3.4, the model (6.1.36) gives the same results, but is more compact. Nevertheless, a more realistic approach to modelling the extrinsic pathway would take into account the action of inhibitors.

In the next section we give a first approach to modelling the intrinsic pathway for thrombin generation based on the previous analysis.

6.2 Building the model for the intrinsic pathway

Our starting point is the model (6.1.34). As already said in Chapter 1, the two pathways are thought to merge at the level of factor X activation. So, we consider the same reactions until the activation of factor X and the remaining reactions will be substituted by the reactions that are thought to occur in the intrinsic pathway. The reaction where factor IX is activated by factor X_a will be not considered here, because, on the one hand, it is not included in none of the articles of the current literature at our disposal concerning the intrinsic pathway (see for instance [Lin95]) and, on the other hand, its existence in the reaction scheme for the extrinsic pathway is rather questionable. Nevertheless, we will refer to this reaction later on.

It is known from the literature that thrombin formation by the extrinsic pathway is circa 30 seconds faster than thrombin formation by the intrinsic pathway. Reducing in 20% the values of the constants used by Mann and Jones in the paper referred above and integrating the system using the same vector of starting values, it is possible to gain a first visual impression and to compare the course of activated thrombin with time in both pathways. This situation is illustrated in Figure 6.5.

We observe that thrombin formation in the extrinsic pathway starts after 30 seconds while in the intrinsic it takes approximately 20 seconds longer. Moreover, the maximum of thrombin concentration is attained at approximately 2'49'' and 3'40'', respectively.

Since no experimental data is available, we will compare the result of our final simulation with these ones.

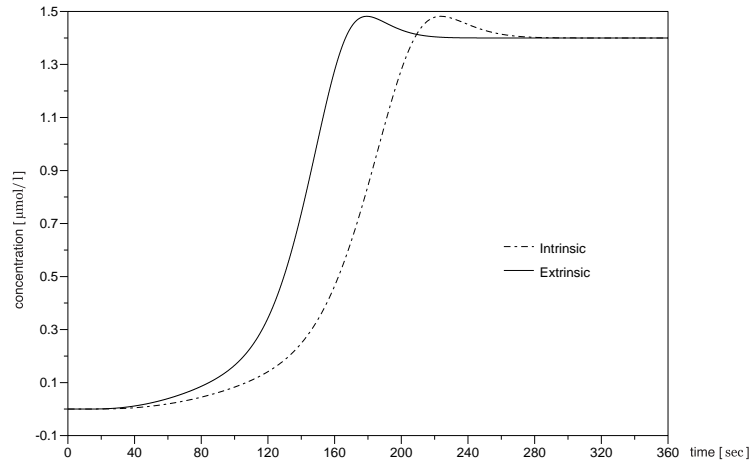
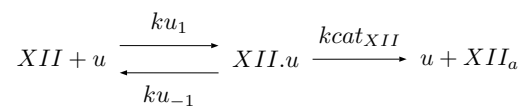


Figure 6.5: Course of activated thrombin with time in both pathways.

The activation of factor XII follows after contact with a foreign substance u . Let us assume that the decay of factor XII concentration with time follows some exponential like behavior so that factor XII will be completely activated. So, the following reaction occurs:



The contribution to the rate of change will be described by:

$$\frac{d[XII]}{dt} = -\frac{kcat_{XII}[XII]u}{km_{XII} + [XII]} \quad \text{and} \quad \frac{d[XII_a]}{dt} = \frac{d[XII]}{dt}$$

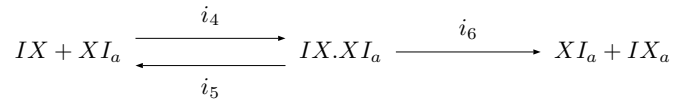
Once activated, factor XII_a activates factor XI in a catalytic reaction, so the following reaction occurs:



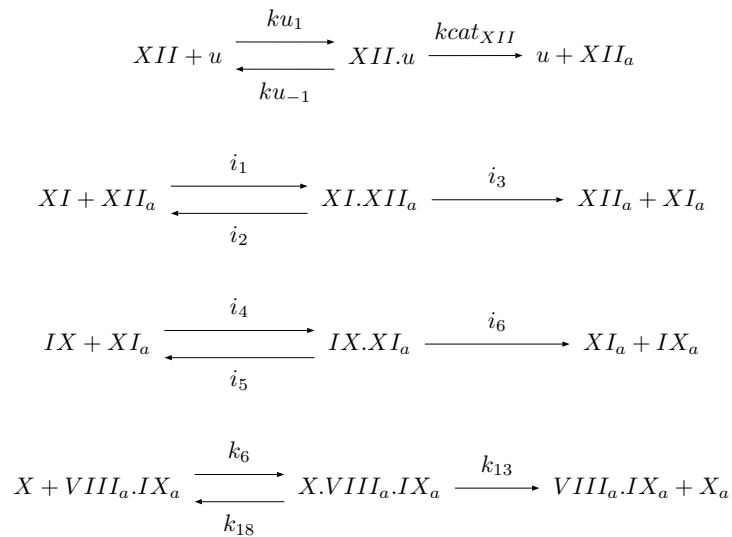
The contribution to the rate of change is then given by:

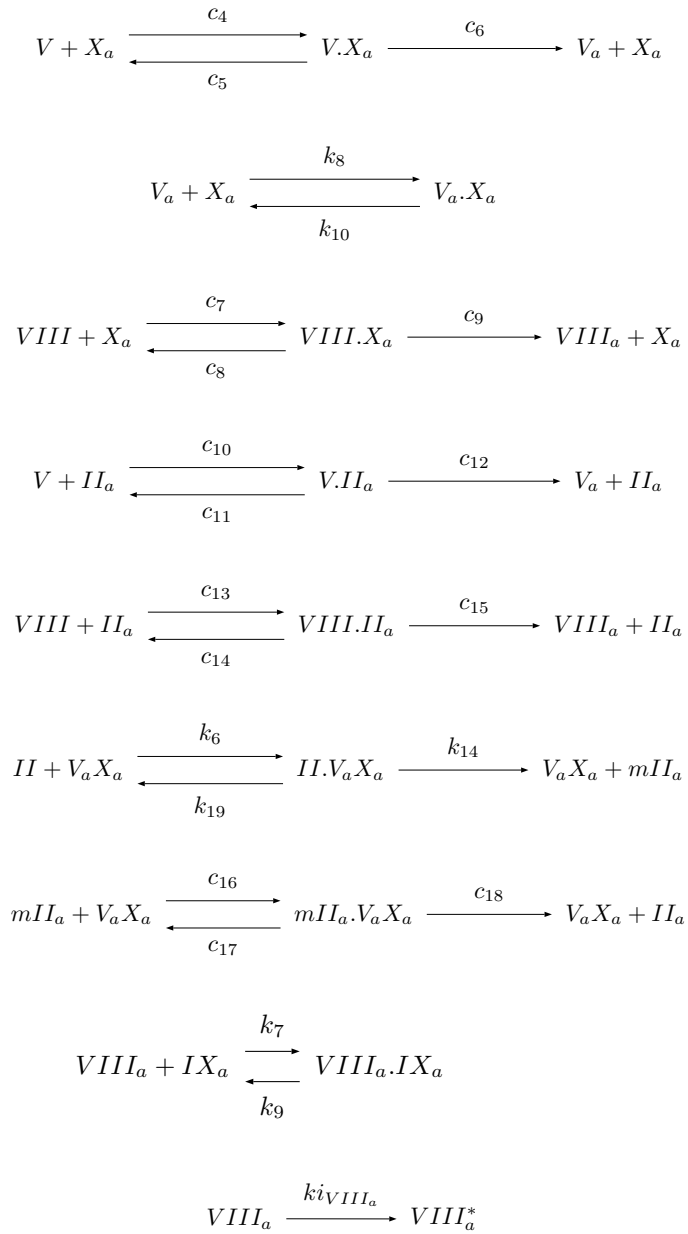
$$\frac{d[XI]}{dt} = -\frac{k_{cat_{XI}}[XI][XII_a]}{k_{m_{XI}} + [XI]} \quad \text{and} \quad \frac{d[XI_a]}{dt} = -\frac{d[XI]}{dt}.$$

In the intrinsic pathway, factor IX is activated by factor XI_a in a catalytic reaction:



The remaining reactions leading to thrombin formation are also part of the extrinsic pathway. Altogether we obtain the following reaction scheme for the intrinsic pathway:





Following the same strategy as for the extrinsic pathway, we derive the corresponding set of differential equations by applying the Michaelis-Menten assumption on catalytic reactions and the law of mass action otherwise.

$$\begin{aligned}
\frac{d[XII]}{dt} &= -\frac{kcat_{XII}[XII]u}{km_{XII} + [XII]} \\
\frac{d[XII_a]}{dt} &= \frac{kcat_{XII}[XII]u}{km_{XII} + [XII]} \\
\frac{d[XI]}{dt} &= -\frac{kcat_{XI}[XI][XII_a]}{km_{XI} + [XI]} \\
\frac{d[XI_a]}{dt} &= \frac{kcat_{XI}[XI][XII_a]}{km_{XI} + [XI]} \\
\frac{d[IX]}{dt} &= -\frac{kcat_{IX}[IX][XI_a]}{km_{IX} + [IX]} \\
\frac{d[IX_a]}{dt} &= -k_7[VIII_a][IX_a] + k_9[VIII_aIX_a] \\
\frac{d[X]}{dt} &= -\frac{kcat_{10}[X][VIII_aIX_a]}{km_{10} + [X]} \\
\frac{d[X_a]}{dt} &= \frac{kcat_{10}[X][VIII_aIX_a]}{km_{10} + [X]} - k_8[V_a][X_a] + k_{10}[V_aX_a] \\
\frac{d[II]}{dt} &= -\frac{kcat_{II}[II][V_aX_a]}{km_{II} + [II]} \\
\frac{d[II_a]}{dt} &= \frac{kcat_2[mII_a][V_aX_a]}{km_2 + [mII_a]} \\
\frac{d[VIII]}{dt} &= -\frac{kcat_{VIII}[VIII][X_a]}{km_{VIII}} - \frac{kcat_8[VIII][II_a]}{km_8} \\
\frac{d[VIII_a]}{dt} &= \frac{kcat_{VIII}[VIII][X_a]}{km_{VIII}} + \frac{kcat_8[VIII][II_a]}{km_8} \\
&\quad - k_7[VIII_a][IX_a] + k_9[VIII_aIX_a] - ki_{VIII_a}[VIII_a] \\
\frac{d[VIII_aIX_a]}{dt} &= k_7[VIII_a][IX_a] - k_9[VIII_aIX_a] \\
\frac{d[V]}{dt} &= -\frac{kcat_V[V][X_a]}{km_V} - \frac{kcat_5[V][II_a]}{km_5 + [V]} \\
\frac{d[V_a]}{dt} &= \frac{kcat_V[V][X_a]}{km_V} + \frac{kcat_5[V][II_a]}{km_5 + [V]} - k_8[V_a][X_a] + k_{10}[V_aX_a] \\
\frac{d[V_aX_a]}{dt} &= k_8[V_a][X_a] - k_{10}[V_aX_a]
\end{aligned}$$

$$\begin{aligned}
\frac{d[mII_a]}{dt} &= \frac{kcat_{II}[II][V_aX_a]}{km_{II} + [II]} - \frac{kcat_2[mII_a][V_aX_a]}{km_2 + [mII_a]} \\
\frac{d[VIII_a^*]}{dt} &= ki_{VIII_a}[VIII_a].
\end{aligned} \tag{6.2.1}$$

6.2.1 Numerical solution

Due to the lack of experimental data, for the simulation, the constants k_7, k_8, k_9 and k_{10} are 20% of the corresponding value given in Table 3.2. The values of the remaining constants are either taken or slightly adjusted from the original values of the constants given in [KKK01] or in [JoMa94]. The adjustment was done within the range of possible values that the constants may take and differ in the literature due to the different circumstances under which the experiments are realized. The constants are summarized in Table 6.2.

Constant	Value [$\mu M^{-1} s^{-1}$]	Constant	Value [s^{-1}]	Reference
km_{XII}	0.51	$kcat_{XII}$	5.7	[KKK01]
km_{XI}	2	$kcat_{XI}$	$9.5 \times 10^{-4}^\dagger$	[KKK01]
km_{IX}	4^\dagger	$kcat_{IX}$	0.4167	[KKK01]
km_{10}	0.19	$kcat_{10}$	29	[KKK01]
km_{II}	0.848	$kcat_{II}$	28.6^\dagger	[JoMa94]
km_2	0.78	$kcat_2$	12	[JoMa94]
km_{VIII}	0.56	$kcat_{VIII}$	0.0184	[JoMa94]
km_8	0.016	$kcat_8$	0.72	[JoMa94]
km_V	0.001144;	$kcat_V$	0.0344	[JoMa94]
km_5	0.5968	$kcat_5$	0.208	[JoMa94]

Table 6.2: Rate constants used for the numerical integration of (6.2.1). The values signalized with \dagger were adjusted.

To trigger the system, we set $u = 0.0058$ and used the following vector of initial concentration values:

$$x_0 = [0.3; 0; 0.025; 0; 0.09; 0; 0.2; 0; 1.4; 0; 0.0007; 0; 0; 0.032; 0; 0; 0]^T.$$

The numerical solution was again obtained using SCILAB. In the following figure, we compare the total amount of thrombin generated by using the model for the intrinsic pathway (6.2.1)- in the figure *Intrinsic2*- to the total amount of thrombin given by the model for the extrinsic pathway (6.1.34) and to the curve for the course of thrombin concentration with time obtained from (6.1.34) by reducing the value of the constants in 20% - in the figure *Intrinsic1*.

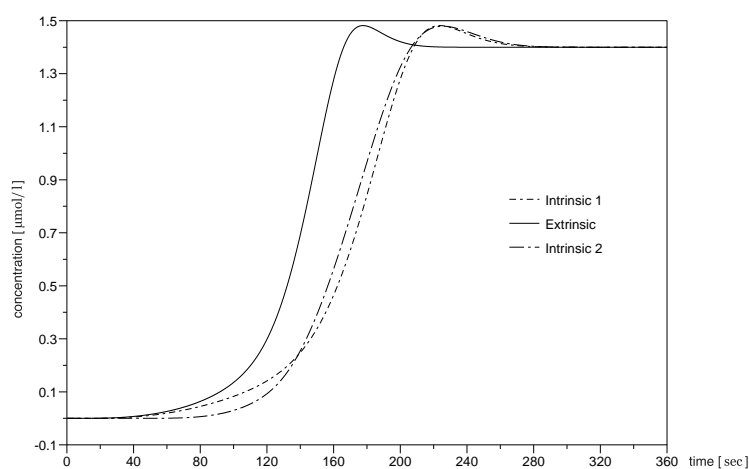
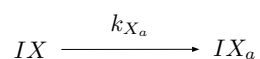


Figure 6.6: Course of thrombin concentration after 4 minutes: intrinsic versus extrinsic.

We observe that the two curves for the intrinsic pathway are rather similar and when compared to the curve for the extrinsic pathway they provide the same qualitative information regarding the time at which maximal concentration of thrombin is achieved. The lag phase is approximately 12 seconds longer. The maximum value for thrombin concentration is attained after 3'40".

Remark 6.2.1. As happened before for different values of $[TF.VII_a]$, for different values of u , we may influence the system by delaying it or by accelerating it.

Remark 6.2.2. We also simulated the course of thrombin concentration with time including the reaction



The result was that the total amount of thrombin was merely generated by the reactions that are thought to belong to the extrinsic pathway. In other words, setting $u = 0$ in this case did not have any effect on the shape of the curve for the course of thrombin with time. Of course, we cannot exclude the possibility that the value of the constants used has an influence on the result.

Remark 6.2.3. As already said for the extrinsic pathway, a more realistic approach to modelling the intrinsic pathway would also include the action of inhibitors.

In the sequel we proceed with the qualitative analysis of the system (6.2.1).

6.2.2 Qualitative analysis

Positivity

Let us start as usual by rewriting the system (6.2.1) of ordinary differential equations in vector form:

$$\mathbf{x} = \mathbf{f}(\mathbf{x}),$$

where the components of \mathbf{x} are given by $x_1 = [XII]$, $x_2 = [XII_a]$, $x_3 = [XI]$, $x_4 = [XI_a]$, $x_5 = [IX]$, $x_6 = [IX_a]$, $x_7 = [X]$, $x_8 = [X_a]$, $x_9 = [II]$, $x_{10} = [II_a]$, $x_{11} = [VIII]$, $x_{12} = [VIII_a]$, $x_{13} = [VIII_a IX_a]$, $x_{14} = [V]$, $x_{15} = [V_a]$, $x_{16} = [V_a X_a]$, $x_{17} = [mII_a]$ and $x_{18} = [VIII^*]$.

The system (6.2.1) can now be written as

$$\begin{aligned} \frac{dx_1}{dt} &= -\frac{kcat_{XII}x_1u}{km_{XII} + x_1} \\ \frac{dx_2}{dt} &= \frac{kcat_{XII}x_1u}{km_{XII} + x_1} \\ \frac{dx_3}{dt} &= -\frac{kcat_{XI}x_3x_2}{km_{XI} + x_3} \\ \frac{dx_4}{dt} &= \frac{kcat_{XI}x_3x_2}{km_{XI} + x_3} \end{aligned}$$

$$\begin{aligned}
\frac{dx_5}{dt} &= -\frac{kcat_{IX}x_5x_4}{km_{IX} + x_5} \\
\frac{dx_6}{dt} &= \frac{kcat_{IX}x_5x_4}{km_{IX} + x_5} - k_7x_{12}x_6 + k_9x_{13} \\
\frac{dx_7}{dt} &= -\frac{kcat_{10}x_7x_{13}}{km_{10} + x_7} \\
\frac{dx_8}{dt} &= \frac{kcat_{10}x_7x_{13}}{km_{10} + x_7} - k_8x_{15}x_8 + k_{10}x_{16} \\
\frac{dx_9}{dt} &= -\frac{kcat_{II}x_9x_{16}}{km_{II} + x_9} \\
\frac{dx_{10}}{dt} &= \frac{kcat_2x_{17}x_{16}}{km_2 + x_{17}} \\
\frac{dx_{11}}{dt} &= -\frac{kcat_{VIII}x_{11}x_8}{km_{VIII}} - \frac{kcat_8}{km_8}x_{11}x_{10} \\
\frac{dx_{12}}{dt} &= \frac{kcat_{VIII}x_{11}x_8}{km_{VIII}} + \frac{kcat_8}{km_8}x_{11}x_{10} \\
&\quad - k_7x_{12}x_6 + k_9x_{13} - ki_{VIII_a}x_{11} \\
\frac{dx_{13}}{dt} &= k_7x_{12}x_6 - k_9x_{13} \\
\frac{dx_{14}}{dt} &= -\frac{kcat_Vx_{14}x_8}{km_V} - \frac{kcat_5x_{14}x_{10}}{km_5 + x_{14}} \\
\frac{dx_{15}}{dt} &= \frac{kcat_Vx_{14}x_8}{km_V} + \frac{kcat_5x_{14}x_{10}}{km_5 + x_{14}} - k_8x_{15}x_8 + k_{10}x_{16} \\
\frac{dx_{16}}{dt} &= k_8x_{15}x_8 - k_{10}x_{16} \\
\frac{dx_{17}}{dt} &= \frac{kcat_{II}x_9x_{16}}{km_{II} + x_9} - \frac{kcat_2x_{17}x_{16}}{km_2 + x_{17}} \\
\frac{dx_{18}}{dt} &= ki_{VIII_a}x_{11}.
\end{aligned} \tag{6.2.2}$$

Let furthermore

$$\mathcal{P} = \{\mathbf{x} \in \mathbb{R}^{18} : x_1 > 0, \dots, x_{18} > 0\}$$

be the positive orthant of \mathbb{R}^{18} .

Proposition 6.2.4. \mathcal{P} and $\overline{\mathcal{P}}$ are positively invariant for the system (6.2.2).

Proof. Similarly as before, the vector \mathbf{x} represents a vector of concentrations, and this means that $x_i \geq 0, i = 1, \dots, 18$. Then, the system is defined in a relative

open subset of \mathcal{P} with $\mathbf{f}(\mathbf{x}) = (f_1(\mathbf{x}), \dots, f_{18}(\mathbf{x}))^T$, where the function \mathbf{f} is C^∞ and the components $f_i, i = 1, \dots, 18$ are defined by the right-hand side of (6.2.2).

For $\mathbf{x} \in \partial\mathcal{P}$, define $C_x = \mathcal{P}$. Setting furthermore $x_k = 0$ in $f_k, k = 1, \dots, 18$ yields $f_i(x_1, \dots, x_{k-1}, 0, x_{k+1}, \dots, x_{18}) \geq 0$ for all $x_j \geq 0, j \neq k$ and $i = 1, \dots, 18$.

Therefore, $\mathbf{f}(\mathbf{x}) \in \overline{\mathcal{P}}$, for all $\mathbf{x} \in \partial\mathcal{P}$. Hence, \mathcal{P} and $\overline{\mathcal{P}}$ are positively invariant for the system (6.2.2). \square

As a consequence of the last proposition, any orbit starting with positive initial values will remain positive for all times.

Linear first integrals and boundedness of the solutions

Proposition 6.2.5. *Given any solution of (6.2.2) with nonnegative initial values, all the components $x_i(t), i = 1, \dots, 18$ are bounded.*

Proof. In this case, since all variables are involved, it is sufficient to prove that the scalar valued functions defined on \mathbb{R}^{18} by

$$\varphi_1(\mathbf{x}) = x_1 + x_2;$$

$$\varphi_2(\mathbf{x}) = x_3 + x_4;$$

$$\varphi_3(\mathbf{x}) = x_5 + x_6 + x_{13};$$

$$\varphi_4(\mathbf{x}) = x_7 + x_8 + x_{16};$$

$$\varphi_5(\mathbf{x}) = x_9 + x_{10} + x_{16};$$

$$\varphi_6(\mathbf{x}) = x_{11} + x_{12} + x_{13} + x_{18};$$

$$\varphi_7(\mathbf{x}) = x_{14} + x_{15} + x_{16}$$

are first integrals of the system (6.2.2).

The proof that $\varphi_j, j = 1, \dots, 7$ are first integrals is immediate by applying Definition A.1.4 as we did in Proposition 6.1.2.

Hence, by directly applying Proposition 6.2.4 and Remark A.1.5, we conclude that the solutions of the system (6.2.2) remain in the level set of $\varphi_j, j = 1, \dots, 7$ in which they start. Hence, $\varphi_j(x_i(t)), j = 1, \dots, 7$ are constant functions of t for all solutions and therefore bounded. Thus, the components $x_i(t), i = 1, \dots, 18$ are bounded. \square

Convergence to a stationary point

The next proposition gives us more conditions that define the set containing the positive limit set $\omega(\mathbf{y})$ of the system (6.2.2). Again, since the proof follows exactly the same pattern as the proof of Proposition 6.1.3 it is immediate and there is no need to repeat it here.

Proposition 6.2.6. *Let $\varphi_8(\mathbf{x}) = x_1$, $\varphi_9(\mathbf{x}) = x_3$, $\varphi_{10}(\mathbf{x}) = x_5$, $\varphi_{11}(\mathbf{x}) = x_7$, $\varphi_{12}(\mathbf{x}) = x_9$, $\varphi_{13}(\mathbf{x}) = x_{14}$ and $\varphi_{14}(\mathbf{x}) = x_9 - x_{10}$ be scalar functions defined on \mathbb{R}^{18} . Then $L_{\mathbf{f}}(\varphi_i)(\mathbf{x}) \leq 0$, $i = 8, \dots, 14$. Equality follows only if $x_1 = x_3 = x_5 = x_7 = x_9 = x_{11} = x_{14} = x_{17} = 0$.*

Remark 6.2.7. Together with Proposition 6.2.4, we conclude that the functions φ_j are non increasing and bounded for $j = 8, \dots, 14$ along a trajectory. That is, there are constants β_j such that $0 \leq \varphi_j(\mathbf{x}) \leq \beta_j$, $j = 8, \dots, 14$. Hence every trajectory remains in the subset of \mathcal{P} defined by $\varphi_j(\mathbf{x}) \leq \beta_j$, which is bounded.

Proposition 6.2.8. *The positive limit set $\omega(\mathbf{y})$ of (6.2.2) is contained in the set*

$$\begin{aligned} N := \{ & \mathbf{x} \in \mathcal{P} : x_1 + x_2 = x_1(0), \quad x_3 + x_4 = x_3(0), \quad x_5 + x_6 + x_{13} = x_5(0), \\ & x_7 + x_8 + x_{16} = x_7(0), \quad x_9 + x_{10} + x_{17} = x_9(0), \quad x_{11} + x_{12} + x_{13} + x_{18} = x_{11}(0), \\ & x_{14} + x_{15} + x_{16} = x_{14}(0), \quad x_1 = 0, \quad x_3 = 0, \quad x_5 = 0, \quad x_7 = 0, \quad x_9 = 0, \\ & x_{11} = 0, \quad x_{14} = 0, \quad x_{17} = 0\}. \end{aligned}$$

Proof. The two previous results together with Theorem A.3.11 imply that the compact sets $M_\alpha := \{\mathbf{x} \in \mathcal{P} : \varphi_j(\mathbf{x}) \leq \alpha, j = 1, \dots, 14\}$ are positively invariant for all $\alpha \in [0, \beta_j)$. Theorem A.3.15 guarantees that the solution of the initial value problem exists on $[0, \infty)$. Moreover, this solution approaches its positive limit set $\omega(\mathbf{y})$, as $t \rightarrow \infty$. So, $\omega(\mathbf{y})$ is nonempty, compact and connected. By Theorem A.3.16 $\omega(\mathbf{y})$ is also invariant.

Hence, by applying LaSalle's principle stated in Theorem A.4.1 we conclude that

$$\omega(\mathbf{y}) \subset N.$$

□

Remark 6.2.9. The conditions defining N are not enough to prove that any solution in N is stationary. This set, like before, contains invariant sets other than the set of stationary points.

In the sequel we derive further conditions to be satisfied by the set of equilibrium points by setting the right-hand side of the system (6.2.2) to zero and solving for \mathbf{x} .

Deriving further conditions for the set of equilibrium points

While trying to find new conditions to be satisfied by an equilibrium point, one observes that the value of the variables x_{13} and x_{16} at the equilibrium depend on the value at the equilibrium of the variables x_6 and x_{12} , and x_8 and x_{15} , respectively. We get

$$E := \left\{ \mathbf{x} \in \overline{\mathcal{P}} : x_1 = 0, x_3 = 0, x_5 = 0, x_7 = 0, x_9 = 0, x_{11} = 0, x_{14} = 0, \right. \\ \left. x_{13} = \frac{k_7 x_6 x_{12}}{k_9}, x_{16} = \frac{k_8 x_8 x_{15}}{k_{10}}, x_{17} = 0 \right\}.$$

The following proposition holds:

Proposition 6.2.10. *If $z_{13} = \frac{k_7 z_6(t) z_{12}(t)}{k_9}$ and $z_{16} = \frac{k_8 z_8(t) z_{15}(t)}{k_{10}}$ then any solution $z(t)$ starting in N given in Proposition 6.2.8 is stationary.*

Proof. Let $z(t)$ be a solution in N satisfying the given conditions, i. e.

$$z(t) = \left(0, z_2(t), 0, z_4(t), 0, z_6(t), 0, z_8(t), 0, z_{10}(t), 0, z_{12}(t), \frac{k_7 z_6(t) z_{12}(t)}{k_9}, \right. \\ \left. 0, z_{15}(t), \frac{k_8 z_8(t) z_{15}(t)}{k_{10}}, 0, z_{18}(t) \right)^T.$$

Thus,

$$\dot{z}(t) = \left(0, \dot{z}_2(t), 0, \dot{z}_4(t), 0, \dot{z}_6(t), 0, \dot{z}_8(t), 0, \dot{z}_{10}(t), 0, \dot{z}_{12}(t), \frac{k_7}{k_9} (\dot{z}_6 z_{12} + z_6 \dot{z}_{12}), \right. \\ \left. 0, \dot{z}_{15}(t), \frac{k_8}{k_{10}} (\dot{z}_8 z_{15} + z_8 \dot{z}_{15}), 0, \dot{z}_{18}(t) \right)^T.$$

The given conditions imply immediately

$$\dot{z}(t) = (0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)^T \text{ and } z(t) \text{ is stationary.} \quad \square$$

Remark 6.2.11. The previous result could also have been stated as: *If $x_6(t)$ and $x_8(t)$ are stationary then any solution $z(t)$ starting in N given in Proposition 6.2.8 is stationary.*

For practical reasons, the stability question will be handled after reducing the number of equations to 11.

Model reduction

Let us now reduce the number of equations of the system (6.2.2) to 11. From the first integrals given in Proposition 6.2.5 we eliminate the variables $x_1, x_3, x_5, x_7, x_9,$

x_{13} and x_{16} .

Setting

$$\begin{aligned} x_1 &= -x_2 + d_1; & x_3 &= -x_4 + d_2; \\ x_5 &= -x_6 - x_{13} + d_3; & x_7 &= -x_8 - x_{16} + d_4; \\ x_9 &= -x_{10} - x_{17} + d_5; & x_{16} &= -x_{14} - x_{15} + d_7 \\ x_{13} &= -x_{11} - x_{12} - x_{18} + d_6, \end{aligned}$$

where $d_1 = x_1(0)$; $d_2 = x_3(0)$; $d_3 = x_5(0)$; $d_4 = x_7(0)$; $d_5 = x_9(0)$; $d_6 = x_{11}(0)$; $d_7 = x_{14}(0)$ are different from zero,

and making the following coordinate transformation:

$$\begin{aligned} y_1 &:= x_2; & y_2 &:= x_4; & y_3 &:= x_6; & y_4 &:= x_8; & y_5 &:= x_{10}; & y_6 &:= x_{11}; & y_7 &:= x_{12}; & y_8 &:= \\ x_{14}; & y_9 &= x_{15}; & y_{10} &= x_{17}; & y_{11} &= x_{18} \end{aligned}$$

we obtain the following system of differential equations:

$$\begin{aligned} \frac{dy_1}{dt} &= \frac{kcat_{XII}(-y_1 + d_1)u}{km_{XII} + (-y_1 + d_1)} \\ \frac{dy_2}{dt} &= \frac{kcat_{XI}(-y_2 + d_2)y_1}{km_{XI} + (-y_2 + d_2)} \\ \frac{dy_3}{dt} &= \frac{kcat_{IX}(-y_3 - (-y_6 - y_7 - y_{11} + d_6) + d_3)y_2}{km_{IX} + (-y_3 - (-y_6 - y_7 - y_{11} + d_6) + d_3)} \\ &\quad - k_7 y_7 y_3 + k_9 (-y_6 - y_7 - y_{11} + d_6) \\ \frac{dy_4}{dt} &= \frac{kcat_{10}(-y_4 - (-y_8 - y_9 + d_7) + d_4)(-y_6 - y_7 - y_{11} + d_6)}{km_{10} + (-y_4 - (-y_8 - y_9 + d_7) + d_4)} \\ &\quad - k_8 y_9 y_4 + k_{10} (-y_8 - y_9 + d_7) \\ \frac{dy_5}{dt} &= \frac{kcat_2 y_{10} (-y_8 - y_9 + d_7)}{km_2 + y_{10}} \\ \frac{dy_6}{dt} &= -\frac{kcat_{VIII}}{km_{VIII}} y_6 y_4 - \frac{kcat_8}{km_8} y_6 y_5 \\ \frac{dy_7}{dt} &= \frac{kcat_{VIII}}{km_{VIII}} y_6 y_4 + \frac{kcat_8}{km_8} y_6 y_5 \\ &\quad - k_7 y_7 y_3 + k_9 (-y_6 - y_7 - y_{11} + d_6) - ki_{VIII_a} y_6 \\ \frac{dy_8}{dt} &= -\frac{kcat_V y_8 y_4}{km_V} - \frac{kcat_5 y_8 y_5}{km_5 + y_8} \\ \frac{dy_9}{dt} &= \frac{kcat_V y_8 y_4}{km_V} + \frac{kcat_5 y_8 y_5}{km_5 + y_8} - k_8 y_9 y_4 + k_{10} (-y_8 - y_9 + d_7) \end{aligned}$$

$$\begin{aligned}
\frac{dy_{10}}{dt} &= \frac{kcat_{II}(-y_5 - y_{10} + d_5)(-y_8 - y_9 + d_7)}{km_{II} + (-y_5 - y_{10} + d_5)} - \frac{kcat_2 y_{10}(-y_8 - y_9 + d_7)}{km_2 + y_{10}} \\
\frac{dy_{11}}{dt} &= ki_{VIII_a} y_6.
\end{aligned}
\tag{6.2.3}$$

This system has a single equilibrium point and its coordinates can be calculated explicitly by setting the right-hand side of (6.2.3) to zero and solving to \mathbf{y} . There are again very complicated terms involved and surely lots of impossible solutions due to the physiological meaning of the variables, so we are going to make use of all the information available about the system once more to exclude some candidates.

$$\frac{y_1}{dt} = 0 \Leftrightarrow y_1 = d_1, \frac{y_2}{dt} = 0 \Leftrightarrow y_2 = d_2 \text{ and } \frac{y_{11}}{dt} = 0 \Leftrightarrow y_6 = 0.$$

This implies in particular that the right-hand side of the sixth equation of (6.2.3) equals zero.

Because of the physiological meaning, $y_4 > 0$ and $y_5 > 0$. Then, $\frac{y_8}{dt} = 0 \Leftrightarrow y_8 = 0$ as the remaining variables involved are strictly positive.

The same kind of argument can be used to infer $y_7 = 0$ and $y_{11} = d_6$ after setting $\frac{y_7}{dt} = 0$.

Substituting these conditions we get furthermore that:

$$\frac{y_3}{dt} = 0 \Leftrightarrow y_3 = d_3.$$

Moreover,

$$\frac{y_5}{dt} = 0 \Leftrightarrow y_{10} = 0 \text{ or } y_9 = d_7.$$

$y_9 = d_7$ implies at the same time that $\frac{y_9}{dt} = 0$ only if $y_4 = 0$. But $y_4 \neq 0$, a contradiction. Hence, $y_{10} = 0$.

This implies in particular that $\frac{y_{10}}{dt} = 0$ only if $y_5 = d_5$.

On the other hand,

$$\frac{y_9}{dt} = 0 \Leftrightarrow -k_8 y_9 y_4 + k_{10}(-y_9 + d_7) = 0 \Leftrightarrow y_9 = \frac{k_{10} d_7}{k_8 y_4 + k_{10}}.$$

Finally,

$$\frac{y_4}{dt} = 0 \Leftrightarrow -y_4 + y_9 - d_7 + d_4 = 0.$$

After substituting the condition for y_9 we obtain the following second degree equation in y_4 :

$$k_8 y_4^2 + (k_{10} + k_8(d_7 - d_4))y_4 - d_4 k_{10} = 0.$$

This equation admits only real roots and the value of y_4 at the equilibrium is uniquely determined by the positive root.

Remark 6.2.12. Substituting the values of the constants involved we obtain for the equilibrium point the same values like the ones that can be deduced by visual inspection of the numerical solution.

Remark 6.2.13. Altogether we obtained the coordinates of the unique equilibrium point towards which the system (6.2.3) converges. Thus, by applying Theorem A.4.1 follows asymptotic stability of the equilibrium point of this system.

6.3 Discussion

Taking into account that there are some anticoagulatory drugs capable of influencing the system only in the presence of a physiological anticoagulant factor³, the analysis of controllability makes more sense in a model including inhibitors.

From the sigmoidal like shape of the numerical solution of some variables, we infer that there are cooperativity effects in the blood coagulation network. So, besides using only Michaelis and Menten kinetics, one could think in modelling these behaviors as explained in Section 2.3.3. Another challenge for forthcoming research.

In [Lin95] we are given two schemes that correspond to two models that postulate that coagulation is initiated by the extrinsic pathway. The main difference is the way factor IX becomes activated. One theory emphasizes furthermore the role of factor XI in blood coagulation and the other not even considers its activation. Both models could provide an explanation for the fact that some patients with factor XI deficiency bleed and some do not but not in a satisfactory way. Nevertheless, one could derive the differential equations governing both systems and compare them with the results given by the models we study in this chapter.

In [Pru00] we are given an approach modelling the intrinsic pathway that is very similar to the model developed above. The main difference is that factor XI is activated by thrombin by means of a feedback reaction instead of factor XII_a directly. So, in order to make another bridge for a more complete understanding one could establish the mathematical model for the reaction scheme published in [Pru00] and make further analysis.

³Heparin is an example of an anticoagulatory drug that is only effective in the presence of $ATIII$ (see Chapter 1)

Chapter 7

Extending Stortelder's Model

In this chapter we present two possibilities for extending the model from Stortelder and Hemker. Although the physiological background has already been given in Section 1.1.2 of Chapter 1, we recall that platelets are an essential component of the blood coagulation process *in vivo*. Aggregated, activated platelets provide procoagulant phospholipid-equivalent surfaces upon which the complex-dependent reactions of the blood coagulation network are localized. Thus, our first approach models thrombocyte contribution for blood thrombin formation in the model concerning the common pathway published in [SHH97]. For the set of constants published in [SHH97], the lag that characterizes the course of thrombin concentration with time is hardly noticed by observing the numerical solution. The reason for that may be the fact that the reaction process starts with a purified enzyme RVV . So, in the second approach, we substitute RVV by introducing the remaining plas-matic factors and chemical reactions occurring in the extrinsic pathway, including the action of inhibitors and having the complex $TF.VII_a$ as an activator. Since no experimental data is available and the value of the constants cannot be estimated by others, we limit our selves to present a possible mechanism of action, together with the set of differential equations governing the system and reduce the order of the system by using first integrals.

7.1 Modelling platelet's contribution

As it has been seen in Section 5.2, there is a possibility of steering the course of thrombin by means of an external control. Experimentally, one may add for instance platelets in form of phospholipids to a blood sample in order to accelerate the process of thrombin formation.

Although controllability of the system is an important feature, we wish to have a model where the action of thrombocytes is considered without external inputs. Therefore, we propose in this section a first approach based on a small modification of the system (3.1.1) by modelling a possible mechanism of action of thrombocytes or platelets after being activated by thrombin. At this stage of the common pathway, the major influence of thrombocytes occurs at the level of factor X activation (see Figure 1.1).

Since this approach is an extension of the model proposed in [SHH97], let us go through some aspects that will help to gain some more insights and to give some hints about how this extension can be done.

Ranging the initial concentration of the phospholipids from 0.05 to 200 μmol does not influence the maximal amount of thrombin generated, much likely due to the third order term appearing for instance in the 5th equation of (3.1.1). The reason why this term was included in the model can be understood by taking a look at the model developed by Nesheim, Russel, Tracy and Mann in [NeTrMa84] for the activation of thrombin by the prothrombinase complex.

As already mentioned, prothrombinase is a multicomponent enzymatic complex involved in blood coagulation that proteolytically converts the vitamin K -dependent zymogen prothrombin to the blood clotting enzyme thrombin. The components are activated factors V_a and X_a . These proteins form a 1:1 complex through Ca^{2+} -dependent interaction on negatively charged phospholipid vesicles ($PCPS$).

Figure 7.1 illustrates the development of the model of prothrombinase action published in [NeTrMa84].

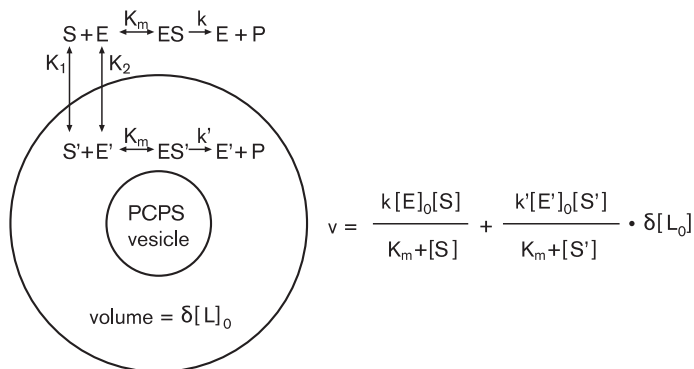


Figure 7.1: Development of the model of prothrombinase taken from [NeTrMa84]

In the figure there are represented three different regions. These regions consist of the bulk solution, the $PCPS$ vesicle and a region in between called *interface*

*shell*¹. As Nesheim and coworkers state, the volume of this region is proportional to the *PCPS* concentration and it is given by

$$V = \delta[L]_0,$$

where V is the summed volume around the vesicles in the reaction, $[L]_0$ is the nominal concentration of *PCPS*, and δ is a constant of proportionality.

Prothrombin is the substrate of this reaction. The model takes the factor X_a as the solution phase catalyst, whereas the complex X_aV_a is the catalyst on the phospholipid surface, what implies that factor V_a is primarily associated with the phospholipid under conditions in which the model is applicable. Comparing with the model (3.1.1), we may identify $[L]$ with $[PL]$ and this interaction is precisely described by the term of third order in the 5th equation. Moreover, in the equation describing the course of thrombin formation, the term

$$\frac{kcat_{II}[II][PT]}{km_{II} + [II]} + \frac{kcat_2[II][X_a]}{km_2 + [II]},$$

where $[PT]$ represents the concentration of the prothrombinase complex, is the sum of the rates in the two components and is of the form of the equation given in Figure 7.1.

7.1.1 The model

In our approach we include the activation of factor X by thrombin at the surface of activated thrombocytes by positive feedback. The reaction is assumed to be of second order with reaction constant k_T :



such that the term $k_T[II_a][X]$ is added to the second equation and subtracted to the eight equation. Moreover, similarly to what was done for $II_a\alpha_2M$ by Stortelder and Hemker, we include two more equations, one for II_aATIII and the other for X_aATIII in order to balance the system.

The reaction scheme is essentially the same as the one in Figure 3.2 together with the reaction (7.1.1).

We obtain the following set of 11 differential equations:

¹The interface shell constitutes an element of volume in which *PCPS*- bound proteins interact

$$\begin{aligned}
\frac{d[X]}{dt} &= -\frac{kcat_X[X][RVV]}{km_X + [X]} \\
\frac{d[X_a]}{dt} &= \frac{kcat_X[X][RVV]}{km_X + [X]} - ki_{X_a}[X_a] \\
&\quad - k_{PT}[V_a][X_a][PL] + k_{PL}[PT] + k_T[II_a][X] \\
\frac{d[V]}{dt} &= -\frac{kcat_V[V][II_a]}{km_V + [V]} \\
\frac{d[V_a]}{dt} &= \frac{kcat_V[V][II_a]}{km_V + [V]} - k_{PT}[V_a][X_a][PL] + k_{PL}[PT] \\
\frac{d[PL]}{dt} &= -k_{PT}[V_a][X_a][PL] + k_{PL}[PT] \\
\frac{d[PT]}{dt} &= k_{PT}[V_a][X_a][PL] - k_{PL}[PT] \\
\frac{d[II]}{dt} &= -\frac{kcat_{II}[II][PT]}{km_{II} + [II]} - \frac{kcat_2[II][X_a]}{km_2 + [II]} \\
\frac{d[II_a]}{dt} &= \frac{kcat_{II}[II][PT]}{km_{II} + [II]} + \frac{kcat_2[II][X_a]}{km_2 + [II]} \\
&\quad - ki_{II_a\alpha_2M}[II_a] - ki_{II_aATIII}[II_a] - k_T[II_a][X] \\
\frac{d[II_a\alpha_2M]}{dt} &= ki_{II_a\alpha_2M}[II_a] \\
\frac{d[II_aATIII]}{dt} &= ki_{II_aATIII}[II_a] \\
\frac{d[X_aATIII]}{dt} &= ki_{X_a}[X_a].
\end{aligned} \tag{7.1.2}$$

We proceed by presenting the results of the numerical integration and with a qualitative analysis of the new system.

7.1.2 Numerical integration

Numerical integration for $t \in [0, 30]$ (in minutes) of the system was again made in SCILAB. RVV was once more set equal to 0.03 and the vector of initial values was given by

$$\mathbf{x}_0 = (0.2, 0, 0.03, 0, 0.05, 0, 1.4, 0, 0, 0, 0)^T.$$

No convergence problems arouse.

In the Figure 7.2 one may observe the course of the amidolytic activity of thrombin with time and compare the original model from Stortelder and Hemker ($k_T = 0$) to the model where $k_T = 5.53$.

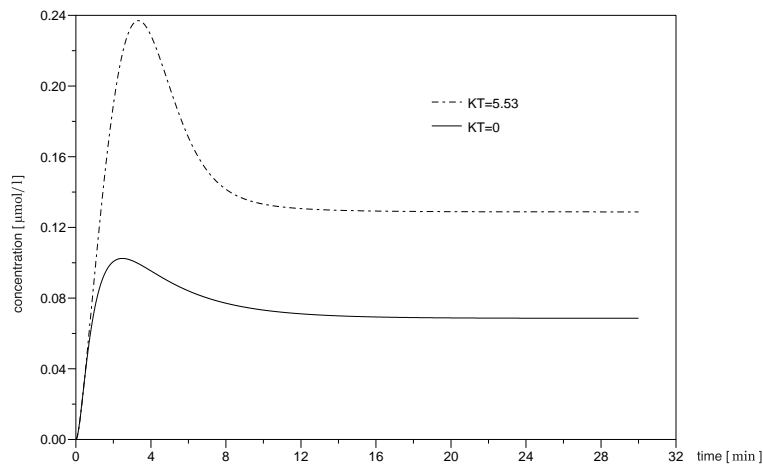


Figure 7.2: Course of the amidolytic activity of thrombin with time with $k_T = 0$ and $k_T = 5.53$.

In the model where $k_T = 5.53$, the concentration increases more than 50 % but the system attains the equilibrium state faster when compared with the original model.

Figure 7.3 illustrates what happens by changing the value $k_T = 5.53$ to the half and to the double for the same value $k_{iII_a\alpha_2M} = 0.2762$.

We observe that with an increase of k_T , the reaction is much faster and that the maximal concentration also increases.

The values of k_T are here fictitious and for a more realistic estimate experimental data should be collected, followed by parameter identification and realization of statistical significance tests.

The SCILAB code and the remaining graphics can be seen in Appendix E.

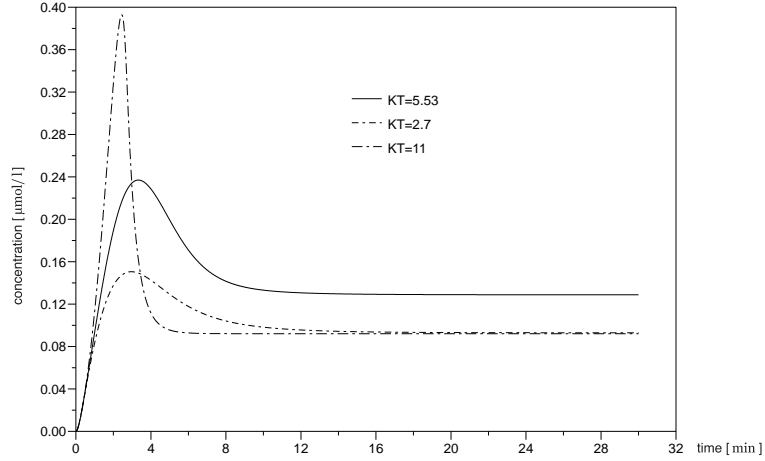


Figure 7.3: Course of the amidolytic activity of thrombin with time where $k_{iII_a\alpha_2M} = 0.2762$ and $k_T = 11, k_T = 5.53$ and $k_T = 2.7$.

7.1.3 Qualitative analysis

Positivity

Let us again rewrite the system (7.1.2) of ordinary differential equations in vector form:

$$\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x}),$$

where the components of \mathbf{x} are given by $x_1 = [X]$, $x_2 = [X_a]$, $x_3 = [V]$, $x_4 = [V_a]$, $x_5 = [PL]$, $x_6 = [PT]$, $x_7 = [II]$, $x_8 = [II_a]$, $x_9 = [II_a\alpha_2M]$, $x_{10} = [II_aATIII]$ and $x_{11} = [X_aATIII]$.

The system (7.1.2) can now be written as

$$\begin{aligned} \frac{dx_1}{dt} &= -\frac{k_1x_1RVV}{k_2+x_1} \\ \frac{dx_2}{dt} &= \frac{k_1x_1RVV}{k_2+x_1} - k_3x_2 - k_4x_4x_2x_5 + k_5x_6 + k_{14}x_8x_1 \\ \frac{dx_3}{dt} &= -\frac{k_6x_3x_8}{k_7+x_3} \\ \frac{dx_4}{dt} &= \frac{k_6x_3x_8}{k_7+x_3} - k_4x_4x_2x_5 + k_5x_6 \end{aligned}$$

$$\begin{aligned}
\frac{dx_5}{dt} &= -k_4x_4x_2x_5 + k_5x_6 \\
\frac{dx_6}{dt} &= k_4x_4x_2x_5 - k_5x_6 \\
\frac{dx_7}{dt} &= -\frac{k_8x_7x_6}{k_9 + x_7} - \frac{k_{10}x_7x_2}{k_{11} + x_7} \\
\frac{dx_8}{dt} &= \frac{k_8x_7x_6}{k_9 + x_7} + \frac{k_{10}x_7x_2}{k_{11} + x_7} - k_{12}x_8 - k_{13}x_8 - k_{14}x_8x_1 \\
\frac{dx_9}{dt} &= k_{12}x_8 \\
\frac{dx_{10}}{dt} &= k_{13}x_8 \\
\frac{dx_{11}}{dt} &= k_3x_2,
\end{aligned} \tag{7.1.3}$$

where the constants $k_i, i = 1, \dots, 13$ have the same meaning as in Section 4.1, and $k_{14} = k_T$.

Let furthermore

$$\mathcal{P} = \{\mathbf{x} \in \mathbb{R}^{11} : x_1 > 0, \dots, x_{11} > 0\}$$

be the positive orthant of \mathbb{R}^{11} .

Proposition 7.1.1. \mathcal{P} and $\overline{\mathcal{P}}$ are positively invariant for the system (7.1.3).

Proof. The vector \mathbf{x} represents a vector of concentrations, and this means that $x_i \geq 0, i = 1, \dots, 11$. Then, the system is defined in a relative open subset of \mathcal{P} with $\mathbf{f}(\mathbf{x}) = (f_1(\mathbf{x}), \dots, f_{11}(\mathbf{x}))^T$, where the function \mathbf{f} is C^∞ and the components $f_i, i = 1, \dots, 11$ are defined by the right-hand side of (7.1.3).

For $\mathbf{x} \in \partial\mathcal{P}$, define $C_x = \mathcal{P}$. Setting furthermore $x_k = 0$ in $f_k, k = 1, \dots, 11$ yields $f_i(x_1, \dots, x_{k-1}, 0, x_{k+1}, \dots, x_{11}) \geq 0$ for all $x_j \geq 0, j \neq k$ and $i = 1, \dots, 11$.

Therefore, $\mathbf{f}(\mathbf{x}) \in \overline{\mathcal{P}}$, for all $\mathbf{x} \in \partial\mathcal{P}$. Hence, \mathcal{P} and $\overline{\mathcal{P}}$ are positively invariant for the system (7.1.3). \square

As a consequence of the last proposition, any orbit starting with positive initial values will remain positive for all times.

First integrals and analysis of stability

Since

$$\begin{cases} \dot{x}_5 + \dot{x}_6 = 0 \\ \dot{x}_3 + \dot{x}_4 + \dot{x}_6 = 0 \\ \dot{x}_1 + \dot{x}_2 + \dot{x}_6 + \dot{x}_{11} + \dot{x}_7 + \dot{x}_8 + \dot{x}_9 + \dot{x}_{10} = 0, \end{cases} \tag{7.1.4}$$

we conclude that there are at least three conserved quantities in the system (7.1.3). In fact, we have the following proposition:

Proposition 7.1.2. *The scalar valued functions $\varphi_1(\mathbf{x}) = x_6 + x_5$, $\varphi_2(\mathbf{x}) = x_3 + x_4 + x_6$ and $\varphi_3(\mathbf{x}) = x_1 + x_2 + x_6 + x_{11} + x_7 + x_8 + x_9 + x_{10}$ defined on \mathbb{R}^{11} are first integrals of the system (7.1.3).*

Corollary 7.1.3. *Given any solution of (7.1.3) with nonnegative initial values, all the components $x_i(t), i = 1, \dots, 11$ are bounded.*

Proof. By directly applying the last proposition and Remark A.1.5 we conclude that the solutions of the system (7.1.3) remain in the level set of φ_1, φ_2 and of φ_3 , in which they start. Hence, $\varphi_1(x_i(t)), \varphi_2(x_i(t))$ and $\varphi_3(x_i(t))$ are constant functions of t for all solutions and therefore bounded. Thus, all the components $x_i(t), i = 1, \dots, 11$ are bounded. \square

Furthermore, the following proposition holds:

Proposition 7.1.4. *The scalar valued functions defined on \mathbb{R}^{11} by $\psi_1(\mathbf{x}) = k_{13}x_9 - k_{12}x_{10}$ and $\psi_2 = \frac{k_6}{k_{12}}x_9 + k_7 \ln x_3 + x_3$ are first integrals of the system (7.1.3).*

Proof. Applying directly the definition we show that $L_{\mathbf{f}}(\psi_i) = 0, i = 1, 2$.

$$\begin{aligned} L_{\mathbf{f}}(\psi_1)(\mathbf{x}) &= D_{\mathbf{x}}(\psi_1)\mathbf{f}(\mathbf{x}) = (0, 0, 0, 0, 0, 0, 0, 0, k_{13}, -k_{12}, 0) \begin{pmatrix} f_1(\mathbf{x}) \\ \vdots \\ f_{11}(\mathbf{x}) \end{pmatrix} \\ &= k_{13}k_{12}x_8 - k_{12}k_{13}x_8 = 0; \end{aligned}$$

$$\begin{aligned} L_{\mathbf{f}}(\psi_2)(\mathbf{x}) &= D_{\mathbf{x}}(\psi_2)\mathbf{f}(\mathbf{x}) = (0, 0, \frac{k_7}{x_3} + 1, 0, 0, 0, 0, 0, \frac{k_6}{x_{12}}, 0, 0) \begin{pmatrix} f_1(\mathbf{x}) \\ \vdots \\ f_{11}(\mathbf{x}) \end{pmatrix} \\ &= \left(\frac{k_7}{x_3} + 1 \right) \left(-\frac{k_6 x_3 x_8}{k_7 + x_3} \right) + \frac{k_6}{k_{12}} k_{12} x_8 \\ &= -k_6 x_8 + k_6 x_8 = 0; \end{aligned}$$

\square

The proof of the next proposition is in all similar to the proof of Proposition 4.3.5 and therefore there is no need to repeat it here. So we state without proof that:

Proposition 7.1.5. *Let $\varphi_4(\mathbf{x}) = x_1, \varphi_5(\mathbf{x}) = -x_{11}, \varphi_6(\mathbf{x}) = -x_{10}$ and $\varphi_7(\mathbf{x}) = x_7$ be scalar functions defined on \mathbb{R}^{11} . Then $L_{\mathbf{f}}(\varphi_i)(\mathbf{x}) \leq 0, i = 4, \dots, 7$.*

Theorem 7.1.6. *The positive limit set $\omega(\mathbf{y})$ of (7.1.3) is contained in*

$$N := \{\mathbf{x} \in \mathcal{P} : x_1 = 0, x_2 = 0, x_4 + x_3 + x_6 = 0.03, x_6 + x_5 = 0.05, x_8 = 0, \\ x_1 + x_2 + x_6 + x_{11} + x_7 + x_8 + x_9 + x_{10} = 1.6\}.$$

Furthermore, any solution in N is stationary and the equilibrium point is stable.

Proof. The two previous results together with Theorem A.3.11 imply that the compact sets $M_\alpha := \{\mathbf{x} \in \mathcal{P} : \varphi_j(\mathbf{x}) \leq \alpha, j = 1, \dots, 7\}$ are positively invariant for all $\alpha \in [0, \beta_j)$. Theorem A.3.15 guaranties that the solution of the initial value problem exists on $[0, \infty)$. Moreover, this solution approaches its positive limit set $\omega(\mathbf{y})$, as $t \rightarrow \infty$. So, $\omega(\mathbf{y})$ is nonempty, compact and connected. By Theorem A.3.16 $\omega(\mathbf{y})$ is also invariant.

Hence, by applying LaSalle's principle stated in Theorem A.4.1 we conclude that

$$\omega(\mathbf{y}) \subset N.$$

Let furthermore $z(t) = (z_1(t), \dots, z_{11}(t))^T$ be a solution of (7.1.3) in N . We prove that $z(t)$ is stationary. It holds,

$z(t) = (0, 0, z_3(t), z_4(t), z_5(t), z_6(t), z_7(t), 0, z_9(t), z_{10}(t), z_{11}(t))^T$. Thus, together with (7.1.3) we have

$$\dot{z}(t) = \begin{pmatrix} 0 \\ 0 \\ \dot{z}_3 \\ \dot{z}_4 \\ \dot{z}_5 \\ \dot{z}_6 \\ \dot{z}_6 \\ 0 \\ \dot{z}_9 \\ \dot{z}_{10} \\ \dot{z}_{11} \end{pmatrix} \stackrel{!}{=} \begin{pmatrix} 0 \\ k_5 z_6 \\ 0 \\ k_5 z_6 \\ k_5 z_6 \\ -k_5 z_6 \\ \frac{k_8 z_7 z_6}{k_9 + z_7} \\ \frac{k_8 z_7 z_6}{k_9 + z_7} \\ 0 \\ 0 \\ 0 \end{pmatrix}.$$

This means, in particular, that $z_6 = 0$ since $\dot{z}_2 = 0$, and

$$\dot{z}(t) = (0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)^T.$$

That is, $z(t)$ is constant and the solution is stationary.

The ω -limit set of (7.1.3) is nonempty. However, we still do not know exactly its representation. To calculate the equilibrium points we set like in Theorem 4.1.6 the

right-hand side of the system equal to zero and solve for \mathbf{x} . The set of stationary points belongs to the set \mathcal{E} given by:

$$E = \alpha_3 \begin{pmatrix} 0 \\ 0 \\ 1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix} + \alpha_4 \begin{pmatrix} 0 \\ 0 \\ 0 \\ 1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix} + \alpha_7 \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix} + \alpha_9 \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 0 \\ 0 \\ 0 \end{pmatrix} + \alpha_{10} \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 0 \end{pmatrix} + \alpha_{11} \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \end{pmatrix},$$

$$\alpha_3, \alpha_4, \alpha_5, \alpha_7, \alpha_9 \in \mathbb{R}_0^+.$$

The points of \mathcal{E} belong to N if $\alpha_3 + \alpha_4 = 0.03 = x_3(0)$, $\alpha_7 = 1.6 - (x_9 + x_{10} + x_{11}) = (x_1(0) + x_7(0)) - (x_9 + x_{10} + x_{11})$. So, we may write instead:

$$P = \begin{pmatrix} 0 \\ 0 \\ 0 \\ x_3(0) \\ x_5(0) \\ 0 \\ x_7(0) + x_1(0) \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix} + \beta_3 \begin{pmatrix} 0 \\ 0 \\ 1 \\ -1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix} + \beta_9 \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ -1 \\ 0 \\ 1 \\ 0 \end{pmatrix} + \beta_{10} \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ -1 \\ 0 \\ 1 \end{pmatrix} + \beta_{11} \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ -1 \\ 1 \end{pmatrix}, \quad (7.1.5)$$

where $0 \leq \beta_3 \leq 0.03$ and $0 \leq \beta_9 + \beta_{10} + \beta_{11} \leq x_1(0) + x_7(0)$ to exclude negative concentrations.

The Jacobian of (7.1.3) at any equilibrium point has the representation:

$$\begin{pmatrix} -\frac{k_1[RVV]}{k_2} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{k_1[RVV]}{k_2} & -k_3 - k_4\alpha_4\alpha_5 & 0 & 0 & 0 & k_5 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & \frac{-k_6\alpha_3}{k_7 + \alpha_3} & 0 & 0 & 0 \\ 0 & -k_4\alpha_4\alpha_5 & 0 & 0 & 0 & k_5 & 0 & \frac{k_6\alpha_3}{k_7 + \alpha_3} & 0 & 0 & 0 \\ 0 & -k_4\alpha_4\alpha_5 & 0 & 0 & 0 & k_5 & 0 & 0 & 0 & 0 & 0 \\ 0 & k_4\alpha_4\alpha_5 & 0 & 0 & 0 & -k_5 & 0 & 0 & 0 & 0 & 0 \\ 0 & -\frac{k_{10}\alpha_7}{k_{11} + \alpha_7} & 0 & 0 & 0 & -\frac{k_8\alpha_7}{k_9 + \alpha_7} & 0 & 0 & 0 & 0 & 0 \\ 0 & \frac{k_{10}\alpha_7}{k_{11} + \alpha_7} & 0 & 0 & 0 & \frac{k_8\alpha_7}{k_9 + \alpha_7} & 0 & -k_{12} - k_{13} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & k_{12} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & k_{13} & 0 & 0 & 0 \\ 0 & k_3 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}.$$

We observe that the Jacobian suffered very little changes if we compare it with the original model from Stortelder and Hemker. In this case zero is an eigenvalue of the matrix with algebraic and geometric multiplicities equal to 7 and that there are conserved quantities present. And, any equilibrium point of (7.1.3) and the singularity is nonisolated.

With the first integrals given in Proposition 7.1.2 and Proposition 7.1.4 we are able to reduce the order of the system to 6. The set of equilibrium points of the reduced system built the triangle

$$P = \begin{pmatrix} 0 \\ 0 \\ 0 \\ c_1 \\ 0 \\ 0 \end{pmatrix} + \mu_3 \begin{pmatrix} 0 \\ 0 \\ 1 \\ 0 \\ 0 \\ 0 \end{pmatrix} + \mu_6 \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \end{pmatrix}, \quad \mu_3, \mu_6 \in \mathbb{R}_0^+, \quad (7.1.6)$$

with $0 \leq \mu_3 \leq [V](0)$ and $0 \leq \mu_6 \leq [X](0)$.

Zero is an eigenvalue with algebraic and geometric multiplicities equal to two. The corresponding eigenvectors are the direction vectors of the hyperplane containing the equilibrium points. The Jacobian at the equilibrium of the reduced system has 2 zero columns and the last line is a linear combination of the first, second and sixth lines and it has the form

$$J_r^* := \left(\begin{array}{ccc|c} & J_r & & \mathbf{0}_{5 \times 1} \\ - & - & - & - \\ 0 & k_3 & 0 & 0 \end{array} \right),$$

where J_r is the matrix (4.2.3).

In Theorem 4.1.6 we proved that the nonzero eigenvalues of J_r have negative real part. Since any solution starting in N is stationary and the system converges to a nonisolated stationary point, a similar argument allows us to conclude stability of the equilibrium point of (7.1.3). The coordinates corresponding to the eigenvalues equal zero are therefore constants that depend on the initial value problem and the remaining ones are equal zero. \square

Remark 7.1.7. Notice that the stability of the linear system $\dot{\mathbf{y}} = J_r^* \mathbf{y}$ can be concluded from the stability of the matrix J_r .

Let $\lambda_1, \lambda_2, \lambda_3$ and λ_4 be the nonzero eigenvalues of J_r . In Theorem 4.1.6 we proved that $\operatorname{Re} \lambda_i < 0, i = 1, \dots, 4$. Thus, the general solution of the linear system

$$\dot{\mathbf{y}} = J_r^* \mathbf{y}$$

can be written as

$$\mathbf{y}(t) = \alpha_1 \mathbf{v}_1 e^{\lambda_1 t} + \alpha_2 \mathbf{v}_2 e^{\lambda_2 t} + \alpha_3 \mathbf{v}_3 e^{\lambda_3 t} + \alpha_4 \mathbf{v}_4 e^{\lambda_4 t} + \alpha_5 \mathbf{v}_5 + \alpha_6 \mathbf{v}_6,$$

with $\mathbf{v}_i, i = 1, \dots, 4$ the corresponding eigenvectors to λ_i and $\mathbf{v}_5 = (0, 0, 1, 0, 0, 0)^T$ and $\mathbf{v}_6 = (0, 0, 0, 0, 0, 1)^T$.

For $t \rightarrow \infty$ we have $\mathbf{y}(t) \rightarrow \alpha_5 \mathbf{v}_5 + \alpha_6 \mathbf{v}_6$, with α_5 and α_6 uniquely determined by the initial values. It holds,

$$|\mathbf{y}(t, \mathbf{y}_0) - \mathbf{y}(t, \tilde{\mathbf{y}}_0)| = |\alpha_1 \mathbf{v}_1 e^{\lambda_1 t} + \alpha_2 \mathbf{v}_2 e^{\lambda_2 t} + \alpha_3 \mathbf{v}_3 e^{\lambda_3 t} + \alpha_4 \mathbf{v}_4 e^{\lambda_4 t} - \tilde{\alpha}_1 \mathbf{v}_1 e^{\lambda_1 t} - \tilde{\alpha}_2 \mathbf{v}_2 e^{\lambda_2 t} - \tilde{\alpha}_3 \mathbf{v}_3 e^{\lambda_3 t} - \tilde{\alpha}_4 \mathbf{v}_4 e^{\lambda_4 t} + (\alpha_5 - \tilde{\alpha}_5) \mathbf{v}_5 + (\alpha_6 - \tilde{\alpha}_6) \mathbf{v}_6|.$$

Since $(\alpha_1 - \tilde{\alpha}_1) \mathbf{v}_1 e^{\lambda_1 t} + (\alpha_2 - \tilde{\alpha}_2) \mathbf{v}_2 e^{\lambda_2 t} + (\alpha_3 - \tilde{\alpha}_3) \mathbf{v}_3 e^{\lambda_3 t} + (\alpha_4 - \tilde{\alpha}_4) \mathbf{v}_4 e^{\lambda_4 t}$ is $o(1)$, as $t \rightarrow \infty$ we have

$$|\mathbf{y}(t, \mathbf{y}_0) - \mathbf{y}(t, \tilde{\mathbf{y}}_0)| \leq K\delta, \text{ with } \delta \rightarrow 0 \text{ as } \tilde{\mathbf{y}}_1 \rightarrow \mathbf{y}_0.$$

And the assertion follows by definition of stability.

Model reduction

From the first integrals given in Proposition 7.1.5 we eliminate the variables x_4, x_6 and x_7 :

$x_6 = -x_5 + c_1$; $x_4 = -x_3 - x_6 + c_2$ and $x_7 = -x_1 - x_2 - x_6 - x_{11} - x_8 - x_9 - x_{10} + c_3$, where $c_1 = x_5(0)$; $c_2 = x_3(0)$ and $c_3 = x_1(0) + x_7(0)$.

Hence, we omit the 4th, 6th and 7th equations.

By Proposition 7.1.4

$$x_9 = \frac{k_{12}}{k_6}(c - k_7 \ln x_3 - x_3), \text{ where } c = k_7 \ln x_3(0) + x_3(0) \text{ and } x_{10} = \frac{k_3}{k_{12}}x_9.$$

Altogether, the order of the system can be reduced to 6 after performing the following coordinate transformation:

$$y_1 := x_1; y_2 := x_2; y_3 := x_3; y_4 := x_5; y_5 := x_8; y_6 := x_{11}$$

and each occurrence of x_4, x_6, x_7, x_9 and x_{10} is substituted respectively by:

$$x_4 = y_4 - y_3 + c'_1 \text{ with } c'_1 = -x_5(0) + x_3(0) < 0;$$

$$x_6 = -y_4 + c_1 \text{ with } c_1 = x_5(0);$$

$$x_7 = -y_1 - y_2 + y_4 - y_5 - (c - k_7 \ln y_3 - y_3) \left(\frac{k_{12} + k_{13}}{k_6} \right) - y_6 + c'_3 \text{ with } c'_3 = -x_5(0) + x_1(0) + x_7(0);$$

$$x_9 = \frac{k_{12}}{k_6}(c - k_7 \ln y_3 - y_3), \text{ where } c = k_7 \ln x_3(0) + x_3(0) \text{ and } x_{10} = \frac{k_3}{k_{12}}x_9.$$

The reduced system comprises the following 6 differential equations.

$$\begin{aligned} \frac{dy_1}{dt} &= -\frac{k_1 y_1 R V V}{k_2 + y_1} \\ \frac{dy_2}{dt} &= \frac{k_1 y_1 R V V}{k_2 + y_1} - k_3 y_2 - k_4 (y_4 - y_3 + c'_1) y_2 y_4 + k_5 (-y_4 + c_1) + k_{14} y_5 y_1 \\ \frac{dy_3}{dt} &= -\frac{k_6 y_3 y_5}{k_7 + y_3} \\ \frac{dy_4}{dt} &= -k_4 (y_4 - y_3 + c'_1) y_2 y_4 + k_5 (-y_4 + c_1) \\ \frac{dy_5}{dt} &= r_1 + r_2 - k_{12} y_5 - k_{13} y_5 - k_{14} y_5 y_1 \\ \frac{dy_6}{dt} &= k_3 y_2, \end{aligned} \tag{7.1.7}$$

where

$$r_1 = \frac{k_8(-y_1 - y_2 + y_4 - y_5 - (c - k_7 \ln y_3 - y_3) \left(\frac{k_{12} + k_{13}}{k_6} \right) - y_6 + c'_3)(-y_4 + c_1)}{k_9 + (-y_1 - y_2 + y_4 - y_5 - (c - k_7 \ln y_3 - y_3) \left(\frac{k_{12} + k_{13}}{k_6} \right) - y_6 + c'_3)};$$

$$r_2 = \frac{k_{10}(-y_1 - y_2 + y_4 - y_5 - (c - k_7 \ln y_3 - y_3) \left(\frac{k_{12} + k_{13}}{k_6} \right) - y_6 + c'_3)y_2}{k_{11} + (-y_1 - y_2 + y_4 - y_5 - (c - k_7 \ln y_3 - y_3) \left(\frac{k_{12} + k_{13}}{k_6} \right) - y_6 + c'_3)}.$$

Notice furthermore that although the last equation of the system (7.1.7) is a simple integration of $y_2(t)$, it cannot be omitted.

7.2 From the common to the extrinsic pathway

As we have seen in Section 6.1.2 changing the value of complex $TF.VII_a$ concentration influences the lag length. This is not the case when we consider different values of RVV , though.

Our approach is now to substitute RVV used in the truncated clotting mechanism by introducing the remaining plasmatic factors and chemical reactions occurring in the extrinsic pathway leading to the formation of thrombin, including the action of inhibitors and having the complex $TF.VII_a$ as initiator. Since Sortelder's model comprises the action of inhibitors, we also account for the action of $TFPI$, which combines with factor X_a to form a strong inhibitor of the complex $TF.VII_a$. We recall that this complex also activates factor IX that together with factor $VIII_a$ is again a strong activator of factor X . This reaction occurs in the presence of phospholipids and the complex will be designated by IT (intrinsic tenase). Factor $VIII_a$ arises from plasmatic factor $VIII$ previously activated by of thrombin. The contribution of $ATIII$ is modelled as a first order reaction and besides factor X_a it will inhibit factor IX_a and $TF : VII_a$ as well.

The mechanism of inhibition by $TFPI$ has been the subject of numerous investigations. According to [HJEM02], the most satisfactory explanation for $TFPI$ behavior is provided by Baugh et. al. [BBK98]. For another approach see [PZA02].

The mechanism of action of $TFPI$ by Baugh et. al. [BBK98] comprises two steps. In a first step, factor X_a is inhibited the reaction between $TFPI$ and X_a . The X_aTFPI complex formed in this initial step reacts efficiently with $TF.VII_a$ in a second step to yield an inhibited complex $X_a.TFPI.TF.VII_a$.

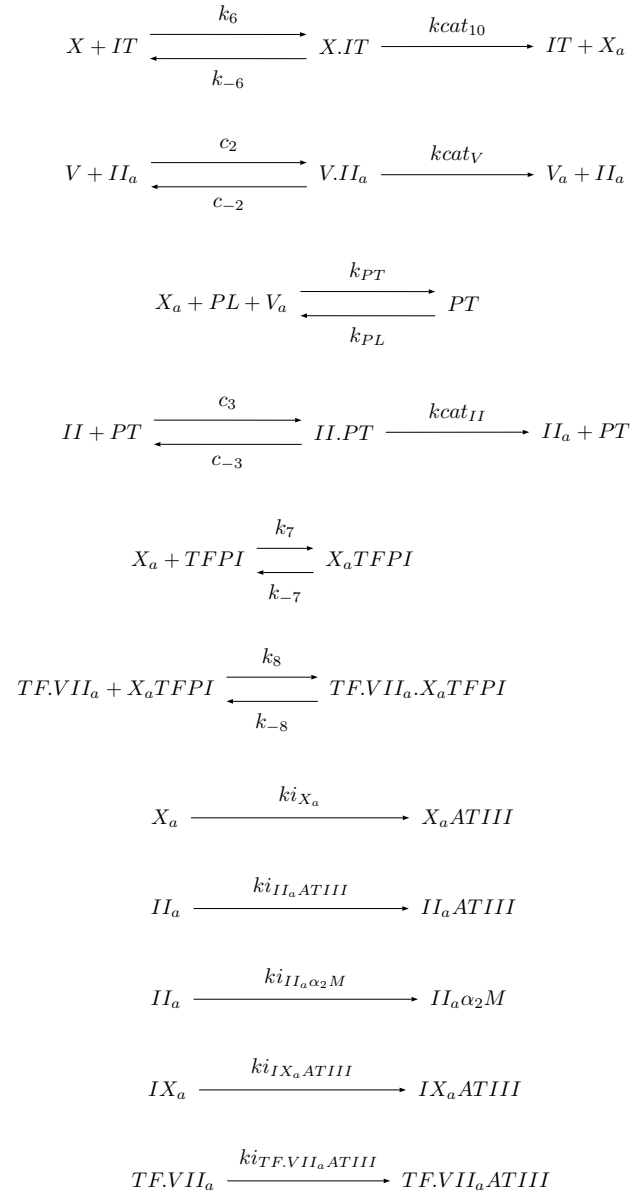


Figure 7.4: Reaction scheme corresponding for the extrinsic pathway with *TFPI*, *ATIII* and α_2M as inhibitors.

We model the catalytic reactions by using the Michaelis and Menten equation and the remaining ones by using the law of mass action. Thus, we obtain the following system of differential equations comprising 22 equations:

$$\begin{aligned}
\frac{d[X]}{dt} &= -\frac{kcat_X[X][TFVII_a]}{km_X + [X]} - \frac{kcat_{10}[X][IT]}{km_{10} + [X]} \\
\frac{d[X_a]}{dt} &= \frac{kcat_X[X][TFVII_a]}{km_X + [X]} + \frac{kcat_{10}[X][IT]}{km_{10}[X_a] + [X]} - ki_{X_aATIII}[X_a] \\
&\quad - k_{PT}[X_a][PL][V_a] + k_{PL}[PT] - k_7[X_a][TFPI] + k_{-7}[X_aTFPI] \\
\frac{d[IX]}{dt} &= -\frac{kcat_{IX}[IX][TFVII_a]}{km_{IX} + [IX]} \\
\frac{d[IX_a]}{dt} &= \frac{kcat_{IX}[IX][TFVII_a]}{km_{IX} + [IX]} - k_5[VIII_a][PL][IX_a] \\
&\quad k_{-5}[IT] - ki_{IX_aATIII}[IX_a] \\
\frac{d[II]}{dt} &= -\frac{kcat_{II}[II][PT]}{km_{II} + [II]} - \frac{kcat_2[X_a][II]}{km_2 + [II]} \\
\frac{d[II_a]}{dt} &= \frac{kcat_{II}[II][PT]}{km_{II} + [II]} + \frac{kcat_2[X_a][II]}{km_2 + [II]} - ki_{II_a\alpha_2M}[II_a] \\
&\quad - ki_{II_aATIII}[II_a] \\
\frac{d[VIII]}{dt} &= -\frac{kcat_{VIII}[VIII][II_a]}{km_{VIII} + [VIII]} \\
\frac{d[VIII_a]}{dt} &= \frac{kcat_{VIII}[VIII][II_a]}{km_{VIII} + [VIII]} - k_5[VIII_a][PL][IX_a] + k_{-5}[IT] \\
\frac{d[IT]}{dt} &= k_5[VIII_a][PL][IX_a] - k_{-5}[IT] \\
\frac{d[V]}{dt} &= -\frac{kcat_V[V][II_a]}{km_V + [V]} \\
\frac{d[V_a]}{dt} &= \frac{kcat_V[V][II_a]}{km_V + [V]} - k_{PT}[X_a][PL][V_a] + k_{PL}[PT] \\
\frac{d[PL]}{dt} &= -k_{PT}[X_a][PL][V_a] + k_{PL}[PT] - k_5[VIII_a][PL][IX_a] + k_{-5}[IT] \\
\frac{d[PT]}{dt} &= k_{PT}[X_a][PL][V_a] - k_{PL}[PT]
\end{aligned}$$

$$\begin{aligned}
\frac{d[TFPI]}{dt} &= -k_7[X_a][TFPI] + k_{-7}[X_aTFPI] \\
\frac{d[X_aTFPI]}{dt} &= k_7[X_a][TFPI] - k_{-7}[X_aTFPI] \\
\frac{d[TFVII_a]}{dt} &= -k_8[TFVII_a][X_aTFPI] + k_{-8}[TFVII_aX_aTFPI] \\
&\quad -ki_{TFVII_aATIII}[TFVII_a] \\
\frac{d[TFVII_aX_aTFPI]}{dt} &= k_8[TFVII_a][X_aTFPI] - k_{-8}[TFVII_aX_aTFPI] \\
\frac{d[X_aATIII]}{dt} &= ki_{X_aATIII}[X_a] \\
\frac{d[II_aATIII]}{dt} &= ki_{II_aATIII}[II_a] \\
\frac{d[II_a\alpha_2M]}{dt} &= ki_{II_a\alpha_2M}[II_a] \\
\frac{d[IX_aATIII]}{dt} &= ki_{IX_aATIII}[IX_a] \\
\frac{d[TFVII_aATIII]}{dt} &= ki_{TFVII_aATIII}[TFVII_a]
\end{aligned} \tag{7.2.1}$$

7.2.1 Qualitative analysis

Positivity

Let us rewrite the system (7.2.1) of ordinary differential equations in vector form:

$$\mathbf{x} = \mathbf{f}(\mathbf{x}),$$

where the components of \mathbf{x} are given by $x_1 = [X]$, $x_2 = [X_a]$, $x_3 = [IX]$, $x_4 = [IX_a]$, $x_5 = [II]$, $x_6 = [II_a]$, $x_7 = [VIII]$, $x_8 = [VIII_a]$, $x_9 = [IT]$, $x_{10} = [V]$, $x_{11} = [V_a]$, $x_{12} = [PL]$, $x_{13} = [PT]$, $x_{14} = [TFPI]$, $x_{15} = [X_aTFPI]$, $x_{16} = [TFVII_a]$, $x_{17} = [TFVII_aX_aTFPI]$, $x_{18} = [X_aATIII]$, $x_{19} = [II_aATIII]$, $x_{20} = [II_a\alpha_2M]$, $x_{21} = [IX_aATIII]$ and $x_{22} = [TFVII_a]$.

The system (7.2.1) can now be written as

$$\begin{aligned}
\frac{dx_1}{dt} &= -\frac{kcat_X x_1 x_{16}}{km_X + x_1} - \frac{kcat_{10} x_1 x_9}{km_{10} + x_1} \\
\frac{dx_2}{dt} &= \frac{kcat_X x_1 x_{16}}{km_X + x_1} + \frac{kcat_{10} x_1 x_9}{km_{10} + x_1} - ki_{X_a ATIII} x_2 \\
&\quad - k_{PT} x_2 x_{12} x_{11} + k_{PL} x_{13} - k_7 x_2 x_{14} + k_{-7} x_{15} \\
\frac{dx_3}{dt} &= -\frac{kcat_{IX} x_3 x_{16}}{km_{IX} + x_3} \\
\frac{dx_4}{dt} &= \frac{kcat_{IX} x_3 x_{16}}{km_{IX} + x_3} - k_5 x_8 x_{12} x_4 + k_{-5} x_9 - ki_{IX_a ATIII} x_4 \\
\frac{dx_5}{dt} &= -\frac{kcat_{II} x_5 x_{13}}{km_{II} + x_5} - \frac{kcat_2 x_5 x_2}{km_2 + x_5} \\
\frac{dx_6}{dt} &= \frac{kcat_{II} x_5 x_{13}}{km_{II} + x_5} + \frac{kcat_2 x_5 x_2}{km_2 + x_5} - ki_{II_a \alpha_2 M} x_6 - ki_{II_a ATIII} x_6 \\
\frac{dx_7}{dt} &= -\frac{kcat_{VIII} x_7 x_6}{km_{VIII} + x_7} \\
\frac{dx_8}{dt} &= \frac{kcat_{VIII} x_7 x_6}{km_{VIII} + x_7} - k_5 x_8 x_{12} x_4 + k_{-5} x_9 \\
\frac{dx_9}{dt} &= k_5 x_8 x_{12} x_4 - k_{-5} x_9 \\
\frac{dx_{10}}{dt} &= -\frac{kcat_V x_{10} x_6}{km_V + x_{10}} \\
\frac{dx_{11}}{dt} &= \frac{kcat_V x_{10} x_6}{km_V + x_{10}} - k_{PT} x_2 x_{12} x_{11} + k_{PL} x_{13} \\
\frac{dx_{12}}{dt} &= -k_{PT} x_2 x_{12} x_{11} + k_{PL} x_{13} - k_5 x_8 x_{12} x_4 + k_{-5} x_9 \\
\frac{dx_{13}}{dt} &= k_{PT} x_2 x_{12} x_{11} - k_{PL} x_{13} \\
\frac{dx_{14}}{dt} &= -k_7 x_2 x_{14} + k_{-7} x_{15} \\
\frac{dx_{15}}{dt} &= k_7 x_2 x_{14} - k_{-7} x_{15} \\
\frac{dx_{16}}{dt} &= -k_8 x_{16} x_{15} + k_{-8} x_{17} - ki_{TFVII_a ATIII} x_{16} \\
\frac{dx_{17}}{dt} &= k_8 x_{16} x_{15} - k_{-8} x_{17}
\end{aligned}$$

$$\begin{aligned}
\frac{dx_{18}}{dt} &= ki_{X_a ATIII} x_2 \\
\frac{dx_{19}}{dt} &= ki_{II_a ATIII} x_6 \\
\frac{dx_{20}}{dt} &= ki_{II_a \alpha_2 M} x_6 \\
\frac{dx_{21}}{dt} &= ki_{IX_a ATIII} x_4 \\
\frac{dx_{22}}{dt} &= ki_{TFVII_a ATIII} x_{16}.
\end{aligned} \tag{7.2.2}$$

Let furthermore

$$\mathcal{P} = \{\mathbf{x} \in \mathbb{R}^{22} : x_1 > 0, \dots, x_{22} > 0\}$$

be the positive orthant of \mathbb{R}^{22} .

Proposition 7.2.1. \mathcal{P} and $\overline{\mathcal{P}}$ are positively invariant for the system (7.2.2).

Proof. The vector \mathbf{x} represents a vector of concentrations, and this means that $x_i \geq 0, i = 1, \dots, 22$. Then, the system is defined in a relative open subset of \mathcal{P} with $\mathbf{f}(\mathbf{x}) = (f_1(\mathbf{x}), \dots, f_{22}(\mathbf{x}))^T$, where the function \mathbf{f} is C^∞ and the components $f_i, i = 1, \dots, 22$ are defined by the right-hand side of (7.2.2).

For $\mathbf{x} \in \partial\mathcal{P}$, define $C_x = \mathcal{P}$. Setting furthermore $x_k = 0$ in $f_k, k = 1, \dots, 22$ yields $f_i(x_1, \dots, x_{k-1}, 0, x_{k+1}, \dots, x_{22}) \geq 0$ for all $x_j \geq 0, j \neq k$ and $i = 1, \dots, 22$.

Therefore, $\mathbf{f}(\mathbf{x}) \in \overline{\mathcal{P}}$, for all $\mathbf{x} \in \partial\mathcal{P}$. Hence, \mathcal{P} and $\overline{\mathcal{P}}$ are positively invariant for the system (7.2.2). \square

As a consequence of the last proposition, any orbit starting with positive initial values will remain positive for all times.

First integrals and convergence to a stationary point

Since

$$\begin{cases}
\dot{x}_1 + \dot{x}_2 + \dot{x}_{13} + \dot{x}_{15} + \dot{x}_{18} = 0 \\
\dot{x}_3 + \dot{x}_4 + \dot{x}_9 + \dot{x}_{21} = 0 \\
\dot{x}_5 + \dot{x}_6 + \dot{x}_{19} + \dot{x}_{20} = 0 \\
\dot{x}_7 + \dot{x}_8 + \dot{x}_9 = 0 \\
\dot{x}_{10} + \dot{x}_{11} + \dot{x}_{13} = 0 \\
\dot{x}_9 + \dot{x}_{12} + \dot{x}_{13} = 0 \\
\dot{x}_{14} + \dot{x}_{15} = 0 \\
\dot{x}_{16} + \dot{x}_{17} + \dot{x}_{22} = 0,
\end{cases} \tag{7.2.3}$$

we conclude that there are at least eight conserved quantities in the system (7.2.2). In fact, we have the following proposition:

Proposition 7.2.2. *The scalar valued functions $\varphi_1(\mathbf{x}) = x_1 + x_2 + x_{13} + x_{15} + x_{18}$, $\varphi_2(\mathbf{x}) = x_3 + x_4 + x_9 + x_{21}$, $\varphi_3(\mathbf{x}) = x_5 + x_6 + x_{19} + x_{20}$, $\varphi_4(\mathbf{x}) = x_7 + x_8 + x_9$, $\varphi_5(\mathbf{x}) = x_{10} + x_{11} + x_{13}$, $\varphi_6(\mathbf{x}) = x_9 + x_{12} + x_{13}$, $\varphi_7(\mathbf{x}) = x_{14} + x_{15}$ and $\varphi_8(\mathbf{x}) = x_{16} + x_{17} + x_{22}$ defined on \mathbb{R}^{22} are first integrals of the system (7.2.2).*

Furthermore, the following proposition holds:

Proposition 7.2.3. *The scalar valued functions defined on \mathbb{R}^{22} by*

$$\psi_1(\mathbf{x}) = \frac{kcat_{IX}}{ki_{TFVII_a ATIII}} x_{22} + km_{IX} \ln x_3 + x_3;$$

$$\psi_2(\mathbf{x}) = \frac{kcat_{VIII}}{ki_{II_a \alpha_2 M}} x_{20} + km_{VIII} \ln x_7 + x_7; \text{ and}$$

$$\psi_3(\mathbf{x}) = \frac{kcat_V}{ki_{II_a ATIII}} x_{20} + km_V \ln x_{10} + x_{10};$$

are first integrals of the system (7.2.2).

Proof. The proof follows immediately by applying the definition of Lie derivative. So, we show that $L_{\mathbf{f}}(\psi_i) = 0, i = 1, 2, 3$.

$$\begin{aligned} L_{\mathbf{f}}(\psi_1)(\mathbf{x}) &= D_{\mathbf{x}}(\psi_1) = (0, 0, \frac{km_{IX}}{x_3} + 1, 0, \dots, 0, \frac{kcat_{IX}}{ki_{TFVII_a ATIII}}, 0, 0) \begin{pmatrix} f_1(\mathbf{x}) \\ \vdots \\ f_{22}(\mathbf{x}) \end{pmatrix} \\ &= \left(\frac{km_{IX}}{x_3} + 1 \right) \left(-\frac{kcat_{IX} x_3 x_{16}}{km_{IX} + x_3} \right) + \frac{kcat_{IX}}{ki_{TFVII_a ATIII}} ki_{TFVII_a ATIII} x_{16} \\ &= -kcat_{IX} x_{16} + kcat_{IX} x_{16} = 0; \end{aligned}$$

Analogously, $L_{\mathbf{f}}(\psi_2) = L_{\mathbf{f}}(\psi_3) = 0$. \square

Corollary 7.2.4. *Given any solution of (7.2.2) with nonnegative initial values, all the components $x_i(t), i = 1, \dots, 22$ are bounded.*

Proof. By directly applying the last proposition and Remark A.1.5 we conclude that the solutions of the system (7.2.2) remain in the level set of $\varphi_i, i = 1, \dots, 8$ in which they start. Hence, $\varphi_j(x_i(t)), j = 1, \dots, 8$ are constant functions of t for all solutions and therefore bounded. Thus, all the components $x_i(t), i = 1, \dots, 22$ are bounded. \square

The proof of the next proposition is in all similar to the proof of Proposition 4.3.5 and therefore there is no need to repeat it here. So we state without proof that:

Proposition 7.2.5. *Let $\varphi_9(\mathbf{x}) = x_1, \varphi_{10}(\mathbf{x}) = x_3, \varphi_{11}(\mathbf{x}) = x_4, \varphi_{12} = -x_{18}, \varphi_{13}(\mathbf{x}) = -x_{19}$ and $\varphi_{14}(\mathbf{x}) = -x_{22}$ be scalar functions defined on \mathbb{R}^{22} . Then $L_{\mathbf{f}}(\varphi_i)(\mathbf{x}) \leq 0, i = 9, \dots, 14$.*

Proposition 7.2.6. *The positive limit set $\omega(\mathbf{y})$ of (7.2.2) is contained in*

$$\begin{aligned} N := \{ \mathbf{x} \in \mathcal{P} : x_1 + x_2 + x_{13} + x_{15} + x_{18} = x_1(0), x_3 + x_4 + x_9 + x_{21} = x_3(0), \\ x_5 + x_6 + x_{19} + x_{20} = x_5(0), x_7 + x_8 + x_9 = x_7(0), x_{10} + x_{11} + x_{13} = \\ x_{10}(0), x_9 + x_{12} + x_{13} = x_{12}(0), x_{14} + x_{15} = x_{14}(0), x_{16} = 0 \\ x_{16} + x_{17} + x_{22} = x_{16}(0) + x_{22}(0), x_1 = 0, x_2 = 0, x_4 = 0, x_6 = 0 \}. \end{aligned}$$

Furthermore, any solution in N is stationary.

Proof. The two previous results together with Theorem A.3.11 imply that the compact sets $M_\alpha := \{ \mathbf{x} \in \mathcal{P} : \varphi_j(\mathbf{x}) \leq \alpha, j = 1, \dots, 14 \}$ are positively invariant for all $\alpha \in [0, \beta_j)$. Theorem A.3.15 guaranties that the solution of the initial value problem exists on $[0, \infty)$. Moreover, this solution approaches its positive limit set $\omega(\mathbf{y})$, as $t \rightarrow \infty$. So, $\omega(\mathbf{y})$ is nonempty, compact and connected. By Theorem A.3.16 $\omega(\mathbf{y})$ is also invariant.

Hence, by applying LaSalle's principle stated in Theorem A.4.1 we conclude that

$$\omega(\mathbf{y}) \subset N.$$

Let furthermore $z(t) = (z_1(t), \dots, z_{22}(t))^T$ be a solution in N . We prove that $z(t)$ is stationary. It holds, $z(t) = (0, 0, z_3(t), 0, z_5(t), 0, z_7(t), z_8(t), z_9(t), z_{10}(t), z_{11}(t), z_{12}(t), z_{13}(t), z_{14}(t), z_{15}(t), z_{16}(t), z_{17}(t), z_{18}(t), z_{19}(t), z_{20}(t), z_{21}(t), z_{22}(t))^T$.

Hence,

$$\begin{aligned} \dot{z}(t) = (0, 0, \dot{z}_3(t), 0, \dot{z}_5(t), 0, \dot{z}_7(t), \dot{z}_8(t), \dot{z}_9(t), \dot{z}_{10}(t), \dot{z}_{11}(t), \dot{z}_{12}(t), \dot{z}_{13}(t), \dot{z}_{14}(t), \\ \dot{z}_{15}(t), \dot{z}_{16}(t), \dot{z}_{17}(t), \dot{z}_{18}(t), \dot{z}_{19}(t), \dot{z}_{20}(t), \dot{z}_{21}(t), \dot{z}_{22}(t))^T. \end{aligned}$$

Thus, together with (7.2.2) we have that $z_2(t) = 0$ implies $\dot{z}_{18} = 0$; $z_4(t) = 0$ implies $\dot{z}_{21} = 0$; and $z_6(t) = 0$ implies $\dot{z}_7 = 0, \dot{z}_{10} = 0, \dot{z}_{19} = 0, \dot{z}_{22} = 0$.

On the other hand, $z_{16} = 0$ implies $\dot{z}_{16} = 0$, i.e. $k_{-8}z_{17} = 0$ or $z_{17} = 0$, because $k_{-8} \neq 0$. That is $\dot{z}_{17} = 0$.

Similarly, $z_4 = 0$ implies $\dot{z}_4 = 0$, i.e. $k_{-5}z_9 = 0$ or $z_9 = 0$, because $k_{-5} \neq 0$. That is, $\dot{z}_9 = 0$. Hence, $\dot{z}_8 = 0$, too.

Finally $z_2 = 0$ implies $\dot{z}_2 = k_{PL}z_{13} + k_{-7}z_{15} = 0$. That is, $z_{13} = z_{15} = 0$, because $k_{PL}, k_{-7} > 0$. Therewith $\dot{z}_{13} = \dot{z}_{15} = 0$. Thus, $\dot{z}_5 = \dot{z}_6 = \dot{z}_{11} = \dot{z}_{12} = \dot{z}_{14} = 0$.

Altogether,

$$\dot{z}(t) = (0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)^T.$$

That is, $z(t)$ is constant and the solution is stationary. \square

Remark 7.2.7. The set of equilibrium points can be obtained once again by setting the right-hand side of the system (7.2.2) to zero and solving for \mathbf{x} . Together with the conditions given in N of Proposition 7.2.6, it turned out that the set of equilibria is a manifold of dimension 4 and it is given by

$$E := \{\mathbf{x} \in \mathbb{R}^{22} : x_1 = 0; x_2 = 0; x_4 = 0; x_6 = 0; x_9 = 0; x_{12} = x_{12}(0); x_{13} = 0; x_{14} = x_{14}(0); x_{15} = 0; x_{16} = 0; x_{17} = 0; x_{18} = x_1(0); x_{21} = x_3(0); x_{22} = x_{16}(0) + x_{22}(0); x_3 + x_{21} = x_3(0); x_5 + x_{19} + x_{21} = x_5(0); x_7 + x_8 = x_7(0); x_{10} + x_{11} = x_{10}(0)\}.$$

Model reduction

From the first integrals given in Proposition 7.2.2 we eliminate the variables $x_{15}, x_9, x_5, x_7, x_{10}, x_{13}, x_{14}$ and x_{17} :

$$x_{15} = -x_1 - x_2 - x_{13} - x_{18} + c_1; x_9 = -x_3 - x_4 - x_{21} + c_2; x_5 = -x_6 - x_{19} - x_{20} + c_3; x_6 = -x_8 - x_9 + c_4; x_{10} = -x_{11} - x_{13} + c_5; x_{13} = -x_9 - x_{12} + c_6; x_{14} = -x_{15} + c_7; \text{ and } x_{17} = -x_{16} - x_{22} + c_8,$$

where $c_1 = x_1(0); c_2 = x_3(0); c_3 = x_5(0); c_4 = x_7(0); c_5 = x_{10}(0); c_6 = x_{12}(0); c_7 = x_{14}(0)$ and $c_8 = x_{16}(0) + x_{22}(0)$.

Hence, we omit equations 15, 9, 5, 7, 10, 13, 14, 17.

By Proposition 7.2.3 we have

$$x_{22} = \frac{ki_{TFVII_a}ATIII}{kcat_{IX}}(c_9 - km_{IX} \ln x_3 - x_3),$$

$$x_{20} = \frac{ki_{II_a} \alpha_2 M}{kcat_{VIII}}(c_{10} - km_{VIII} \ln x_7 - x_7) \text{ and}$$

$$x_{19} = \frac{ki_{II_a}ATIII}{kcat_V}(c_{11} - km_V \ln x_{10} - x_{10})$$

$$\text{where } c_9 = km_{IX} \ln x_3(0) + x_3(0) + \frac{kcat_{IX}}{ki_{TFVII_a}ATIII} x_{22}(0),$$

$$c_{10} = km_{VIII} \ln x_7(0) + x_7(0) \text{ and } c_{11} = km_V \ln x_{10}(0) + x_{10}(0).$$

Then the order of the system can be reduced to 11 after performing the following coordinate transformation:

$$y_1 := x_1; y_2 := x_2; y_3 := x_3; y_4 := x_4; y_5 := x_6; y_6 := x_8; y_7 := x_{11}; y_8 := x_{12}$$

$$y_9 := x_{16}; y_{10} := x_{18}; y_{11} := x_{21}.$$

We obtain the following system of 11 differential equations.

$$\begin{aligned}
\frac{dy_1}{dt} &= -\frac{kcat_X y_1 y_9}{km_X + y_1} - \frac{kcat_{10} y_1 r_2}{km_{10} + y_1} \\
\frac{dy_2}{dt} &= \frac{kcat_X y_1 y_9}{km_X + y_1} + \frac{kcat_{10} y_1 r_2}{km_{10} y_2 + y_1} - ki_{X_a ATIII} y_2 \\
&\quad - k_{PT} y_2 y_8 y_7 + k_{PL} r_3 - k_7 y_2 r_4 + k_{-7} r_1 \\
\frac{dy_3}{dt} &= -\frac{kcat_{IX} y_3 y_9}{km_{IX} + y_3} \\
\frac{dy_4}{dt} &= \frac{kcat_{IX} y_3 y_9}{km_{IX} + y_3} - k_5 y_6 y_8 y_4 + k_{-5} r_2 - ki_{IX_a ATIII} y_4 \\
\frac{dy_5}{dt} &= \frac{kcat_{II} r_{10} r_3}{km_{II} + r_{10}} + \frac{kcat_2 r_{10} y_2}{km_2 + r_{10}} - ki_{II_a \alpha_2 M} y_5 - ki_{II_a ATIII} y_5 \\
\frac{dy_6}{dt} &= \frac{kcat_{VIII} r_5 y_5}{km_{VIII} + r_5} - k_5 y_6 y_8 y_4 + k_{-5} r_2 \\
\frac{dy_7}{dt} &= \frac{kcat_V r_6 y_5}{km_V + r_6} - k_{PT} y_2 y_8 y_7 + k_{PL} r_3 \\
\frac{dy_8}{dt} &= -k_{PT} y_2 y_8 y_7 + k_{PL} r_3 - k_5 y_6 y_8 y_4 + k_{-5} r_2 \\
\frac{dy_9}{dt} &= -k_8 y_9 r_1 + k_{-8} r_{11} - ki_{TFVII_a ATIII} y_9 \\
\frac{dy_{10}}{dt} &= ki_{X_a ATIII} y_2 \\
\frac{dy_{11}}{dt} &= ki_{IX_a ATIII} y_4
\end{aligned} \tag{7.2.4}$$

with,

$$\begin{aligned}
r_1 &= -y_1 - y_2 - y_3 - y_4 - y_{11} + y_8 - y_{10} + c_2 - c_6 + c_1; \\
r_2 &= -y_3 - y_4 - y_{11} + c_2; \quad r_3 = y_3 + y_4 - y_8 - c_2 + c_6; \\
r_4 &= y_1 + y_2 + y_3 + y_4 + y_{11} - y_8 + y_{10} - c_2 + c_6 - c_1 + c_7; \\
r_5 &= -y_6 + y_3 + y_4 + y_{11} - c_2 + c_4; \\
r_6 &= -y_7 - y_3 - y_4 - y_{11} + y_8 + c_2 - c_6 + c_5; \quad r_7 = \frac{ki_{TFVII_a ATIII}}{kcat_{IX}} (c_9 - km_{IX} \ln y_3 - y_3); \\
r_8 &= \frac{ki_{II_a \alpha_2 M}}{kcat_{VIII}} (c_{10} - km_{VIII} \ln r_5 - r_5); \quad r_9 = \frac{ki_{II_a ATIII}}{kcat_V} (c_{11} - km_V \ln r_6 - r_6); \\
r_{10} &= -y_5 - r_9 - r_{20} + c_3; \quad r_{11} = -y_9 - r_7 + c_8.
\end{aligned}$$

Although the number of equations is reduced, the complexity of the right-hand side of the dynamical system has increased. It is however possible that the complexity diminishes if some values of the constants are known. On the other hand, there are constants that are dimensionless and others that are not. Therefore, the next step before handling questions of stability would be to make a dimension analysis. A work to be accomplished in a future investigation. The same can be said to what the numerical solution is concerned.

Chapter 8

Summary and Concluding Remarks

The first chapter is a brief description of the blood coagulation system. Besides making an excursion through the nomenclature and the principal properties of this system we were confronted with the pertinence of some questions that are nowadays object of discussion among the scientific community investigating the process of thrombin formation. As a matter of fact, we are in the presence of a network of chemical reactions involving a series of positive and negative feedback loops and Chapter 1 gave an insight into what is known about this complex system and the problems arising while modelling such a system mathematically.

The second chapter is an excursion on what is known about the mathematical modelling of biochemical networks. There we presented some of the formalism developed by Martin Feinberg and Rutherford Aris regarding structural aspects of such networks. This allowed easy reference to some of the best known concepts and theories in this field, like stoichiometry or the defect of a network, that are not standard for mathematicians. Although this formalism does not include explicitly mathematical descriptions of reactions where a substance is activated by another, like in the blood coagulation system when an inactive proenzyme is converted into its active form, this chapter was useful, in particular, to understand what determines the structure of a chemical network.

In the third chapter we described two of the most cited mathematical models for modelling a part of the blood coagulation system. These models are due to Stortelder, Hemker and Hemker [SHH97] and to Jones and Mann [JoMa94]. The model from Stortelder and Hemker models the so called common pathway and the one from Jones and Mann the so called extrinsic pathway. The models comprise

systems of nonlinear differential equations, where the reaction constants are taken as parameters and the physiological concentration of the different factors involved in the blood coagulation process are taken as initial values. To the description done in this chapter it belongs the presentation of the numerical solution and the analysis of the stoichiometry. From the numerical analysis of the system by Stortelder we could identify some of the parameters that influence both the concentration of thrombin at the equilibrium and the total amount of thrombin formed. Since the reaction scheme of Stortelder and Hemker does not fit the formalism presented in the second chapter, the stoichiometric analysis was only performed for the model due to Jones and Mann. Moreover, we interpreted the reaction scheme as a graph and we observed the existence of loops and determined the number of connected components. Since the network has deficiency 4 and is not weakly reversible, we could not conclude or exclude, with the formalism presented in chapter two, the existence of multiple positive equilibria in each stoichiometric class. Due to inconsistencies to the law of mass action, we traced back the equations corresponding to reaction scheme of Jones and Mann and obtained a new set of equations.

The fourth chapter is devoted to the analysis of the dynamics of both systems by doing a qualitative analysis using results of the local theory of differential equations and nonlinear dynamical systems. For the readers that are not familiar with the main results of the qualitative theory, we present a survey of known results in appendix. We concluded that the positive orthant and its closure are positively invariant for Stortelder's system, that the solutions are bounded and that every solution starting in the set containing the positive ω -limit set is stationary. Although been relatively compact, we were also able to reduce the dimension of the system from 9 to 5 after identifying three linear first integrals and one nonlinear first integral. It turned out that the equilibrium points of the reduced system belonged to a segment of line and that the Jacobian at any equilibrium point had one eigenvalue equal to zero and that the remaining ones have negative real parts. The stability of this point was demonstrated therefore with the help of a result from Bibikov. To finalize, we made a numerical simulation and observed that the course of thrombin concentration with time is very well described by its linearization at the equilibrium point toward which the system converges. The qualitative analysis of the system proposed by Jones and Mann should have followed the same pattern as before, however we prove that the system does not remain positive for all times for a given set of positive initial values and the qualitative analysis was performed for the corrected version presented in the third chapter. This model comprises 18 equations and the positive orthant is positively invariant and the solution bounded. In contrast with the model from Stortelder and Hemker, the sum of all the components is again a first integral. However, with the results at hand we were not able to prove that any solution starting in the set containing the positive ω -limit set is stationary. There were some conditions missing and we could deduce them after calculating some of the coordinates of the equilibrium

point explicitly. Using 6 linear independent first integrals we were able to reduce the dimension of the system to 12. The equilibrium point was determined explicitly and asymptotic stability of the reduced model followed.

Since the application of a drug can be of interest to reestablish hemostatic equilibrium, it is very important to address the question of the controllability of the system. This was done in the fifth chapter. There we started by analyzing the controllability of the linearized system from Stortelder and Hemker. It turned out that the linearized system was not completely controllable, however we identified a controllable subspace of dimension 4 and give a numerical example illustrating the hypothetical inhibition of anti-thrombin. The controllability of the non-linear system followed by the identification of a flat output. For the readers that are not familiar with these mathematical concepts, we made a summary of the principal definitions and results and it can be found in appendix.

In chapter three we derived the set of differential equations corresponding to the reaction scheme published in the paper [JoMa94] following the same strategy as the authors by using the law of mass action. However, one should keep in mind that we are in the presence of enzyme catalyzed reactions and that the Michaelis and Menten approach may be more appropriate for the modelling of such kind of networks. So, in chapter six, we rewrite the system by using this approach every time we are in the presence of a catalysis. While deriving the set of differential equations one notices that there is a huge number of candidate models and from these we selected the one that better approximated the curve obtained by numerical integration of the system modelled using the law of mass action and a qualitative analysis was done yielding the same qualitative information as before. Testing every empirical result would be very exhaustive, however one should at least verify whether the system is responsive to changes in concentration of some of the factors involved. In our case, we checked the effect of changing different concentrations of the process input and compared the course of thrombin concentration with time in the presence and absence of hemophilia. This model for the extrinsic pathway served furthermore as a basis for building a model for the intrinsic pathway. Although there is some controversy regarding this pathway, its contribution for thrombin formation and the occurrence or not of a determined reaction, one should keep in mind that the presence of foreign substances, like artificial valves, in the organism may activate the intrinsic cascade and damage vital organs. So, while there is not a consensus among the scientific community investigating the blood coagulation system regarding this, it makes sense to introduce new mathematical approaches to model a part of this mechanism in order to gain more insights. These should be validated with experimental data of course. Unfortunately, by the time this thesis has been written, no experimental data was available and therefore no validation or refutation of our approach was possible. Nevertheless, we proposed a possible mechanism of action and provided a numerical solution that agreed with the theoretical knowledge in the field. Besides that,

we did a qualitative analysis, identified conserved quantities and reduced the order of the system and, at least for the set of constants available, asymptotic stability followed as before. Anyway, a more realistic approach should include in both cases the action of inhibitors.

Since physiologists may influence the system by adding thrombocytes to a sample of blood, we describe in chapter seven a possible mechanism of action for the platelets based on the knowledge gained from the previous analysis extending the model from Stortelder and Hemker as a first approach to modelling platelet action. Qualitative analysis and numerical simulation were also done in this chapter, following the same pattern as before. The lag present in the course of thrombin concentration with time is imperceptible in the numerical results. Without excluding the possibility that this may be the case when a purified enzyme like *RVV* is used to trigger the system, we finished this chapter by proposing a new scheme for the extrinsic pathway as an extension of Stortelder's model by replacing the purified enzyme *RVV* by the reactions that are thought to occur in the extrinsic pathway, including also inhibitory reactions. No numerical solution was presented because we could not identify from experimental data the value of the dimensionless reaction constants

At this point it is important to notice that the models presented in this thesis cannot be compared without some previous adaptations that could be realized in future investigations. In fact, besides that they model different parts of the blood coagulation system, they are significantly different. For instance, since the reaction schemes based on the original reaction scheme of Mann and Jones do not include the action of inhibitors and Stortelder's model does, one should first account for inhibitory activity before comparing them. Moreover, this model should use dimensionless variables, because the constants present in Stortelder's model are also dimensionless. However, Stortelder's model should be extended first and the value of dimensionless variables must be gained and this cannot be done without experimental data.

All the numerical solutions are calculated with Scilab 3.0 and for symbolic calculations we used Maple 9.

We hope that this work will help people working in the mathematical modelling of biological or physiological systems that are highly nonlinear to be aware of the difficulties inherent to such a task and to learn that apparently simple questions regarding mathematical aspects like stability or controllability of more general systems with several unknown parameters have to be handled properly. Moreover, we saw how classical mathematical theorems can be useful to gain more insights into complex phenomena that arise from the current practice of investigation of other disciplines.

Appendix A

General Methods of Qualitative Theory

The main goal here is to present some definitions and results that elucidate the understanding of the qualitative behavior of flows, induced by ordinary differential equations or by systems of ordinary differential equations near critical points. This problem is closely related to the long-time behavior of solutions or, in other words, to the so-called stability theory. Two of the central concepts are the stability of equilibrium points in the sense of Lyapunov and a stability criteria due to J. P. LaSalle. Although these concepts are standard for mathematicians, they are included here as a guideline for other scientists to whom these concepts are not so standard.

A.1 Basic definitions and criteria

In this work, we deal with so called *autonomous differential equations*. These equations have the right-hand side not depending upon the time t and therefore they have the form

$$\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x}). \tag{A.1.1}$$

We assume that the function \mathbf{f} is defined on $\mathcal{U} \subseteq \mathbb{R}^n$, nonempty, open and connected, and that \mathbf{f} is locally Lipschitz (\mathbf{f} is k times continuous differentiable or of class C^k , where $k \geq 1$).

The fundamental existence-uniqueness theorem for a nonlinear autonomous system

of ordinary differential equations asserts that the initial value problem $\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x}), \mathbf{x}(t_0) = \mathbf{y}$ with $t_0 \in \mathbb{R}, \mathbf{y} \in \mathcal{U}$, has exactly one solution on an open interval, which is called the *maximal interval of existence* and denoted by I , and $t_0 \in I$. A proof of this result can be found in any standard literature for differential equations like [Ama90], [Arn80] or [Per96].

Let us denote the solution of the initial value problem $\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x}), \mathbf{x}(0) = \mathbf{y}$, with $\mathbf{y} \in \mathcal{U}$, by $S(t, \mathbf{y})$ and the maximal interval of existence of this solution by $I_{\mathbf{y}}$.

- (i) $S(t, \mathbf{y}) = \mathbf{y}$ for all t if and only if $\mathbf{f}(\mathbf{y}) = \mathbf{0}$. Points with this property are called *stationary points*, or *equilibrium points*, of the equation.
- (ii) S is also called *general solution* or *local flow* of $\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x})$.

In general, we need to know properties of solutions even if they are not given explicitly. And, one of the first tasks to do is therefore to obtain information about the maximal interval of existence. Since this interval will be generally different from $(-\infty, +\infty)$, we need criteria that guarantee that the maximal interval of existence extends to infinity.

Proposition A.1.1. *Consider the autonomous equation $\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x})$ and $\mathbf{y} \in \mathcal{U}$. If $K \subseteq \mathcal{U}$ is compact and if the positive semitrajectory $S^+(\mathbf{y}) = \{S(t, \mathbf{y}) : t \in I_{\mathbf{y}} \text{ and } t \geq 0\}$ is contained in K then $S(t, \mathbf{y})$ exists for $t \in [0, \infty)$. Likewise, if the negative semitrajectory $S^-(\mathbf{y}) = \{S(t, \mathbf{y}) : t \in I_{\mathbf{y}} \text{ and } t \leq 0\}$ is contained in K then $S(t, \mathbf{y})$ exists for $t \in (-\infty, 0]$.*

Instead of simply using curves to describe a vector field one may also use derivatives of functions. This allows us to investigate how a given scalar-valued function changes along solutions of a differential equation. For that matter, two basic concepts are those of *Lie derivative* and of *first integral*¹.

Theorem A.1.2. *Let $\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x})$ be given on \mathcal{U} , $\mathcal{U}^* \subseteq \mathcal{U}$ be a nonempty open set and $\varphi : \mathcal{U}^* \rightarrow \mathbb{R}$ a C^1 -function. Then the function $L_{\mathbf{f}}(\varphi) : \mathcal{U}^* \rightarrow \mathbb{R}, (L_{\mathbf{f}}(\varphi)(\mathbf{x})) := D_{\mathbf{x}}(\varphi)\mathbf{f}(\mathbf{x})$ is called the Lie derivative of φ with respect to \mathbf{f} . If $z(t)$ solves $\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x})$ then*

$$\dot{\varphi}(\mathbf{x}) = \frac{d}{dt}(\varphi(z(t))) = (D_z \varphi) \dot{z}(t) = (D_z \varphi) \mathbf{f}(z(t)) = L_{\mathbf{f}}(\varphi)(z(t)).$$

Remark A.1.3. If $\dot{\varphi}(\mathbf{x})$ is negative in \mathcal{U} then $\varphi(\mathbf{x})$ decreases along the solution $z(t_0)$ through $\mathbf{x}_0 \in \mathcal{U}$ at $t = 0$.

Definition A.1.4. Let $\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x})$ be given on \mathcal{U} , $\mathcal{U}^* \subseteq \mathcal{U}$ be a nonempty open set and $\varphi : \mathcal{U}^* \rightarrow \mathbb{R}$ a C^1 -function. Then, we call φ a *first integral* of $\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x})$ if φ is not constant and $L_{\mathbf{f}}(\varphi) = 0$.

¹First integrals are conserved quantities. Thus, any autonomous system having a non trivial first integral is called *conservative system*.

Remark A.1.5. If φ is a first integral of $\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x})$ then the solutions of $\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x})$ remain in the level set of φ in which they start. This means that $\varphi(z(t))$ is a constant function of t for all solutions.

For further properties of Lie derivatives and of first integrals we refer to [Arn80], p. 77-83.

A.2 Lyapunov's stability theory

Let us start by formally defining stability after [Son90]:

Definition A.2.1. Let $\mathbf{y} \in U$ such that the solution $z(t) = S(t, \mathbf{y})$ of the system (A.1.1) for $t \in [0, \infty)$ exists. This solution is called *stable* if for all $\varepsilon > 0$ there exists $\delta > 0$ such that for all $\mathbf{y}^* \in U$ with $|\mathbf{y} - \mathbf{y}^*| < \delta$ the solution $S(t, \mathbf{y}^*)$ in $[0, \infty)$ exists and $|S(t, \mathbf{y}^*) - z(t)| < \varepsilon$ for all $t > 0$. If the solution is not stable it is called *unstable*. Further, the solution $z(t)$ is *asymptotically stable* if $z(t)$ is stable and besides that if ρ exists such that

$$\lim_{t \rightarrow \infty} |S(t, \mathbf{y}^*) - z(t)| = 0$$

for all $\mathbf{y}^* \in U$ with $|\mathbf{y}^* - \mathbf{y}| < \rho$.

Remark A.2.2. The equilibrium point \mathbf{y}^* is stable if the trajectories do not depart to far from \mathbf{y}^* whenever the initial state is chosen close enough to \mathbf{y}^* . Asymptotic stability of \mathbf{y}^* requires stability and implies that the solution $S(t, \mathbf{y}^*)$ always approaches the equilibrium point \mathbf{y}^* provided that the initial deviation is within a certain region around \mathbf{y}^* .

Lyapunov's stability theory is one of the methods at hand that help to establish stability.

The so called *first method* of Lyapunov, or Lyapunov's *indirect method*, presupposes a known stationary solution and uses the linearization of the system at stationary points. Since the linearization is made locally, this method can only be used to prove local stability.

The stability on nonhyperbolic equilibrium points (see Remark A.2.4) may be decided by using Lyapunov's *second method*, also called Lyapunov's *direct method*, that does not require the knowledge of the solutions themselves [LaSa61]. This method makes use of a function, known as the Lyapunov function.

A.2.1 Linearization principle for stability

Following [Per96], let \mathbf{x}^* be an equilibrium point of the system (A.1.1). Linearization of the function $\mathbf{f}(\mathbf{x})$ near \mathbf{x}^* means to approximate $\mathbf{f}(\mathbf{x})$ by its first order Taylor polynomial:

$$\mathbf{f}(\mathbf{x}) = \mathbf{f}(\mathbf{x}^*) + D_{\mathbf{f}}(\mathbf{x}^*)(\mathbf{x} - \mathbf{x}^*) + o(\mathbf{x}) = D_{\mathbf{f}}(\mathbf{x}^*)(\mathbf{x} - \mathbf{x}^*) + o(\mathbf{x}),$$

since $\mathbf{f}(\mathbf{x}^*) = \mathbf{0}$.

Moreover, $D_{\mathbf{f}}(\mathbf{x}^*)$ denotes the Jacobian matrix at the equilibrium point and $o(\cdot)$ denotes the Landau symbol, i. e. $f(x) = o(g(x)) \Leftrightarrow |f(x)|/|g(x)| \rightarrow 0$ as $x \rightarrow 0$. Thus, for nearby solutions it is expected that the deviation from equilibrium $\mathbf{y}(t) = \mathbf{x}(t) - \mathbf{x}^*$ will be governed by the linearized system

$$\dot{\mathbf{y}} = A\mathbf{y}, \text{ where } A = D_{\mathbf{f}}(\mathbf{x}^*).$$

Theorem A.2.3. *Let \mathbf{x}^* be an equilibrium point of the system (A.1.1). If all the eigenvalues of the Jacobian $D_{\mathbf{f}}(\mathbf{x}^*)$ have negative real part then \mathbf{x}^* is asymptotically stable. If $D_{\mathbf{f}}(\mathbf{x}^*)$ has at least an eigenvalue with positive real part then \mathbf{x}^* is an unstable equilibrium point.*

Remark A.2.4. An equilibrium point \mathbf{x}^* is called *hyperbolic* if none of the eigenvalues of the matrix $D_{\mathbf{f}}(\mathbf{x}^*)$ have zero real part. Problems occur when one or more of the eigenvalues is either zero or purely imaginary while the remaining ones are negative. In other words, if we are in the presence of a nonhyperbolic equilibrium point. In this cases, Theorem A.2.3 is inconclusive and more detailed information is needed to make assertions about the nature of the equilibrium. This happens to be the case when dealing with conservative systems.

A.2.2 Lyapunov functions

To study the stability of a nonhyperbolic equilibrium point we may use furthermore the following theorem:

Theorem A.2.5 (Stability theorem). *Let \mathcal{U} be an open subset of \mathbb{R}^n containing \mathbf{x}_0 . Suppose that \mathbf{f} is defined in \mathcal{U} and is of class C^1 and that $\mathbf{f}(\mathbf{x}_0) = \mathbf{0}$. Suppose further that there exists a function φ on \mathcal{U} and of class C^1 satisfying $\varphi(\mathbf{x}_0) = 0$ and $\varphi(\mathbf{x}) > 0$ if $\mathbf{x} \neq \mathbf{x}_0$. Then*

- (i) *if $L_{\mathbf{f}}(\varphi)(\mathbf{x}) \leq 0$, for all $\mathbf{x} \in \mathcal{U}$ then \mathbf{x}_0 is stable;*
- (ii) *if $L_{\mathbf{f}}(\varphi)(\mathbf{x}) < 0$, for all $\mathbf{x} \in \mathcal{U} \setminus \{\mathbf{x}_0\}$ then \mathbf{x}_0 is asymptotically stable;*

A proof of this theorem can be found in [Per96].

Definition A.2.6. A function $\varphi : \mathbb{R}^n \rightarrow \mathbb{R}$ satisfying the hypotheses of Theorem A.2.5 is called *Lyapunov function*.

A Lyapunov function is continuously defined on the state space, bounded from below, and non-increasing along trajectories. Such a function is constant over the limit set of each trajectory; that is, its derivative with respect to time vanishes on every limit set.

Remark A.2.7. A strict or strong Lyapunov function - that is, a strictly decreasing function over nonstationary trajectories - causes every trajectory to approach asymptotically a set of equilibria. The system is convergent if each limit set contains a single equilibrium point. Thus, Theorem A.2.5 gives a criterion that can be applied if we know an appropriate Lyapunov function φ . However, there is no general method of finding Lyapunov functions, from general non-linear equations.

In the sequel we treat briefly the particular case in which the Jacobian of the right-hand side of a differential autonomous nonlinear system at the equilibrium has only one zero eigenvalue and the remaining eigenvalues have negative real part. For more details we refer to [Bib79] or to [LaSa61].

A.2.3 Critical case of one zero eigenvalue

Suppose that the basic system is

$$\dot{\mathbf{x}} = P\mathbf{x} + \mathbf{q}(\mathbf{x}), \quad (\text{A.2.1})$$

where P is a constant matrix of the linear terms and $\mathbf{q}(\mathbf{x})$ is a vector whose components q_1, q_2, \dots are convergent power series in those components $x_i, i = 1, \dots, n$ of \mathbf{x} which begin with terms of degree at least 2.

If the matrix P has eigenvalues with zero real parts and has no eigenvalue with positive real part. Then, we are confronted with a case, which Lyapunov called *critical*.

In particular, one of the cases Lyapunov discussed extensively is the case of one characteristic real root equal to zero and this is also the case we are interested in.

So, let us suppose that the system (A.2.1) has one zero eigenvalue with the others having negative real parts.

Following Bibikov [Bib79] (page 75) there is a non singular transformation that reduces the system (A.2.1) to a system of the form

$$\begin{aligned} \dot{\mathbf{y}} &= F(\mathbf{y}, \mathbf{z}) \\ \dot{\mathbf{z}} &= Q\mathbf{z} + \tilde{F}(\mathbf{y}, \mathbf{z}), \text{ where} \end{aligned} \quad (\text{A.2.2})$$

$\mathbf{y} = x_1$; $F(\mathbf{y}, \mathbf{z})$ and $\tilde{F}(\mathbf{y}, \mathbf{z})$ are formal power series (the symbol \sim means the expansion of the corresponding power series may contain terms linear in \mathbf{y}). \mathbf{z} is a $(n - 1)$ -dimensional vector and Q is a constant matrix whose characteristic roots $\kappa_2, \dots, \kappa_n$ are the same as those of P which are not zero, and therefore have negative real parts.

Theorem 3.2 of [Bib79] (page 18) gives sufficient conditions for the formal transformation of (A.2.2) into its normal form on invariant surface (*NFIS*) to converge.

The *NFIS* has the form

$$\begin{aligned}\dot{\mathbf{y}}' &= Y(\mathbf{y}') \\ \dot{\mathbf{z}}' &= Q\mathbf{z}' + Z'(\mathbf{y}, \mathbf{z}').\end{aligned}\tag{A.2.3}$$

If $Y(\mathbf{y}') = 0$ then we are in the *transcendental case* and for the solution of stability problems one has to take into account all the terms of the expansion of the right-hand part of (A.2.1). Here, we typically have a non-isolated singularity.

If $Y(\mathbf{y}') = g\mathbf{y}'^N + \dots, g \neq 0$ then we are in the algebraic case and the stability of the origin depends on a finite number of terms in the expansion of the right-hand side of (A.2.1).

Theorem 12.2 of [Bib79], page 79, says that in the transcendental case the zero solution of the system (A.2.1) is stable.

Theorem 12.1 of [Bib79], page 77, gives a solution for the stability problem dependent on the sign of g .

Altogether, the solutions of the stability problem in the critical case of one zero eigenvalue is reduced to either calculate the sign of g , or to proving that the case is transcendental.

Because it is important to handle the stability problem in some of the systems considered in this thesis, we summarize in the following theorem (S. Walcher, private statement) the information regarding the transcendental case:

Theorem A.2.8. *Let $\mathbf{x}^* = (x_1^*, \dots, x_n^*)$ be a stationary point of*

$$\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x}),$$

where \mathbf{f} is an analytic function in $\mathcal{U} \subset \mathbb{R}^n$. Furthermore, $D_{\mathbf{f}}(\mathbf{x}^)$ has a simple eigenvalue $\lambda_1 = 0$ and $n - 1$ eigenvalues $\lambda_2, \dots, \lambda_n$, with negative real parts. If the stationary point is not isolated, then the transformation into *NFIS* converges in $x_2 - x_2^* = x_3 - x_3^* = \dots = x_n - x_n^* = 0$. The *NFIS* has the representation*

$$\begin{aligned}\dot{x}_1 &= \frac{d}{dt}(x_1 - x_1^*) = 0 \\ \dot{x}_2 &= \frac{d}{dt}(x_2 - x_2^*) = \lambda_2(x_2 - x_2^*) + \dots \\ &\vdots \\ \dot{x}_n &= \frac{d}{dt}(x_n - x_n^*) = \lambda_n(x_n - x_n^*) + \dots\end{aligned}\tag{A.2.4}$$

Moreover, it holds that \mathbf{x}^* is stable but not asymptotically stable. The equilibrium point has coordinates $x_1 = c$ and $x_2 = \dots = x_n = 0$.

A.3 Invariance. Limit sets

In general, we are interested in studying the asymptotic behavior of solutions. But, sometimes it happens that a system is asymptotically stable in theory, but actually unstable in practice. This means that, in order to have "true" asymptotic stability, one should be sure that when the perturbations are not too large the system tends to return to equilibrium [LaSa61], particularly if the system is nonlinear. Concepts like *limit set* and *invariance* constitute an attempt to understand properties and long-time behavior of solutions .

In the sequel we introduce these concepts and some properties.

A.3.1 Invariance

Using the same notation as in [Wal01], we have:

Definition A.3.1. Let $\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x})$ be given on \mathcal{U} , and let Y be a subset of \mathcal{U} .

- (i) If $S(t, \mathbf{y}) \in Y$ for all $t \in I_{\mathbf{y}}$ whenever $\mathbf{y} \in Y$ then Y is called an *invariant set* of $\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x})$.
- (ii) The set Y is called *positively invariant* with respect to $\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x})$ if for all $\mathbf{y} \in Y$ the positive semitrajectory $S^+(\mathbf{y})$ is contained in Y .
- (iii) The set Y is called *negatively invariant* with respect to $\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x})$ if for all $\mathbf{y} \in Y$ the negative semitrajectory $S^-(\mathbf{y})$ is contained in Y .

A proof of the following result can also be found in [Wal01].

Proposition A.3.2. Let $\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x})$ be given on \mathcal{U} , and let $K \subseteq \mathcal{U}$ be compact and homeomorphic to a convex set such that, for all $\mathbf{y} \in K$ the positive semitrajectory $S^+(\mathbf{y})$ is contained in K . Then K contains a stationary point of the differential equation. Moreover, K is positively invariant.

Remark A.3.3. (i) Invariant subsets are unions of trajectories $S(\mathbf{y}) = \{S(t, \mathbf{y}) : t \in I_{\mathbf{y}}\}$ and vice-versa.

- (ii) Let $\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x})$ be given on \mathcal{U} and consider $Y \subseteq \mathcal{U}$ invariant. Then, the closure \overline{Y} , the interior $\text{int}(Y)$ and the boundary ∂Y are also invariant.
- (iii) Any subset of $S(\mathbf{y})$ containing only stationary points of the differential equation is invariant.

We now present some criteria for invariance without actual knowledge of solutions in order to develop some strategies to determine invariant sets with respect to the flow induced by \mathbf{f} . The proofs can be found in [Ama90] (pages 215–219).

Theorem A.3.4. *Let $M \subseteq \mathcal{U}$ be closed. Then M is positively invariant if and only if for every $\mathbf{x} \in M$, the sub-tangent condition*

$$\liminf_{t \rightarrow 0^+} \frac{\text{dist}(\mathbf{x} + t\mathbf{f}(\mathbf{x}), M)}{t} = 0 \quad (\text{A.3.1})$$

is satisfied.

Remark A.3.5. Condition (A.3.1) is obviously satisfied for $\mathbf{x} \in \text{int}(M)$. It is therefore only a condition for \mathbf{f} on the boundary of M . Notice furthermore that $\text{int}(M)$ can be empty and as a consequence $\partial M = M$ is possible.

This result can be put in other terms:

Theorem A.3.6. *Let $\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x})$ be given on \mathcal{U} and $\varphi : \mathcal{U} \rightarrow \mathbb{R}$ be a C^1 -mapping such that $\nabla\varphi(\mathbf{x}) \neq 0$ for all $\mathbf{x} \in \varphi^{-1}(0)$, i. e., assume that 0 is a regular value of φ . Then, $M := \varphi^{-1}(-\infty, 0]$ is positively invariant if and only if*

$$\langle \nabla\varphi(\mathbf{x}), \mathbf{f}(\mathbf{x}) \rangle \leq 0, \text{ for all } \mathbf{x} \in \partial M = \varphi^{-1}(0). \quad (\text{A.3.2})$$

Remark A.3.7. Here it is stated that if M is a manifold with boundary and $\dim(M) = n$, then condition (A.3.1) is equivalent to requiring that the vector field \mathbf{f} "points inward" or "lies below the tangent plane" along ∂M . This also explains the name *sub-tangent condition*.

Corollary A.3.8. *Let $\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x})$ be given on $\mathcal{U} \subseteq \mathbb{R}^n$ and let $\varphi_1, \dots, \varphi_k$ be C^1 -mappings from \mathcal{U} to \mathbb{R} . Moreover, assume that 0 is a regular value of each $\varphi_j, j = 1, \dots, k$, and let*

$$M := \bigcap_{j=1}^k \varphi_j^{-1}(-\infty, 0].$$

If

$$\langle \nabla\varphi_j(\mathbf{x}), \mathbf{f}(\mathbf{x}) \rangle \leq 0, \text{ for all } \mathbf{x} \in \varphi_j^{-1}(0), j = 1, \dots, k, \quad (\text{A.3.3})$$

then M is positively invariant. If

$$\langle \nabla\varphi_j(\mathbf{x}), \mathbf{f}(\mathbf{x}) \rangle = 0, \text{ for all } \mathbf{x} \in \varphi_j^{-1}(0), j = 1, \dots, k, \quad (\text{A.3.4})$$

then M and all the hypersurfaces $\varphi_j^{-1}(0), j = 1, \dots, k$, are invariant.

Remark A.3.9. If 0 is a regular value of the C^1 -mapping $\varphi : \mathcal{U} \rightarrow \mathbb{R}$, then we know that $\varphi^{-1}(0)$ is a hypersurface in \mathbb{R}^n , which bounds the n -dimensional C^1 -manifold $\varphi^{-1}(-\infty, 0]$. Moreover, $\nabla\varphi(\mathbf{x})$ is a vector in the direction of the outward unit normal at $\mathbf{x} \in \varphi^{-1}(0)$. Hence, Corollary A.3.8 states in particular that M is invariant if at every \mathbf{x} , \mathbf{f} is a tangent vector on every hypersurface $\varphi^{-1}(0)$ through that point.

The following criterion holds for convex sets and is a special case of Theorem A.3.4.

Proposition A.3.10. *Let $\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x})$ be given on \mathcal{U} and let V be convex with nonempty interior. Furthermore, for $\mathbf{x} \in \partial V$ define*

$$C_{\mathbf{x}} := \{Z \in \mathbb{R}^n : \mathbf{x} + sZ \in \text{int}(V) \text{ for sufficiently small } s > 0\}.$$

If $\mathbf{f}(\mathbf{x}) \in \overline{C_{\mathbf{x}}}$ for all $\mathbf{x} \in \partial V$ then \overline{V} is positively invariant.

The following theorem is also proved in [Ama90] and shows that Lyapunov functions can be used to determine positively invariant sets:

Theorem A.3.11. *Let $-\infty \leq \gamma < \beta$ and assume that $\varphi : \mathcal{U} \rightarrow \mathbb{R}$ is a Lyapunov function on*

$$\{\mathbf{x} \in \mathcal{U} \mid \gamma < \varphi(\mathbf{x}) < \beta\}.$$

Then

$$M_{\alpha} := \{\mathbf{x} \in \mathcal{U} \mid \varphi(\mathbf{x}) \leq \alpha\}$$

is positively invariant for each $\alpha \in [\gamma, \beta)$.

Remark A.3.12. Theorem A.3.6 and Theorem A.3.11 are closely related. However, while Theorem A.3.6 involves a condition that must be satisfied only on the boundary of M_{α} , Theorem A.3.11 requires φ to be a Lyapunov function in a whole neighborhood of ∂M .

A.3.2 Limit sets

The following definition and the proofs of the subsequent theorems can be found in [Wal01].

Definition A.3.13. Let $\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x})$ be given on \mathcal{U} and let $\mathbf{y} \in \mathcal{U}$ be such that $S(t, \mathbf{y})$ exists for $0 \leq t < \infty$, respectively for $-\infty < t \leq 0$. Then $Z \in \mathcal{U}$ is called an ω -limit point, respectively an α -limit point, of \mathbf{y} if there is a sequence $(t_k)_{k \in \mathbb{N}}$ of positive numbers with $t_k \rightarrow \infty$ as $k \rightarrow \infty$, respectively if there is a sequence $(t_k)_{k \in \mathbb{N}}$ of negative numbers with $t_k \rightarrow -\infty$ as $k \rightarrow \infty$, such that

$$\lim_{k \rightarrow \infty} S(t_k, \mathbf{y}) = Z.$$

The collection of all ω -limit points, respectively of all α -limit points, is called ω -limit set, respectively α -limit set, and is denoted by $w(\mathbf{y})$, respectively by $\alpha(\mathbf{y})$.

Remark A.3.14. (i) Intuitively if $z(t)$ is a solution of the initial value problem $\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x}), \mathbf{f}(\mathbf{x}_0) = \mathbf{0}$ its ω -limit set is whatever the curve $z(t)$ tends to with infinite time [LaSa61].

- (ii) Limit sets are properties of solution orbits rather than individual points.
- (iii) Limit sets may be empty.

Theorem A.3.15. For $\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x})$ given on \mathcal{U} , let $\mathbf{y} \in \mathcal{U}$ and K be a compact set such that $S(t, \mathbf{y}) \in K$, i. e., the solution is bounded, for all sufficiently large t . Then, $S(t, \mathbf{y})$ exists for all positive real t , and $\omega(\mathbf{y})$ is nonempty, compact and connected.

Theorem A.3.16. Let $\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x})$ be given on $\mathcal{U} \subseteq \mathbb{R}^n$, and $\mathbf{y} \in \mathcal{U}$ such that $S(t, \mathbf{y})$ exists for all $t \in [0, \infty)$. Then $w(\mathbf{y})$ is an invariant set.

A.4 LaSalle's invariance principle

We are now in a position to state the following theorem, which gives a useful tool to determine limit sets for certain classes of functions. This proposition is known as *LaSalle invariance principle*. A proof can be found either in [Ama90] or in [LaSa61] or in [KnoKa74].

Theorem A.4.1 (LaSalle). Let $M \subseteq \mathcal{U}$ be closed and assume that $\varphi : M \rightarrow \mathbb{R}$ is a C^1 Lyapunov function on $M \subseteq \mathcal{U}$. If $\mathbf{y} \in M$ is such that $S^+(\mathbf{y}) \in M$ for all $t \in [0, \infty)$, there exists some $\alpha \in \mathbb{R}$ such that $\omega(\mathbf{y}) \subseteq \varphi^{-1}(\alpha)$. In particular,

$$\omega(\mathbf{y}) \subseteq N := \{\mathbf{x} \in M : L_{\mathbf{f}}(\varphi)(\mathbf{x}) = 0\}.$$

- Remark A.4.2.* (i) If we impose that $L_{\mathbf{f}}(\varphi)(\mathbf{x}) < 0$ for all $\mathbf{x} \neq 0$ in M , then the equilibrium point is stable and all positive semitrajectories converge to the equilibrium point as $t \rightarrow \infty$ [LaSa61].
- (ii) LaSalle's principle can be used if the system has an equilibrium set rather than an isolated equilibrium point and it does not require the function $\varphi(\mathbf{x})$ to be positive definite.

Besides equilibrium points and periodic orbits, a dynamical system can have strange attractors as limit sets. However, not every limit set of a trajectory of (A.1.1) is an attracting set of (A.1.1) [Per96].

Appendix B

Results from Control Theory

We start with a general definition for a controlled dynamical system and state in the sequel important properties such as controllability, stabilizability, observability and detectability. For the proofs of the results refer to [Zab92], [Son90], [Bar75], [KnKw80] and [NivdSc90]. The last section concerns flat systems and their application to motion planning. For literature on flat systems see [Rot97] and [FLMR95].

B.1 Basic definitions and criteria

Definition B.1.1 (Control System). We say that

$$\Sigma = (\mathcal{T}, X, U, \mathcal{U}, Y, s, o)$$

is a control system if

- (i) \mathcal{T} is a nonempty subset of \mathbb{R} ,
- (ii) X, Y and U are nonempty topological spaces,
- (iii) \mathcal{U} is a nonempty subset of $\{u|u : \mathcal{T} \rightarrow U\}$,
- (iv) s is a mapping defined on a subset \mathcal{D}_s of the set

$$\mathcal{D} = \{(t_0, t_1, \mathbf{x}, \mathbf{u}) | t_0, t_1 \in \mathcal{T}, t_0 \leq t_1, \mathbf{x} \in X, \mathbf{u} \in \mathcal{U}\}$$

into X with the following properties:

- (a) For all $t_0 \in \mathcal{T}$ and $\mathbf{x} \in X$ there exists $t_1 \in \mathcal{T}$ with $t_1 > t_0$ and $\mathbf{u} \in \mathcal{U}$ such that $(t_0, t_1, \mathbf{x}, \mathbf{u}) \in \mathcal{D}_s$.

(b) $(t_0, t_0, \mathbf{x}, \mathbf{u}) \in \mathcal{D}_s$ and it is $s(t_0, t_0, \mathbf{x}, \mathbf{u}) = \mathbf{x}$, for all $t_0 \in \mathcal{T}$, $\mathbf{x} \in X$ and $\mathbf{u} \in \mathcal{U}$.

(c) For all $t_0, t_1 \in \mathcal{T}$ with $t_0 < t_1$ and $\mathbf{u}, \mathbf{u}^* \in \mathcal{U}$ with $\mathbf{u}(t) = \mathbf{u}^*(t)$, for all $t \in [t_0, t_1] \cap \mathcal{T}$ and for each $\mathbf{x} \in X$ such that $(t_0, t_1, \mathbf{x}, \mathbf{u}) \in \mathcal{D}_s$,

$$(t_0, t_1, \mathbf{x}, \mathbf{u}^*) \in \mathcal{D}_s \text{ and } s(t_0, t_1, \mathbf{x}, \mathbf{u}) = s(t_0, t_1, \mathbf{x}, \mathbf{u}^*) \text{ hold .}$$

(d) For all $t_0, t_1 \in \mathcal{T}$ with $t_0 < t_1$ and for all $\mathbf{x}_0 \in X, \mathbf{u} \in \mathcal{U}$ with $(t_0, t_1, \mathbf{x}_0, \mathbf{u}) \in \mathcal{D}_s$,

$$(t_0, t, \mathbf{x}_0, \mathbf{u}) \in \mathcal{D}_s \text{ and } (t, t_1, \mathbf{x}, \mathbf{u}) \in \mathcal{D}_s$$

hold for each $t \in [t_0, t_1] \cap \mathcal{T}$ with $\mathbf{x} = s(t_0, t, \mathbf{x}_0, \mathbf{u})$.

(e) For all $t_0, t_1, t_2 \in \mathcal{T}$ with $t_0 \leq t_1 \leq t_2$ and for all $\mathbf{x} \in X, \mathbf{u} \in \mathcal{U}$ such that $(t_0, t_2, \mathbf{x}, \mathbf{u}) \in \mathcal{D}_s$,

$$s(t_1, t_2, \mathbf{x}_1, \mathbf{u}) = s(t_0, t_2, \mathbf{x}, \mathbf{u}) \text{ with } \mathbf{x}_1 = (t_0, t_1, \mathbf{x}, \mathbf{u}) \text{ holds.}$$

(v) o is a mapping from $\mathcal{T} \times X \times U$ into Y .

Remark B.1.2. In the former definition, X is called the *state space*, Y the *output space* and U is the *control variable space*; \mathcal{T} denotes the *time-domain* or *time horizon*, s the *system transfer matrix* and o the *output mapping*.

In this thesis we deal with the so called *finite dimension differential control systems*.

Definition B.1.3. Let $\mathcal{D}^* \subset \mathcal{T} \times X \times \mathcal{U}$ and let $\mathbf{f} : \mathcal{D}^* \rightarrow X$ with:

(i) $\mathbf{f}(t, \mathbf{x}, \mathbf{u})$ is piecewise continuous in \mathbf{u}, t for all $\mathbf{x} \in X$.

(ii) $\mathbf{f}(t, \mathbf{x}, \mathbf{u})$ is Lipschitz-continuous in $\mathbf{x} \in X$ for $(t, \mathbf{u}) \in (\mathcal{T} \times U) \cap \mathcal{D}_\mathbf{x}^*$.

$\sigma = (\mathcal{T}, X, Y, U, \mathcal{U}, \mathbf{f}, \mathbf{o})$ is a finite dimensional differential control system if

(i) \mathcal{T} is a real interval;

(ii) X, Y, U are finite dimensional Euclidean spaces;

(iii) \mathcal{U} is a set of piecewise continuous functions on \mathcal{T} .

(iv) $\mathbf{f} : \mathcal{D}^* \subset \mathcal{T} \times X \times \mathcal{U} \rightarrow X$ fulfilling the conditions mentioned above.

(v) $\mathbf{o} : \mathcal{T} \times X \times U \rightarrow Y$ is the output map function.

From this class of system, *continuous time linear differential control systems* deserve our special attention.

Definition B.1.4. If

$$\mathbf{f}(t, \mathbf{x}, \mathbf{u}) = A(t)\mathbf{x} + B(t)\mathbf{u}, \quad \mathbf{o}(t, \mathbf{x}, \mathbf{u}) = C(t)\mathbf{x} + D(t)\mathbf{u}$$

then σ is a linear differential control system with $A \in \mathbb{R}^{(n,n)}$, $B \in \mathbb{R}^{(n,m)}$, $C \in \mathbb{R}^{(k,n)}$ and $D(t) \in \mathbb{R}^{(k,m)}$. Furthermore, $\mathbf{x}(t) \in \mathbb{R}^n$ and $\mathbf{u}(t) \in \mathbb{R}^m$.

B.1.1 Controllability and observability

Controllability and observability can be checked in a purely algebraic way. Controllability essentially means that it should be always possible to reach an arbitrary state from another arbitrary state in a finite time. Closely related to the idea of controllability is that of *observability*, which in general terms means that it is possible to determine the state of a system by solely measuring the output. This section is a survey of the results on controllability and observability that can be found for instance in [Son90], [Bar75] and in [KnKw80].

Let us formalize first the definition of controllability.

Definition B.1.5. Let Σ be a control system. Then,

- (i) The pair $(t_1, \mathbf{x}_1) \in \mathcal{T} \times X$ can be steered to some point $(t_2, \mathbf{x}_2) \in \mathcal{T} \times X$, $t_2 > t_1$, if there exists a control function $\mathbf{u} \in \mathcal{U}$ such that $(t_1, t_2, \mathbf{x}_1, \mathbf{u}) \in \mathcal{D}_s$ and $s(t_1, t_2, \mathbf{x}_1, \mathbf{u}) = \mathbf{x}_2$.
- (ii) $\mathbf{x}_1 \in X$ can be steered to \mathbf{x}_2 in time $T > 0$ if for any $t \in \mathcal{T}$ with $t + T \in \mathcal{T}$, (t, \mathbf{x}_1) can be steered to $(t+T, \mathbf{x}_2)$. Symbolically, we write $(t, \mathbf{x}_1) \rightsquigarrow (t+T, \mathbf{x}_2)$.
- (iii) $\mathbf{x}_1 \in X$ can be steered to \mathbf{x}_2 if for any $t_1 \in \mathcal{T}$ there exists $t_2 \in \mathcal{T}$ with $t_2 > t_1$ such that $(t_1, \mathbf{x}_1) \rightsquigarrow (t_2, \mathbf{x}_2)$. Moreover, the state (t_2, \mathbf{x}_2) is said to be *reachable*.

In practice, we have a more common definition:

Definition B.1.6. Let Σ be a control system. Then,

- (i) Σ is (completely) controllable on the interval $[t_0, t_1]$ if for all state points $\mathbf{x}_1, \mathbf{x}_2 \in X$ it holds $(t_0, \mathbf{x}_1) \rightsquigarrow (t_1, \mathbf{x}_2)$.
- (ii) Σ is (completely) controllable in time interval T if for all state points $\mathbf{x}_1, \mathbf{x}_2 \in X$ and for all $t \in \mathcal{T}$ it holds $(t, \mathbf{x}_1) \rightsquigarrow (t+T, \mathbf{x}_2)$.
- (iii) Σ is (completely) controllable if $\mathbf{x}_1 \rightsquigarrow \mathbf{x}_2$, for all $\mathbf{x}_1, \mathbf{x}_2 \in X$.

The following theorem, due to Hautus and Kalman, gives necessary and sufficient conditions for controllability of time invariant systems.

Theorem B.1.7. Consider the time invariant system

$$\dot{\mathbf{x}} = \mathbf{A}\mathbf{x} + \mathbf{B}\mathbf{u}.$$

The following conditions are equivalent

- (i) The pair $[A, B]$ is completely controllable
- (ii) For each eigenvector ν of A^T holds $\nu^T B \neq 0$.
- (iii) The Kalman controllability matrix

$$U = [B, AB, A^2B, \dots, A^{n-1}B]$$

has maximal rank n .

Corollary B.1.8. If $\text{rank } B = p < n$, then the condition in Theorem B.1.7 reduces to

$$\text{rank}[B, AB, A^2B, \dots, A^{n-p}B] = n.$$

If a system is not completely controllable, it can be shown that it is possible to transfer it from an initial state x_0 to a final state x_f provided that both belong to the column space of the controllability matrix. The proof of the following result, often called the *Kalman controllability decomposition*, can be found in [Son90]. Let $GL(n)$ denote the group of all invertible $n \times n$ matrices over a field \mathcal{K} .

Lemma B.1.9. Assume that the pair $[A, B]$ is not controllable. Let $r < n$ be the dimension of the controllable subspace $\mathcal{R}[A, B]$. Then, there exists $T \in GL(n)$ such that the matrices $\tilde{A} := T^{-1}AT$ and $\tilde{B} := T^{-1}B$ have the block structure

$$\tilde{A} = \begin{pmatrix} A_1 & A_2 \\ 0 & A_3 \end{pmatrix} \quad \tilde{B} = \begin{pmatrix} B_1 \\ 0 \end{pmatrix},$$

where A_1 is $r \times r$ and B_1 is $r \times m$. Furthermore, the pair $[A_1, B_1]$ is controllable.

Definition B.1.10. The control system Σ is *completely observable* if for any t_0 and any initial state $\mathbf{x}(t_0) = \mathbf{x}_0$ there is a finite time $t_1 > t_0$ such that the knowledge of $\mathbf{u}(t)$ and $\mathbf{y}(t)$ for $t_0 \leq t \leq t_1$ suffices to determine \mathbf{x}_0 .

In fact, the concept of observability tells something about the dynamical relationships between the components of $\mathbf{x}(t)$ in the sense that deviations in the state at the time t_0 are reflected on deviations in the output $\mathbf{y}(t)$, where t belongs to an interval.

The following theorem gives a necessary and sufficient condition for observability.

Theorem B.1.11. The system Σ is completely observable if and only if for any t_1 there exists $t_0 < t_1$ such that the observability matrix

$$V(t_0, t_1) = \int_{t_0}^{t_1} \Phi(t, t_1)^T C(t)^T C(t) \Phi(t, t_1) dt$$

is positive definite. $\Phi(t, \tau)$ is the state transition matrix of the homogeneous differential equation $\dot{\mathbf{x}} = A(t)\mathbf{x}$.

A proof of this result can be found in both [Bar75] and [KnKw80]. For time-invariant systems we have the following theorem corresponding to the controllability criterion of Theorem B.1.7.

Theorem B.1.12. *The time invariant system*

$$\dot{\mathbf{x}}(t) = A\mathbf{x}(t) + B\mathbf{u}(t), \mathbf{y} = C\mathbf{x} \quad (\text{B.1.1})$$

is observable if and only if one of the following conditions hold:

(i) *The rank of the observability matrix*

$$V := (C^T, A^T C^T, (A^2)^T C^T, \dots, (A^{n-1})^T C^T) \quad (\text{B.1.2})$$

is equal to n .

(ii) *If p is an eigenvector of the matrix A then $Cp \neq 0$.*

For time invariant systems it holds furthermore:

Theorem B.1.13. *If the system σ is time-invariant then if the observability matrix (B.1.2) has rank $n_1 < n$ there exists a system algebraically equivalent to S obtained by a transformation $\mathbf{x} \rightarrow P\mathbf{x}$, $\mathbf{y} \rightarrow Q\mathbf{y}$ such that A and C have respectively the following block structure*

$$A = \begin{pmatrix} A_{11} & 0 \\ A_{21} & A_{22} \end{pmatrix} \quad C = \begin{pmatrix} C_{11} \\ 0 \end{pmatrix},$$

and the system

$$\dot{\mathbf{x}}_1 = A_{11}\mathbf{x}_1, \quad \mathbf{y}_1 = C_{11}\mathbf{x}_1$$

is completely observable. Furthermore, $\dim \mathbf{x}_1 = n_1$.

Remark B.1.14. The vector with the remaining states \mathbf{x}_2 of dimension $n - n_1$ is said to be *unobservable* and the state space has been divided into two parts regarding observability. This aspect is used to define *detectability*.

Notice furthermore that controllability and observability are dual properties in the sense that the pair $[A, B]$ is controllable if and only if the pair $[B^T, A^T]$ is observable and the pair $[C, A]$ is observable if and only if $[A^T, C^T]$ is controllable.

As a final remark, notice that a linear time-invariant system can be split up into four mutually exclusive parts, respectively (1) completely controllable but unobservable; (2) completely controllable and completely observable; (3) uncontrollable and unobservable and (4) completely observable but not controllable.

B.1.2 Stabilizability and detectability

The general definition of stabilizability and detectability can also be found in books like [Zab92], [Son90], [Bar75], [KnKw80]. Here we give special attention to the time invariant case and introduce the following definitions:

Definition B.1.15. The ordered pair $[A, B]$ is called *stabilizable* if there exists $F \in \mathbb{R}^{(m,n)}$ such that $A + BF$ is asymptotically stable.

Definition B.1.16. The ordered pair $[A, B]$ is called *detectable* if there exists $K \in \mathbb{R}^{(m,k)}$ such that $A + BK$ is asymptotically stable.

It is clear by definition that these are dual properties, since $[A, B]$ is stabilizable if and only if the pair $[B^T, A^T]$ is detectable and the pair $[A, C]$ is detectable if and only if $[A^T, C^T]$ is stabilizable.

Remark B.1.17. (i) The system is stabilizable if and only if the non-controllable subspace is stable.

(ii) The system is called detectable if its unobservable subspace is contained in its stable subspace.

If a system is not completely observable then it is impossible to uniquely determine the state of the system from the output. However, if the system is detectable then it can at least be asymptotically reconstructed from the past behavior of the output.

B.2 Second order Taylor expansions

There are several approaches to find the second-order approximation to a dynamical system. In this work we make use of the Magnus and Neudecker [MaNeu99] definition of the Hessian matrix, since it can be easily implemented in a software for symbolic calculations like MAPLE, MUPAD or MATLAB.

Then, as stated in [MaNeu99], the second order Taylor expansion of a twice differentiable function $\mathbf{f} : \mathbb{R}^n \rightarrow \mathbb{R}^m$ around \mathbf{x}_0 is given by

$$\mathbf{f}(\mathbf{x}) \approx \mathbf{f}(\mathbf{x}_0) + \mathbf{D}\mathbf{f}(\mathbf{x} - \mathbf{x}_0) + \frac{1}{2}(I_m \otimes (\mathbf{x} - \mathbf{x}_0)^T) \mathbf{H}\mathbf{f}(\mathbf{x}_0)(\mathbf{x} - \mathbf{x}_0),$$

where \otimes denotes the Kronecker Product and

$$\mathbf{H}\mathbf{f}(\mathbf{x}_0) = \frac{\partial^2 \mathbf{f}(\mathbf{x}_0)}{\partial \mathbf{x} \partial \mathbf{x}^T} = \mathbf{D} \operatorname{vec}((\mathbf{D}\mathbf{f}(\mathbf{x}_0))^T)$$

is the Hessian matrix.

Observing moreover that

$$\mathbf{Df}(\mathbf{x}) = \begin{pmatrix} \mathbf{D}f_1(\mathbf{x}) \\ \mathbf{D}f_2(\mathbf{x}) \\ \vdots \\ \mathbf{D}f_m(\mathbf{x}) \end{pmatrix}$$

and that

$$\mathbf{Hf}(\mathbf{x}) = \begin{pmatrix} \mathbf{H}f_1(\mathbf{x}) \\ \mathbf{H}f_2(\mathbf{x}) \\ \vdots \\ \mathbf{H}f_m(\mathbf{x}) \end{pmatrix},$$

we conclude that the Hessian $\mathbf{Hf}(\mathbf{x})$ is of dimension $mn \times n$ and consists of m vertically concatenated symmetric $n \times n$ matrices.

Remark B.2.1. Given an equilibrium of a function, one computes the eigenvalues of the Hessian matrix and determines the type of equilibrium by computing the *index*¹ of that equilibrium. If the Hessian matrix is positive definite, the equilibrium point is a minimum, and if the Hessian is negative definite it is a maximum. If the Hessian matrix has eigenvalues zero or has positive and negative eigenvalues then the equilibrium point is a saddle point.

B.3 About flat systems

In this section, we introduce briefly the fundamental concepts of *flatness* based on differential geometric and algebraic methods in view of the practical application within the scope of the present work. For these and for other aspects of the analysis of flatness please refer to [MMR97], [Rot97] and [FLMR95], where the theory is formalized and illustrated with some examples.

In many modern systems, one typical use of control theory is to invert the dynamics of a system to compute the inputs required in order to perform a specific task. The inversion problem may consist in determining appropriate inputs to steer a control system from a state to another or may involve finding inputs to follow a *desired* trajectory for some or all state variables of the system.

Since the use of linear structures alone is oft not sufficient to solve the control problems that are arising in applications, control theorists look for other type of

¹Number of negative eigenvalues

structures that in addition to simple linear ones help to understand the complexity of the system. To this class of system belong the so called *flat systems*, for which the structure of the trajectories of the nonlinear dynamics can be completely characterized.

Roughly speaking, the system is said to be flat if it is possible to find a set of variables, called the *flat outputs*, such that the system is algebraic over the differential field generated by the set of flat outputs. In other words, the system is flat if the state and input variables can be directly expressed in terms of the flat outputs (equal in number to the number of inputs) and finite number of its derivatives without integrating any differential equation.

One of the properties of flat systems is that they can be feedback linearized using dynamic feedback. However, because it is a property inherent to the system, flatness does not imply that the principal intention is to transform the system to a single linear system via a dynamic feedback and appropriate change of coordinates. In fact, flatness is a geometric property independent of coordinate choice and is an indicator that the nonlinear structure of the system is well characterized and one can exploit that structure designing control algorithms for motion planning, desired trajectory generation and stabilization.

Moreover, the flat output has usually a physical meaning and they might be regarded as providing another nonlinear extension of Kalman's controllability condition.

Formally speaking, let us consider the non-linear dynamical system defined by

$$\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x}, \mathbf{u}), \mathbf{x}(0) = \mathbf{x}_0 \in M_n, \mathbf{u} \in \mathbb{R}^m, \text{rank} \frac{\partial \mathbf{f}(\mathbf{x}, \mathbf{u})}{\partial \mathbf{u}} = m, \quad (\text{B.3.1})$$

where \mathbf{x} is the n -dimension state variable defined in a manifold of dimension n , isomorphic to \mathbb{R}^n in a neighborhood of the origin (for the purpose of this work, actually no distinction will be made between M_n and \mathbb{R}^n), and \mathbf{u} is the m -dimension input.

Following the definition given in [Rot97], we have:

Definition B.3.1. A non-linear dynamical system (B.3.1) is called (*differential²*) *flat* if there is a fictive output $\mathbf{y} = (y_1, \dots, y_m)$ with $m = \dim \mathbf{u}$ satisfying the following conditions:

- (i) The variables $y_i, i = 1, \dots, m$ can be expressed as a function of the state variables $x_j, j = 1, \dots, n$ and $u_i, i = 1, \dots, m$, and a finite number of time

²The word *differential* is used to emphasize that all states of the system can be obtained by differentiating \mathbf{y}

derivatives $u_i^{(k)}$, $k = 1, \dots, \alpha_i$. That is,

$$\begin{aligned}\mathbf{y} &= \varphi(\mathbf{x}, u_1, \dots, u_1^{(\alpha_1)}, \dots, u_m, \dots, u_m^{(\alpha_m)}) \\ &= \varphi(\mathbf{x}, \mathbf{u}, \dot{\mathbf{u}}, \dots, \mathbf{u}^{(\alpha)}).\end{aligned}$$

- (ii) The variables x_i , $i = 1, \dots, n$, respectively u_j , $j = 1, \dots, m$ can be expressed as a function of y_i , $i = 1, \dots, m$ and a finite number of their time-derivatives $y_i^{(k)}$, $k = 1, \dots, \beta_i + 1$, i. e.

$$\begin{aligned}\mathbf{x} &= \psi_1(y_1, \dots, y_1^{(\beta_1)}, \dots, y_m, \dots, y_m^{(\beta_m)}) \\ &= \psi_1(\mathbf{y}, \dot{\mathbf{y}}, \dots, \mathbf{y}^{(\beta)}); \\ \mathbf{u} &= \psi_2(y_1, \dots, y_1^{(\beta_1+1)}, \dots, y_m, \dots, y_m^{(\beta_m+1)}) \\ &= \psi_2(\mathbf{y}, \dot{\mathbf{y}}, \dots, \mathbf{y}^{(\beta+1)}).\end{aligned}\tag{B.3.2}$$

- (iii) The components of \mathbf{y} are differential independent, i. e., they do not satisfy a differential equation of the form:

$$\varphi(\mathbf{y}, \dot{\mathbf{y}}, \dots, \mathbf{y}^{(\gamma)}) = 0.$$

If these conditions are at least locally satisfied, then the fictive output \mathbf{y} is flat and the system is also called flat.

Remark B.3.2. If condition (ii) holds then condition (iii) is equivalent to $\dim \mathbf{y} = \dim \mathbf{u}$.

Like this, we may speak about a *finite parametrization* of the system by a flat output. This parametrization facilitates the determination and the analysis of stationary points $(\mathbf{x}_s, \mathbf{y}_s)$ of the system B.3.1. For that, we only need to consider equations (B.3.2) evaluated at $\mathbf{y} = \mathbf{y}_s$ and $\mathbf{y}^{(k)} = 0$, $k \geq 1$ to obtain:

$$\begin{aligned}\mathbf{x}_s &= \psi_1(\mathbf{y}_s, 0, \dots, 0) = \bar{\psi}_1(\mathbf{y}_s) \\ \mathbf{u}_s &= \psi_2(\mathbf{y}_s, 0, \dots, 0) = \bar{\psi}_2(\mathbf{y}_s).\end{aligned}\tag{B.3.3}$$

Notice that an explicit representation of the relation $\mathbf{x}(\mathbf{u}_s)$ is not always possible. By using the equations (B.3.3), we can for instance calculate the equilibrium point as a function of \mathbf{y}_s .

Remark B.3.3. (i) A flat output is not uniquely determined;

(ii) A system without controls cannot be flat;

- (iii) The distance to flatness is measured a non-negative integer called the *defect*. Thus, a system is flat if and only if its defect is zero.

The defect of a linear system is equal to the dimension of its torsion submodule, i. e., to the dimension of its Kalman uncontrollable subspace. The following theorem holds:

Theorem B.3.4. *A linear system is flat if and only if it is controllable.*

If a system is not flat, the general solution cannot be expressed without the integration of at least one of the differential equations. Though we can obtain more information about the system by checking for flatness. Namely, which are the components that are responsible for the non-flatness and how they can be approached and have some hints for the adequate choice of the inputs.

Noteworthy is that there is no general computable test for checking whether a system is flat. One of the main difficulties is moreover that a candidate flat output may *a priori* depend on derivatives of the control function of arbitrary order, since it remains up to now an open problem whether the order of the derivatives admits an upper bound.

Appendix C

Technical Remarks

C.1 Solving systems of stiff differential equations using SciLab

Roughly speaking, a stiff problem is one in which the stability is associated with big eigenvalues (with negative real parts) of the Jacobian matrix of the function on the right-hand-side of the ODE. This implies that the Jacobian has a big norm, which in its turn is associated to a big Lipschitz constant. As a consequence, the process described by the ODE contains components operating on different time scales. This kind of problem should be solved by implicit methods of integration, in which the step size is chosen to be very small for assuring stability giving the desired accuracy. A survey on numerical methods suitable to approach the solution of stiff systems is given in [Hemk72].

In this thesis, the numerical solution of the different systems was obtained using SCILAB- 3.0.

SCILAB is a numerical programming and graphics environment available for free from the French Government's "Institut Nationale de Recherche en Informatique et en Automatique - INRIA". It is similar in operation to MATLAB, GNU OCTAVE, or other existing numerical/graphic environments and it can be run using a variety of operating systems including UNIX, WINDOWS, Linux, etc.

SCILAB is available for free from the SCILAB web page: <http://scilabsoft.inria.fr> together with several documentation files in several formats that help to get started with the program.

Developed for system control and signal processing applications, SCILAB con-

tains high quality solvers for ODEs [Sall04]. In the simplest use of SCILAB to solve ODEs, one only needs to give the initial value problem and the interval of integration. If the user does not specify something else, the solver chooses between stiff and non stiff methods. However, if the system is stiff, it is recommended the user to provide the Jacobian of the function on the right-hand side because of accuracy reasons. Otherwise, it will be computed internally by the finite differences method. Furthermore, stability of the system is also required and this is achieved if the Jacobian matrix of the function defined by the right-hand side of the system is stable at the equilibrium point.

C.2 The modules TriSer and Tsolve

The functions available in these modules allow one to decompose efficiently any polynomial system into fine triangular systems and into irreducible triangular systems, to decompose any algebraic variety into unmixed or irreducible subvarieties, and to solve any system of polynomial equations and inequalities [Wang04]. For the decomposition of ordinary differential polynomial systems into irreducible differential triangular systems one can use furthermore the module dTriser.

Appendix D

Original Model from Stortelder and Hemker

SciLab code:

```
;getf("C : /DokumenteundEinstellungen/Sandra/Doutoramento  
/SciLabHemker/Sys9/BCHemk.sci");
```

\mathbf{x}_0 is the vector containing the initial values and it corresponds to the physiological concentration in the blood given in $\mu\text{M/l}$.

```
x0=[0.2;0;0.03;0;0.05;0;1.4;0;0];
```

```
t0=0;
```

The interval of integration corresponding to 30 minutes is represented by

```
T=[0:0.01:30];
```

The solution is obtained by calling the routine `ode`. The argument `BCHemkj` indicates that the jacobian was provided.

```
sol=ode(x0,t0,T,BCHemk,BCHemkj);
```

The amydolytic activity of thrombin is given by

```
AmAct=sol(8,;)+0.556*sol(9,;);
```

The function, BCHemk is given by

```

function xdot=BCHemk(t,x)
RVV=0.03;
k1=2.391d2;
k2=2.365d1;
k3=4.531;
k4=1.229d2;
k5=8.014d2;
k6=7.844;
k7=1.497d2;
k8=4.387d1;
k9=6.225d1;
k10=1.240d1;
k11=6.148d-2;
k12=7.859d-1;
k13=1.762d-1;
//reactions on the right-hand side
r1=(k1*x(1)*RVV)/(k2+x(1));
r2=k3*x(2);
r3=k6*x(3)*x(8)/(k7+x(3));
r4=k4*x(4)*x(2)*x(5);
r5=k5*x(6);
r6=(k8*x(7)*x(6))/(k9+x(7));
r7=(k10*x(7)*x(2))/(k11+x(7));
r8=k13*x(8);
r9=k12*x(8);
//system of ODE's
xdot=[-r1;..
r1-r2-r4+r5;..
-r3;..
r3-r4+r5;..
-r4+r5;..
r4-r5;..
-r6-r7;..
r6+r7-r8-r9;..
r8] //correction according to the reaction scheme instead of r9
endfunction

```

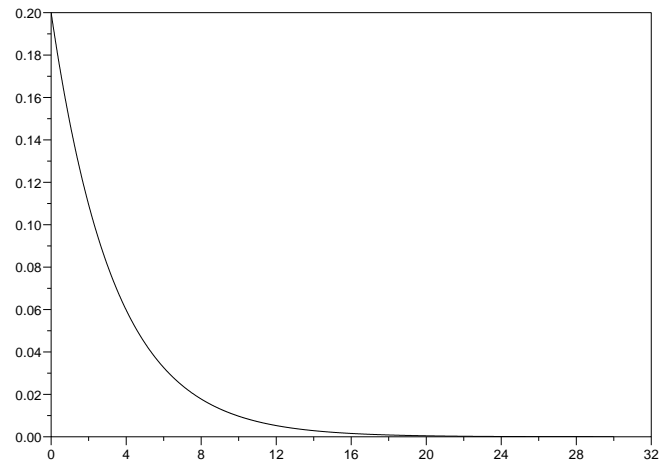
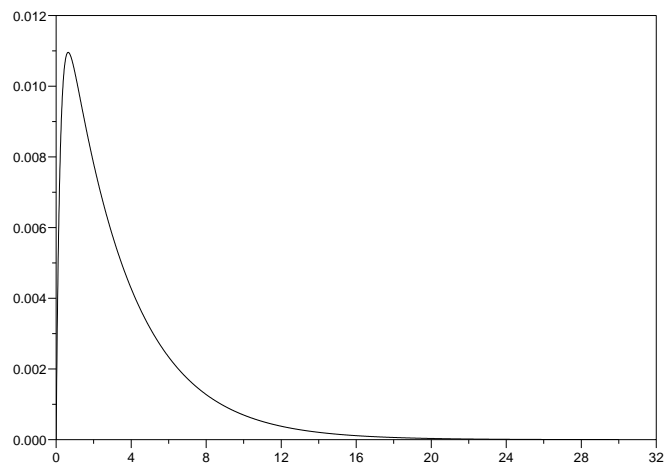


Figure D.1: Factor X

Figure D.2: Factor X_a .

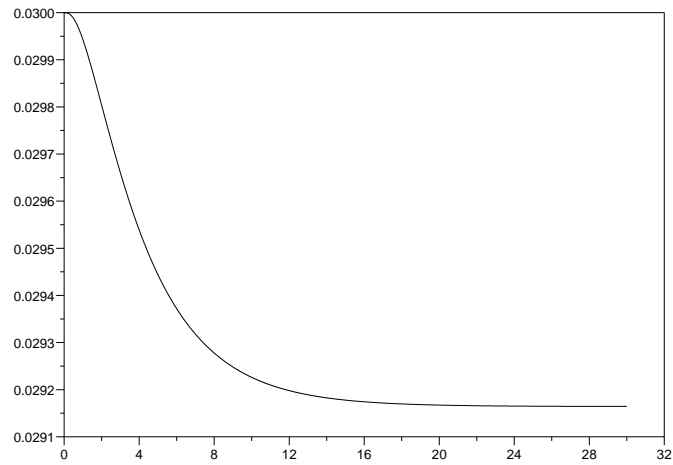


Figure D.3: Factor V

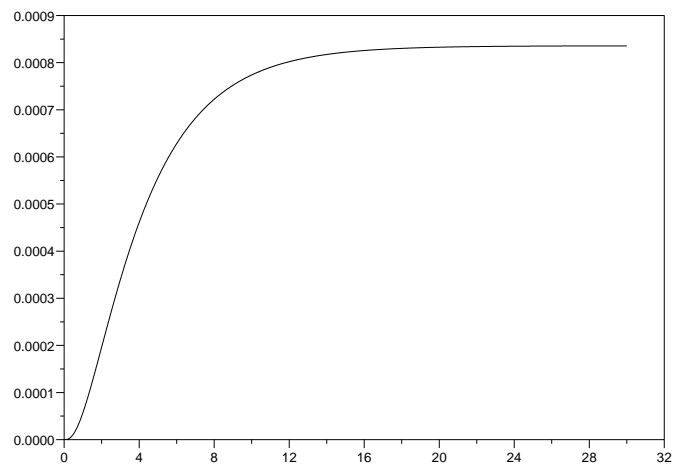


Figure D.4: Factor Va

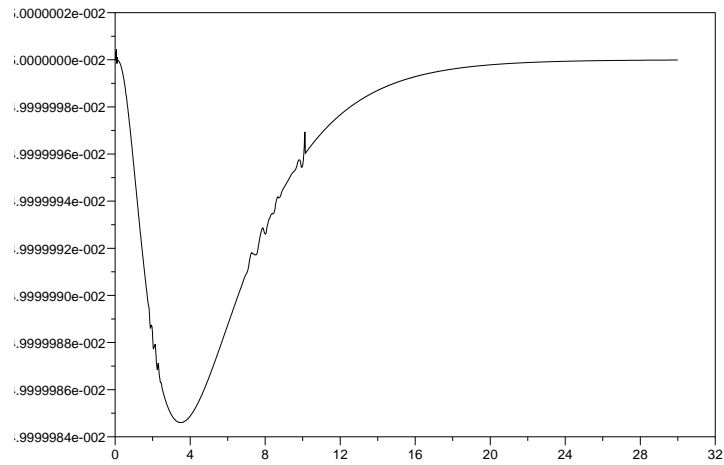


Figure D.5: Phospholipids.

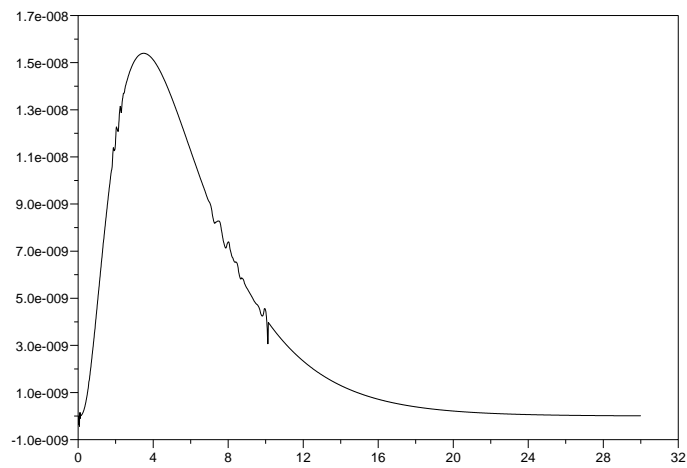


Figure D.6: Prothrombinase complex.

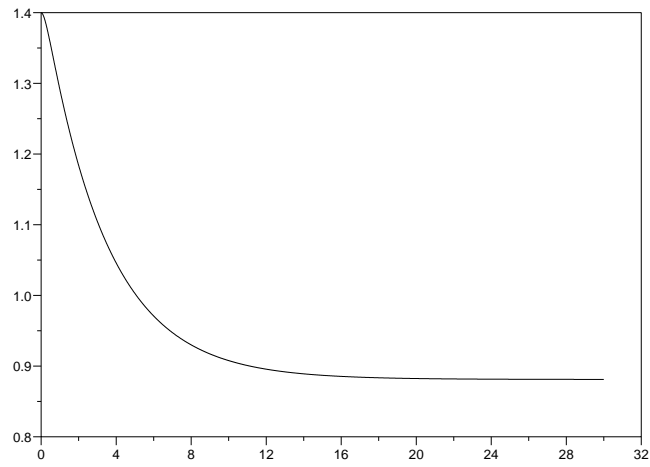
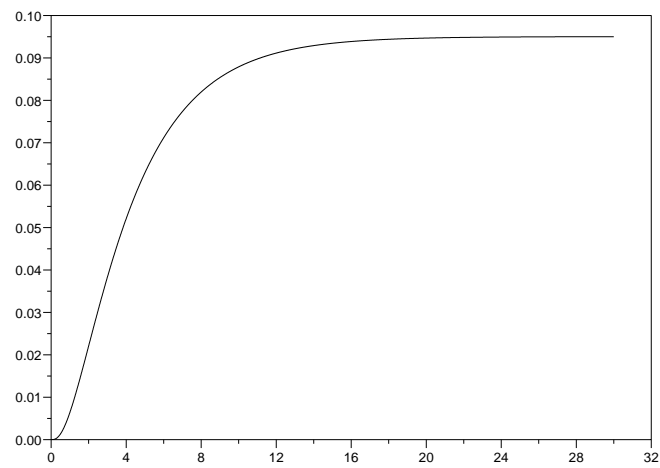


Figure D.7: Prothrombin

Figure D.8: Factor II α_2 M

Appendix E

Platelets Contribution - An Extension of Stortelder's Model

First approach to model the influence of platelets - adapted from [SHH97].

```
;getf("C : /DokumenteundEinstellungen/Sandra/Doutoramento  
/SciLabHemker/Sys9Mod/BCHemkThromb.sci");
```

```
T=[0:0.01:30];  
xt=[0.2;0;0.03;0;0.05;0;1.4;0;0;0;0];  
t0=0;  
solt=ode(xt,t0,T,BCHemkThromb,BCHemkThromb);
```

```
function xdot=BCHemkThromb(t,x)
```

```
RVV=0.03;  
k1=2.391d2;  
k2=2.365d1;  
k3=4.531;  
k4=1.229d2;  
k5=8.014d2;  
k6=7.844;  
k7=1.497d2;  
k8=4.387d1;  
k9=6.225d1;
```

```

k10=1.240d1;
k11=6.148d-2;
k12=7.159d-1; changing this value changes the equilibrium level of AmAct
k13=1.762d-1;
kT=5.53; constant for thrombocyte action

```

```

r1=(k1*x(1)*RVV)/(k2+x(1));
r2=k3*x(2);
r3=k6*x(3)*x(8)/(k7+x(3));
r4=k4*x(4)*x(2)*x(5);
r5=k5*x(6);
r6=(k8*x(7)*x(6))/(k9+x(7));
r7=(k10*x(7)*x(2))/(k11+x(7));
r8=k13*x(8);
r9=k12*x(8);
r10=kT*x(8)*x(1); first order reaction modeling the activation of factor X
by thrombin at the surface of activated platelets

```

```

xdot=[-r1;..
r1-r2-r4+r5+r10;..
-r3;..
r3-r4+r5;..
-r4+r5;..
r4-r5;..
-r6-r7;..
r6+r7-r8-r9-r10;..
r9;..
r8]
endfunction

```

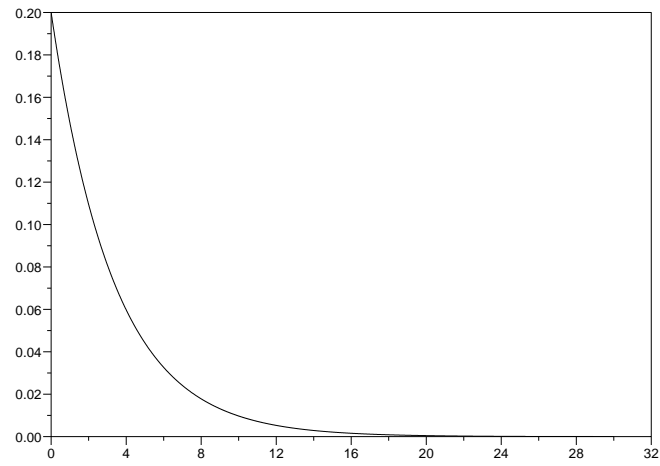


Figure E.1: Factor X

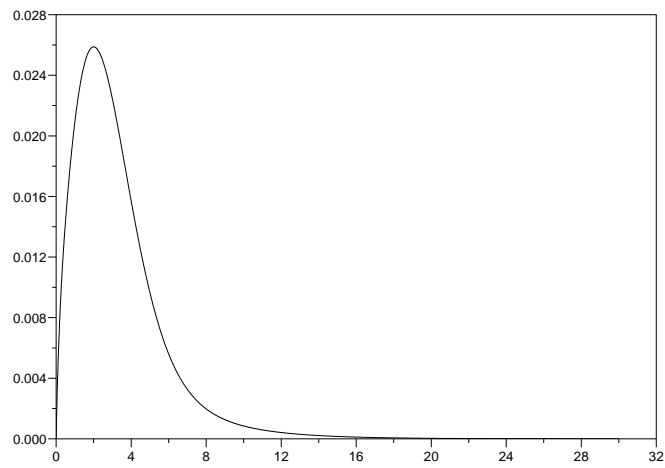


Figure E.2: Factor Xa

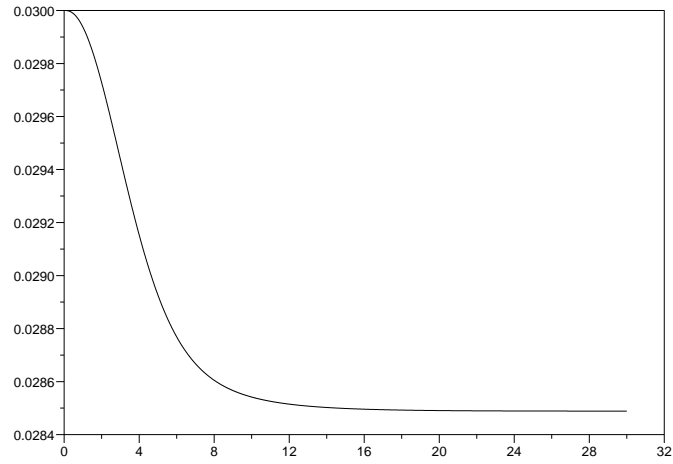


Figure E.3: Factor V

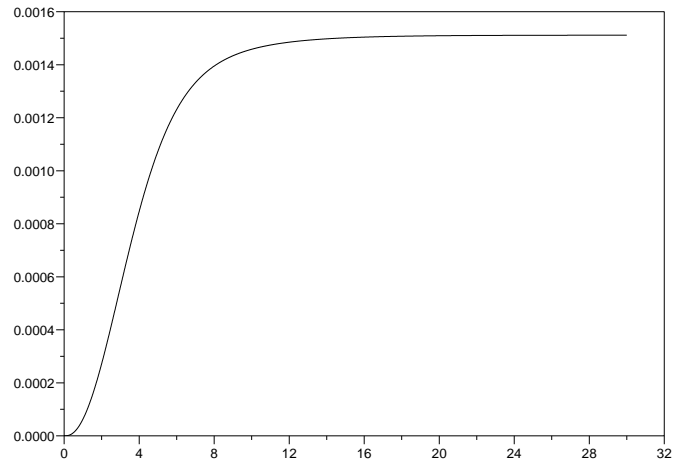


Figure E.4: Factor Va

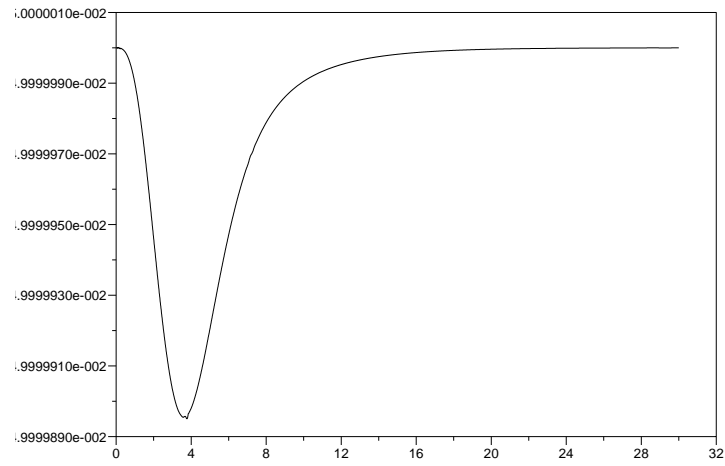


Figure E.5: Phospholipids

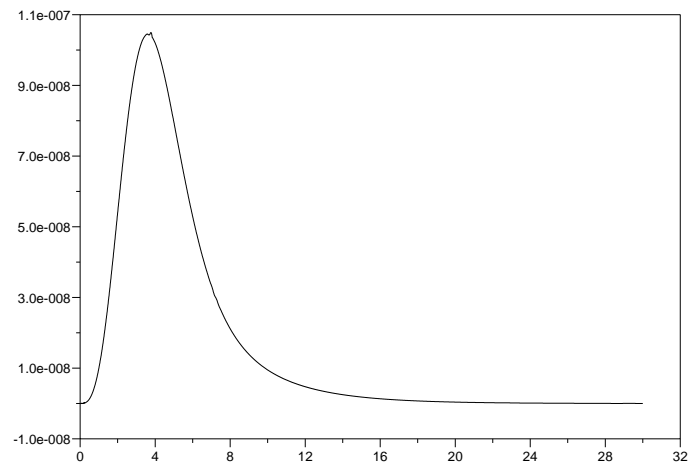


Figure E.6: Prothrombinase

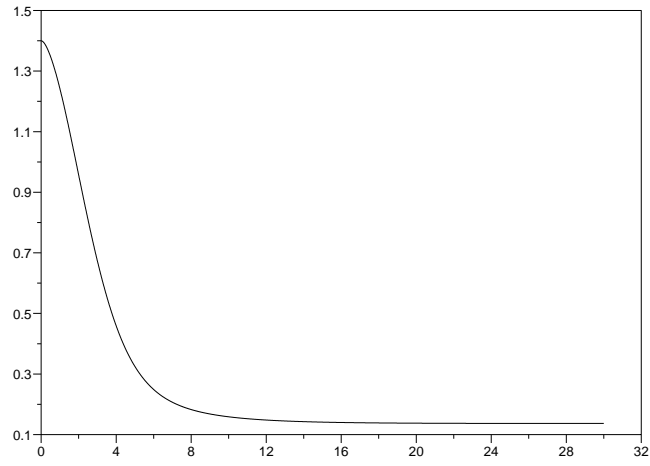


Figure E.7: Factor II

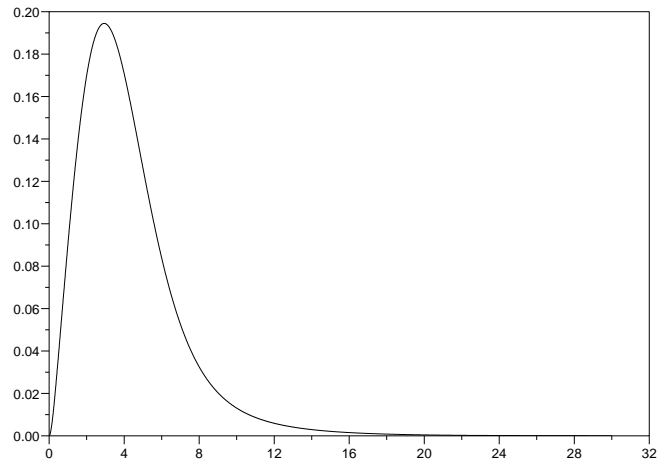


Figure E.8: Factor IIa

Appendix F

Corrected Model from Mann and Jones

SciLab code:

```
;getf("C : /DokumenteundEinstellungen/Sandra/Doutoramento/
```

```
SciLabMann/BCMannCorr.sci");
```

\mathbf{x}_0 is the vector containing the initial values and it corresponds to the physiological concentration in the blood given in μM .

```
x0=[0.000005;0.09;0.2;0.032;0.0007;1.4;0;0;0;0;0;0;0;0;0];
```

```
t0=0;
```

The interval of integration, where the time of 16 minutes is given in seconds is represented by

```
T=[0:0.1:960];
```

The solution is obtained by calling the routine `ode`. The argument `BCMannCorrj` indicates that the jacobian was provided.

```
sol=ode(x0,t0,T,BCMannCorr,BCMannCorrj);
```

The amount of activated thrombin in blood is given by

```
a=sol(9,:)+1.2*sol(11,:);
```

In the function, BCMannCorr the constants are reduced to the appropriate dimension.

```

function xdot=BCMannCorr(t,x)
k1=20;
k2=20;
k3=10;
k4=20;
k5=10;
k6=100;
k7=10;
k8=400;
k9=0.005;
k10=0.4;
k11=0.3;
k12=1.15;
k13=8.2;
k14=32;
k15=0.1;
k16=25;
k17=44;
k18=0.001;
k19=70;
k20=0.02;
xdot=[k11*x(12)-k6*x(1)*x(2)+k16*x(12)+k12*x(13)-k6*x(1)*x(3)+k17*x(13);..
k16*x(12)-k6*x(1)*x(2)-k15*x(2)*x(16);..
k17*x(13)-k6*x(1)*x(3)-k6*x(7)*x(3)+k18*x(14);..
-k1*x(4)*x(16)-k2*x(4)*x(9);..
-k3*x(5)*x(16)-k4*x(5)*x(9);..
k19*x(10)-k6*x(8)*x(6);..
k7*x(18)*x(15)-k9*x(7)-k6*x(7)*x(3)+k18*x(14)+k13*x(14);..
k8*x(16)*x(17)-k10*x(8)+k19*x(10)-k6*x(8)*x(6)+k14*x(10);..
k5*x(8)*x(11);..
k6*x(8)*x(6)-k19*x(10)-k14*x(10);..
k14*x(10)-k5*x(8)*x(11);..
k6*x(1)*x(2)-k16*x(12)-k11*x(12);..
k6*x(1)*x(3)-k17*x(13)-k12*x(13);..
k6*x(7)*x(3)-k18*x(14)-k13*x(14);..
k9*x(7)-k7*x(18)*x(15)+k11*x(12)+k15*x(2)*x(16);..
k10*x(8)-k8*x(16)*x(17)+k12*x(13)+k13*x(14);..
k10*x(8)-k8*x(16)*x(17)+k1*x(4)*x(16)+k2*x(4)*x(9);..
k9*x(7)-k7*x(18)*x(15)+k3*x(5)*x(16)+k4*x(5)*x(9)]
endfunction

```

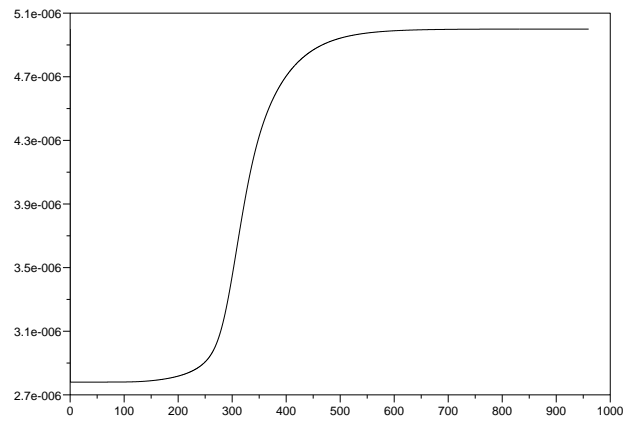



Figure F.1: ComplexTFVIIa

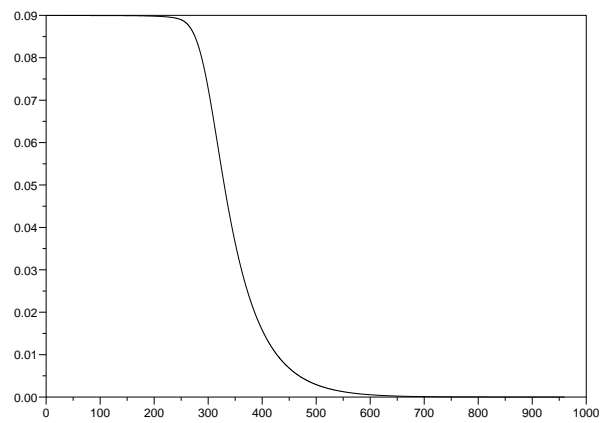


Figure F.2: FactorIX

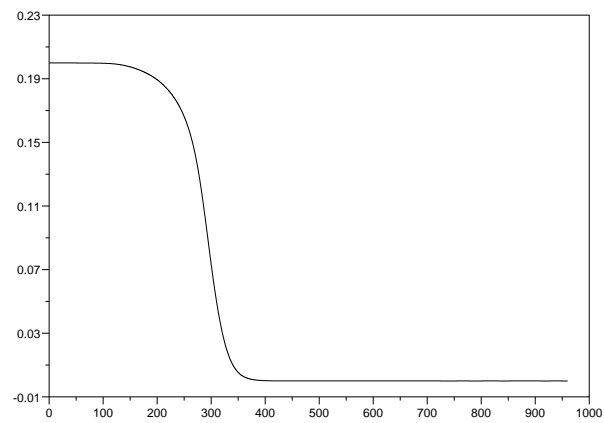


Figure F.3: Factor X

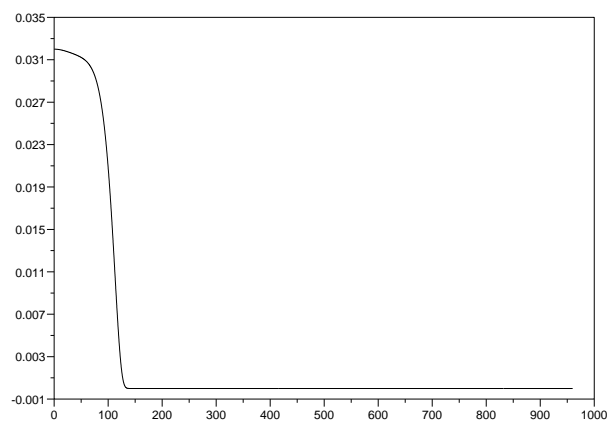


Figure F.4: Factor V

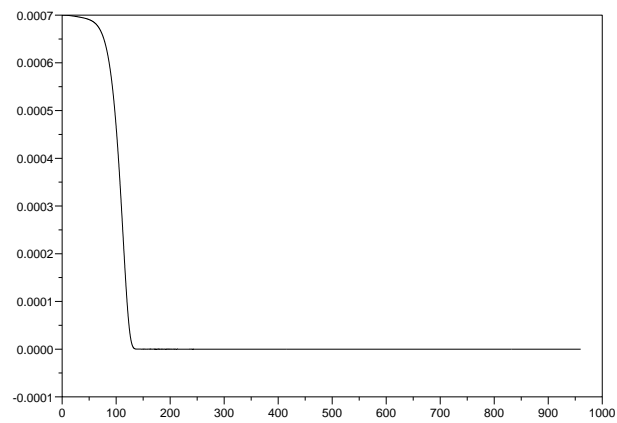


Figure F.5: Factor VIII

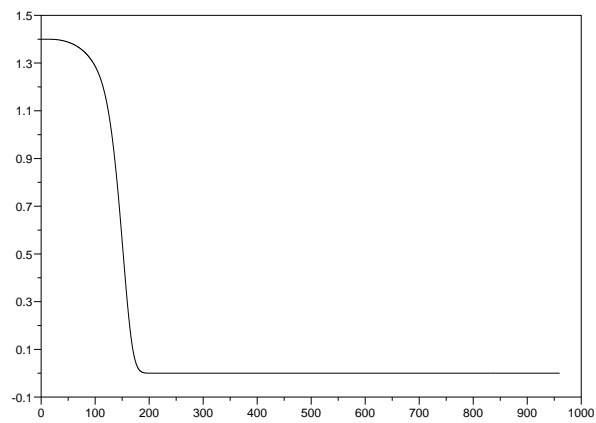


Figure F.6: Factor II

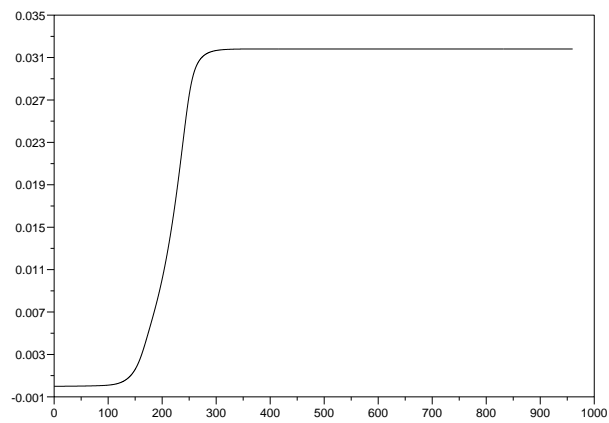


Figure F.7: ComplexVaXa

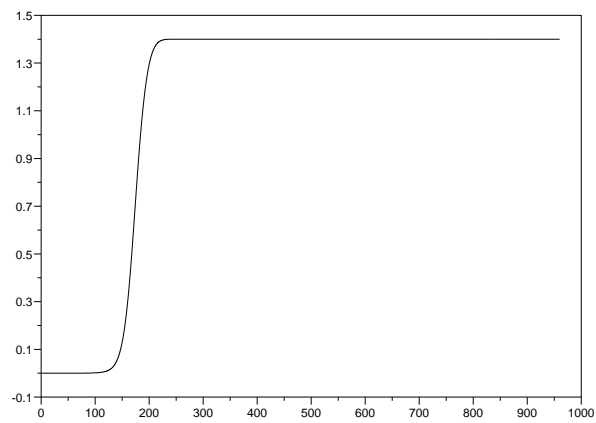


Figure F.8: Factor IIa

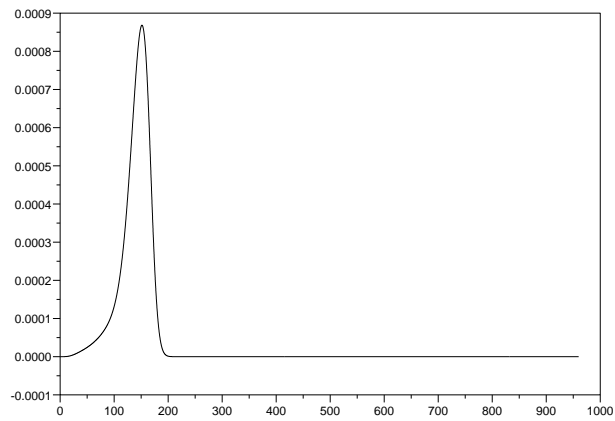


Figure F.9: ComplexVaXaII

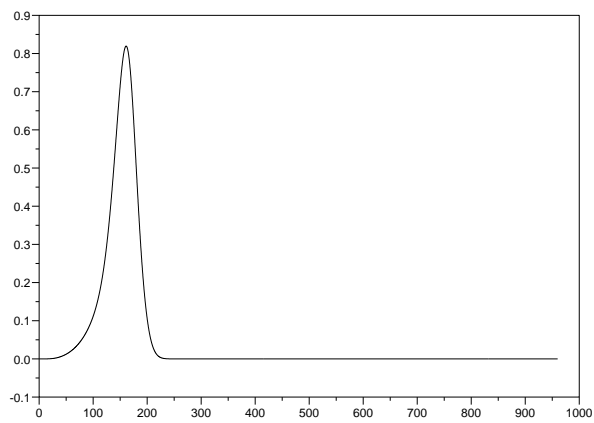


Figure F.10: Factor mIIa

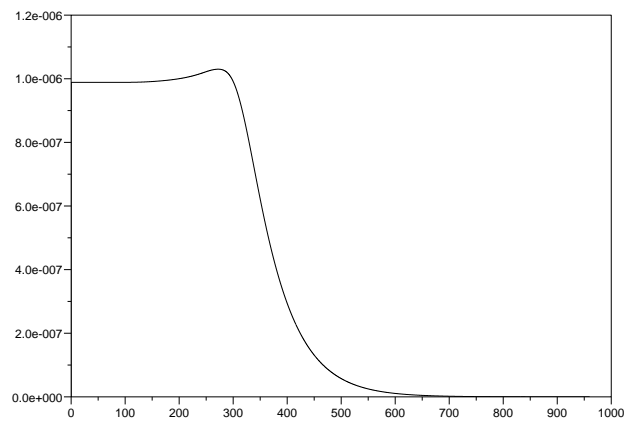


Figure F.11: ComplexTFVIIaX

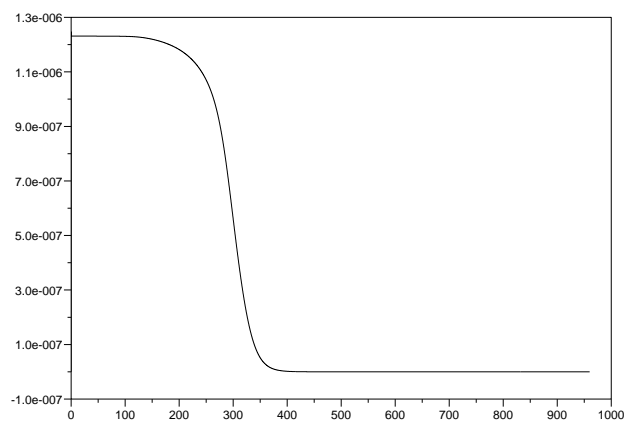


Figure F.12: ComplexTFVIIaX

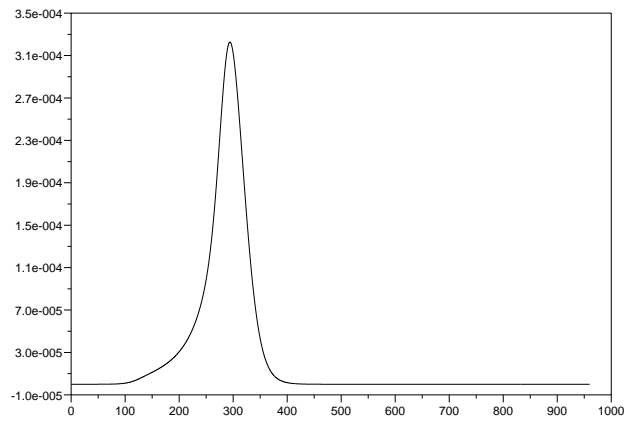


Figure F.13: ComplexVIIIaIXaX

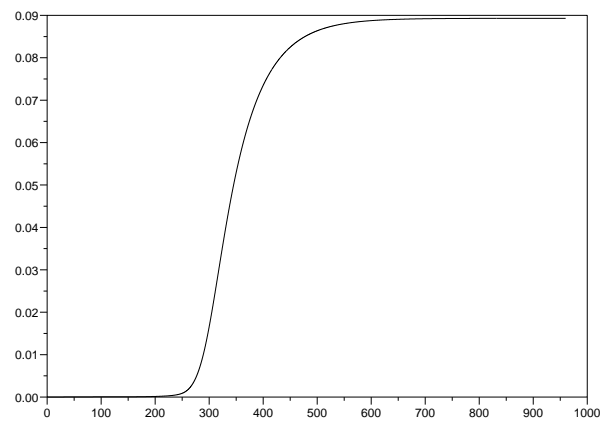


Figure F.14: Factor IXa

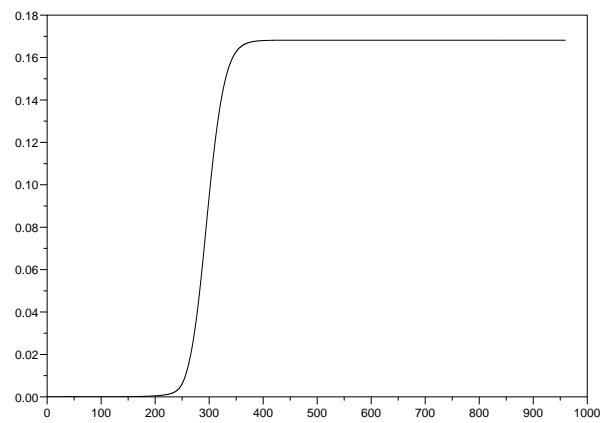


Figure F.15: Factor Xa

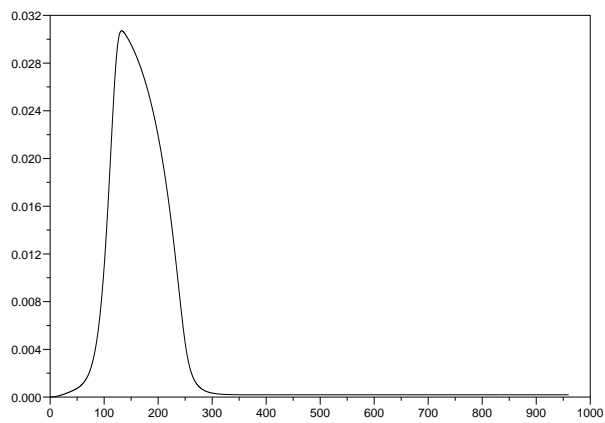


Figure F.16: Factor Va

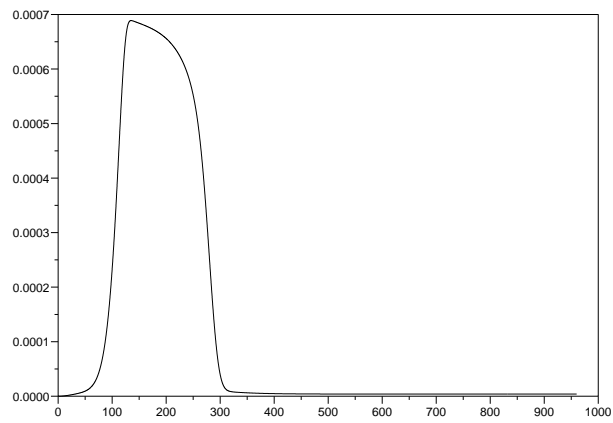


Figure F.17: Factor VIIIa

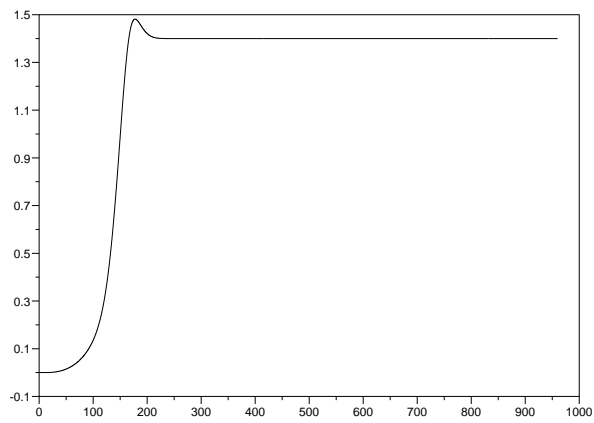


Figure F.18: Activated Thrombin (time=960 seconds=16 min)

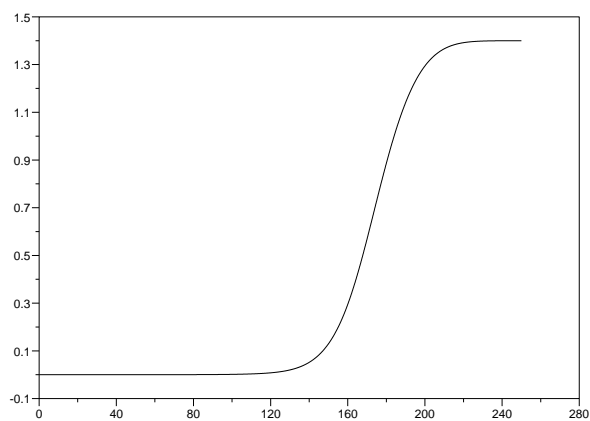


Figure F.19: Factor IIa (time=250 seconds \approx 4 min)

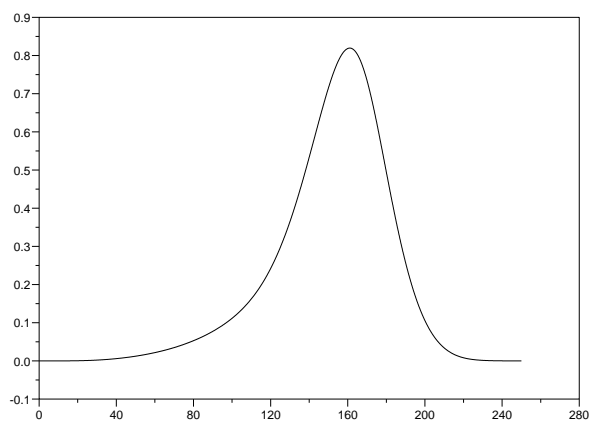


Figure F.20: mIIa (time=250 seconds \approx 4 min)

Bibliography

- [Ama90] Amann H (1990). Ordinary Differential Equations - An Introduction to Nonlinear Analysis. Walter de Gruyter, Berlin - New York.
- [Aris65a] Aris R (1965). Prologomena to the rational analysis of chemical reactions. *Archive for rational mechanics and analysis*, **19**(2),81–99.
- [Aris65b] Aris R (1965). Introduction to the analysis of chemical reactors. Prentice-Hall, Inc., Englewoos Cliffs, New Jersey.
- [Arn80] Arnol'd VI (1980). Gewöhnliche Differentialgleichungen. Springer-Verlag, Berlin - Heidelberg - New York.
- [AtPa05] Ataullakhanov FI, Pantelev MA (2005). Mathematical modeling and computer simulation in blood coagulation. *Pathophysiol Haemost Thromb*,**34**,60–70.
- [Bar75] Barnett S (1975). Introduction to mathematical control theory. Clarendon Press, Oxford - Great Britain.
- [BBK98] Baugh RJ, Broze GJ Jr, Krishnaswamy S (1998). Regulation of extrinsic pathway factor Xa formation by tissue factor pathway inhibitor. *J. Biol. Chem*,**273**,4378–4386.
- [BeJe95] Beltrami E, Jesty J (1995). Mathematical analysis of activation thresholds in enzyme-catalyzed positive feedbacks: Application to the feedbacks of blood coagulation. *Proc. Natl. Sci. USA*, **92**,8744–8748.
- [BdBS96] Borghans JAM, de Boer RJ, Segel LA (1996). Extending the quasi-steady state approximation by changing variables. *Bulletin of Mathematical Biology*, **58**, No. 1,23–63.
- [Bib79] Bibikov YN(1979). Local Theory on Nonlinear Analytic Ordinary Differential Equations. *Lecture Notes in Mathematics*. Springer-Verlag Berlin Heidelberg.
- [BriHal25] Briggs GE, Haldane JBS (1925). A note on the kinetics of enzyme action. *Biochem. J.*, **19**,338–339.

- [BuMa02] Butenas S, Mann KG (2002). Blood Coagulation. *Biochemistry*, **67**, 3–12.
- [BvVM99] Butenas S, van't Veer C, Mann KG (1999). "Normal" thrombin generation -plenary paper. *Blood*, **94**, No. 7, 2169–2178.
- [BWHL95] Billy D, Willems GM, Hemker HC, Lindhout T (1995). Prothrombin Contributes to the Assembly of the Factor Va-Factor Xa Complex at Phosphatidylserine-containing Phospholipid Membranes. *American Society for Biochemistry and Molecular Biology, Inc.*, **270**, No 45, 26883–26889.
- [B-ZVBM05] Brummel-Ziedins KE, Vossen CY, Butenas S, Mann KG, Rosendaal FR (2005). Thrombin generation in deep venous thrombosis. *J Thromb Haemost*, **3**, 2497–2505.
- [ChaRu94] Char BW, Russo MF (1994). Automatic Identification of Time Scales in Enzyme Kinetics Models. *ISAAC 94-7/94 Oxford England Uk*, 74–83.
- [CSM04] Crampin EJ, Schnell S, McSharry PE (2004). Mathematical and computational techniques to deduce complex biochemical reaction mechanisms. *Progress in Biophysics & Molecular Biology*, **86**, 77–112.
- [Davie05] Davie EW (2005). A Brief Historical Review of the Waterfall/Cascade of Blood Coagulation. *J. Biol. Chem.*, **278**, 50819–50832.
- [Fein79] Feinberg M (1979). Lectures on chemical reaction networks. *4,5 out of 9 lectures delivered at the Mathematics Research Center, Univ. of Wisconsin.*
- [FLMR95] Fliess M, Lèvine J, Martin P and Rouchon P (1995). Flatness and defect of non-linear systems: introductory theory and examples. *Int. J. Control*, **61**(6), 1327–1361.
- [FoKu98] Fogelson A, Kuharsky AL (1998). Membrane Binding-site Density can Modulate Activation Thresholds in Enzyme Systems. *J. theor. Biol.*, **193**, 1–18.
- [Gaw99] Gawaz M (1999). Das Blutplättchen. Georg Thieme Verlag, Stuttgart.
- [HJEM02] Hocking MF, Jones KC, Everse SJ, Mann KG (2002). A model for the Stoichiometric Regulation of Blood Coagulation. *J. Biol. Chem.*, **277**, No. 21, 18322–18333.
- [Hemk72] Hemker PW (2002). Numerical methods for differential equations in system simulation and in parameter estimation. In H.C. Hemker and B. Hess, editors, *Analysis and Simulation of Biochemical Systems*. North Holland Publ. Comp. 59–80.
- [JeBeWi93] Jesty J, Beltrami E, Willems G (1993). Mathematical analysis of a proteolytic positive feedback loop: dependence of a lag time and enzyme yields on the in initial conditions and kinetic parameters. *Biochemistry*, **32**, 6266–6274.

- [Jesty05] Jesty J (2005). Blood Coagulation. *Encyclopedia of Life Sciences*. John Wiley & Sons, Ltd: Chichester <http://www.els.net/>[doi: 10.1038/npg.els.0003984].
- [JoMa94] Jones KC, Mann KG (1994). A Model for the Tissue Factor Pathway to Thrombin- A Mathematical Simulation. *The Journal of Biological Chemistry*, **Vol. 269, No. 37** , 23367–23373.
- [KeSn98] Keener J, Sneyd J (1998). *Mathematical Physiology*. Springer-Verlag New York, Inc.
- [KKK01] Kogan AE, Kardakov DV, Khanin MA (2001). Analysis of the Activated Partial Thromboplastin Time Test Using Mathematical Modeling. *Thrombosis Research*, **101** ,299–310.
- [KnoKa74] Knobloch HW, Kappel F (1974). *Gewöhnliche Differentialgleichungen*. B. G. Teubner, Stuttgart.
- [KnKw80] Knobloch HW, Kwakernaak H (1980). *Lineare Kontrolltheorie*. Springer-Verlag Berlin Heidelberg New York Tokyo. Stuttgart.
- [LaMa91] Lawson JH, Mann KG (1991). Cooperative activation of human factor IX by the human extrinsic pathway of blood coagulation. *J. Biol. Chem.*, **266** ,11317–11327.
- [LBRD95] Leipold RJ, Bozarth TA, Racanelli AL, Dicker IB (1995). Mathematical Model of Serine Protease Inhibition in the Tissue Factor Pathway to Thrombin. *J. Biol. Chem.*, **270** ,25383–25387.
- [LoPa91] Lollar P, Parker ET (1991). Structural basis for the decreased procoagulant activity of human factor VIII compared to the porcine homolog. *J. Biol. Chem.*, **266** ,12481–12486.
- [LinSe88] Lin CC, Segel LA (1988). *Mathematics applied to deterministic problems in the natural sciences*, 2nd ed. Philadelphia: Society for Industrial and Applied Mathematics (SIAM).
- [Lin95] Lind Stuart E. (1995). *The Hemostatic System. Blood: Principles and Practice of Hematology*. J. B. Lippincott Company, Philadelphia, 949–973.
- [LKSM94] Lawson JH, Kalafatis M, Stram S, Mann KG (1994). A Model for the Tissue Factor Pathway to Thrombin- An Empirical Study *The Journal of Biol. Chem*, **Vol. 269, No. 37** , 23357–23366.
- [LaSa61] La Salle J, Lefschetz S (1961). *Stability by Lyapunov's Direct Method with Applications*. New York Academic Press Inc.
- [MaMo74] Martorana F, Moro A (1974). On the Kinetics of Enzyme Amplifier Systems with Negative Feedback. *Mathematical Biosciences*, **21** ,77–84.

- [MaNeu99] Magnus J, Neudecker H (1999). Matrix Differential Equations With Applications in Statistics and Econometrics. John Wiley and Sons.
- [MMR97] Martin Ph, Murray RM, Rouchon P (1997). Flat Systems *Mini-Course ECC'97* Brussels ,211–264.
- [MNCHK90] Mann KG, Nesheim ME, Church WR, Haley P, Krishnaswamy S (1990). Surface-Dependent Reactions of the Vitamin K-Dependent Enzyme Complexes - Review Article. *Blood*.**76**, No. 1 ,1–16.
- [MoTr90] Monkovic DD, Tracey PB (1990). Activation of human factor V by factor X_a and thrombin *Biochemistry*.**29**(5) ,1118–1128.
- [Mur93] Murray JD (1993). Mathematical Biology. Springer-Verlag Berlin Heidelberg. John Wiley and Sons.
- [NeMa79] Nesheim ME, Mann KG (1979). Thrombin-catalyzed activation of single chain bovine factor V. *J. Biol. Chem.*.**254** ,1326–1334.
- [NeTrMa84] Nesheim ME, Tracy RP, Mann KG (1984). "Clotspeed" a mathematical simulation of the functional properties of prothrombinase *J. Biol. Chem.*.**259**, No 3 ,1447–1453.
- [NivdSc90] Nijmeijer H, van der Schaft A (1990). Nonlinear Dynamical Control Systems. Springer Verlag- New York Inc.
- [NoeWa05] Nöthen L, Walcher S (2005). Quasi-steady state in the Michaelis-Menten system. *Preprint*.
- [NTM79] Nesheim ME, Taswell JB, Mann KG (1979). The contribution of bovine Factor V and Factor V_a to the activity activity of prothrombinase. *J. Biol. Chem.*.**254** ,10952–10962.
- [PZA02] Panteleev MA, Zarnitsina V, Ataulakhanov F (2002). Tissue factor pathway inhibitor-A possible mechanism of action. *Eur. J. Biochem.*.**269** ,2016–2031.
- [Per96] Perko L (1996). Differential equations and Dynamical Systems. Springer-Verlag New York, Inc.
- [Pru00] Pruthi, RK (2000). An Overview of the Hemostatic System and Interpretation of Common Screening Coagulation Tests. *Primary Hematology edited by Ayalew Tefferi*. Humana Press, Totowa, New Jersey, 291–302.
- [Rot97] Rothfuß R (1997). Anwendung der flachheitsbasierten Analyse und Regelung nichtlinearer Mehrgrößensysteme. *Fortschritt-Berichte*, Reihe 8: Meß-, Steuerungs- und Regelungstechnik Nr. **664**.
- [SaIn04] Sauro HM, Ingalls B (2004). Conservation Analysis in Biochemical Networks - Computational issues for software writers. *Biophys. Chem.*,**109**(1),1–15.

- [Sall04] Sallet G (2004). Ordinary Differential Equations with Scilab. *WATS lectures - provisional notes Université de Saint-Louis*, Université de Metz INRIA Lorraine.
- [SchMai00] Schnell S and Maini P (2000). Enzyme kinetics at high enzyme concentration. *Bull. Math. Biol.*, **62**, 483–499.
- [SchMai03] Schnell S and Maini P (2003). A Century of Enzyme Kinetics: Reliability of the K_M and v_{max} Estimates. *Comments on Theoretical Biology*, **8**, 169–187.
- [SchMen97] Schnell S and Mendoza C (1997). Closed form solution for time-dependent enzyme kinetics. *J. Theor. Biol.*, **187**, 207–212.
- [Seg88] Segel LA (1988). On the validity of the steady-state assumption of enzyme kinetics. *Bull. Math. Biol.*, **50**, 579–593.
- [Seg91] Segel LA (1991). Biological Kinetics. *Cambridge Studies in Mathematical Biology*, Cambridge University Press.
- [SeSl89] Segel LA, Slemrod M (1989). The quasi steady-state assumption: A case study in perturbation. *SIAM Rev.*, **31**, 446–477.
- [SHH97] Stortelder WJH, Hemker PW, Hemker HC (1997). Mathematical Modelling in Blood Coagulation MAS-R9720 September 30.
- [Son90] Sontag ED (1990). Mathematical Control Theory. Springer-Verlag New York, Inc.
- [Son01] Sontag ED (2001). Structure and Stability of Certain Chemical Networks and Applications to the Kinetic Proofreading Model of T-Cell Receptor Signal Transduction. *IEEE Transactions on Automatic Control*, **46/7**, 1028–1047.
- [Son05a] Sontag ED (2005). Molecular Systems Biology and Control. *European J. of Control.*, **11**, 1–40.
- [Son05b] Sontag ED (2005). Lecture Notes in Mathematical Biology. *Rutgers University*, Rutgers University.
- [StFr79] Stayton MM, Fromm HJ (1979). A computer analysis of the validity of the integrated Michaelis-Menten equation. *J. Theor. Biol.*, **78**, 309–323.
- [Sw84] Swan GW (1984). Applications of optimal control theory in biomedicine. Marcel Dekker, Inc, New York.
- [vDTRH81] van Diejtin G, Tans G, Rosing J, Hemker HC (1981). The role of phospholipid and factor $VIII_a$ in the activation of bovine factor X. *J. Biol. Chem.*, **256**, 3433–3442.
- [Wal01] Walcher S (2001). Nonlinear Dynamics. *TUM lecture notes*, Technische Universität München.

- [Wang04] Wang D (2004). Elimination practice - Software Tools and Applications. Imperial College Press, London.
- [Zab92] Zabczyk J (1992). Mathematical Control Theory: An Introduction. Birkhäuser, Boston.
- [Zer00] Zerz E (2000). Topics in multidimensional linear systems theory. *Lecture notes in control and information sciences*, **256**. Springer-Verlag London Limited.

Lebenslauf

Zur Person

Name: Cunha Órfão, Sandra Maria
Geburtsdatum: 03.08.1974
Geburtsort: Quelimane (Mosambik)
Familienstand: ledig
Nationalität: portugiesisch

Ausbildung

1980-1992: Schulbildung in Chaves (Portugal) mit Erwerb des Abiturs 1992
1992-1997: Studium der Mathematik an der Universität Coimbra (Portugal) mit Ablegen des Staatsexamens 1997

Berufstätigkeit

09.1996-08.1997: Referendariat an der Escola Secundária Infanta D. Maria (Coimbra)
09.1997-12.2006: Studienrätin für Mathematik an der Escola Secundária Alfredo Reis Silveira (Lisboa)
11.2001-09.2002: Wissenschaftliche Hilfskraft im Institut für Medizinische Statistik der RWTH Aachen
10.2002-04.2004: Wissenschaftliche Hilfskraft am Lehrstuhl II für Mathematik und in der Klinik für Anästhesie des Universitätsklinikums der RWTH Aachen
05.2004-02.2005: Wissenschaftliche Hilfskraft in der Klinik für Anästhesie des Universitätsklinikums der RWTH Aachen
seit 03.2005: Wissenschaftliche Angestellte am Lehrstuhl II für Mathematik der RWTH Aachen