HIV-1 Infection Model Analysis and Therapy Design using a Nonlinear Control approach

João Gonçalo Silva Marques *

Under supervision of João Miranda Lemos, Emília Valadas and Susana Vinga

November 4, 2011

Abstract

There is currently an increasing interest in the study of Human Immunodeficiency Virus 1 (HIV-1) infection dynamics, with several mathematical models developed with this objective. In some of them, there is the attempt of considering the action of antiretroviral drugs. However, in most cases, the concern is only in the relation between drug effect and viral load. In addition, the drug effect is usually seen as a continuous variable. In order to bring the analysis of HIV-1 dynamic models closer to the reality, this work deals with drug doses instead of drug effects, which are administered to patients in the form of a series of discrete events. These drug doses are then processed by a Pharmacokinetics (PK) and Pharmacodynamics (PD) model, in order to compute drug effect from the doses administered to the patient. Moreover, an adherence and virus resistance model is also included, so that their impact can be analyzed in simulations, since they play an important role in real patients. After this analysis, a sensitivity and local identifiability analysis is performed for the proposed model, and it is concluded that there are time periods, especially in early stages of the infection, that are far more informative than others, which may help in the planning of measurements. In addition, it is seen that the model is not identifiable, and a subset of unidentifiable parameters is obtained. Different therapy combinations are used to test the developed model, and it shown that the outcome of a regular therapy, but with patient adherence of 50%, is far better than that of a therapy with 100% patient adherence, but with drug doses being administered with half the frequency. Finally, using a nonlinear control approach, it is also demonstrated that it is possible to achieve an undetectable viral load with drug doses far below those currently used in practice. However, the developed algorithm does not take into account resistance development, which may lead the therapy predicted by the controller to eventually fail.

Keywords: Human Immunodeficiency Virus 1, Infection Dynamic Models, Antiretroviral Drugs, Pharmacokinetics/Pharmacodynamics, Sensitivity and Identifiability Analysis, Nonlinear Control Theory

*This work was supported by FCT under the project *HIVCONTROL - Control based on dynamic modeling of HIV-1 infection for therapy design*, contract PTDC/EEA-CRO/100128/2008, and INESC-ID multiannual funding through the PIDDAC Program funds.

1 Introduction

Acquired Immune Deficiency Syndrome (AIDS) is a disease caused by HIV-1 that affects the human immune system, reducing its effectiveness and leaving it susceptible to other diseases. Statistics show that, during 2009 only, about 2.6 million people were newly infected with HIV-1, and that there were around 1.8 million HIV-1 related deaths. Nowadays, HIV-1 infection is treated with Highly Active Antiretroviral Therapy (HAART), and the improvement in the life expectancy and quality of infected people is considerable [1]. However, there are some drawbacks in the efficacy of HAART. Causes such as low availability of antiretroviral drugs or appearance of side-effects can lead to the interruption of the therapy, compromising its success.

These problems associated with HAART, together with the high costs involved in this kind of therapy, have led the scientific community into the development and analysis of mathematical models that can mimic the HIV-1 infection. Some of these models also incorporate a way of reproducing drug effects, and their impact in the infection dynamic system. This work has the objective of studying some of this methods, and incorporating into one of them the action of Reverse Transcriptase Inhibitors (RTIs) and Protease Inhibitors (PIs), making use of their pharmacokinetic and pharmacodynamic properties, in order to allow the simulation of discrete drug doses. Furthermore, the patient adherence situation is also addressed, and a resistance model is proposed and simulated.

Once the dynamic modeling is achieved, the next natural step is to understand if it is possible to use an automatic control on the models. This may prove to be a powerful tool in therapy design, making it possible to adapt the treatment to the patient.

This work starts with a description of the HIV-1 infection model in Section 2. In Section 3, a local sensitivity and identifiability analysis is performed. After that, the kinetic and dynamic models for two of the most used antiretroviral drugs are further detailed in Section 4. Finally, in Section 5, a control approach is developed to face the challenge of building a personalized therapy that can adjust itself based on the patient's response to the treatment.

2 HIV-1 Infection Model

The HIV-1 infection model considered in this work allows to describe the infection dynamics (CD4+ T cell count and virus load), taking into account specific initial conditions, as well as the drug doses that the patient takes. The given model has four main blocks: the adherence model, the PK model, the PD model, and the virus dynamics. The model is summarized in Figure 1.

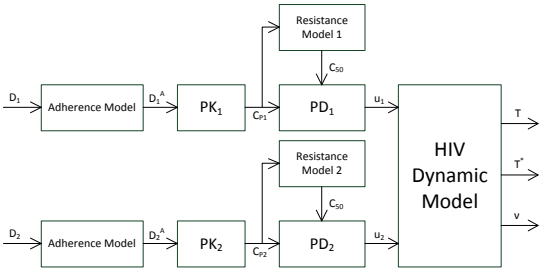


Figure 1: General HIV-1 infection model, including PK+PD, adherence and resistance.

In Figure 1, D_1 and D_2 represent the RTIs and PIs doses, respectively. These two inputs then go through an Adherence Model, which transforms them according to whether the patients follows the prescription or not. The drug doses that are actually taken by the patient are represented by D_1^A and D_2^A. After preprocessing the drug inputs, it is necessary to convert these doses into drug effect, which will range from 0 to 1. For this, a PK+PD model is considered. The first converts the input into plasma concentration of the drug over time (C_{P1} and C_{P2}), while the second will take this concentration and output the drug ef-

fect (u_1 and u_2). It is also considered that drug resistances may develop, which will result in lower drug effects. These effects will serve as input for the HIV-1 dynamic model that allows the infection simulation.

The drug's PK is modeled using a two compartment system model for each drug [2], and its representation is given by

$$\dot{\mathbf{x}}(t) = \mathbf{A}\mathbf{x}(t),\ \mathbf{x}(0) = \mathbf{x}_0,\ t \geq 0 \tag{1}$$

where

$$\mathbf{A} = \begin{bmatrix} -(a_{12}+a_{10}) & a_{21} \\ a_{12} & -(a_{21}+a_{20}) \end{bmatrix},\ \mathbf{x} = \begin{bmatrix} x_1 \\ x_2 \end{bmatrix} \tag{2}$$

The constants a_{ij} in (2) are specific for each drug, and are therefore said to be kinetic parameters of the drug.

Drug PD is modeled using the Hill equation

$$E(C_P) = E_{\max}\frac{C_P}{C_P + C_{50}} \tag{3}$$

where E represents the drug effect, $E_{\max}$ is the maximum drug effect, and C_{50} is the drug concentration that corresponds to half the maximum drug effect [2]. This value is said to be a pharmacodynamic parameter, and is specific for each drug.

Patient adherence is represented using a very simple model, that intends to assess whether the patient follows his prescription or not. This model is given by

$$A = \begin{cases} 1, & \text{if all doses are administered} \\ R_k, & \text{if } 100R_k\% \text{ doses are administered.} \end{cases} \tag{4}$$

Drug resistance intends to simulate the possible appearance of patient resistances to drug effects. In this work, the resistance model is given by

$$C_{50}(t) = \left(1 + K_R\int_0^t \max\left[0, L_R - C_P(\tau)\right] d\tau\right) C_{50_{base}} \tag{5}$$

Each time the drug concentration falls below L_R (a model parameter), C_{50} increases irreversibly. The model also depends on another parameter, K_R, that defines the resistance development rate [3].

The dynamic model used in this work is fully described in [4–7] and is given by

$$\begin{cases} \dot{T} &= s - dT - (1-u_1)\beta T\nu \\ \dot{T}^* &= (1-u_1)\beta T\nu - \mu T^* \\ \dot{\nu} &= (1-u_2)kT^* - c\nu \end{cases} \tag{6}$$

The three populations considered in this model are healthy CD4+ T cells (T), infected CD4+ T cells (T^*), and free virus particles (ν). The drug action influences the model via u_1 and u_2. For simplicity, T, T^* and ν shall be represented by X_1, X_2 and X_3, respectively, and all the parameters needed will be included in $\boldsymbol{\theta}$, so that one has:

$$\mathbf{X} \triangleq (X_1, X_2, X_3)' \tag{7}$$

$$\boldsymbol{\theta} \triangleq (s, d, \beta, \mu, k, c, u_1, u_2)' \tag{8}$$

$$\dot{\mathbf{X}} = \mathbf{f}(\mathbf{X}, \boldsymbol{\theta}) \tag{9}$$

The model steady states are obtained computing $\mathbf{f}(\mathbf{X}, \boldsymbol{\theta}) = \mathbf{0}$, and are given by

$$\begin{aligned} \overline{\mathbf{X}}^1 &= \left(\tfrac{s}{d}, 0, 0\right) \\ \overline{\mathbf{X}}^2 &= \left(\tfrac{\mu c}{\beta k}, \tfrac{s}{\mu} - \tfrac{dc}{\beta k}, \tfrac{sk}{\mu c} - \tfrac{d}{\beta}\right) \end{aligned} \tag{10}$$

3 Model Sensitivity and Local Identifiability Analysis

3.1 Model Sensitivity Analysis

Sensitivity computation is a tool that may help design clinical experiences, in order to achieve better outcomes. Algebraic manipulation of (9) allows to

obtain the following differential equation [5]

$$\dot{\mathbf{S}} = \left.\frac{\partial \mathbf{f}}{\partial \mathbf{X}}\right|_{\boldsymbol{\theta}=\boldsymbol{\theta}_0} \cdot \mathbf{S} + \left.\frac{\partial \mathbf{f}}{\partial \boldsymbol{\theta}}\right|_{\boldsymbol{\theta}=\boldsymbol{\theta}_0} \tag{11}$$

where

$$\mathbf{S} = \left.\frac{\partial \mathbf{X}}{\partial \boldsymbol{\theta}}\right|_{\boldsymbol{\theta}=\boldsymbol{\theta}_0} \tag{12}$$

The sensitivity simulations are performed using the parameter set in [5] by solving (9) and (11) simultaneously. The results obtained for one of the parameters (β) is represented in Figure 2.

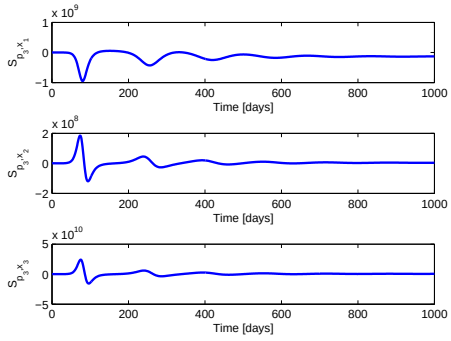


Figure 2: Time evolution of the sensitivities for all three states, for parameter β.

The results obtained allow to conclude that the most informative time period is between the 50^{th} and 100^{th} day, and that after 600 days measurements carry little or no information about the parameters, since the sensitivity values are practically 0.

3.2 Local Identifiability Analysis

After these sensitivity simulations, a local identifiability analysis is performed. Two different methods are used: a classical method, the analysis of the sensitivity correlation matrix [8, 9], and a proposed method, the Singular Value Decomposition (SVD) of the sensitivity matrix [10–12]. This analysis intends to determine whether the model is identifiable or not, and if possible, which parameters are identifiable or not.

The computation of the sensitivity correlation matrix from the simulation shown previously yields

$$R = \begin{bmatrix} 1 & -0.98745 & 0.98991 & -0.98514 & 0.9995 & -0.99681 \\ -0.98745 & 1 & -0.98396 & 0.958 & -0.98564 & 0.97801 \\ 0.98991 & -0.98396 & 1 & -0.95447 & 0.98885 & -0.9782 \\ -0.98514 & 0.958 & -0.95447 & 1 & -0.98777 & 0.99541 \\ 0.9995 & -0.98564 & 0.98885 & -0.98777 & 1 & -0.99814 \\ -0.99681 & 0.97801 & -0.9782 & 0.99541 & -0.99814 & 1 \end{bmatrix}$$

Figure 3: Sensitivity Correlation Matrix

From these results, it becomes evident that the model is not identifiable, since all non-diagonal values are very close to either 1 or -1. However, it is not possible to distinguish the least identifiable parameters with this method. For that, another method is proposed, based on the decomposition of the sensitivity matrix $\mathbf{S} = \mathbf{U}\Sigma\mathbf{V}^{\mathrm{T}}$.

In Figure 4 are represented the sensitivity matrix singular values, for the model presented in (9) and (11). The cutoff used was 10^{-6}.

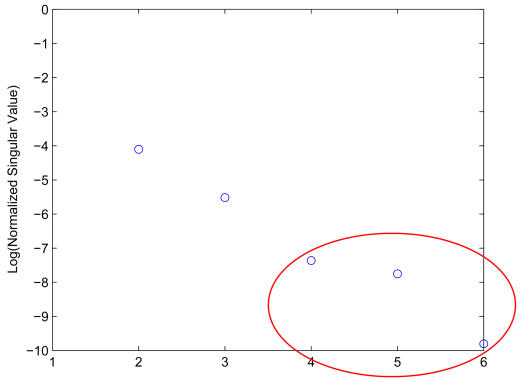


Figure 4: Normalized Singular Values of the sensitivity matrix $\mathbf{S}$, in a logarithmic scale. In red, the singular values below the cutoff value.

As it is possible to see, there are unidentifiable parameters. To assess which are these parameters, it is necessary to inspect the last columns of $\mathbf{V}$.

$$V = \begin{bmatrix} 0.0000 & -0.0005 & -0.0205 & 0.1565 & \mathbf{0.9875} & -0.0056 \\ 0.0004 & 0.9999 & -0.0172 & 0.0011 & 0.0000 & 0.0000 \\ -1.0000 & 0.0004 & 0.0000 & 0.0000 & 0.0000 & 0.0000 \\ 0.0000 & 0.0155 & 0.9219 & 0.3848 & -0.0419 & -0.0011 \\ 0.0000 & -0.0001 & -0.0024 & 0.0090 & 0.0042 & \mathbf{0.9999} \\ 0.0001 & 0.0076 & 0.3864 & \mathbf{-0.9096} & 0.1522 & 0.0085 \end{bmatrix}$$

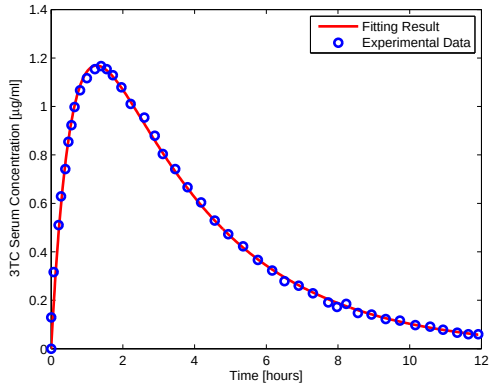
Figure 5: Matrix $\mathbf{V}$ from the SVD of $\mathbf{S}$.

Analyzing these results, it is possible to see that

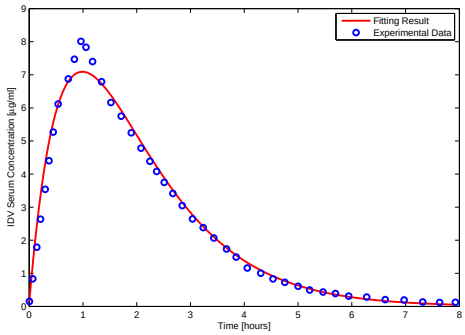
the least unidentifiable parameter is the 5^{th}, k, followed be the 1^{st}, s, and the last one, c. This happens because the highest values in the last three columns (that correspond to the three singular values below the cutoff value considered) are for these three parameters.

4 Drug Kinetic and Dynamic Models

For each of the drugs used, it is necessary to find a PK and a PD model. Using experimental data, a fitting for the model presented earlier is performed, and the results obtained are represented in Figure 6.



(a) Lamivudine (3TC)

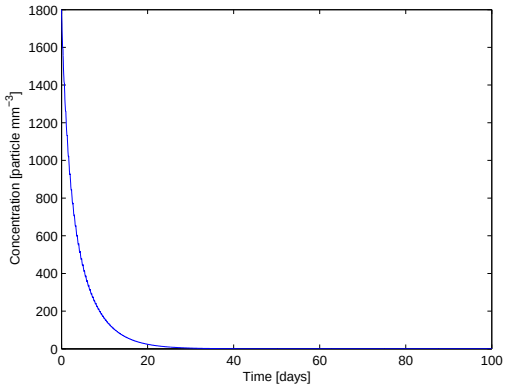


(b) Indinavir (IDV)

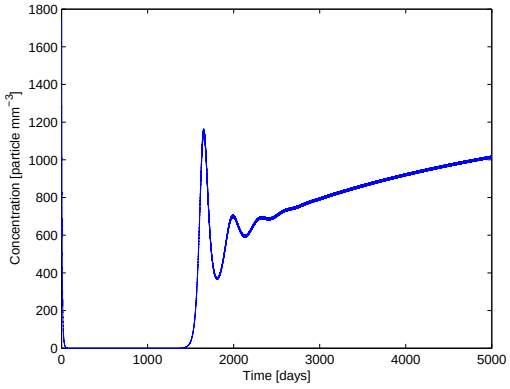
Figure 6: Pharmacokinetic data fit for both 3TC and IDV.

For the pharmacodynamic model, only one parameter is needed: C_{50}. In the case of 3TC, this value is 0.1674 mg/L [13]. For IDV, the value for C_{50} is 0.2639 mg/L [14].

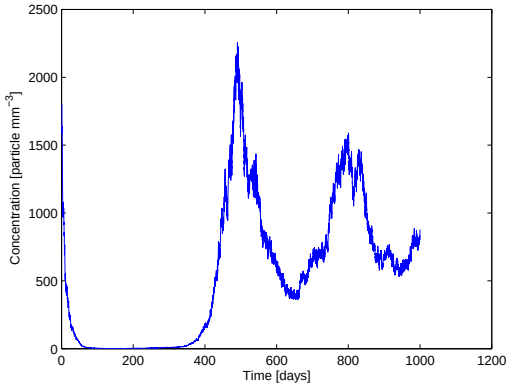
After the estimation for the PK parameters, it is possible to perform simulations using the complete model described in Section 2. Four simulations were computed, for four different experimental conditions. Results are shown in Figure 7.



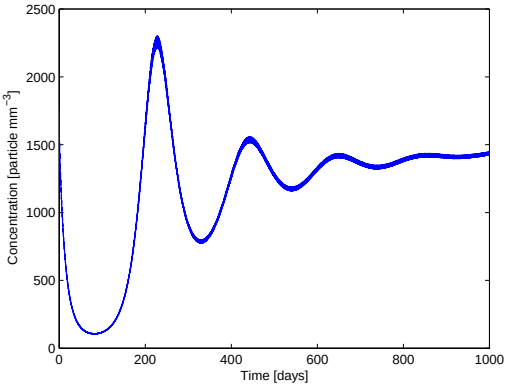
(a) Normal therapy, 100% adherence



(b) Reduce dosages, 100% adherence



(c) Normal therapy, 50% adherence



(d) Half dosage frequency, 100% adherence

Figure 7: Dynamic HIV-1 full model simulation, including drug effect, for different experimental conditions.

From the results obtained, several conclusion can be drawn. First of all, the prescribed therapy works if patient adherence is high. However, for reduced drug dosages, resistance eventually builds, and after about 5 years, the viral load rebounds, and the therapy fails. Still, the most surprising conclusion is reached when the other two simulations are compared. Even though they are equivalent in terms of drug dosage administered, the results when the pills are taken randomly are far better than when they are taken with half the frequency, but 100% adherence. This may be explained in a simple way: if the drugs are administered randomly, there is a high probability that in the beggining of the treatment, there are a few days where adherence is very high. Therefore, the virus is correctly suppressed in this period, and is almost fully cleared. At this point, the amount of drug necessary to keep it that way is smaller, justifying the fact that the random prescription works better.

5 Nonlinear Control of HIV-1 Infection Model

Control is a branch of engineering and mathematics, and consists of a mathematical study of how to manipulate the parameters affecting the behavior of a system to produce the desired or optimal outcome [15]. For the HIV-1 infection, the system to be controlled is the infection dynamic model presented earlier. This control is performed by a doctor (controller), that may adjust the treatment based on the viral load (sensor), when measurements are performed. This closed-loop mechanism is summarized in Figure 8.

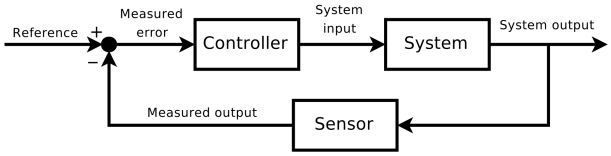


Figure 8: Closed-loop feedback of a dynamic system.

In this work, the main control objective is to maintain the viral load ν at a predefined value r. At every iteration (with each iteration representing one day), the purpose is to minimize the difference between the viral load at the next iteration and r. Additionally, another objective is set, which is to minimize the amount of drug used in the attempt of fulfilling the main objective.

Control is achieved by finding the inputs ($D_1(k)$ and $D_2(k)$) that best fit the objectives presented above. However, at any iteration k, the viral load at the next iteration $(k+1)$ is not know. Therefore, it needs to be estimated as closely as possible. In this work, a simple relation between $\nu(k+1)$, $\nu(k)$, $u_1(k)$ and $u_2(k)$ is used, given by

$$\nu(k+1) = a\nu(k) + b_1 u_1(k) + b_2 u_2(k) \tag{13}$$

This expression assumes that viral load is measured everyday, and therefore the viral load in the following day is defined as being proportional to the viral load today, as well as to the drug effects. In order to estimate a, b_1 and b_2, a Recursive Least Squares (RLS) algorithm is used [16] alongside with the control algorithm. However, the inputs that the

system allows are $D_1(k)$ and $D_2(k)$, while (13) uses the drug effects $u_1(k)$ and $u_2(k)$. Because the relationship between $D_i(k)$ and $u_i(k)$ $(i = 1, 2)$ is not simple, an approximation is used. Whenever it is necessary to compute $u_i(k)$, the PK/PD model is simulated for the drug doses $D_i(k)$, and the average drug efficacy $\overline{u}_i(k)$ is computed. Then, it is this value that is used in (13), which results in the expression

$$\nu(k+1) = a\nu(k) + b_1\overline{u}_1(k) + b_2\overline{u}_2(k) \tag{14}$$

Two control algorithms are implemented in this work. First, a control algorithm using only drug efficacies u_i (bypassing drug doses D_i) is shown. After that, a more complete model using drug doses is designed. The first one is simpler to control, and may still provide with interesting results on the levels of drug effect that are necessary to control the infection successfully. However, it is the second model that allows to control drug doses, making it very similar to real cases.

5.1 Model Control using Drug Efficacies

The first algorithm has the objective of driving the viral load to a predefined value, while minimizing the amount of drug used. Since this algorithm uses drug efficacies instead of drug doses, the cost function at iteration k is given by

$$J(k) = \frac{1}{2}\left[(\nu(k+1) - r)^2 + \rho_1 u_1(k)^2 + \rho_2 u_2(k)^2\right] \tag{15}$$

where $\nu(k+1)$ is given by 13. The evolution of the viral load for the simulation of this algorithm is represented in Figure 9.

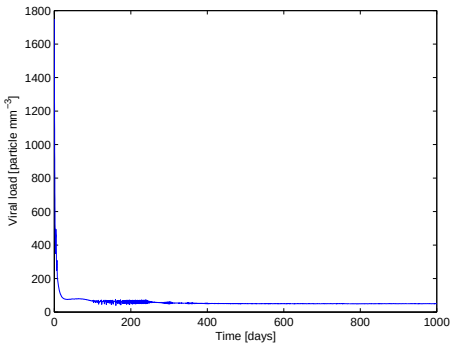
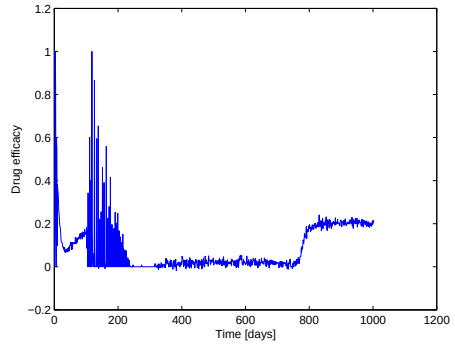
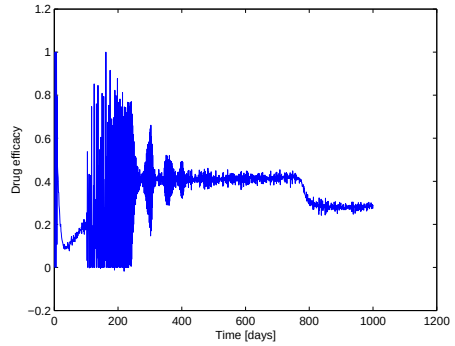


Figure 9: Viral load evolution with time, for the control algorithm comprising drug efficacies.

It is possible to see in Figure 9 that the viral load quickly drops to 50 copies/ml, and remains at that level for the rest of the simulation. This means that the main control objective is clearly achieved with this algorithm. As to the second objective, the time evolution of the drug efficacies is show in Figure 10.



(a) RTI efficacy



(b) PI efficacy

Figure 10: Drug efficacy evolution with time, for the first control algorithm.

From this results, it can be seen that the drug ef-

ficacies are, for the most of the simulation, in very low values. Even though the efficacies are high in the beginning (when the viral load is still high), they quickly drop once the infection is controlled. This shows that the amount of drugs (or at least their effect) does not need to remain high through the entire treatment. Moreover, around day 800, there is a sudden increase in the RTI efficacy, while the PI efficacy decreases. Since there is no visible change in the viral load, it can be said that the two types of drugs are, at least to some extent, complementary and interchangeable. The results obtained are satisfactory, but it is still not known how much drug is needed to maintain these efficacies, even at low levels. The next algorithm allows to address this matter further.

5.2 Model Control using Drug Doses

This simulation intends to be an upgrade of the one presented previously, because it uses drug doses as inputs, rather than drug efficacies. This represents an improvement since it is not possible for a doctor to prescribe drug efficacy. The only available input for the treatment of the HIV-1 infection are the drug doses, and therefore it only makes sense to include them in the control algorithm. Besides, when using drug efficacies, it is not possible to include the PK/PD model and the resistance development model in the simulation.

For this algorithm, the cost function is given by

$$J(k) = \frac{1}{2}\left[(\nu(k+1) - r)^2 + \rho_1 \overline{u}_1(k)^2 + \rho_2 \overline{u}_2(k)^2\right] \tag{16}$$

where $\nu(k+1)$ is given by (14).

The viral load evolution with time for this algorithm is represented in Figure 11.

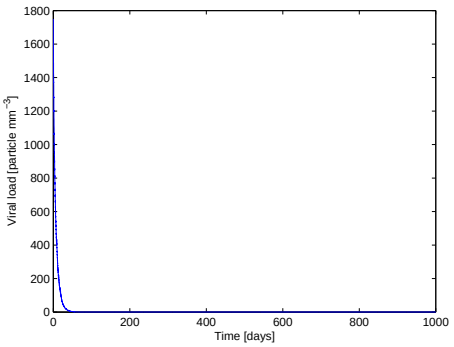
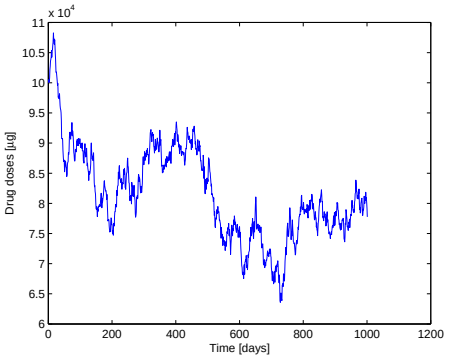
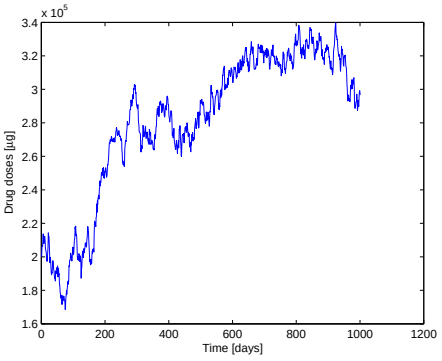


Figure 11: Viral load evolution with time, for the control algorithm comprising drug doses.

It is possible to see in Figure 9 that, like in the previous simulation, the viral load is quickly controlled, and remains at low levels for the entire simulation, meaning that the main objective is achieved. For the second control objective, it is now possible to assess the amount of drug used in this treatment. These are represented in Figure 12.



(a) RTI dose



(b) PI dose

Figure 12: Drug efficacy evolution with time, for the second control algorithm.

Analyzing the results in Figure 12, it is possible to see that drug doses as high as 110 mg (for RTI) and 340 mg (for PI) are sufficient to keep the viral load undetectable for the entire simulation. This means that drug doses lower than those currently prescribed still maintain high success rates in the infection control. However, one fact that this control algorithm does not focus on is the drug plasma concentration not falling below the resistance threshold. In fact, after the simulation is concluded, the values for C_{50}^{RTI} and C_{50}^{PI} are, respectively, 0.2128 mg/L and 0.7467 mg/L (compared to the initial values of 0.1674 mg/L and 0.2639 mg/L). This means that resistance is slowly developing due to the low drug doses, which will eventually result in treatment failure. In conclusion, the algorithm shows great potential as a tool for lowering drug dosage without compromising the treatment, but it still needs to be tuned to take into account the resistance model developed in this work, so that the antiretroviral drugs work equally effectively throughout the entire treatment.

6 Conclusions and Future Work

Throughout this dissertation, several conclusions about HIV-1 dynamics can be drawn. From the sensitivity analysis, it can be concluded that, for the studied model, the infection's most informative time period is between the 50^{th} and the 100^{th} day, and that sensitivities quickly drop to negligible values. After this analysis, a local identifiability study is performed, and a traditional approach (correlation matrix) shows that the proposed model is not identifiable. However, the proposed model for the identifiability analysis, based on SVD analysis, shows not only that the model is not identifiable, but also that the parameters which are not identifiable are k, s and c.

After the assembly of the full model, considering not only the PK+PD model, but also the adherence and resistance models, several simulations are performed using a therapy composed of two antiretroviral drugs currently available on the market. Analyzing the results from these simulations, the first conclusion that can be reached is that the currently indicated posology is effective in leading the viral load to undetectable values, and maintaining it that way, if patient adherence is 100%. However, if lower dosages are reduced, the therapy fails after approximately 5 years, due to drug resistance. Still, the most conclusive analysis performed was the one in which a model considering 50% adherence was compared with a model with 100% adherence but half the sampling frequency of drug administration. From these simulations, it is clear that the first therapy can maintain the viral load undetectable far longer than the second one.

The last part of this work consisted in the development of control algorithms that can be applied to HIV-1 dynamic models. Two algorithms are developed, one calculating the drug efficacies necessary to control the infection, and another that computes the drug doses with the same objective. The second algorithm is not only more complex, but also more realistic, since only drug doses can be controlled in real patients. Both algorithms succeed in maintaining the viral load at low levels, while minimizing the amount of drug used. The second algorithm, that considers drug doses, shows that it is possible to control the HIV-1 infection longer that three years with 3TC doses lower than 110mg, and IDV doses lower than 340mg. When compared to the currently recommended posology (300mg and 800mg, respectively), it is possible to conclude that the therapies can be greatly optimized, and drug doses reduced. However, there is one factor that is not considered in these control simulations, and that can influence the results greatly. This is the resistance modeling, that is certain to appear eventually and that will lead to therapy failure.

References

[1] F. Palella, K. Delaney, A. Moorman, M. Loveless, J. Fuhrer, G. Satten, D. Aschman, and S. Holmberg, "Declining Morbidity and Mortality among Patients with Advanced Human Immunodeficiency Virus Infection," *New England Journal of Medicine*, vol. 338, no. 13, pp. 853–860, 1998.

[2] J. M. Bailey and W. M. Haddad, "Drug dosing control in clinical pharmacology," *Control Systems, IEEE*, vol. 25, no. 2, pp. 35 – 51, april 2005.

[3] J. Pinheiro, J. M. Lemos, and S. Vinga, "Nonlinear mpc of hiv-1 infection with periodic inputs," in *50th IEEE Conference on Decision and Control and European Control Conference, to be held in Orlando, FL, USA*, december 2011.

[4] I. Craig, X. Xia, and J. Venter, "Introducing HIV/AIDS Education Into the Electrical Engineering Curriculum at the University of Pretoria," *IEEE Transactions on Education*, vol. 47, no. 1, pp. 65–73, Feb. 2004.

[5] V. Costanza, P. Rivadeneira, F. Biafore, and C. D'Attellis, "A closed-loop approach to antiretroviral therapies for HIV infection," *Biomedical Signal Processing and Control*, vol. 4, no. 2, pp. 139–148, Apr. 2009.

[6] I. Craig and X. Xia, "Can hiv/aids be controlled? applying control engineering concepts outside traditional fields," *Control Systems, IEEE*, vol. 25, no. 1, pp. 80 – 83, feb. 2005.

[7] P. Nelson and A. Perelson, "Mathematical analysis of delay differential equation models of HIV-1 infection." *Mathematical biosciences*, vol. 179, no. 1, pp. 73–94, 2002.

[8] J. Jacquez and P. Greif, "Numerical parameter identifiability and estimability: Integrating identifiability, estimability, and optimal sampling design," *Mathematical Biosciences*, vol. 77, no. 1-2, pp. 201–227, 1985.

[9] D. Zak, G. Gonye, J. Schwaber, and F. Doyle, "Importance of Input Perturbations and Stochastic Gene Expression in the Reverse Engineering of Genetic Regulatory Networks: insights from an Identifiability Analysis of an *in silico* Network." *Genome Research*, vol. 13, no. 11, pp. 2396–2405, 2003.

[10] G. Golub and W. Kahan, "Calculating the Singular Values and Pseudo-Inverse of a Matrix," *Journal of the Society for Industrial and Applied Mathematics: Series B, Numerical Analysis*, vol. 2, no. 2, pp. 205–224, 1965.

[11] S. Vinga, K. Thomaseth, J. M. Lemos, A. Neves, H. Santos, and A. T. Freitas, "Structural analysis of metabolic networks: A case study on lactococcus lactis," in *CONTROLO 2008, 8th Portuguese Conference on Automatic Control*, July 2008, pp. 566 – 571.

[12] J. Jacquez and T. Perry, "Parameter estimation: local identifiability of parameters." *The American journal of physiology*, vol. 258, no. 4, pp. E727–E736, Apr. 1990.

[13] M. Carten and H. Kessler, "Zidovudine, Lamivudine, and Abacavir," in *Reverse Transcriptase Inhibitors in HIV/AIDS Therapy*, ser. Infectious Disease. Humana Press, 2006, pp. 33–76.

[14] G. Williams and P. Sinko, "Oral Absorption of the HIV Protease Inhibitors: a current update," *Advanced Drug Delivery Reviews*, vol. 39, no. 1-3, pp. 211–238, 1999.

[15] J. Zabczyk, *Mathematical control theory: an introduction*. Birkhäuser, 2007.

[16] M. Hayes, *Statistical Digital Signal Processing and Modeling*. John Wiley & Sons, 1996.